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Dopaminergic Modulation of the Neuron Activity in the Cerebral Cortex of the Wakeful Animal

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ABSTRACT: Specific effects of the dopamine synaptic transmission modulator on the activity of sensomotor cortical neurons in a wakeful animal, performing a conditioned reflex are discussed. First, specific responses in the neocortical neurons after application of glutamate agonists and antagonists and gamma aminobutyric acid are described and then the effect of dopamine, its agonists and antagonists and amantadine, a dopamine releaser, on the background and induced pulse activities in the cortical neurons, as well as on specific characteristics of conditioned reflex motor responses, such as latency and intensity are analyzed in detail.

KEY WORDS: human cerebral cortex, dopamine synaptic transmission, cerebral cortical neuron function, dopamine agonists and antagonists, neuron activity, anesthetics, sensomotor cortex, dopaminergic innervation, latency, dopamine releaser, synaptically active substances

I. INTRODUCTION

The proposed publication discusses the specific effects of the dopamine synaptic transmission modulator on the activity of sensomotor cortical neurons in the wakeful animal, performing a conditioned reflex of putting a paw on the support. The issues of how dopamine, noradrenalin, serotonin, and acetylcholine affect the cerebral cortical neuron function in a wakeful animal as well as in humans have long been discussed by physiologists, neuropharma-

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THE LIST OF LATINIZED WORDS AND ABBREVIATIONS

ACPD	excitatory amino acid agonist of metabotropic glutamate receptors
Amantadine	dopamine releaser
AMP	excitatory amino acid, selectively interacting with central glutamate quisqualate receptors
AP-3	metabotropic glutamate antagonist
AP-4, AP-5, AP-7	NMDA receptor antagonists
Bicuculline	GABA(A) receptor antagonist
c	adenosine cyclo-3′,5′-phosphate
CNQX	kainate-quisqualate glutamate receptor antagonist
D1–D5	types of dopamine receptors
EPSP	excitatory postsynaptic potential
FS interneuron	interneuron with fast spikes
GABA	gamma aminobutyric acid
IPSP	inhibitory postsynaptic potential
L-Dopa	levodopa, dopamine precursor
MCPG	α-methyl-4-carboxy phenyl glycine, antagonist of metabotropic glutamate receptors
mGluR1, mGluR2	metabotropic glutamate receptors
MK-801	NMDA receptor antagonist
NMDA	*n*-methyl-*D*-aspartate acid
Quinpirole	D2 receptor agonist
S-4C-PG	metabotropic glutamate receptor antagonist mGluR1 and metabotropic glutamate receptor agonist mGluR2
SCH 23388	D1 receptor antagonist
SCH 23390	selective D1 receptor antagonist
S-HT	serotonin receptor
SKF38393	D1 receptor agonist
SKF83566	selective D1 receptor antagonist
Sulpiride	D2 receptor antagonist
	tetrodotoxin
VAT	vesicular amino transporter
VTA	ventral thalamic area

cologists, and practical clinicians and analyzed in numerous experiments.

Unfortunately, researchers are not always able to correctly evaluate the role of different synaptic transmission modulators. One of the main obstacles to this, in our opinion, is that they try to make

such evaluation based on research in conditions of acute experiments that are convenient for the researcher. They are experimenting on animals under general anesthesia, on brain sections or on neuron cultures, when not only the specific natural modulating associations between the nerve elements are eliminated altogether or substantially modified, but the natural relations in the brain modulating systems themselves are disturbed from the beginning.

Above all, this fact forced us to select a way for analyzing the character of modulating effects of dopamine in the brain of the wakeful animal in a chronic experiment, when this animal performs the newly acquired conditioned reflex response of putting a paw on the support, developed through series of training. Of course, to analyze the role of dopamine, naturally released in the brain of the wakeful animal, the experimenter has to evaluate, first, the effect of transmitters of excitatory and inhibitory synaptic interneuron relations on the cortical neuron activity, and only then analyze a number of dopamine agonists and antagonists in order to have an idea about the real role of natural dopamine effects and the possibilities for adjusting its activity by means of synaptically active substances. We hope that this publication will be useful for understanding the depth of the problem and for finding the ways to counteract the disturbance of dopaminergic modulation of neuron activity in the human cerebral cortex.

II. DOPAMINE AS A NEUROPHYSIOLOGY PROBLEM

The role of dopamine in the natural function of the brain has been evaluated since the time of its discovery by Carlsson[1–4], who was awarded the Nobel Prize for a series of studies analyzing the dopamine problem in 2000. This delayed appreciation of the previous studies was a confirmation of the fact that it took a time to identify the vital role of the dopamine as a neuromodulator in the brain functions and that disturbances of its activity are associated with numerous disorders, *inter alia*, schizophrenia, parkinsonism, substance addiction, and other psychiatric and neurological disor-

ders, as well as age-related changes in the performance of the central nervous system.

It is well known that glutamate supports excitatory associations, while gamma aminobutyric acid (GABA) supports blocking or inhibitory associations between neurons of various structures in the central nervous system, including the cerebral cortex; these functionally opposing types of synaptic associations are the basis of the organized activity of the central nervous system in higher animals. Depolarization of presynaptic membranes, arising spontaneously or as a result of stimulating irritation, helps the entry of calcium ions through voltage-dependent calcium channel into nerve terminals and the outflux (exocytosis) of various neurotransmitters. Thus, the glutamate accumulated in the synaptic cleft cooperates with several types of receptors, located in the postsynaptic membrane. As a result, postsynaptic ionotropic glutamate receptors, directly connected with cation-specific channels, and metabotropic glutamate receptors, connected with G proteins, are activated. According to pharmacological properties, NMDA, AMPA and kainate ionotropic receptors, as well as a group of metabotropic receptors (mGluR) are identified.

GABA is the basic inhibitory neurotransmitter in the mammals' nervous system. Fast responses to synaptic release are effected by two classes of receptors, i.e., GABA(A) and GABA(C), which represent ligand gates of the chlorine channels. Activation of these types of receptors is accompanied by hyperpolarization of the postsynaptic membrane and conditions a fast and early component of the GABA inhibitory response. The later and slower components of the inhibitory transmission are mediated by GABA(B) receptors, which inhibit adenylyl cyclase and mediate hyper-polarization of postsynaptic membranes through activation of calcium.

Beside the basic synaptic mediators, synaptic active substances, exerting modulating effects on the neuron networks, participate in the organization of the neuron structures, *inter alia*, the cerebral cortex. Primarily, they represent a group of neuromodulators, including acetylcholine, noradrenaline, serotonin, and dopamine. They clearly manifest their effects in natural physiological conditions and badly, or not always clearly, in general anesthesia, in

experiments on sections, in tissue culture when the natural medium surrounding the studied neurons is significantly modified in an artificial manner, although the experimenter tries to bring the experimental conditions close to a physiologically comfortable state. Numerous studies of synaptic plasticity in the cerebral cortex confirm that glutamate is the main excitatory transmitter of intracortical associations, modulated during training. This modulation can be induced by the glutamate, or by other synaptic transmitters and modulators, contained in respective neuron axon terminals of some subcortical structures, sending their axons to the neocortex. The excitatory associations between neurons, supported by GABA, are also modulated.

The dopaminergic system draws attention of numerous researchers, especially in the recent time, because the disturbance of its functions is related to a series of pathological disorders. For many years, it has been believed that dopaminergic associations are extensive, basically, in basal ganglions and other subcortical structures, but are very insignificant in the cerebral cortex, where they are limited by its limbic area, the prefrontal cortex. In part, this idea has been related to intensive experiments on rats, including, basically, investigations of dopamine in these structures. However, later it was found that dopaminergic projections in higher animals significantly differ from projections in rats.[5,6] Dopaminergic synaptic associations were identified also in the surface and deep layers of different neocortex areas. It turned out that the deep layers of the somatosensory cortex, for example, receive projections from the ventrolateral thalamic area (VTA) (A10), whereas the surface layers from the black matter (substantia nigra) (A9). During experimental investigations of the dopamine effect on the nervous system activity, special attention was paid to the role of the dopaminergic system in the forebrain, its nigrostriatal and mesocortical projections. The mesocortical tract begins in VTA and goes to the prefrontal cortex and neocortex. In turn, a part of neurons in the deep layers of the prefrontal cortex project fibers to VTA.

The density and layer distribution of dopaminergic fibers in different cortical regions in various species of animals substantially

differ. In rodents, they predominantly concentrate in the prefrontal cortex. In monkeys, the motor cortex has the highest density of dopaminergic fibers, whereas primary cortical receiving areas are innervated comparatively weakly. Dopaminergic fibers of the motor cortex in cats are distributed in different layers in a sufficiently even manner. The interim density of these fibers is found in the frontal, temporal and parietal cortex, where they are located in layers I–III and V–VI. In the primary somatosensory cortex, the highest density of dopaminergic terminals is also found in layer V. In the neocortex, these terminals are projected to stems of apical and basal dendrites of pyramidal neurons, their aciculae and interneuron dendrites. It is interesting to note that varix dilatations of dopaminergic fibers do not always establish synaptic contacts, though they are in close asynaptic contact with target cells.[7] There are three intraneuronal dopamine pools:

- dopamine pool, released during depolarization of the nerve terminal by exocytosis;
- vesicular pool, the place of dopamine conservation in the terminal, which does not participate in the release of the transmitter, but restores the reserves of the dopamine, released by exocytosis; and
- cytoplasmic pool in which dopamine is connected to release and reverse trapping mechanisms, involving the carrier.[8]

Following the release to the synaptic slit, a part of the neurotransmitter interacts with dopaminergic receptors and is inactivated in the extracellular space after the trapping by the nearest glial cells. A part of dopamine goes back from the synaptic slit to the neuron and is affected by intraneuronal metabolism (Fig. 1).

According to some researchers, the mesocortical tract is involved into neurophysiologic functions, such as attentiveness, working memory, reinforcement, and stress.[9] Durstewitz et al.[10] noted that physiological effects of dopamine, produced by different researchers, may vary significantly in different regions of the brain, i.e., hippocampus, striatum, and prefrontal cortex. Therefore, in studying a specific structure, the obtained facts can be, and should be, compared only with data recorded on the same object and in similar or close experimental conditions. It is assumed that when

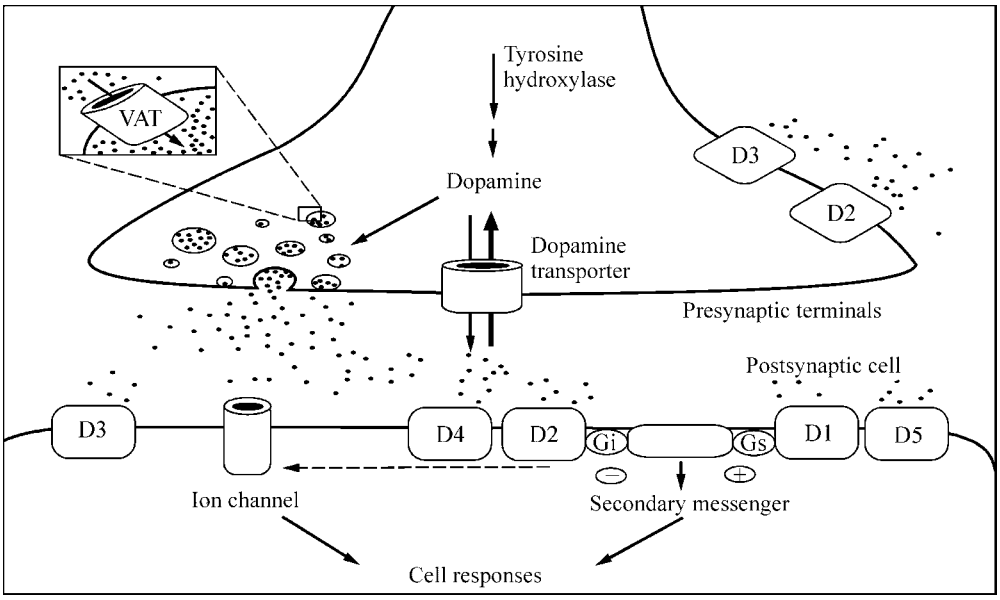


FIGURE 1. Schematic summary model of dopaminergic synapse: D1–D5 — dopamine receptor subtypes, Gi — inhibiting regulatory proteins, Gs — stimulating regulatory proteins, VAT — vesicular amino transporter.[8]

the working memory is investigated in animal behavioral reactions, it is necessary to concentrate, basically, on agonists and antagonists of D1, rather than D2 receptors. Some researchers believe that short-term effects, related to D2 receptors, possibly, perform other functions that are not related directly to retention of traces in the working memory.

In-depth analytical investigations confirm that dopamine:

- shifts the sodium current activation threshold towards a greater hyperpolarization of the membrane and changes the characteristics of slowly inactivating currents;
- reduces the slowly inactivating potassium current in pyramidal cells of the prefrontal cortex;
- reduces the duration and the amplitude of dendrite calcium spikes; and
- increases NMDA-type synaptic currents in the prefrontal cortex via D1 receptors; at the same time, AMPA-type currents are reduced both in the cortex and striatum under the impact of dopamine. The total effect of combined changes of AMPA- and NMDA-type currents leads to a reduced ampli-

tude of the excitatory postsynaptic potential (EPSP), but makes it longer. Besides, dopamine directly affects the inhibitory interneurons of the cortex. The investigations of dopaminergic regulation in the prefrontal cortex, using microdialysis, showed that systemic application of apomorphine, a mixed agonist of D1/D2 receptors, helps increase the extracellular GABA level in the prefrontal cortex, but does not affect the glycine level. The same effect is caused by application of quinpirole, a D2 receptor antagonist. This effect is blocked by sulpiride. Application of D1 agonist SKF38393 does not change the level of GABA. The above experimental data allowed Grobin and Deutch[11] to conclude that dopamine agonists increase the release of GABA in the prefrontal cortex via D2 receptors. It is assumed that changes of functions of the prefrontal cortex GABA system in schizophrenia are the result of changes in the dopamine cortex function.

A comparative study of D1 and D2 receptor distribution in the cerebral cortex of rats, cats and monkeys identified some specific features.[12] It was found that the regional density for D1 receptors is higher in cats and monkeys: it is higher in layers I–II and lower in layers III–IV; the density was average in layers V and VI. Nevertheless, the density of D1 receptors is higher than the density of D2 receptors in all cerebral cortical areas and layers in cats and monkeys and higher in most cortical regions and layers in rats. D2 receptors are denser in the surface cortical layers than in deeper layers in rats, but they are more homogeneous in different cortical layers in cats and monkeys. Mechanisms of dopaminergic effects on the cortex neurons, *inter alia*, on pyramidal neurons, in addition to direct effect, also include an activation mechanism of rapidly discharging interneurons, which cause inhibition in the prefrontal cortex in rats.[13] Thorough experimental studies showed that dopamine modulates excitability of pyramidal neurons not only directly, but also indirectly, affecting the local chains of GABAergic interneurons. Dopaminergic modulation of their function is included into the nominal properties of the prefrontal network during cognition processes. Various types of GABA interneurons,

differing both morphologically and electrophysiologically, have been investigated in layers II–V of the prefrontal cortex in rats. The experimental results, including the recording of voltage in whole cells, showed that dopamine induces reverse tetrodotoxin (TTX)-insensitive depolarization of the membrane and increases excitability of interneurons with fast spikes. Dopamine-induced depolarization of the membrane significantly decreases when D1–D5 receptor antagonist SCH 23390 is applied, but not by D2 receptor antagonist sulpiride or other antagonists. D1–D5 receptor agonists, SKF or dihydroxydine (but not D2 agonist quinpirole), also induce positive depolarization of the membrane. These facts show that dopamine polarizes rapidly discharging interneurons by inhibiting volt-independent leakage of the potassium current (via the D1/D5 receptor mechanism), and the delayed-rectification potassium current by unknown dopaminergic mechanisms. Additional inhibition of slowly inactivating potassium current leads to increase of renewed discharges in response to activation of depolarizing inputs. This increase of interneuron excitability, induced via D1 receptors, increases GABAergic transmission to the pyramidal neurons of the prefrontal cortex and can represent a mechanism by which dopamine inhibits the stable pulse activity of pyramidal neurons *in vivo*.

Bergson et al.[14] tried to evaluate regional, cellular, and subcellular variations in the distribution of specific types of dopaminergic receptors, in particular, D1 and D5 receptors. It was shown that in pyramidal neurons of the cerebral cortex, D1 receptors are located predominantly on dendrite aciculae, while D5 receptors are located directly on their stems. Anatomic segregation of D1 and D5 receptors at the subcellular level of the cortex and at the cellular level of the subcortical structures suggests that their close receptor groups are predominantly associated with different elements of the network and play different regulatory roles in synaptic transmission. Researchers emphasize that the mechanism, underlying different effects of dopamine observed in experiments, depends basically on its concentration and specific impacts of this concentration on different types of receptors. Low dopamine concentrations (<500 nm) increase inhibitory postsynaptic currents

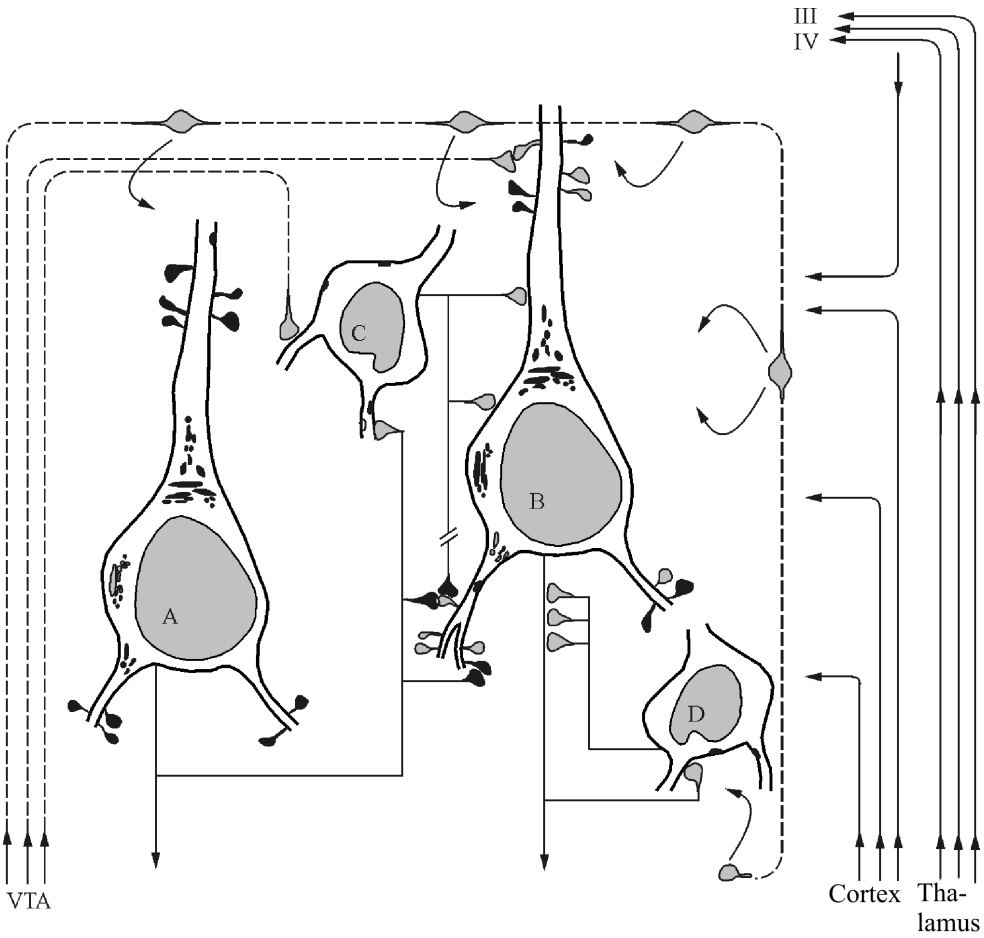


FIGURE 2. Relations between pyramidal neurons and association interneurons and the impact of dopaminergic projections via synaptic and extra-synaptic pathways.[16]

via D1 receptors, protein kinase A and adenosine cyclo-3,5-phosphate (cAMP). Its higher concentrations (>1 μm) decrease the inhibitory postsynaptic potential (IPSP) via the following loop: D2-Gi receptor growth factor — phospholipase C-IP3-Ca^{2+} dopamine and cAMP-regulated phosphoprotein-32 — protein phosphatase 1/2A-GABA(A). Blocking of any molecule in the D2 receptor loop facilitates D1-mediated increase of IPSP. Thus, the dopamine concentration, acting in both directions, can define the relative quantity of cortex inhibition and, thereby, differentially regulate the cortical network.[15] On the one hand, a low dopamine concentration acts via D1 receptors and protein kinase A, facilitating higher

release of GABA. However, it is not quite clear whether such effect is pre- or postsynaptic. On the other hand, a low dopamine concentration activates D1 receptors and enhances excitability of interneurons with fast spikes (FS neurons).

Paspalas and Goldman-Rakic[16] gave a brief review of relations between pyramidal neurons and FS association interneurons, the impact by dopaminergic projections VTA, complex and not fully explicit principles of neocortex organization (Fig. 2). For clarification, two pyramidal neurons (A and B) and two association neurons with parvalbumin, i.e., PV neurons (C and D), are illustrated. Synaptic and extra-synaptic (curved arrows) VTA dopaminergic effects are projected on pyramidal and association neurons of the cortex. Thalamic afferents cross the infra-granular cortex and end in layers IV–III. Neuron A forms axoacicular and axodendrite synapses with neurons B and C, respectively, which represents a sample of targeted expression of the presynaptic D1 heteroreceptor. Almost 80% of such axoacicular contacts can reflect both pre- and postsynaptic D1 receptors. PV interneurons ensure a synaptic input to the perisomatic region (synapses from C to B) or to the original segment of the pyramidal cell axon (synapses between neurons D and B).

Of great interest are also data on the properties and functionalities of VTA dopaminergic neurons *per se*. It was found that infusion of NMDA, kainate, carbachol in the VTA area facilitates the increase of extracellular dopamine, while application of NMDA receptor antagonist AP-5 reduces its concentration in the prefrontal cortex in rats. Increasing the quantity of extracellular dopamine in the prefrontal cortex was also recorded after infusion of bicuculline in the VTA area. These observations suggest that at the VTA level the dopaminergic neurons have tonic excitation by glutamate neurons, affecting NMDA and non-NMDA receptors, and tonic inhibition by GABA and dopamine, affecting GABA(A) and D2 receptors.

Not only the effects of impacts by dopaminergic synapses, but also the specific functioning of dopaminergic neurons per se can present some interest. A study of the ventrothalamic nucleus in monkeys analyzed the activity of 58 dopaminergic and 200

non-dopaminergic neurons.[17] Dopaminergic neurons (neurons with operation potentials over 2.5 msec, responsible for application of apomorphine) had a low background activity frequency of 1–8 pulse/sec. Non-dopaminergic neurons recorded in the same area had the background activity frequency of 10–30 pulse/sec. Two-thirds of all neurons responded to pressing by a touch stimulus; however, dopaminergic neurons responded, basically, by a tonic change of the frequency, while non-dopaminergic neurons had phase responses. Fifteen neurons (5%) had phase responses even when the hand was stretched towards the touch stimulus. Among 124 neurons the following responses were noted: 88 by excitation, and 36 by inhibition. When pressed by a touch stimulus, 91 neurons responded: 32 by excitation and 59 by inhibition. When food was added, 33% of neurons activated. Most of the responses to a touch stimulus (84/124, or 68%) were tonic during the entire period of touching, without correction to every pressing. During this activity, 14 neurons (14/124, or 11%) manifested a specific variation: a short-term early response to the touch was intermittent with stable tonic responses. Application of apomorphine reduced the pulse activity in the first 5–15 min, and then increased this activity.

In general, VTA neurons manifested mostly stable tonic responses, and some of them manifested specific phase responses. They were involved not only in motor responses, but also in changes related to motivations. Neuron responses were excitatory during refeeding, but were depressed during or after food was given. It is assumed that this modulation of pulse activity of dopaminergic neurons may be important for the beginning and continuation of motions and/or motivated behavior. Specific features of VTA neuron activation are discussed in more detail in special studies. For example, Georges and Aston-Jones[18] demonstrated that when these neurons were activated by stimulation of ventromedial and ventrolateral aspects bed nucleus of the stria terminals (vBNST), 84.8% of neurons activated synaptically in response to a single pulse. Such stimulation did not affect the activity of presumed inhibitory VTA neurons. The authors noted three features of dopaminergic VTA neurons: their extracellular action potentials had the shape of two- and three- phase

oscillations with the mean duration of 3 msec and the mean frequency of 4.2 pulse/sec. Application of dopaminergic receptor agonist, apomorphine, inhibited spontaneous pulse activity of these neurons, whereas the subsequent application of D2 receptor antagonist, eticlopride, recovered this activity. In response to vBNST stimulation, 65 of 118 neurons activated synaptically with the latency of less than 25 msec, while the rest had the latency over 65 msec. Among neurons, responding with a higher latency, 29 cells manifested the initial inhibition. Extracellular recording of VTA neuron activation in a wakeful animal shows that dopamine inhibits neurons in the prefrontal cortex, although recent biophysical *in vitro* investigations indicated that dopamine increased their excitation for a long period of time. Besides, though dopaminergic neurons, undoubtedly, code the stimulus arousal by a temporal change in the pulse frequency, the time properties of the dopaminergic signal in the prefrontal cortex, related to different behaviors, are often unusually protracted.

Investigations of activity of mesocortical dopaminergic neurons *in vivo* show that the mesocortical system causes fast postsynaptic responses in the prefrontal cortex that are mediated by non-dopaminergic neutrons; such responses are, probably, initiated by glutamate. And, vice versa, brief stimulations of mesocortical dopaminergic neurons, causing short-term (<4 sec) release of dopamine, also facilitate the reduction of spontaneous discharges (matching with *in vitro* recording) and form dopamine-induced potentiation at which the caused discharge is enhanced for a period of tens of minutes.

Lavin et al.[19] believe that the system can transmit fast signals about the presence or absence of reinforcement via glutamate, while dopamine-mediated modulation of pulse activity affects the long-term dynamics of the prefrontal cortex neuron network. Some researchers attempted to define the impact of VTA neuron activation on the pyramidal neurons of the prefrontal cortex in rats in conditions of an acute experiment. They tried to identify whether stimulation of VTA, the source of dopaminergic innervation of the prefrontal cortex, can cause various responses in it, depending on the level of the membrane potential, which

pyramidal neurons have when recorded *in vivo*, and whether VTA stimulation can perform the controlling role of transmission between these states. It was found that the VTA electrical stimulation led to different effects, in many cases depending on the level of the membrane potential. A series of stimuli resembling a burst of discharges caused a protracted depolarization. Its effect was blocked by D1 antagonist and simulated by VTA chemical stimulation. The investigation results showed that projections from VTA to the prefrontal cortex may be involved in the controlling of the membrane potential states, which define the pyramidal neuron ensembles in this specific area.

Dopaminergic receptors interface with G protein, via which the intracellular signal is transmitted. The receptor represents a polypeptide of seven transmembrane domains with a cellular N end and a cytoplasmic C end. Connection of the ligand molecules with the receptor leads to activation of the effector system via G protein and formation of second messengers with the subsequent transmission of the intracellular signal. Kababian and Calne[20] classified dopaminergic receptors as types D1 (D1, D5) and D2 (D2–D4), depending on their positive or negative connection to adenylate cyclase. It is important that the first group of receptors, as mentioned above, is activated by micromolar dopamine concentrations and the second by nanomolar dopamine concentrations. In such case, D1-type receptors are associated to Gs protein and activate adenylate cyclase, while D2-type receptors are associated to Gi/o protein and, on the contrary, inhibit background activity frequency.

The objects (targets) of dopaminergic innervation in the cortex of rats, monkeys, and cats are both interneurons and pyramidal neurons. Both types of neurons may express various types of receptors, which provides for the different dopaminergic modulations of the cortical neuron activity. D1 receptors were found in all cortex layers in these animals. However, in rats, they are distributed more or less uniformly, and in cats and monkeys they are distributed non-uniformly, sometime with a three-layer concentration: the highest in layers I–II, the lowest in layers III–IV and the interim in layers V–VI. At the cell level, D1 receptors are

located on the soma, apical dendrites, especially on aciculae, on axon terminals of pyramidal neurons, as well as on distal dendrites and presynaptic terminals of GABA interneurons. D1 receptors manifest an expressed affinity of connection to benzodiazepines SCH 23390, SCH 23388, and SKF38393.

A high density of D2 receptors is found in the black matter, VTA, as well as in some cortex areas, the prefrontal and cingular areas. In the mammal's motor cortex they are concentrated in layer V, where, according to some researchers, they exceed the quantity of D1 receptors. D2 receptors are most often located on the soma of pyramidal cells and interneurons. A high density of D3 receptors, localized predominantly in a presynaptic manner, is also found in somatosensory and prefrontal cortex. D4 receptors with a similar structure are located in the frontal cortex, basically in the postsynaptic manner.

The cortex structures expressing D2 receptors are considered to be important locus for antipsychotic agents. Combining immunohistochemical methods with electron microscopy, Negeessy and Goldman-Rakic[16] investigated the ultra-structural localization of D2 receptors in the prefrontal cortex of primates and compared it with the ultra-structural localization of the neuron calcium sensor 1 (NCS-1), a neuron-specific, calcium-bound and D2-interacting protein. D2 pulse reactivity, defined by means of immune peroxidase in individual samples, localizes on cell bodies with ultra-structural characteristics and neurons, and astrogliae. D2 receptors localize in the pre- and postsynaptic structures, including directly dendrites and their aciculae, as well as on excitatory and inhibitory axon terminals. Immunoreactivity of the neuron calcium sensor 1 (NCS-1) was intensive in the pre- and postsynaptic structures, being localized together with D2 receptors. It was observed in 10% of D2 immunopositive aciculae and, to a smaller degree, in immunopositive dendrites. According to the authors, these facts prompt that the role of NCS-1 is to desensitize D2 receptors in the prefrontal cortex.

Based on immunohistochemical and electron microscopy investigations of the cortex in primates, Bergson et al.[14] presumed that dopaminergic fibers, together with glutamate axon terminals form

synaptic triads on dendrite aciculae of pyramidal neurons. Therefore, activation of the former can activate or inhibit excitatory synaptic inputs to pyramidal neurons.

Zhou and Hablitz[21] showed that dopamine modulates the properties of both synapses and the interneuron membrane of the cerebral cortex in rats. Different opinions were offered about the way dopamine modulates excitation of cortical neurons. It was assumed that dopaminergic innervation of dendrite aciculae regulates glutamatergic inputs to pyramidal neurons; however, no experiments have been made to check this idea. Recording neuron activity by visual control, the authors showed that dopamine increases excitability of the inhibitory neuron, but decreases excitability of the pyramidal cell due to depolarization in the first case and hyperpolarization in the second. Accordingly, it also increases the frequency and the amplitude of spontaneous IPSP (sIPSP). When a blocker of the TTX sodium channels is present, dopamine does not affect the frequency, amplitude and kinetics of the miniature IPSP (mIPSP) and the excitatory postsynaptic currents in inhibitory interneurons or pyramidal cells. These results, probably, indicate that dopamine can directly excite cortical interneurons. Unfortunately, dopaminergic gates that would regulate spontaneous release of GABA and glutamate or the properties of postsynaptic GABA and glutamate receptors directly have not been found. The results of investigation of modulation of the membrane and synaptic properties of cortex interneurons in rats[16] showed that dopamine directly excites inhibitory neurons and that this leads to reduced excitability of cortical pyramidal neurons.

Mechanisms underlying numerous dopamine effects on excitability of layer V neurons of the prefrontal cortex in rats were also investigated by Gulledge and Jaffe.[22] It was found that in the control it reduced the output membrane resistance and inhibited generation of the action potential.

When the GABA(A) receptor antagonist, bicuculline, is applied, the dopamine-induced depression of the action potential generation is reduced, manifesting a delayed increase of excitability that is preserved during the recording up to 20 min, till the dopamine is washed. As opposed to disinhibition of the pulse generation

process, the input resistance of the neuron membrane under the impact of dopamine does not decrease, which, probably, testifies to its independent impact on the pulse generation and the input resistance of the cell membrane. Since dopamine increases the frequency of spontaneous inhibitory postsynaptic currents, when TTX is absent or present, the GABAergic mechanism and, according to the hypothesis, dopamine reduce the excitatory output of the pyramidal cell on other neurons. Besides, the focal application of GABA in the perisomatic region of the neuron simulates the inhibitory effect of dopamine on the pulse activity, irrespective of the value of the neuron input resistance.

Dopamine regulates excitability of neurons, located at the output of layers V–VI of the prefrontal cortex, including those that give projection to *n*. accumbens. Dopamine or D1 receptor agonist SKF38393 (but not the D2 receptor agonist quinpirole) reduces the latency and the threshold of neuron discharges in the prefrontal cortex in response to depolarization current surge. Besides, D1 receptor stimulation weakens high-threshold calcium currents, appearing mostly in apical neuron dendrites of this region. Stimulation of D1 receptors in the prefrontal cortex neurons limits the effects of inputs to epical dendrites of these neurons.

In their work, Seamans et al.[23] showed the bidirectional dopaminergic modulation of GABAergic inhibition in the cortical pyramidal neurons. In this study, the authors used methods of voltage recording on cells *in vitro* to characterize dopamine effects. It was found that in the majority of pyramidal neurons it caused a temporary IPSP-produced two-phase effect, thereby facilitating a sharp decrease of its amplitude followed by a delayed increase. Using specific agonists and antagonists of different receptor subtypes, it was found that the initial sharp decrease of the IPSP amplitude was mediated by D2 receptors, while the subsequent slow increase of its amplitude, by D1 receptors. The linearly combined effects of the two agonists could reproduce the two-phase dopamine effect. This is explained by the fact that D1 receptors increased sIPSP, but did not affect mIPSP. Probably, it caused a high IPSP, increasing the inner excitability of interneurons and their axons. And, vice versa, D2 agonist did not affect sIPSP,

but caused a significant reduction of mIPSP. This led to a reduced probability of GABA release. In addition, D2 agonists reduced postsynaptic responses to GABA(A) agonist.

Thus, D1 and D2 receptors regulate GABAergic activity in the opposite direction by means of various mechanisms of pyramidal neurons in the prefrontal cortex. It is interesting to note that the two-phase effect of dopamine effect is manifested not only on neurons, but also on glial cells. It cannot be ruled out that explanation of this phenomenon is related to different sensitivities of the two groups of dopaminergic receptors to the dopamine concentration. However, it is also true that, acting via one and the same receptor, dopamine may cause a bidirectional modulation via different intercellular loops. According to some researchers, dopamine ensures a dynamic bidirectional switching of dendrite calcium potentials due to activation of protein kinase A and protein kinase C.[24]

Henze et al.[25] studied modulation of neuron networks in the dorsomedial prefrontal cortex in rats, which is assumed to play an important role in information processes during activation of the working memory. They analyzed the properties of layer III neurons in field 46 of a toque, using a patch clamp on neurons in the prefrontal cortex sections. All recorded neurons manifested a regular pulse activity coinciding with a pyramidal neuron activity. It was shown that dopamine did not affect noticeably the steady-state membrane potential and the input neuron resistance; however, in 0.5 μM concentration it increased excitability of the cells in response to the depolarization current surge, injected in the soma. An increase of excitability has been related to a hyper-polarization shift in the activity potential threshold and the shortening of the first inter-spike interval. Such effects are needed for activation of D1-type, but not D2-type receptors, since they were inhibited by the D1 receptor antagonist SCH 23390, though changed insignificantly by D2 antagonist. These results show that dopamine modulates the activity of pyramidal neurons and enhances their response to the excitation current. It is known that both excitatory and inhibitory interneurons can be found among neurons that have direct synaptic associations with the studied

neuron and whose bodies are located near such pyramidal neuron. At the same time, it is established that the ratio between inhibitory and excitatory synapses on the body of the pyramidal neuron makes about 4:1.[26] Dopaminergic terminals, leading from the mesencephalon structures,[17,27] form predominantly (87%) symmetrical synapses on apical dendrites of cortical pyramidal neurons.[7,28]

It is believed that substances applied through ionophoresis near the body of the pyramidal cell cannot propagate by more than 200–300 μm from the cell body at a regular current intensity. It can be assumed that the functional structure is comprised of two interneurons, causing excitation or inhibition of the inhibitory interneuron and a direct inhibition of the pyramidal neuron. Bicuculline, blocking GABA release, also blocks the effects of the excitatory metabotropic receptors on the inhibitory neurons, excited by application of α-methyl-4-carboxyphenyl glycine (MCPG). In this case, the inhibitory effect of MCPG is lost. Thus, bicuculline blocks the effect on inhibitory interneurons on the activity of pyramidal neurons directly. It is known that dopamine can increase excitability of the excitatory interneurons just as it enhances excitability of interneurons in the surface neocortex layers.[21] However, dopamine can also depress inhibitory interneurons directly as a result of presynaptic depolarization and depression of metabotropic excitatory effects, thus decreasing their background and induced activities, whereas the pulse activity of pyramidal neurons increases.

The literature sources contain references to the localization of dopaminergic synapses on the bodies of cortical interneurons.[29,30] As opposed to predominantly [3H]raclopride binding in layer V, the bilaminar character of [3H]SCH binding has been observed in the majority of cytoarchitectonic fields with the highest concentration in supragranular layers I–III and infragranular layers V and VI. In human neocortex, 60% of dopaminergic synapses contact dendrite aciculae and only 40% contact directly the dendrite stems. It was mentioned before that these synapses are, as a rule, symmetrical (type II according to Grey) and only 13% of them are asymmetrical.[7] It is shown that D1 receptors are present not only on pyramidal neuron dendrites, but also on parvalbumin and

calretinin-containing interneurons.[31] From other sources[30] it is known that in the medial prefrontal cortex in rats the highest density of dopaminergic receptors is found in layers V–VI on neuron bodies. However, the joint localization of D1 and D2 receptors on the same neurons can be seen only in 25% of cases. Cells that have only D1 receptors normally are not pyramidal neurons. Cells that have only D2 receptors are big interneurons and small pyramidal neurons.[28,31]

Electrical stimulation of the black substance and VTA causes responses of the EPSP–IPSP types in the studied cortical neurons. Only 50% of intracellular tested neurons of the frontal cortex in rats respond to application of dopamine. Experiments on brain sections with application of this substance with intracellular leads from individual neurons showed no direct excitatory effect on the membrane potential level and synaptic potentials.

In selecting the animal species for experiments, it is necessary to consider the specific features of comparative distribution of D1 and D2 receptors in the cerebral cortex of rats, cats, and monkeys. Special investigations[12] showed the following features. The regional density of D1 receptors was higher in cats and monkeys: it was higher in layers I–II, lower in layers III–IV, and mean in layers V and VI. The density of D1 receptors is higher than the density of D2 receptors, in all regions and layers of the cortex in cats, and monkeys. D2 receptors localize in all layers of the brain cortex in rats, cats and monkeys and are highly homogeneous in the regional distribution. The layer-by-layer distribution is comparable for all three species of the animals; however, these receptors have a higher density in the surface cortical layers in rats and are more homogeneous in different cortical layers in cats and monkeys.

It should be noted that, in addition to a direct synaptic effect of dopamine on pyramidal and association neuron, excitatory and inhibitory neurons, some researchers[32] assume possible depolarization of the neurons in the prefrontal cortex without synopsis, for example, under the effect of dopamine. This conclusion was based on the fact that in experiments on sections the depolarization effect of dopamine could not be blocked by alpha and beta antagonists of phentolamine and alprenolon. The antipsychotic drug clozapine

did not have any effect as well. Therefore, the authors assumed that the dopamine-induced depolarization of the neuron was, possibly, mediated by nonspecific mechanisms too.

Initially, it was reported that exogenous dopamine had a depressive effect. As a result, some authors believed that it was an inhibitory transmitter. According to current ideas, the effect of dopamine on neuron activity is assessed as modulating effect. By definition, the modulator should not change the basic neuron activity, but it can weaken or potentiate responses caused by other synaptically active substances. Some research groups showed *in vivo* and *in vitro* that dopamine, released synaptically by stimulating VTA or applied by ionophoresis, depresses glutamatergic synaptic inputs and glutamate-induced excitability. In the case of joint application with glutamate or GABA in very small ionophoretic doses, dopamine potentiates glutamate excitation in a series of subcortical structures and in the somatosensory cortex.[33] However, it is known that the latency and the number of spikes in response to depolarization, caused by a current surge, vary when affecting dopamine receptors in the premotor cortex receptors due to effect on calcium currents.[30] The effect of dopamine agonists and antagonists on the background and caused activities of the neocortex neurons has also been demonstrated in experiments on intact brain neurons in studying callose responses,[34] investigating the conditioned reflex, and analyzing delayed responses of premotor cortex neurons.[35] In one of the works[36] we suggested that dopamine effects on cortex neurons are the result of its modulating effect on Gi proteins and interference into the operation of glutamate metabotropic receptors. Trying to confirm this point of view, we initiated this study in which we tried to investigate, in a more systemic and thorough way, the interaction between glutamate and dopamine agonists and antagonists, identify the effect of such interaction on the background and induced activities in the sensomotor cortex activity in a wakeful animal when it performed a real function, i.e., an instrumental conditioned reflex to a sound.

It should be noted that the majority of investigations, devoted to analysis of the dopamine role in the central nervous system and the brain cortex, were made on animals in acute experiments

under general anesthesia or on sections. Many researchers obtained a huge, very interesting and important material. Nevertheless, facts noted in acute experiments do not always clarify the function of the dopaminergic system in natural physiological conditions.

Many works analyze the role of the glutamate synaptic transmission in plastic changes of interneuron connections in training processes. However, this has recently more often pertained to such training models as a protracted postsynaptic depression. These phenomena are considered as the components required for changing the nervous system function in the training process, for consolidating active or weakening inactive neuron connections. It is believed that further alleviation requires calcium ions entering the cell, mediated by NMDA receptors. On the contrary, homosynaptic depression, induced by low-frequency tetanus in hippocampus *in vitro*, is related to a moderate activation of these receptors and a moderate influx of calcium. Therefore, now researchers discuss, in particular, the role of postsynaptic calcium in the formation of engrams during specific training tasks. Much less is known about specific features of activation of glutamate connections during real operation of brain cortex neurons, related to natural plastic reconstruction, for example, when a conditioned reflex is developed and applied.

In our opinion, such a natural object, suitable for analysis of reconstruction in receptor systems, related to training, can be a model of motor response to a conditioned signal with retention of the real natural function of the neocortex and simultaneous recording of the pulse neuron activity before, during and after ionophoretic application of one active substance or a combination of several synaptically active substances. Some studies have shown that dopamine increases the amplitude of EPSP in the prefrontal cortex in rats. In such case, EPSPs caused by activation of AMPA and NMDA receptors increase. It is believed that this effect is conditioned by activation of D1 receptors and postsynaptic calcium-dependent mechanisms. Peptide inhibitors, protein kinase A or Ca MKII, block the alleviating effect of dopamine. It is suggested that EPSP increase can be related to a signal loop, involving calcium, protein kinase A and Ca MKII.

III. EXPERIMENTAL METHODS

For real assessment of the dopamine role in the brain activity, given the available valuable data on its impact on the brain structures produced in acute experiments, it was necessary to bring experiments very close to the natural and physiologically adequate animal behavior, when a specific natural functions was used and it was possible to identify to which extent the changes in the neuron activity were accompanied by functional changes.

In the book "Assays on Higher Nervous Activity", published in 1977, Asratyan[37] wrote: "We believe that experiments using electrophysiological methods to study conditioned reflexes must be made in ordinary or traditional conditions: a relative isolation of the experimental animal, objective observations and recording of conditioned reflexes in their natural effector manifestation, a relative freedom of movement in the experimental machine (provided this does not recode the studied reflexes), and without the use of sleeping and immobilizing neurotropic drugs". This statement of the outstanding researcher in physiology of higher nervous activity is very topical today. Nowadays, when neurophysiologic mechanisms of higher integrative brain functions are studied, specific difficulties occur, above all, because most neurophysiologists wish to investigate in-depth cellular and molecular mechanisms of an individual neuron activity. This is, of course, very important; however, we should not forget that numerous properties of specific features which can be found and investigated in individual neurons are not always used to perform specific physiological functions, or, if they are used, then in such functional combinations that cannot be reproduced in tissue culture, in brain section preparations or in an animal under general anesthetics.

One of the proofs of this statement can be the experiments investigating the role of individual synaptic systems in plastic reconstructions of the neuron network in the training process with a protracted synaptic potentiation and depression.

What real training model and what organization of the function can be talked about, if we make experiments on sections,

are ruled out? Approximately the same fictitious effect can be obtained by a researcher studying a given phenomenon on an animal under general anesthetics, when the effects of reticular formation are switched off or significantly depressed and all extrathalamic projections in the neocortex are substantially modified in terms of their functions or they stop their modulating effect on the cortical neuron activity. Of course, experimenters know this all too well; however, many of them believe that modulating effects are called "modulating", because their effects do not have a crucial significance for operation of the cortical structures, though physiology of the brain, as well as clinic of many disorders related to disturbance of the modulating brain systems provide reach materials, confirming the importance of extrathalamic modulating effects.

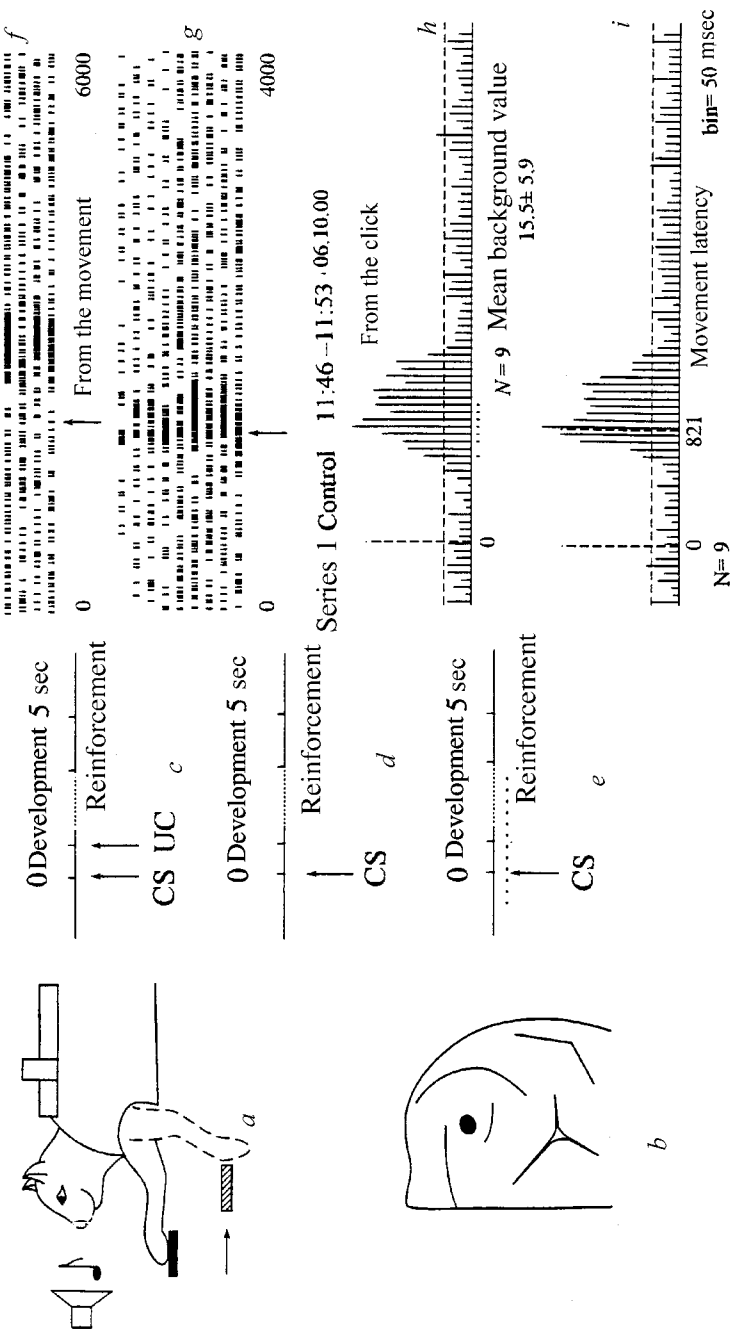
Significant advantages of the recording and analysis of neuron activity in a wakeful animal have been stressed by many researchers. Thus, Goldman-Rakic, a well-known researcher of the dopamine role in the brain operation, highly assessed the method of recoding the pulse activity of individual neurons in a wakeful animal during the performance of a behavioral task.[38] In her opinion, this method brings us very efficiently to understanding the neuron ground of the behavior. As opposed to a cellular analysis *in vitro* on sections, experiments *in vivo* allow a close direct correlation of cell activity and natural physiologic processes, because they are combined by a behavioral response. Since brain mechanisms are studied in natural conditions, the conclusions on the dynamic ground of the information process are direct and straightforward. The limitations of such approximation include the impossibility to unambiguously identify the investigated neuron. However, a number of factors and techniques allow the experimenter, even in a chronic experiment, to assess the localization, functional features and the belonging of the neuron to pyramidal neurons, and, sometimes, to state that we record activity of a pyramidal neuron, directly involved in the performance of a specific motor conditioned reflex response.

In our experiments, we first trained animals to sit in a hammock with loosely hanging forelimbs and an unfixed head. Then, we developed an instrumental conditioned reflex to put a paw on

the support in response to a sound signal (a click 4 msec, 60 dB), followed by food reinforcement. It should be noted that this type of conditioned reflex response is investigated and used as a methodological technique by a number of domestic and foreign laboratories. A conditioned signal (a click) with a 2-sec in-advance start (lead) of the oscillograph beam was send automatically by a computer program. Unconditional irritation, by touching the dorsal surface of the front paw, which caused a response of putting a paw on the support, was performed by the experimenter manually, 1–1.5 sec after the click. Following the motion, food reinforcement was also provided manually, after 2–3 sec. It required up to 50 combinations of sound irritations and touches to make the animal perform the response by putting a paw on the support after a click, without waiting for a touch to the paw. Several days of training were required to reinforce the response. The latent period for these conditioned motor responses in experimental animals varies from 0.4 to 1.5 sec, depending on the individual features of the animal, its satiation and in some cases, as shown below, on the character of the applied substance. Termination of the reinforcement rapidly led to the dying of the reflex (Fig. 3).

Following the development of a conditioned reflex with general anesthesia by nembutal, 50 mg/kg of body weight, intraperitoneal surgery was made. In sterile conditions, skin was cut over the frontal bone, in the area above the sensomotor cortex, where the muscle sensitivity projection goes to the motor cortex.[39–44] A 10-mm hole was drilled by a cutter; the underlying area of the pachymeninx was removed above the sensomotor cortex in the area of the forelimb muscle projection and pyramidal neurons, involved in the initiation of the response of putting a paw on the support. A metal cylinder with a plastic plug was screwed into the hole. Then, 5–10 days after the surgery, the cat was placed into a hammock where it had been trained before, and the plug was replaced by a guiding jugular cannula of a micro-manipulator with a multi-channel glass microelectrode.

One of the microelectrode channels with 5–7 MΩ resistance, filled with 3 M NaCl, was used for extracellular lead of pulse activity from the sensomotor cortex neurons between the pit and


From the click
0 Development 5 sec
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f
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Reinforcement
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0
4000
g
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0 Development 5 sec
Reinforcement
CS
e
N = 9 Mean background value
15.5± 5.9
h
0
821
Movement latency
821± 32
bin= 50 msec
N= 9
i
a
b

FIGURE 3.

the cruciform sulcus (6–8 mm from the sagittal line, on the border between fields 3 and 4), which corresponded to the nerve projection of the contralateral forelimb.[40,41,43] The other two microelectrode channels were filled with synaptically active substance solutions. Afterwards, they were used for microionophoretic application. Substances manufactured by the company "Sigma" were used; these substances were dissolved in distilled water adding, as required, NaOH or Cl: amantadine (10 mM, pH 5.0), dopamine (100 mM, pH 4.0), sulpiride (150 mM, pH 5.0), quinpirole (5 mM, pH 5.0), SCH 23390 (10 mM, pH 4.0), SKF38393 (10 mM, pH 4.0), SKF83566 (10 mM, pH 4.0), GABA (0.5 M, pH 4.0), bicuculline (10 mM, pH 5.0), glutamate (0.5 M, pH 7.4), glutamate transmission agonists and antagonists: ACPD (5 mM, pH 5.0), AMPA (10 mM, pH 5.0), NMDA (20 mM, pH 8.0), CNQX (10 mM, pH 8.0), S-4C-PG (10 mM, pH 10.0), A-4 (10 mM, pH 8.0), AP-7 (20 mM, pH 8.0), MCPG (10 mM, pH 10.0), and MK801 (4 mM, pH 8.0).

Ionophoresis was made using 10–20-nA currents, while the maintenance resistance current was 5–10 nA. The pulse activity of individual neurons in each case was recorded 2 sec before the transmission of a conditional stimulus and then for 30 sec after the

FIGURE 3. Schematic diagram of the conditioned reflex development and recording and initial processing of the experimental material: a) location of the animal in the hammock during development of the conditioned reflex and the experiment; b) the neuron activity lead area; c) scheme of presentation of the conditioned stimulus (CS) and unconditioned stimulus (US) and reinforcement for development of reflex; d) combination of the conditioned stimulus and reinforcement during the experiment; e) the dotted line shows the time of microionophoretic application; f) point distribution of pulse activity during application of a sound stimulus, designated by the arrow; g) the same implementations with the movement start designated by the arrow, which allows evaluating the time the pulse response of a specific neuron can lead, coincide with, or delayed in relation to the start of the conditioned reflex motor response; h) the post-stimulus histogram, correspondent to the pulse distribution in *f* (zero point in the application of the stimulus); *n* — number of implementations; i) the same implementations, as in *g*: the first dashed vertical line indicates the zero point in supply of the sound stimulus; the second vertical line is located in the starting point of the conditioned reflex movement. The histogram shows that the neuron response started 20 msec before the movement (each bin in histogram is 50 msec). The horizontal dotted line corresponds to the mean background value $\pm 2\delta$.

stimulus. As a rule, subsequent analysis was made using only parts of the records, beginning 2 sec before and ending 4 sec after the stimulus application. Ionophoretic application began 1 h before the conditional stimulus and continued for 4 sec in total. In one experiment, effects of two synaptically active substances, applied jointly or individually, were monitored. Normally, the researchers studied series of responses of the same neuron, composed of 10 implementations with 50–60-sec intervals between the implementations, which totally took 10–15 min for one series. After a series of applications was over, a 10-min brake was made; then the character of the same neuron response was verified in the control, and only after that the effects of application of the next synaptically active substance or a combined action of two substances on the same neuron were studied. It should be underlined that all 6–8 series of responses in one experiment belonged to one and the same neuron. If during the experiment the neuron was lost and a new neuron was found, then a new experiment was organized.

Data on the pulse activity of neurons, the time of the sound stimulus, and the beginning of the conditioned reflex movement, as well as the time of ionophoretic application were entered into the computer. The initial analysis of the induced pulse activity (summing of 10 implementation data) in relation to the background activity level, the construction of peristimulus histograms and histograms built in relation to the beginning of the conditioned reflex movement were made using two special software developed by our programmers Batluk and Potyagailo. Further analysis of the materials was made using standard computer software Exel, Origin, and Sigma Plot.

Some specific features of experimental methods and principles of the initial statistical estimation of the experimental data are illustrated in Figure 3.

In assessing methodological prerequisites and possibilities of this investigation, we should emphasize the following specific features. Firstly, in a chronic experiment the animal, whose movements are significantly limited for 1.5–2 h, performs a series of motor reactions: it places a paw on the support in response to a sound click; it can volitionally move its head when it receives,

chews and swallows "refeeding". In this case, it is very important that all such movements should not displace the tips of the microelectrode in relation to the recorded neuron. It is known that large cortical pyramidal neurons have a comparatively big external electrical field of 100–150 μm in radius, while small association neurons have a field of not more than 30–40 μm. In the first case, the tip of the microelectrode can be located at a significant distance away from the cell body, which gives a better chance for a long-term recording of the pulse activity, despite the motor response of the animal related to the putting of a paw on the support and its movements during chewing and swallowing of food. Therefore, it is correct to find neurons in deep cortical layers (1.5–2.0 mm from the surface), where bodies of large pyramidal neurons are located. It is assumed that a long-term recorded neuron is always a pyramidal neuron, and our experiment showed that in about one-third of all cases this was a pyramidal tract neuron. This is proved, in particular, by the fact that in one-third of recorded neurons the pulse response leads the beginning of the conditioned reflex movement by 150 msec or more. Besides, when specific synaptically active substances are applied, some of the neurons that in the control respond only after the beginning of the movement responded much in advance of the movement; hence, it was a neuron that initially did not participate in a specific movement but later involved into the motor response. Of course, limiting the recording of activity by only large pyramidal neurons, the experimenter loses a lot of valuable information about the performance of the neuron network in the investigated cerebral cortex. However, because the recorded pyramidal neurons are located at the system exit, the researcher can, nevertheless, obtain data on the function of a specific neocortical area.

Observations of other authors also confirm that neurons of the motor cortex can widely participate in motor responses. For example, one of the works investigated neuron activity of the primary motor and dorsal premotor cortex in monkeys performing targeted movements by a contralateral or ipsilateral limb. During these movements, more than half of the cells in area M1 (41/74, or 59%) were related to the movements of both fore limbs; however,

the dominant directions of their motor functions more often were very different. It is interesting to note that most of the neurons in the dorsal premotor cortex (55/90, or 61%) were related to activity of both limbs, though their more intensive pulse activity was related mostly to the contralateral limb. These observations allowed a conclusion that the activity in the dorsal premotor cortex was more abstract and independent.

It should be underlined that the sensomotor cortical area, selected for these electrophysiological studies, as shown in Figure 3, was investigated by Moliner[45–47] in more detail. This author identifies the following in the rear sigmoid gyrus of cats:

- an outside layer containing a small number of nerve cells, a lot of dendrites and axons; the fibers in this layer belong to horizontal Cajal cells, ascending axons of Martinotti spindle cells and aberrant branches of afferent plexus;
- an outside layer of small and medium pyramidal cells;
- an outside Baillarger band, poor in dendrites and bodies of nerve cells, but rich in stellate cells and terminals of specific axon plexus;
- a layer of big pyramidal neurons rich in dendrites;
- an inside Baillarger band, poor in dendrites, but with a great number of heterogeneous axon terminals; and
- a layer of spindle cells with a great number of dendrites and axon terminals.

According to Moliner,[45–47] this cortical region has well-expressed vertical components of neuron chains:

- descending axons of pyramidal neurons and their ascending apical dendrites;
- stellate cells with descending axons; and
- ascending Martinotti cell axons and recurrent collaterals of pyramidal neurons, U-shaped axons of small pyramidal cells which, having reached the white matter, return back to the upper cortical layers.[48]

Moliner[45–47] also underlines the importance of horizontal associations: branches of specific afferent fibers diverse along the horizontal up to 1 mm; Cajal cell axons in the outside layer

propagate approximately by 1.5 mm; numerous collaterals of descending axons propagate along the horizontal by more than 700 μm.

It is known that functionally the cortex is composed of vertical neuron chains. The most important place is taken by input neurons, to which specific afferent fibers and neurons of outside cortical layers lead. Descending axons of the latter send pulses to neurons of layers V and VI, which, in turn, send pulses not only to the underlying structures, but also back to cortical layers II–III. Circulation of pulses in such chain can be modified in layers I–III and VI due to activity of the leading association fibers.

Investigating the development of the motor cortex in humans in ontogenesis, Marin-Padilla[49,50] identified a basket-and-pyramidal system of neurons. According to his data, axon branches of cortical basket cells localize within flat vertical grey matter plates, located orthogonally to the gyrus length. They cover all pyramidal cells of this plate. Each such plate, about 200-μm thin and 1–2-mm long, occupies the vertical space from layer II to layer VI. Rows of horizontal neuron chains are formed between branches of basket cells on different layers and pyramidal neurons of the same layers. In the process of ontogenesis, both systems of neurons (basket and pyramidal) develop and mature in parallel, following the growth of afferent fibers to the respective cortical layer. Pyramidal neurons of layer V and big basket neurons of layer IV mature first. The maturing of pyramidal neurons in the lower third of layer II is accompanied by the maturing of respective basket cells of this layer and their pericellular baskets around pyramidal neuron bodies of the same sublayer and partially around pyramidal neurons of layer V. In functional terms, basket cells inhibit the activity of pyramidal neurons of layers III–V.

A neurophysiologist, who attempts to analyze activity of sensomotor cortical neurons in a wakeful animal, should have clear ideas about the depth of inserting the microelectrode, how the activity of the studied neuron is related to the motor activity of the animal, and how the activity of a specific neuron is affected by excitatory, inhibitory or modulating synaptically active substances that can be applied during the experiment.

In cooperation with the skilled histologist Kosareva,[51] we attempted to assess, above all, the specific distribution of neurons in the investigated sensomotor cortical region. The purpose of this study was to identify the relative number of active neurons at different functional states in the sensomotor cortical region with accurately determined density of neurons and to clarify how evenly the background active neurons were distributed along the cortex vertical.

For this study, we selected the cortical region in the rostral part of the rear sigmoid gyrus, in front of the pit, which corresponds to motor sensory area I, related to the fore limb muscles.[52] The density of neuron elements, the number of neurons per 100 μm, was studied using 8 preparations treated according to Nissl. We took into account only cells with a nucleus and a nucleolus. In 20-μm-thick cuts, we counted the number of cells in a column with 100-μm cross section passing through the entire cortex, using an eyepiece micrometer.

The mean neuron density values at different cortical levels, obtained as a result of treatment, are presented in Table 1. It was found that the total mean neuron density in the investigated region made 40.7 ± 2.3. The mean density for level 0–200 μm was underestimated, because the calculations included the volume of cortical layer I free from neurons. In reality, the density of elements in layer II for histological preparations was equal to 100 ± 2.2.

In the investigated region, we also analyzed the character of neuron distribution by their maximum cross-sectional dimensions. To this end, we used a drawing device to redraw the outlines of neuron bodies, containing a nucleus and a nucleolus, included in columns 100 × 100 μm, crossing the entire cortex vertical. Following the measurement of the maximum cross-sectional dimensions of each neuron, we calculated the mean cross-sectional dimensions of neurons after each 200 μm of depth. They turned out to be very close: 0–200 μm — 10.6 μm; 200–400 μm — 10.4; 400–600 μm — 10.1; 600–800 μm — 9.5; 800–1000 μm — 10.4; 1000–1200 μm — 10.1; 1200–1400 μm — 10.4; and 1400–1600 μm — 11.6 μm.

Separation of individual neurons by their cross-sectional dimensions into three groups (5–10, 10–15, and over 15 μm)

TABLE 1. Distribution of the density of neurons along the sensomotor cortex vertical

Cortical depth *h* in the preparation, m	Neuron density in the histological preparation	Root-mean-square deviation	Recalculated depth in the native cortex (*h*/0.763)	Neuron density in the native cortex
200	68.7	1.1	262	39.2
400	47.2	2.3	524	26.9
600	39.8	1.5	786	22.7
800	36.8	3.8	1048	20.7
1000	33.5	2.4	1310	18.1
1200	40.1	1.6	1573	22.9
1400	29.1	5.2	1835	16.6
1600	13.6	2.1	2097	7.8

showed that cells with the smallest cross-sectional dimensions were located almost evenly through the cortex depth, the majority of neurons with cross-sectional dimensions of 10–15 μm were concentrated in the surface layers, while cells with cross-sectional dimensions over 15 μm had a two-hunch distribution and their number was small (Fig. 4). Of course, cross-sectional dimensions do not give a full idea about the true size of the neuron, since it depends, at least equally, on the vertical extension of the body. However, they are significant for evaluation of the electrophysiological results, when the microelectrode is submerged orthogonally to the cortex surface.

In order to determine the compression of the studied cortical region for the applied histological treatment of the surface of the investigated sigmoid gyrus and in the cortex depth by means of a silver point electrode, serving as anode, we applied black markings by electrical deposition: 0.2-mA current was passed for 30 sec. These markings had strong retention during subsequent treatment. The distance between the markings was measured by an eyepiece micrometer till the preparation was fixed and after it was filled with paraffin. The vertical cortex compression was determined by the markings made inside the cortex. Following the preparation cut, a depth of the markings determined by the eyepiece micrometer was compared to the electrode insertion depth, pre-measured by the

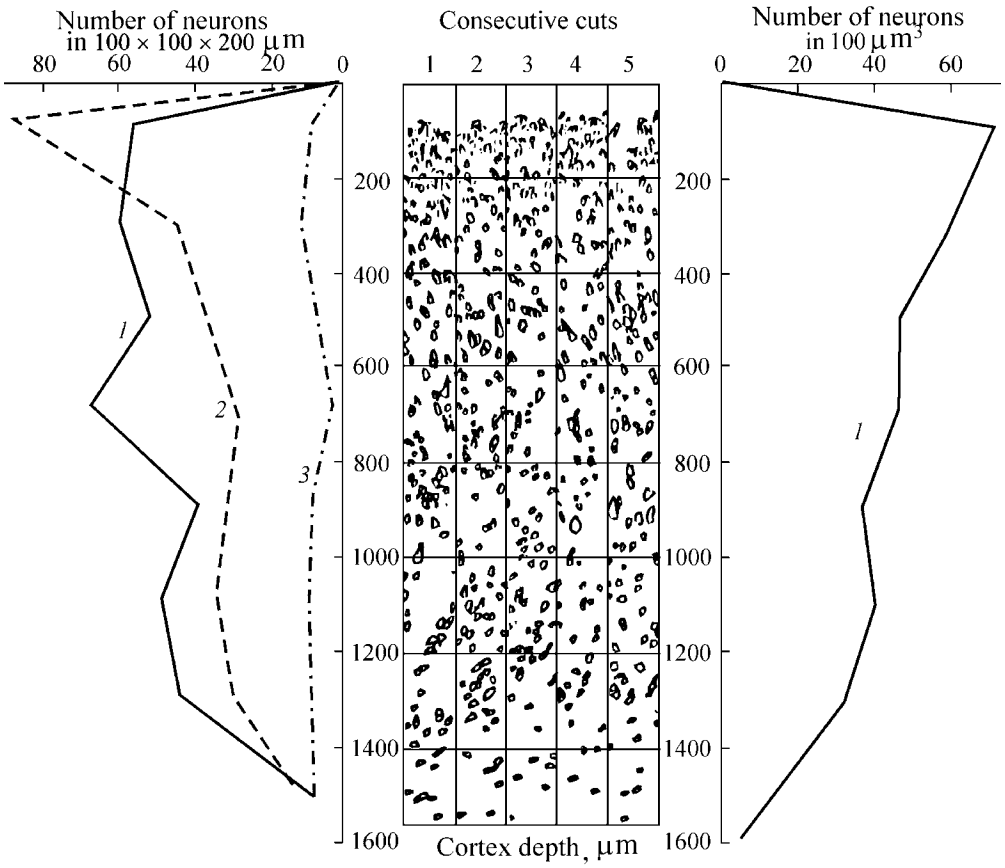


FIGURE 4. Vertical distribution in the cortex of neurons with different cross-sectional dimensions. In the center, sketches of the neuron body outlined in five consecutive sections (section thickness 20 μm, strip thickness in each section 100 μm). On the right, a cortex vertical density graph; on the left, graphs of the background active neurons of different diameters: 1) neurons with the cross-sectional dimensions 5–10 μm, 2) 10–15 μm, 3) over 15 μm.

scale of the beat indictor mounted into the micromanipulator. It was found that the linear dimensions in the rear sigmoid gyrus decreased in the sagittal direction to 85.1 ± 2.2%, in the frontal direction to 88.7 ± 2.2%, and in cortex vertical to 76.3 ± 7.1%. The first two values were obtained by averaging 40 measurements in each direction. The compression of the brain along the cortex vertical was determined by studying 4 preparations with 11 markings, made at the depth of 2000 μm. On histological preparations, the depth of the markings was 1527 ± 142.5 μm (1274–1750 μm) on the average.

The obtained data were used to calculate the volume after compression, which made 57% of the native cortex volume, i.e., the bulk modulus ρ was 0.57. This allows calculating the density of neurons in the native cortex (Table 1). The mean density of neurons for the entire studied region makes 23.2 ± 1.4. A decrease of the volume by 43% in our studies was somewhat higher than the data given in literature. Usually, for similar compression followed by paraffin filling, the brain is compressed by 37–40%.[53]

The mean cortex width in the histological preparation of the investigated region was 1672 μm (1430–1855 μm), while the average width of individual layers from I to VI inclusive made 112 ± 36; 113 ± 12; 388 ± 53; 212 ± 7; 275 ± 39; and 572 ± 82 μm. In order to determine the width of the native cortex in connection to vertical tissue compression up to 76.3%, these values should be divided by 0.763.

Thus, the mean depth of individual neuron layers of the native preparation is 0–146; 146–294; 294–803; 803–1079; 1079–1439, and 1439–2191 μm, respectively.

In our previous electrophysiological experiments, we determined the number of inserted active neurons with the pulse activity in one tract, when the microelectrode was inserted to 2200 μm. In a series of experiments under a light general anesthesia by nembutal with one passage across the entire cross-sectional dimension of the cortex, 6.2 neurons, on the average, with a background pulse activity (268 neurons in 43 tracts) were found. In a series of acute experiments, after the surgery under nembutal general anesthesia and application of d-tubocurarine followed by artificial respiration, as well as in experiments on animals operated under ether general anesthesia followed by curarization and artificial ventilation, 10 neurons in the tract, on the average (812 neurons in 81 tracts) were found.

It should be taken into consideration that, in fact, only a small part of the cortical neurons in a wakeful animal are neurons with spontaneous background activity and that these neurons take active part in responses to peripheral irritations, as well as to signals fed from other nerve structures. This capacity of individual background active neurons to respond by pulse activity to the

coming signals seems to be the main functional meaning of the background rhythms of the cortical neurons; therefore, identification of the number of background active neurons is essential when we attempt to estimate the function role of specific neocortex areas or the effects of pharmacological active substances. We attempted to make a similar quantitative estimation of the distribution of background active neurons along the cortex vertical, using literature data and our own experimental materials, in our previous studies.[41] It is known that at small microelectrode resistances, pulses with initially negative values and the total amplitude of 0.2–0.3 mV are mostly recorded.[54] In such case, the radius of recording of active neurons reaches 100 μm or more. Recording by microelectrodes with the tip diameter less than 1 μm allowed, though rarely, recording pulse activity up to 100 μm.[40] It was also found that it belonged to large neurons, most often to neurons which begin the pyramidal tract.

In order to determine the mean radius of electrical fields of cortical neurons, Livanov[55] proposed a radius calculation formula by the mean chord. Using this method in our previous experiments, we measured the distances at which pulse activity was recorded for 138 neurons in the investigated cortical area in cats. It was found that the mean chord was equal to 62 μm. This corresponded to a mean radius of the electrical field equal to 39.5 μm ($62 = \pi r/2$). In calculations by Livanov, based on the data about the mean chord in the visual, auditory, and somatosensory cortex of a rabbit, this indicator was 38 μm.

In our present experiments, we used three-channel microelectrodes with the total tip diameter 3–5 μm. Two channels were filled with synaptically active substances, used for iontophoretic application. One of the three channels, used for extracellular lead of pulse activity, had the diameter not more than 1 μm. As it was mentioned before, resistance in this channel usually made 5–7 MΩ. In evaluating the mean radius of the electrical field of the neuron, it is necessary to take into account the ideas of Lorente de No[56] about neurons with closed and open electrical fields. Stellate neurons which are presumed to have closed electrical fields have small radii of the external electrical

field. Besides, different dimensions of cells and their surfaces with the same membrane potential can lead to significant differences in the discharges of individual cortical neurons, which should, probably, have an effect on the radius of their electrical field. Analysis of the field potential of pyramidal neurons using two-stem electrodes whose tips were distant along the vertical allowed a conclusion that these potentials could lead predominantly at the level of its soma and proximal departments of apical dendrites.[57] However, the experimenter should not move the electrode too close to the neuron with background activity, if the experimenter intends to monitor its pulse activity for a long time, especially on an animal that can move both a paw and the head, as was the case in our experiments.

All these considerations as well as other considerations not mentioned here allowed us to assume that the radius of the extracellular lead of neurons' pulse activity in conditions of our experiments was within, at least, 40–100 μm. However, given the above data on the density of neurons in the investigated cortical region of a wakeful animal, the number of neurons with background pulse activity, encountered by the inserted microelectrode, and the number of neurons responding to the increased pulse activity during a conditioned reflex movement, we should acknowledge that in a normally functioning sensomotor cortex of a wakeful animal, in its deep layers, the number of responding neurons does not exceed even 5%. According to Melekhova and Shulgina,[58] only about 2% of neurons have spontaneous activity in the cortex of a wakeful rabbit. Thus, only a small share of sensomotor cortex neurons, basically manifesting background pulse activity, participates in the generation of pulse responses to the irritation, *inter alia*, to a conditional stimulus. It can be assumed that this capacity to respond to a conditional (or unconditional) external signal, probably, represents the functional significance of the background rhythms of cortical neurons. However, earlier studies showed that only 67.6% of neurons with background activity responded to stimulation in the visual cortex.[59] In the auditory cortex, the percentage of neurons responding to a sound that did not manifest background activity before stimulation was higher.[60]

Histograms of the distribution of intervals between pulses of individual cortical neurons as well as summary graphs of the mean values of these intervals and of their dispersion for the analyzed sample of neurons in the sensomotor cortex can be used as the basis to argue about a significant deviation of the distribution of such intervals during the background activity from the normal Poisson distribution. Such facts testify against a spontaneous character of their background activity or, in an extreme case, allow us to argue that spontaneous rhythms are overlapped by the activity related to the arrival of pulses from numerous mechanical receptors of skin, muscles, tendons, and internals, caused by continuous physiological processes in a living body. These effects can be influenced not only due to arrivals through specific projection paths, but also in relation to tonic effects on the reticular formation of the brain stem.[61,62] It should be underlined that in the following experiments, made on wakeful cats, actively participating in the conditioned reflex response by putting a paw on the support, where the lead is accompanied by application of synaptically active substances, the number of encountered neurons with the background pulse activity in a track per insertion usually does not exceed 5–10. We used the background activity of such neurons as an evidence of their capacity to respond to a conditional stimulus in the control and when synaptically active substances are applied.

Trying to achieve a protracted stable recording of the background and induced activities of the neuron that would allow a thorough estimation of its relation to the application of specific synaptically active substances, we intentionally inserted the microelectrode into a depth of 1500–2200 μm. Recording of the activity of single neurons during 6–7 series with 5–10-min brakes between the series was made for 1.5–2 h totally. This allowed us to record the background and induced activities in the control case, when a conditioned reflex was made, and to measure these activities, conditioned by application of individual synaptically active substances and their combinations. Of course, these were large neurons of deep cortical layers, most probably, pyramidal neurons. The pulse response of one-third of such neurons led the beginning of the limb movement to a conditional signal by 150 msec or more, which,

according to the general opinion of many researchers, allows us to refer such neurons to pyramidal tract neurons. Responses of the rest of the recorded neurons, which also had a large electrical field, but did not lead the beginning of the movement, were not identified as pyramidal tract neurons; nevertheless, probably, they belonged to pyramidal neurons. Since pyramidal neurons, and especially pyramidal tract neurons, are located in deep cortical layers and they are related to the activity of association excitatory and inhibitory neurons, the recording of activity of such neurons in a wakeful animal, which also performs the learned conditioned reflex movements in response to a conditional stimulus, allows the experimenter to analyze the work of the brain at the neuron level. Of course, recording the activity of a pyramidal neuron during 6–8 series and applying synaptically active substances, the experimenter is fully aware of the fact that this neuron is subject to significant effects by association excitatory and inhibitory interneurons. Therefore, in assessing experimental results it is important to remember about possible participation of association neurons in the observed system responses.

IV. EFFECT OF GLUTAMATE AND GAMMA AMINOBUTYRIC ACID ON ACTIVITY OF NEURONS IN THE SENSOMOTOR CORTEX

Before analyzing the modulating effects of dopamine on the neocortex excitation and inhibition processes, it is expedient first to have a look at specific properties and features of the excitation and inhibition processes occurring in the cortex of a wakeful animal with behavior close to natural conditions, as well as when it performs conditioned reflex movements. Also, it is interesting to clarify the specific features of neuron responses when glutamate and GABAergic interneuron associations are directly affected by ionophoretic applications of glutamate and GABA, their agonists and antagonists.

It is known that glutamate represents a dominant excitatory synaptic transmitter in the cerebral cortex. In such case, 50% of cortex neurons represent glutamate immunoreactive neurons,

whereas their share in the surface cortex layers increases up to 80–90%. Electrophysiological and pharmacological studies helped identify three basic subtypes of glutamate receptors in the neocortex: ionotropic NMDA receptors, ionotropic non-NMDA receptors, which include AMPA and kainate receptors, as well as metabotropic receptors, including several specific subgroups. When activated, ionotropic receptors open cation channels, which allow sodium, potassium, and calcium ions inside the cell. AMPA and kainate receptors in the neocortex have low calcium permeability, except for a subpopulation of interneurons. In addition to sodium and calcium transmission, NMDA receptors are also the principal calcium carrier in the cerebral cortex. These receptors differ from other glutamate receptors in that they also serve as a gate ligand and remain sensitive to voltage variations. Synaptic currents, mediated by them, increase slowly, but decrease rapidly. They are characterized by a rapid desensitization which depends on the reduction-defined capacity to responses, caused by the previous activation. Metabotropic glutamate receptors either activate G protein phosphoinositide path or inhibit and loop potassium channels when glutamate is activated.

Metabotropic receptors are connected to the system of intracellular messengers. Their stimulation activates phospholipase C, which catalyzes the production of inositol triphosphate and leads to mobilization of calcium from inner reserves, as well as diacyl glycerol, activating protein kinase. One of the subtypes of these receptors inhibits the cAMP loop. Stimulation of metabotropic receptors by the specific agonist, t-ACPD, leads to a series of physiological effects: it inhibits potassium conduction, causes slow depolarization and, simultaneously, blocks the trace depolarization. It is known that metabotropic receptors cause presynaptic influence both with inhibitory and excitatory effects. However, it is shown that activation of mGluR via protein kinase C increases glutamate release. Physiological properties of glutamate receptors in the neocortex are characterized in a rather detailed way in one of the reviews.[63]

Activation of NMDA receptors in the cortex causes slow and protracted EPSP, whose amplitude increases in the case of mem-

brane depolarization. When magnesium is absent, this voltage dependence is eliminated. The NMDA receptor represents a gate-related ion channel with high calcium permeability. It activates in the case of moderate shifts of the membrane potential, rather than as a result of "all or nothing" reaching of the activation threshold. Both weak and strong responses contain an NMDA component, whereas NMDA application increases the induced response. AMPA and kainate receptors represent ligand-gate ion channels. Their activation opens cation sodium and potassium channels, and the synaptic current, mediated by them, increases and decreases rapidly. They are also characterized by rapid desensitization, which is defined as the reduction of the response capacity, induced by the preceding L-Glu activation. AMPA and kainate receptors are difficult to differentiate, because of the absence of selective agonists suitable for such purposes. Kainate activates both kainate and AMPA receptors, though with different efficiencies, while AMPA affects predominantly AMPA receptors. It is believed that AMPA receptors are involved into mediation of all forms of the rapid glutamate transmission. Functions of kainate receptors are less known. Kainate activates slow-conducting ion channels that are difficult to investigate by electrophysiological methods. Any systemic or intracerebral kainate impact causes damaging effect, and high-affinity kainate receptors may be involved into kainate-mediated neurotoxicity.[64]

Metabotropic glutamate receptors are typically connected with a system of secondary intracellular transmitters. Metabotropic impact of glutamate on phosphoinositide metabolism was demonstrated on the culture of striate neurons[65] and on neurons of hippocampus sections.[66]

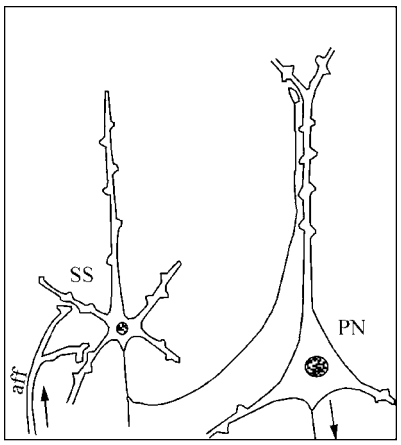
The cerebral cortex has a high concentration of glutamate receptors. It is known from numerous literature sources that the blocking of AMPA and NMDA receptors by CNQX and APV antagonists, respectively, fully eliminates the induced responses of all neocortex neurons. At the same time, AMPA and NMDA application has an excitatory effect on the cortex neurons. The *in vivo* experiments showed[67] that activity of the majority of layer IV neurons in the visual cortex of cats, for example, is blocked by

application of kinurenate, a nonspecific glutamate receptor blocker, but not by application of APV. It was also shown that NMDA receptors controlled only a small quantity (10%) of neurons in this cortex layer in adult cats. The same authors found that APV reduced activity of layer IV neurons. Other researchers, using intracellular lead from motor cortex neurons in a cat without anesthetization, showed that thalamic cortex EPSPs can be transmitted predominantly, but not exclusively, by non-NMDA receptors.[68] Following a thorough comparison of data on the impact of glutamate on neocortex neurons, Kaczmarek et al.[63] concluded that the majority of cortical neurons are responsible for application of both NMDA and non-NMDA agonists. Often, these two types of receptors can be found in the same synapse. In layer IV, thalamic cortical synapses are, probably, switched in adult animals by means transmission that is dependent on non- NMDA receptors. Interneurons in nongranular cortical layers also receive input from pyramidal cells not via these receptors. The intracortical transmission to pyramidal and stellate cells has both NMDA- and non-NMDA-dependent components. Neurons of supragranular and granular layers are predominantly inhibited when metabotropic mGluR agonists are applied, while infragranular neurons, and especially layer V neurons, exhibit excitatory responses.

Afferent projections to neocortex pyramidal neurons have excitatory character and are concentrated predominantly in cortex layers III–IV. Both pyramidal and stellate neurons are located in this area. Unlike pyramidal neurons, interneurons include both excitatory and inhibitory neurons. Part of excitatory interneurons has dendrites with a high quantity of aciculae, and excitatory afferent fibers contact with these aciculae. It is considered that cortical basket neurons with a long axon (up to 1.5 mm) and stellate neurons with smooth dendrites belong to inhibitory neurons.[49] The first neurons are located in cortex layer IV, while their axons form inhibitory synapses on layer V neurons. Stellate neurons with smooth dendrites are located in different cortical layers, but their highest density can be found in layer IV. A great number of inhibitory synapses in this layer, where afferent signals are received initially, probably, indicate that inhibition takes a very

active part in processing of sensor information as soon as it enters the cortex. A significant part of inhibitory neurons, located in this cortical region, is excited monosynaptically. Pulses of these neurons, in turn, can cause inhibition of the neighboring pyramidal and stellate neurons. This is a direct afferent disynaptic inhibition. Part of inhibitory neurons is excited via reverse collaterals of axons of input pyramidal neurons and via collaterals of axons of stellate neurons, which leads to reverse inhibition. Inhibition, occurring in input neurons, facilitates the reduction of neuron chains, involved in the cortical irritation response, i.e., leads to concentrated excitation. In the second case, the propagation of excitation via neuron cortical chains is limited, which facilitates termination of the cortical response. Batuev and Babmindra[69] made a detailed review of reference sources on morphology and function of interneurons in the sensomotor cortex of cats, as well as their own new data. The authors finally identified two interneuron groups in the somatosensory cortex, which take part in the formation of synaptic chain loops with excitatory and inhibitory end effects. Excitatory chains are formed with participation on stellate neurons with aciculae, cells with double bundles of dendrites and stellate neurons without aciculae, in addition to pyramidal neurons. Inhibitory chains are formed with participation of basket neurons and axoaxonic cells, in addition to pyramidal neurons and acicular stellate neurons (Fig. 5).

GABA is known as the main mediator of postsynaptic inhibition in the cerebral cortical neurons. Electrophoretic application of this compound on the cortical neurons is accompanied by depression of their background and induced activity. It was found that changes in the membrane potential and the ion conductance of the cortical neuron when GABA is applied are similar to changes when a real IPSP is developed. Application of bicuculline, a GABA antagonist, together with GABA increases the frequency of neuron pulse activity, exceeding the initial background activity; therefore, it is assumed that, in addition to the blocking effect on GABA receptors, bicuculline also causes a direct excitatory impact on cortical neurons, enhancing their responses to efferent irritation. Replacement of a Ringer solution by a calcium-free solution during



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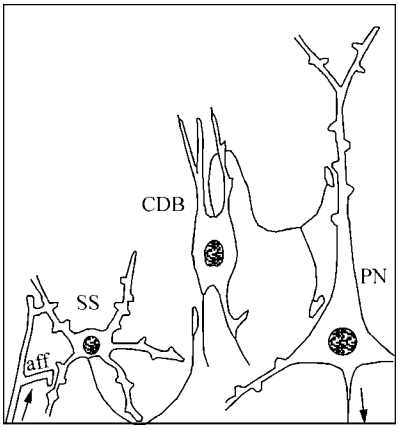

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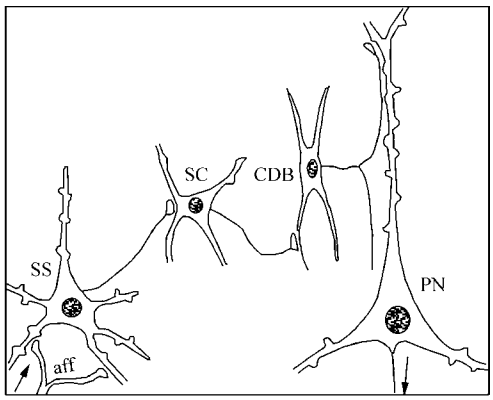

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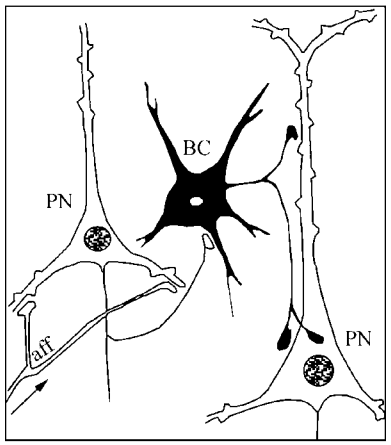
FIGURE 5.



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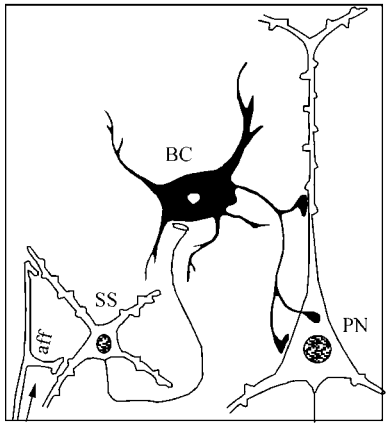

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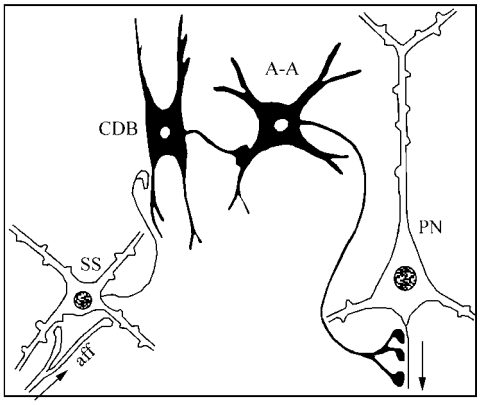

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FIGURE 5. Interneurons which form excitatory (a–c) and inhibitory (d–f) loops of synaptic chains in the sensomotor cortex. SS — a stellate acicular neuron, PN — a pyramidal neuron, CDB — a cell with a double bundle of dendrites, SC — a stellate neuron without aciculae, BC — a basket cell, A–A — an axoaxonic cell, aff — afferent.[69]

perfusion facilitates the termination of GABA exit to the solution. As a result, the inhibitory action of irritation on activity of cortical neurons is stopped.[70]

Inhibitory neurons make up to 20% of the total number of neurons in the cerebral cortex.[71] Dendrites of large basket cells branch in all cortical layers, and their axons form synapses predominantly on the bodies of pyramidal neurons.[72] Axons of small basket and spider neurons terminate in cortex layer IV on dendrites, their aciculae and rarely on the axon hillock. Spider neurons form a thick network of terminals around neuron bodies,[73] and chandelier neurons form synapses on axon hillocks of pyramidal neurons.[72] GABAergic receptors are divided into three types: GABA(A), GABA(C), and metabotropic GABA(B).[74,75] GABA(A) receptors, most widely spread in the cortex, refer to the class of ligand-controlled ion channels. Most native receptors are composed of α, β, and γ subunits. GABA(A) receptors are ligand-controlled chlorine channels. Their excitation leads to opening of these channels and entry of chlorine ions into the cell. This results in hyperpolarization of the membrane and increases its sensitivity threshold, which also reduces the neuron sensitivity to excitatory synaptic effects: the membrane depolarization decreases and an inhibitory response develops. A high density of GABA(A) receptors has been noted in cortical layers I–IV, and a low density in layers V–VI. This type of receptors is located on the soma, apical dendrites, and the initial segment of the axon.[76,77] Metabotropic GABA(B) receptors in the cortex are located predominantly on dendrites and are activated not only by GABA, but also by baclofen, and are blocked by phaclofen. They are connected to calcium and potassium channels and can be located on both pre- and postsynaptic membranes. On the postsynaptic membrane, their activation opens potassium channels connected to G protein,[78] which leads to hyperpolarization and depression of the inositol phospholipid cycle. Presynaptic inhibition is made via axoaxonic synapses between the interneuron and the neighboring excitatory fiber. In such case, the neuron-exciting fiber itself is affected by the neighboring fiber which releases GABA during activation; this depresses the excitatory postsynaptic potential due to depolarization of excitatory presynaptic

terminals. When presynaptic GABA(A) receptors are excited, the permeability of the excitatory nerve terminals to chlorine ions increases. This leads to decreased depolarization of the presynaptic terminal of the excitatory neuron when the nerve pulse is received.

Different subgroups of interneurons have different origins. The process of GABA release depends on the presence of calcium ions in the medium. In particular, it was shown that parvalbumin- and somatostatin-expressive interneurons initially develop in the medial gangliac protuberance of telencephalon, while calretinin-expressive interneurons originate from its caudal protuberance.[79] Cortical interneurons can be classified by their function. Gao et al.[80] identified neurons with fast spiking (FS) and non-fast spiking. The latter include neurons with regular spikes (RS), low-threshold spikes (LTS), and later spikes (LS). It was found that FS neurons represent inhibitory neurons that predominantly innervate the soma or the initial segment of the pyramidal neuron axon and, due to this, actually control initiation of the action potential. At the same time, other interneurons regulate excitation of dendrites and efficiency of excitatory inputs.[81] Using a coupled whole-cell recording, the authors investigated the effect of dopamine on the local inhibitory loop, involving FS and non-FS neurons, respectively. It was shown that dopamine depressed the inhibitory transmission between FS interneuron and the pyramidal neuron, but increased inhibition between the FS interneuron and the pyramidal cells. The FS inhibitory transmission manifests properties related to presynaptic effect on D1 receptors.

To learn the specific functions of the glutamate and GABAergic cerebral cortex system in a wakeful animal in conditions close to natural conditions, we performed a series of experiments. In one of the series, we investigated 112 sensomotor cortical neurons, where we analyzed changes in the background and induced pulse activities under the impact of agonists and antagonists of ionotropic and metabotropic glutamate transmission.

When selective ionotropic glutamate agonists (AMPA and NMDA) were applied, the level of background and induced pulse activities, as a rule, increased.

However, when applications of these two agonists were added, the level of neuron activity did not increase notably. When the concentration of the applied substances was increased, the added action of the two glutamate agonists did not enhance the response intensity, as could have been expected, but, often, decreased (Fig. 6).

Figure 6 shows that application increases the intensity of induced pulse response with a slight decrease of the background activity. During subsequent control, the background neuron activity returns to the initial level, which is accompanied by the recovery of the initial pulse response intensity. NMDA application caused an increased background level with a moderate increase of the pulse response intensity, compared to the control level. When these ionotropic glutamate transmission agonists were applied together, we noted a significant increase in the background pulse activity and a moderate increase in the pulse response, compared to the initial level. Unexpectedly, after a new control, the double increase of the current, when these 2 glutamate transmission agonists were applied, did not result in a further growth of the pulse activity, but, on the contrary, was accompanied by a clear decrease in the induced pulse activity of the neuron (histogram 8). Statistical assessment of the intensity of pulse responses following one after another showed a high reliability ($P < 0.001$) of the intensity differences between histograms 1–2, 2–3, 3–4, 4–5, 5–6, and 6–7. The difference in the response intensities in histograms 7–8 is not reliable.

Usually, it is believed that advanced occurrence of the pulse neuron response by 150 msec or more before the beginning of the movement is the evidence that the neuron belong to pyramidal tract neurons. These particular neurons participate in the initiation and performance of the movement. We tried to estimate how stably in time the beginning of the studied neuron pulse response occurred before the beginning of the movement. As shown in the histograms on the right (Fig. 6), initially, in the control, the beginning of the neuron pulse response advanced the movement by 250 msec, i.e., the neuron refers to the pyramidal tract neurons. AMPA application increased this advance up to 500 msec; however, after its cancellation, the advance of the pulse response

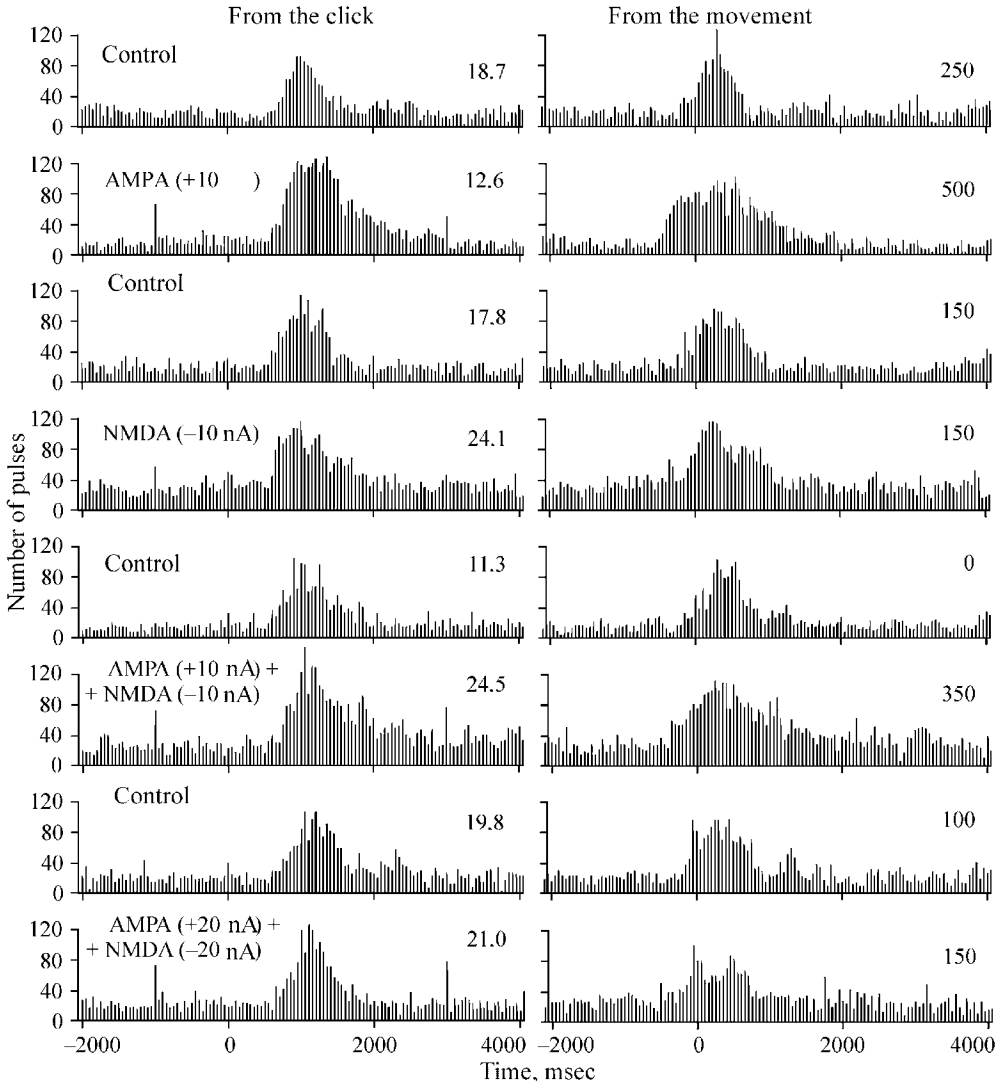


FIGURE 6. Effect of application of ionotropic glutamate transmission agonists AMPA and NMDA on the background and induced pulse activities of the neuron (post- and peristimulus histograms). Each histogram is built on the sum of 10 implementations. In the left column, the point of the sound click coincides with the zero point in the histogram, while in the right column, the zero point coincides with the beginning of the conditioned reflex movement. In this and all subsequent histograms, 1 bin corresponds to 50 msec. The left column shows the applied substance and the force of the applied current on the left and the frequency of the background activity (pulse/sec) on the right. The right column shows, above each histogram, the time (msec) by which the neuron response leads the movement.

returned to the previous value and remained at this level during NMDA application. In the subsequent control, the advance value approximated zero, and in the case of joint and NMDA application increased to 350 msec. The increase of their application intensity was accompanied by reduction of the advance up to 150 msec. Comparing oscillations of the advance pulse response latencies, depending on the type of the applied glutamate agonists, testifies to the fact that AMPA and kainate receptors are, probably, localized on the neuron body near the axon hillock, while NMDA receptors are located predominantly in the area of dendrite branching.

We also compared the glutamate agonist effects with application of respective selective glutamate transmission agonists, CNQX and MK 801 (Fig. 7).

As shown in Figure 7, ionophoretic application of CNQX is accompanied by almost a double increase on the background pulse activity and an expressed enhancement of the pulse response to the conditional stimulus. Application of MK-801, although less expressed, also increases the background level and the response intensity, compared to the previous control response. The specific features of this response are that in the subsequent control both the background level and the response intensity do not return at once to the initial level, as it occurs after CNQX application, but, on the contrary, somewhat increase (histogram e). In the case of joint application of the two glutamate transmission agonists, as shown in histogram e, the background activity level doubles at a high level of response intensity.

It should be noted that the two glutamate ionotropic transmission blockers were applied by opposite-sign currents. This allows excluding the direct increase of electrical polarization effect on the pulse activity of the neuron. Histogram h shows the comparative intensity of pulse responses of the previous seven histograms. It is interesting to note that in the case of joint CNQX and MK-801 application the intensity of the background and induced activities sharply drops, and in the case of control stimulation it returns immediately to the initial level. The differences in the neuron pulse response intensities between the histograms are highly reliable ($P << 0.001$), except for histograms d and e.

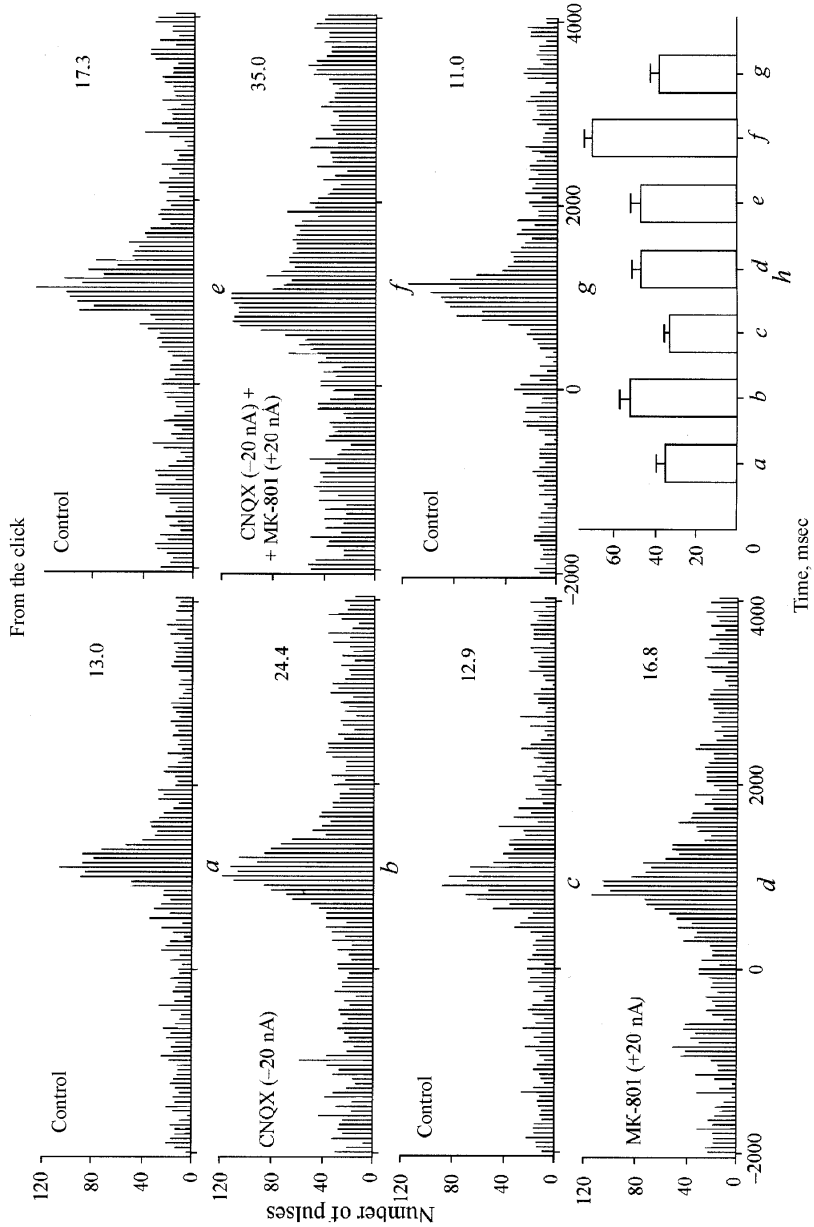


FIGURE 7. Effect of the application of glutamate transmission antagonists CNQX and MK-801 on the background and induced pulse activities of neurons in the sensomotor cortex (*h* — the comparative pulse response intensity, represented in a–g). The point of the conditional stimulus coincides with the zero point. The name of the applied substance and the current value are given on the left; the frequency of the background activity is on the right (pulse/sec).

Given that in conditions of our experiments, against expectations, the selective ionotropic glutamate transmission antagonists increased the background and induced pulse activities in the sensomotor cortex, it was necessary to investigate the possibility of the inhibitory GABAergic system in these paradoxical responses.

The character of changes in the background and induced pulse activities under the impact of the ionotropic glutamate synaptic transmission antagonist AP-7 and its interaction with the inhibitory cerebral cortex system was tracked in a small sample of neurons with AP-7 application, on the one hand, and GABA and bicuculline, on the other (Fig. 8).

Histogram a (Fig. 8) shows the depressive effect of GABA on the background and induced neuron activities. Application of the ionotropic glutamate antagonist FP-7 causes a paradoxical and unexpected response, i.e., a sharp increase of the background and, especially, induced pulse activities. This character of the background and induced activities in the subsequent control to some extent decreased, though, in general, it retained at a rather high level. In case of joint GABA and AP-7 application, the effect caused by the latter is fully eliminated; both the background and induced neuron responses become almost the same as during the initial GABA application.

The second neuron (Fig. 8b) was used to compare the effect caused by AP-7 application, with changes caused by bicuculline application. Both types of application facilitate the increase of the background and induced activities. However, in the case of joint application of these two synaptically effective blockers of glutamate and GABAergic transmission the induced pulse response returned to the initial control level, though the background activity remains slightly increased.

Thus, it is evident that the apparent unexpected sharp increase of the background and induced pulse activities of the studied pyramidal neuron, caused by AP-7 application, can be, to a significant degree, related to the blocking, primarily, of the synaptic glutamate activation not of the pyramidal neurons, whose pulse activity is recorded, but rather to the activity of the excitatory synaptic inputs to the inhibitory interneurons which control the

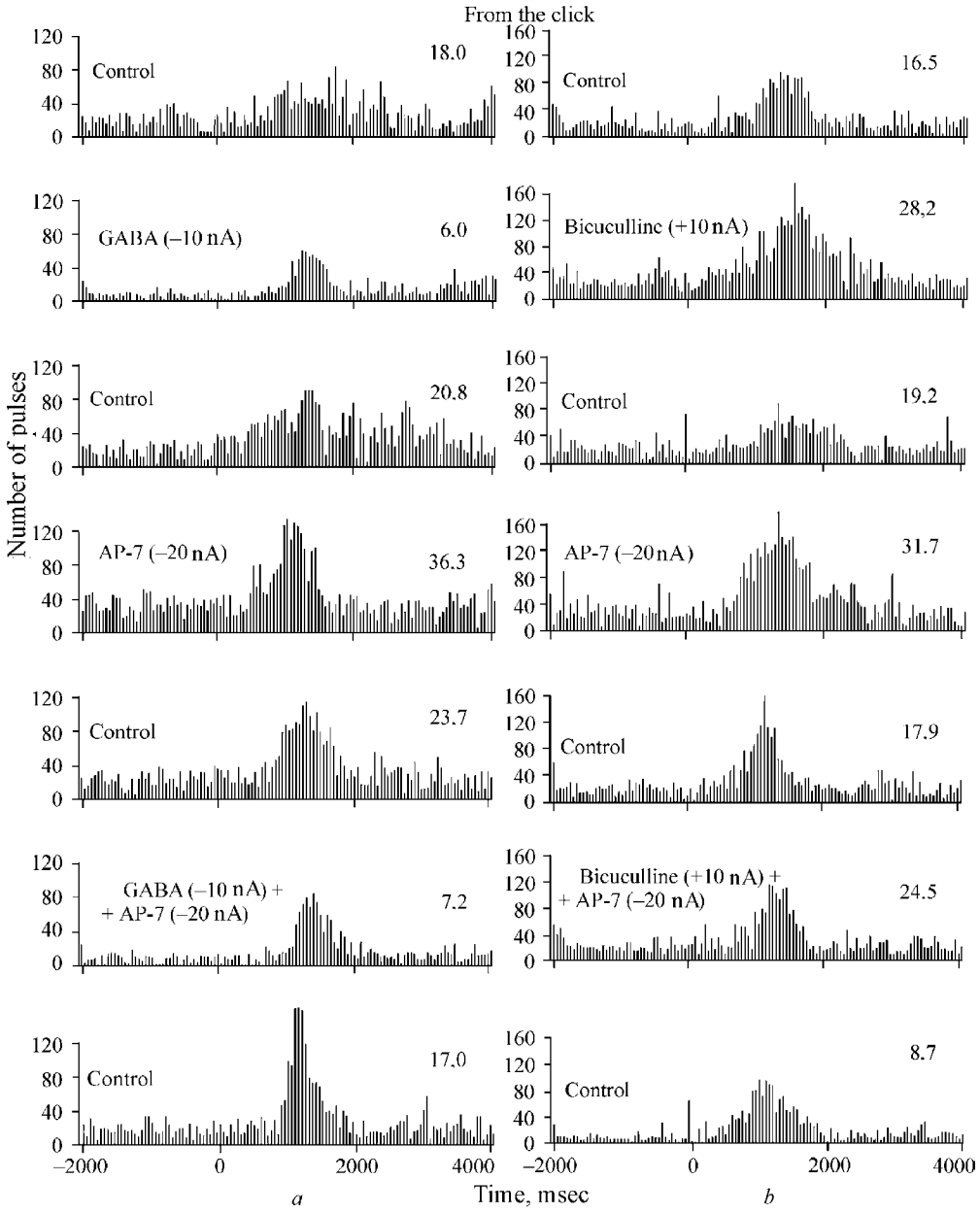


FIGURE 8. Effect of GABA (a) and bicuculline (b) on the background (figures on the right, pulse/sec) and induced activities of two neurons in the sensomotor cortex and on the effects caused by NMDA glutamate receptor antagonist AP-7.

studied pyramidal neuron. If bicuculline is applied, then it directly eliminates IPSP, caused by activation of inhibitory interneurons in the recorded pyramidal neuron. As a result, we obtain approximately identical character of changes in the pulse response, as in the case of GABA application. Most probably, this is why in the sixth histogram in Figure 8a (for GABA and AP-7 application) and the sixth histogram in Figure 8b (for bicuculline and AP-7 application) the character of pulse responses is actually the same, although the background activity level significantly differs.

The fact that the changes demonstrated in Figure 8 are not a casual observation, but a natural phenomenon is confirmed by statistical analysis of the activity of 17 neurons tested by applying bicuculline and AP-7 (Fig. 9), and 11 neurons tested by applying AP-7 and GABA (Fig. 10).

It is shown that AP-7 application exerts a moderate effect on the increase of the background pulse activity and with a better reliability enhances the induced neuron activity. This considerably occurs not only due to increased frequency of pulse activity during the response, but also due to its increased duration. Changes in the pulse response and movement latencies were not reliable.

During statistical estimation of the effects produced by combined application of AP-7 and GABA, the interaction effect is less evident. However, it is proved that GABA depresses the background and induced activities, while AP-7 application is accompanied by a reliable increase of the background activity and the frequency of the induced pulse activity. In the case of joint application with GABA, all reliable changes caused by GABA, namely, the reduced background and induced activities and the increased pulse response latency, the application of AP-7 decreases or totally eliminates these changes.

Qualitatively differing changes in the character of neuron responses and the latency of conditioned reflex motor responses were observed in experiments with application of metabotropic glutamate transmission agonists and antagonists (Fig. 11).

Figure 11 illustrates changes in the background and induced pulse activities of two neurons under the impact of glutamate metabotropic transmission agonist (D) and antagonist (G). It is seen

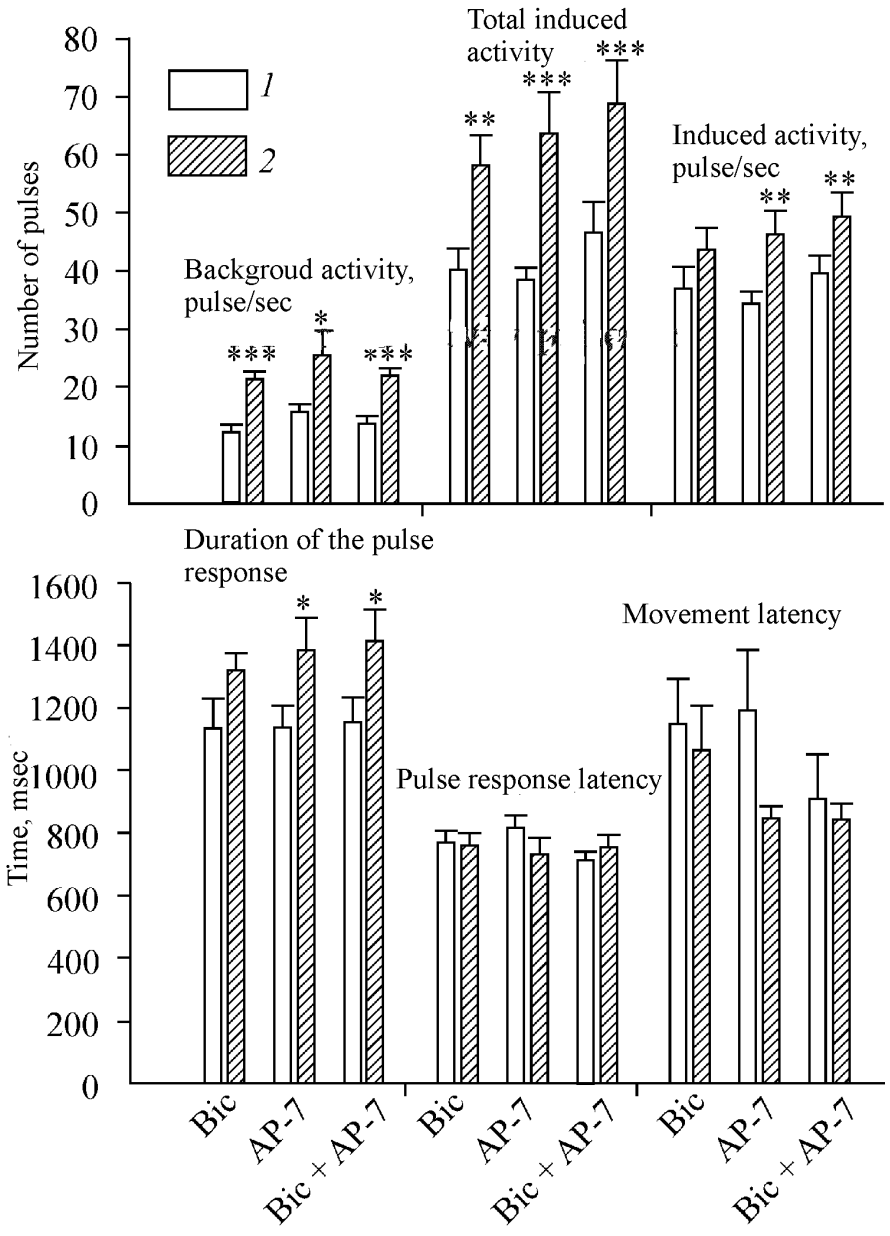


FIGURE 9. Statistical estimation of the effect of AP-7 and bicuculline application on the background and induced pulse activities of neurons in the sensomotor cortex: 1) control, 2) applied substance; asterisks denote the reliability of results: one asterisk — $P < 0.05$, two asterisks — $P < 0.01$, three asterisks – $P < 0.001$.

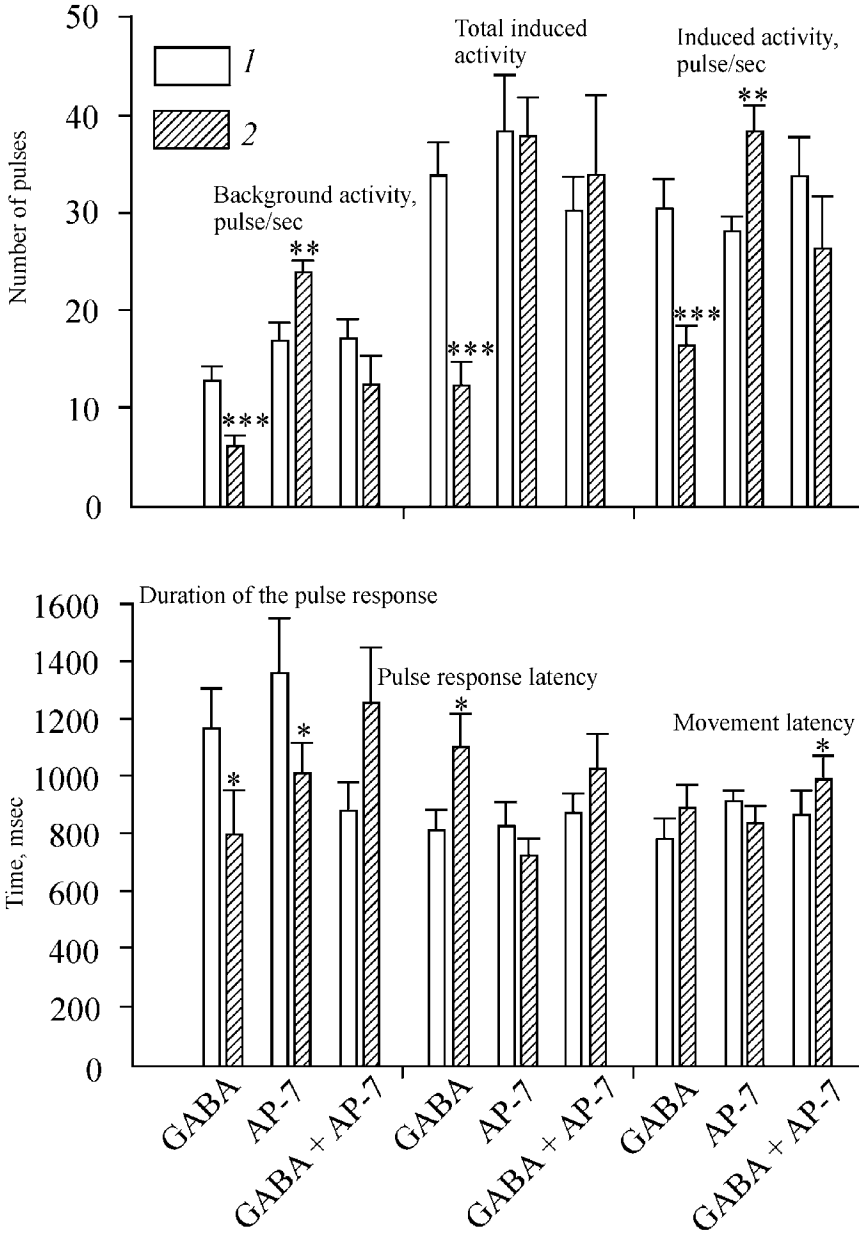


FIGURE 10. Statistical estimation of AP-7 and GABA effect on the pulse activity of two neurons in the sensomotor cortex. For symbols see Figure 9.

that D application is accompanied by the enhanced background activity from 6.2 to 10.8 and from 6.2 to 21.3 pulse/sec. In the case of ACPD application, the pulse response also enhances to some extend, and the response latency is shortened (by 61 and 113 msec, respectively). On the contrary, application of the glutamate metabotropic transmission antagonist MCPG causes a sharp decrease of the background level and the induced response intensity, compared to the previous control. At the same time, the conditioned reflex movement latency significantly increases, compared to the previous control — in specified cases from 887 to 976 and from 488 to 628 msec. In the case of joint D and MCPG application, changes in the background and induced activities due to MCPG application were partly leveled. This is confirmed by the statistical analysis of the effects of the glutamate metabotropic transmission antagonist agonist and antagonist, D and MCPG. The agonist causes a moderate increase in the background and induced activities of the studied neurons. It is definitely proven that the antagonist of metabotropic MCPG receptors depresses both the background and induced pulse activities and the latency of neuron pulse response and the latent period of the conditioned reflex response increase. In the case of joint application of the glutamate metabotropic transmission agonist and antagonist, the background and induced activities increased, so that the blocker effect was actually eliminated.

Assuming that effects, caused by MCPG application, can be related to the involvement of GABAergic effects, we attempted to clarify the impact of bicuculline on the responses, caused by MCPG application (Fig. 12).

As shown by an example of two neurons, MCPG application was accompanied by decrease of the background and induced pulse activities of neurons and a sharp increase of the conditioned reflex response latency, from 675 to 1268 and from 579 to 1138 msec. In the subsequent control, the return to initial values for the first neuron was very slow, and for the second neuron it was faster. Bicuculline increased the background and induced pulse activities; however, no substantial change in the motor response latency was found. In the subsequent control, the background and induced pulse activities differed from the initial ones slightly.

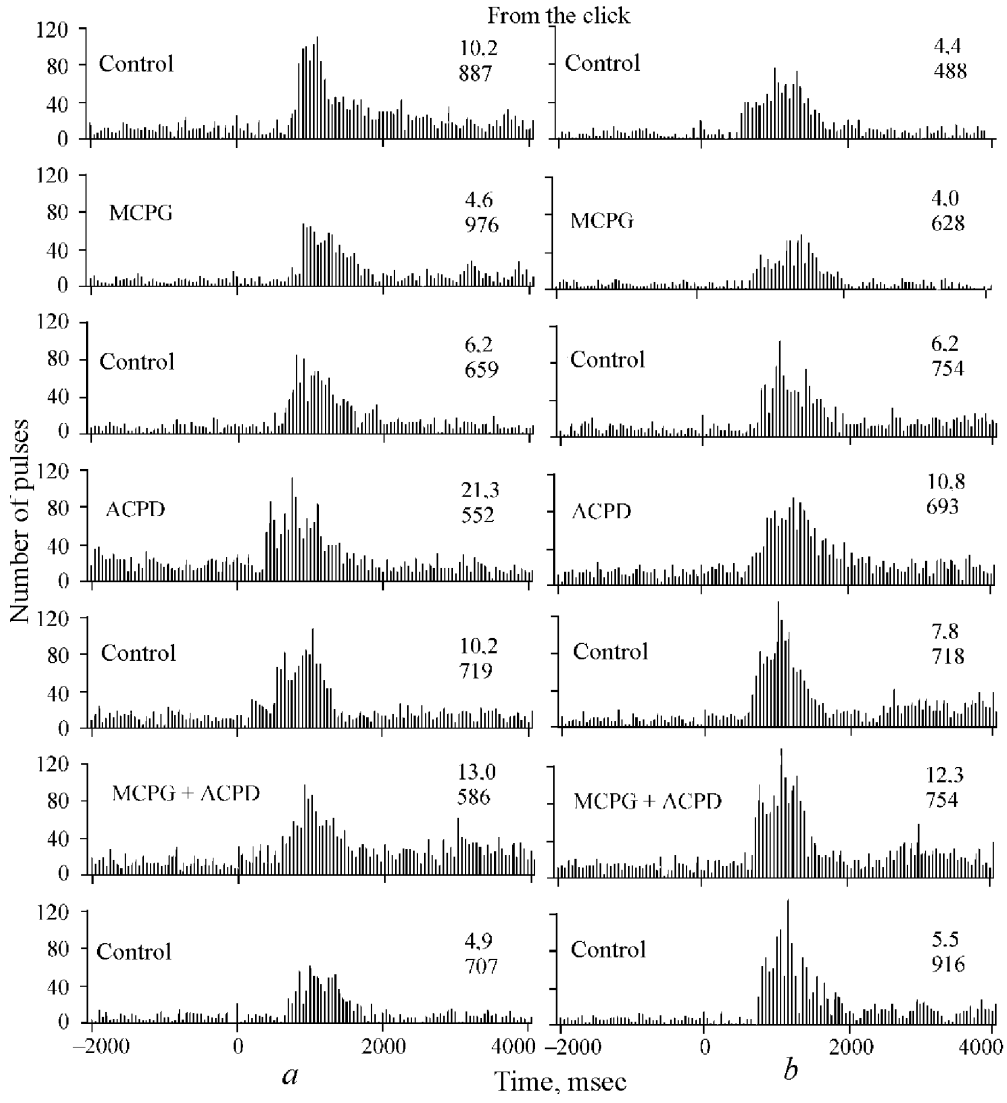


FIGURE 11. Effect of ACPD and MCPG on the background (figures at the top right, pulse/sec) and induced pulse activities of two neurons (a, b) in the sensomotor cortex and on the latency of the conditioned reflex motor response (figures at the bottom right, pulse/sec).

Application of MCPG sharply depressed the background and induced pulse activities. In parallel, the motor response latency increased substantially, almost 2 times. In control after the application, the first neuron (Fig. 12a) recovered the background level and the response intensity, but the motor response latency period decreased very slowly, not reaching the initial level even by the end of the experiment. For the second neuron (Fig. 12b), the return to the initial motor response latency after G application occurred in the second control. This suggests that in this case, the depressive effect of CPG is, possibly, exerted through the blocking of glutamate metabotropic receptors on the interneurons in the studied sensomotor cortex area.

It is seen in Figure 13 that bicuculline *per se* and in combined application with MCPG reliably increases the background activity level. It is important to underline that MCPG application with a high reliability facilitates the increase of the pulse response latency and the conditioned reflex movement latency. The effects caused by MCPG application are totally eliminated by bicuculline. This is a serious indication that bicuculline, blocking GABAergic transmission to pyramidal neurons, whose activity we mostly record, protect these neurons against inhibitory inputs. Consequently, it can be assumed that the blocking effect of MCPG is exerted via inactivation of metabotropic glutamate receptors, located on the bodies of inhibitory interneurons.

Such differences in the effects of the ionotropic and metabotropic glutamate transmission blockers on the background and induced pulse activities of the neocortex neurons, predominantly pyramidal neurons, are related to the fact that the majority of ionotropic glutamate synapses are concentrated directly on pyramidal neurons, while metabotropic glutamate receptors localize not only on pyramidal neurons, but also on association inhibitory neurons. Therefore, it would be interesting to learn the specific features of distribution of ionotropic and metabotropic receptors on neocortex neurons.

Specific summary results of activation and depression of neuron ionotropic and metabotropic associations in the sensomotor cortex are given in Table 2.

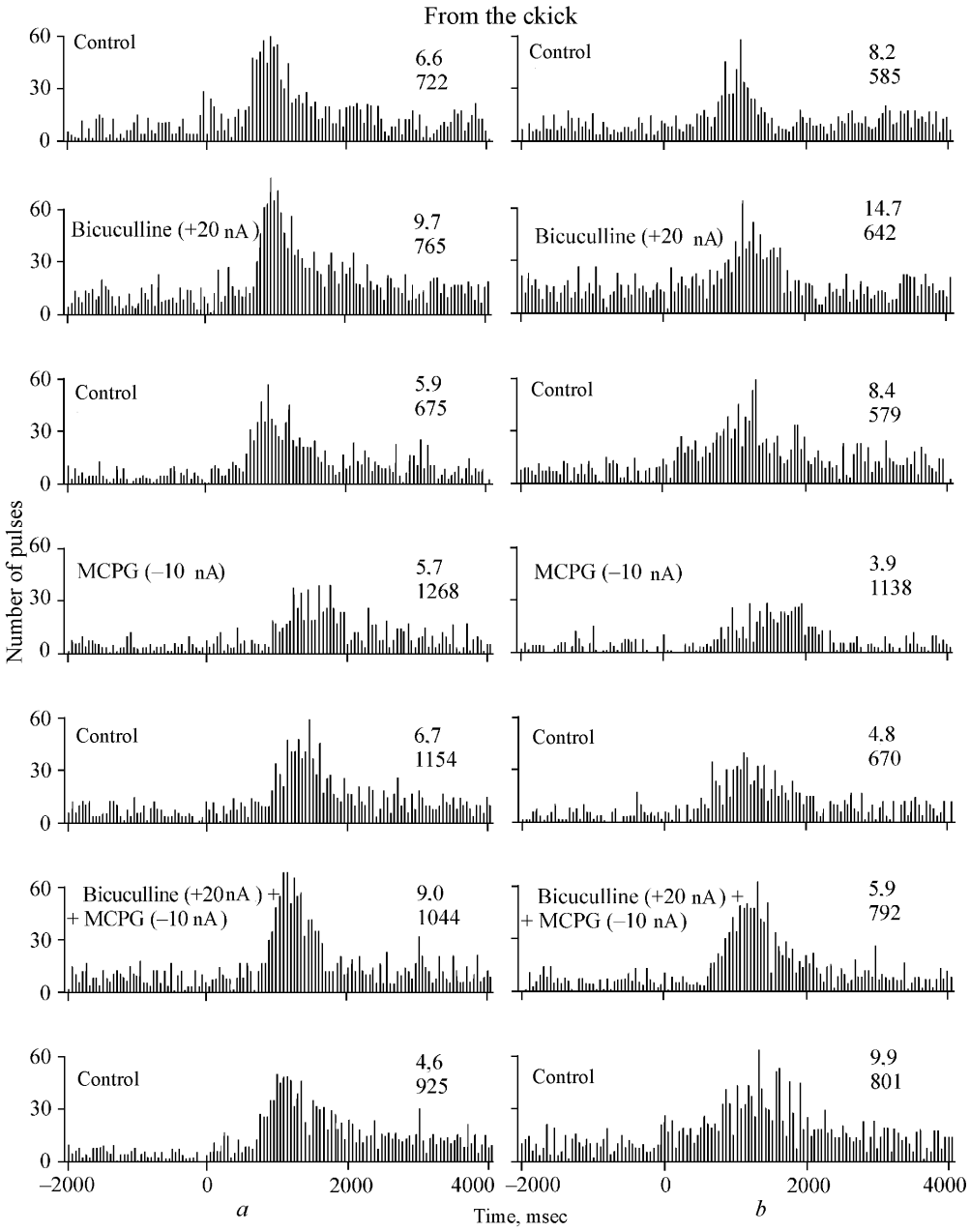


FIGURE 12. Restorative effect of bicuculline on the background (figures at the top right, pulse/sec) and induced pulse activities of two neurons (a, b) and on the conditioned reflex motor response latency (figures at the bottom right, pulse/sec), caused by MCPG application.

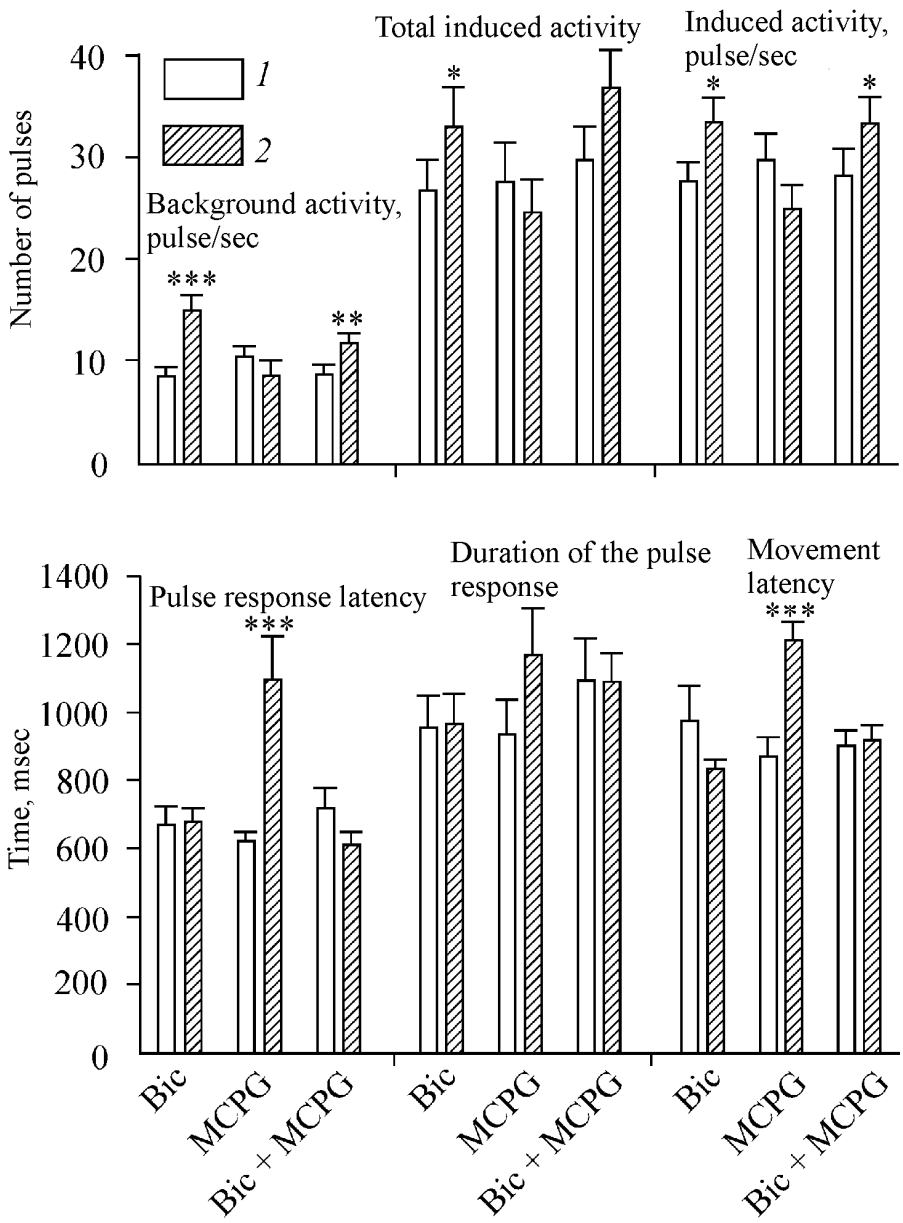


FIGURE 13. Statistical estimation of MCPG and bicuculline effect on the pulse activity of 17 neurons in the sensomotor cortex and on the conditioned reflex motor response latency.

In the case of application of CNQX, an agonist of ionotropic kainate and quisqualate receptors, the background neuron activities increased in 31 out of 34 studied neurons, sometimes 2–3 times compared to the initial background level. The conditional signal response latency shortened in 26 neurons and its duration increased in 22 neurons. At the same time, the intensity of pulse charges notably increased in the majority of cells. Normally, this increase on the average did not exceed 30–50% compared to the control response, though in some neurons the response intensity was 2 times higher.

A similar picture was seen for the application of NMDA receptor antagonist AP-7. In 24 out of 29 studied neurons, the background activity level increased during the application. For some neurons this background level increased 2–3 times. At the same time, the response intensity increased for 23 neurons of the same sample. Application of another NMDA receptor blocker, MK-801, caused nearly the same changes in the pulse activity as in the case of AP-7. Application of MK-801 caused an increase of the background activity in 15 out of 19 recorded neurons, whereas the induced response latency shortened in 12 neurons and the response duration increased in 11 neurons.

Since MCPG, just as GABA, depresses the background and induced pulse activities of neurons, it was interesting to investigate interaction of effects by the metabotropic transmission antagonist MCPG and bicuculline, a blocker of GABA. It was mentioned before that bicuculline increases the background and induced pulse activities, without substantial effect on the motor response latency. This is well demonstrated in Figure 12. As shown in histograms, MCPG application is accompanied by the reduced background and induced pulse activities. In parallel, the pulse response latency increases with a high reliability. It is interesting to note that in the subsequent control it was proved that the pulse response latency in one case remained higher than the initial values. In another neuron, it immediately returned to the initial values. Joint application of bicuculline and MCPG is accompanied by recovery of the background and induced pulse activities. The motor response latency approximates the initial value. All this leads to a significant

TABLE 2. Effects of application of ionotropic and metabotropic glutamate transmission agonists and antagonists on the background and induced activities in sensomotor cortical neurons, as well as the latency of neuron and summary motor responses during a conditioned reflex

Substance	Number of neurons	Background activity, pulse/sec		Pulse response latency, msec		Pulse response duration, msec		Movement latency, msec	
		C	A	C	A	C	A	C	A
AMPA	16	15.8 ± 4.5	19.5 ± 5.6*	796.9 ± 221.7	740.6 ± 189.0	862.5 ± 396.4	1108.0 ± 329.0**	971.7 ± 290.9	964.2 ± 370.8
NMDA	15	14.6 ± 2.8	22.2 ± 5.1**	906.7 ± 306.4	796.7 ± 260.8	788.8 ± 236.7	933.3 ± 240.1*	831.5 ± 202.6	867.5 ± 128.1
CNQX	34	11.9 ± 4.2	19.2 ± 5.1**	787.4 ± 302.0	671.0 ± 231.0**	729.4 ± 263.4	857.3 ± 297.5*	930.7 ± 313.7	1003.9 ± 402.8
AP-7	29	14.7 ± 5.5	21.8 ± 5.1***	874.0 ± 252.7	800.0 ± 202.7	924.1 ± 228.4	989.7 ± 223.1	975.2 ± 337.5	830.9 ± 267.9
MK-801	19	15.1 ± 4.0	18.6 ± 5.6**	831.6 ± 162.6	700.0 ± 213.5*	786.8 ± 270.0	928.9 ± 366*	1055.3 ± 336.9	1148.5 ± 366.7
ACPD	20	13.5 ± 1.1	15.7 ± 1.0	680.0 ± 53.1	667.5 ± 31.5	21.6 ± 2.2	25.7 ± 3.5	890.3 ± 62.7	862.7 ± 68.7
MCPG	72	9.1 ± 0.8	6.1 ± 0.4***	540.4 ± 35.8	671.0 ± 43.0***	23.2 ± 1.5	20.7 ± 1.5*	764.8 ± 34.0	975.5 ± 36***
S-4C-PG	21	11.2 ± 1.6	9.3 ± 0.9	580.7 ± 53.2	725.1 ± 53.5**	25.2 ± 4.9	20.7 ± 3.8	859.8 ± 61.9	1003.1 ± 74.8*

Note. Intensity of neuron response is expressed as the number of pulses in the response above the background level. Here and in Tables 3 and 4: C — control, A — application; asterisks denote the reliability of results calculated by the Student *t*-criterion: one asterisks — $P < 0.05$, two asterisks — $P < 0.01$, three asterisks — $P < 0.001$.

increase of both the background and, especially, the induced pulse activities.

The complex preparation S-4C-PG, which is considered as a competitive agonist, affecting mGluR1 and also acting as mGluR2 agonist, does not manifest a clearly directional effect on the neuron activity during the development of the conditioned reflex responses.

The obtained experimental material is difficult for discussion, since the effects of the applied substance are seen both on the activity of a whole group of neurons surrounding the neuron, whose pulse activity is to be recorded. As discussed above, sustainable and long-term recording in our conditions was possible and conducted from large neurons with an open electrical field, at a depth of 1.5–2.0 mm from the cortex surface. The beginning of the pulse response in 1/3 of the studied neurons led the beginning of the movement by 150 msec or more, which is estimated as their belonging to pyramidal tract neurons. The rest of the neurons could also belong to pyramidal cells, but were connected directly not with the biceps, but rather with other muscles of the limb which, possibly, were activated as participants in the organization of a later motor-response phase. It is known that a sustainable and protracted intercellular recording of pulse activity by a glass 4–10-MΩ microelectrode is possible when the tip of the microelectrode is at least 50–100 μm away from the body of the recorded neuron. Possibilities of the microionophoretic propagation of the applied synaptically active substance with the current of 10–20 nA, 4-sec duration, which we normally used in our experiments, also do not exceed 100 μm in radius.

The fact that selective antagonists of ionotropic glutamate transmission in our experimental conditions resulted in increased background and induced activities of neurons in the sensomotor cortex pushed us to pay attention to possible involvement of the GABAergic system, in these paradoxical responses. We followed the character of changes in the background and induced pulse activities during effects on the brain cortex inhibitory system using a small sample of neurons with GABA and bicuculline application. These substances were also investigated with selective agonists and

TABLE 3. Effects of application of GABA and bicuculline on the background and induced activities of sensomotor cortical neurons during a conditioned reflex

Substance	Number of neurons	Background activity, pulse/sec		Pulse response latency, msec		Pulse response duration, msec		Movement latency, msec	
		C	A	C	A	C	A	C	A
GABA	18	13.0 ± 4.5	5.4 ± 2.0**	825.0 ± 220.6	1025.0±337.0*	1063.0 ± 317.0	868.8 ± 527.5	782.6 ± 171.9	983.2 ± 256.0**
Bicuculline	20	11.1 ± 4.8	20.5 ± 5.9**	787.5 ± 135.6	772.5 ± 162.6	927.5 ± 205.5	1083.0 ± 308.3	875.3 ± 290.1	872.6 ± 311.1

antagonists of the ionotropic glutamate transmission in relation to the background and induced activities of cortical neurons. In 14 out of 16 neurons affected by GABA, the background activity was reduced 2 times on the average, compared to the initial activity. The pulse response latency to conditional sound irritation increased, while the duration of the responses shortened. The intensity of neuron pulse responses and their frequency to the conditional stimulus were sometime decreased 2 times (Table 3).

It should be noted that GABA application decreased the background activity level of some neurons 2–3 times, compared to the initial control level. Latent response periods increased, while their duration decreased. Often, application of this substance led to a substantial, statistically reliable, increase of the pulse response latent period and the motor response latency. It is interesting that the alleviating effects of some ionotropic glutamate transmission antagonists in the case of joint GABA application disappeared, thus sharply decreasing the background and induced neuron activities. On the contrary, application of the GABA receptor blocker bicuculline was accompanied by a significant increase of the background activity in 18 out of 20 studied neurons. The latent period of responses to the conditional sound decreased only for 7 neurons and, on the contrary, increased for 8 neurons. The pulse response intensity increased for all neurons, despite the decrease of the response duration for a part of neurons (7 out of 18).

It is interesting to investigate the interaction between the effects of the NMDA receptor antagonists, AP-7, and bicuculline. As shown in Table 4, during application of bicuculline the neuron properties were not changed, except for a clear increase of the background activity. However, when AP-7 and bicuculline were applied together, the alleviating action effect was eliminated; therefore, the response intensity was not higher than in the initial control case. In other cases, with a more intensive initial response to bicuculline than to AP-7, the pulse response did not manifest occlusion, though it was more intensive than the control case. It was mentioned above, that in experiment in a wakeful animal the long-term stable extracellular recording of pulse activity is possible only if the tip of the microelectrode is located not very close to the

neuron body. This condition is observed for leads from large pyramidal neurons that have an open electrical field, unlike small association neurons with closed electrical fields.

It is known that the ratio of inhibitory and excitatory synapses on pyramidal neurons is about 4:1, while the bulk of excitatory synapses are on the stems of apical dendrites and their aciculae. Application of synaptically active substances in our experiments can only insignificantly propagate towards apical dendrites, since the possibilities of the microionophoretic propagation of the applied substance with a current of 10–20 nA, 4 sec, which we usually applied in our experiments, do not exceed 100 μm in radius, as mentioned above. At the same time, interneurons, *inter alia* short-axon inhibitory interneurons, can be located close to the pyramidal neuron bodies.

Based on the fact that the effect of activity amplification in the recorded pyramidal neurons, when selective glutamate antagonists are applied, is, probably, connected to the blocking of the synaptic excitation of inhibitory interneurons, but to their synaptic contacts on the studied neuron. As a consequence, this leads to alleviation of their inhibitory effect on the background and induced activities of large pyramidal neurons. Although activation of excitatory glutamate effects in the region of the pyramidal neuron body for this application can also be eliminated, but powerful excitatory effects on synapses of remote apical dendrites are fully retained, because the applied substances in the effective concentration in our experiments do not go there. Therefore, depression of an insignificant quantity of effective excitatory synapses on the pyramidal neuron bodies is leveled by the blocking of a great number of effective inhibitory terminals on the body and the axon hillocks of the same cells. Retention of the excitatory effects of dendrites covers the switching off of excitatory effects on the cell body. The fact that the character and manifestation of inhibitory effects on neurons, whose activity we record, is related to activation or, vice versa, blocking of inhibitory synapses on the cell body, near the pickup electrode, is confirmed by the experimental results with GABA and bicuculline application. Effects of application of these substances fully coincide with the expected responses during

TABLE 4. Impact of bicuculline on the effects of ionotropic and metabotropic blockers of glutamate transmission

Substance	Number of neurons	Background activity, pulse/sec		Total induced activity		Induced activity		Pulse response duration, msec		Pulse response latency, msec		Movement latency, msec	
		C	A	C	A	C	A	C	A	C	A	C	A
Bicuculline	17	12.5 ± 1.2	21.4 ± 1.4***	40.5 ± 3.6	58.3 ± 5.2*	37.3 ± 3.6	44.2 ± 3.6	1113.2 ± 88.4	1320.5 ± 55.9	767.4 ± 35.1	761.1 ± 36.7	1147.0 ± 140.8	1064.0 ± 152.2
AP-7	17	16.1 ± 1.1	25.6 ± 4.3*	38.7 ± 2.2	64.2 ± 7.2**	34.6 ± 2.0	46.7 ± 4.2**	1134.6 ± 68.47	1384.6 ± 101.6*	819.2 ± 38.2	730.8 ± 49.2	1192.3 ± 189.8	846.1 ± 41.8
Bicuculline + AP-7	17	14.1 ± 0.9	22.1 ± 1.2***	47.0 ± 5.2	69.3 ± 7.5***	40.6 ± 2.5	49.7 ± 4.3**	1155.9 ± 75.2	1414.7 ± 97*	717.6 ± 20.5	755.8 ± 35.3	908.8 ± 137.4	844.1 ± 46.5
Bicuculline	15	10.2 ± 0.8	14.1 ± 1.4***	28.7 ± 3.9	36.9 ± 4.2*	27.5 ± 2.4	32.4 ± 2.2*	1190.9 ± 235.8	1227.2 ± 177.3	653.8 ± 56.4	657.6 ± 43.4	1242.3 ± 240.3	1030.7 ± 151.8
MCPG	15	11.2 ± 1.1	7.1 ± 0.9***	28.3 ± 4.6	28.1 ± 3.9	27.6 ± 3.2	21.3 ± 1.6*	1066.6 ± 163.4	1312.5 ± 149.1	589.3 ± 38.2	925.0 ± 50.4***	930.7 ± 96.6	1273.0 ± 53.2***
Bicuculline + MCPG	15	8.2 ± 1.1	10.9 ± 1.1*	31.4 ± 4.1	40.8 ± 5.4*	26.1 ± 2.3	28.5 ± 2.5	1268.7 ± 163.9	1450.0 ± 313.9	720.0 ± 74.9	675.0 ± 40.8	1030.0 ± 143.6	1245.2 ± 244.3

activation and depression of GABAergic terminals of the inhibitory neurons. The best proof that glutamate antagonists alleviate the responses specifically via blocking glutamate activation of the system of inhibitory interneurons is the fact that paradoxical enhancement of pulse responses, caused by separate application of bicuculline and AP-7, in the case of joint application is totally eliminated (Fig. 8b).

Table 4 presents the experimental results for application of the ionotropic glutamate transmission NMDA receptor agonist AP-7 and the metabotropic glutamate receptor antagonist MCPG, and the effects of these antagonists when the GABA blocker is used. In each series of experiments, we made a tentative estimation of the effects of bicuculline on the background and induced pulse activities of these neurons. In two series of experiments, we studied 32 neurons. The use of bicuculline increased the background activity and increased the number of pulses in the induced response with a high degree of reliability. The neuron response latency, the motor response latency, and the response duration did not change substantially.

Though it seems strange, the ionotropic glutamate transmission antagonist AP-7 also caused an increase in the background and induced pulse activities and together with bicuculline increased the proved increase of both the background and induced pulse activities. However, this did not affect the neuron response latency. On the contrary, it was proved that the metabotropic antagonist MCPG indeed decreased the background activity, increased the neuron response latency period and, with a high reliability, increased the motor response latency.

Effects of the ionophoretic application of these antagonists of ionotropic and metabotropic glutamate transmission (AP-7, MCPG), when applied together with bicuculline, were partially leveled: the pulse response period and its latent period, the motor response latency and the background and induced pulse activities approximated the initial values. Application of bicuculline together with MCPG fully eliminated the proven increase of latency caused by this metabotropic receptor antagonist.

Given that bicuculline eliminates or decreases the effects of inhibitory neurons, it is interesting to study the character of its

interaction on the effects of two types of antagonists of glutamate transmission, i.e., ionotropic and metabotropic. It was found that joint application of AP-7 and bicuculline increases the background activity, but, to some extend, alleviates the induced activity. At the same time, application of bicuculline together with MCPG fully eliminates the proved increase of the neuron response latency caused by this metabotropic receptor antagonist and the latent period of conditioned reflex movement.

It is known that inhibitory interneurons are rich in parvalbumin and calretinin. The first is identified with basket cells, while the second with acicular stellate cells. Part of association neurons in the cortex has an excitatory effect. Changes that we observed in neuron responses such as depression of the background and induced activities by glutamate transmission antagonists can be related to the blocking of these receptors on the effective glutamate excitatory synapses in the region of pyramidal neuron bodies. The alleviating role of bicuculline can be explained by the blocking of inhibitory synapses on pyramidal neuron bodies, whereas the elimination of inhibition, caused by the blocking of glutamate transmission, by bicuculline, can be explained by the fact that elimination of inhibitory effects on the pyramidal neuron body enhances the efficiency of excitatory synaptic inputs in the region of its apical dendrites, where ionophoretic application of glutamate transmission blockers is not always found.

By the experimental results presented in this chapter, we can draw the following conclusions:

1. The background activity of neurons in the sensomotor cortex and the induced activity in response to the conditional stimulus increase not only in the case of microionophoretic application of glutamate ionotropic transmission antagonists, but also in the case of application of their ionotropic antagonists, such as CNQX, AP-7, MK-801.
2. The background and induced pulse activities of neurons in the case of application of glutamate metabotropic antagonists, such as ACPD, S-4C-PG, moderately enhance. Application of MCPG antagonist of the metabotropic glutamate receptors reduces the background level, significantly de-

presses the induced pulse response, and is accompanied, as a rule, by a noticeable increase of latent periods of conditioned reflex responses.

3. Application of GABA leads to depression of the background activity and activity induced by the conditional stimulus of the sensomotor cortex neurons, while application of bicuculline, on the contrary, enhances the background and induced neuron activities.
4. GABA eliminates the alleviating effect of the glutamate transmission antagonists when they are applied together. Joint application of ionotropic glutamate transmission antagonists together with bicuculline is accompanied by full elimination of their alleviating effect on the pulse responses or occlusion of effects, which testifies to involvement of ionotropic glutamate agonists in activation of association inhibitory neurons.
5. Discussion of the specific features of application effects of synaptically active substances makes it possible to conclude that a wakeful animal has a constant tonic inhibitory control of pyramidal neuron pulse responses to a conditional stimulus.

V. DOPAMINERGIC EFFECTS ON NEURON ACTIVITY IN THE SENSOMOTOR CORTEX

Following a preliminary familiarization with the effects of glutamate and GABA agonists and antagonists on the sensomotor cortex neuron activity, we attempted to evaluate the character of dopamine effects on their activity. The task was not only to study the dopamine effects on the pulse activity of specific neuron groups. Above all, we tried to identify specific effects of this substance on neuron responses caused by glutamate and GABA agonists and antagonists in conditions close to natural conditions, with real performance of physiological functions.

The modulating alleviating and inhibitory effects of dopamine on the background and induced activities of the recorded neurons, presumably pyramidal neurons, can be explained from the literature sources not only by a direct impact on the studied

neuron, but also by activation of excitatory and inhibitory interneurons.

As mentioned before, dopaminergic innervation is sent to the sensomotor cortex from VTA. The dopaminergic fibers form synapses both on pyramidal neurons and interneurons. It is known that the return excitatory transmission between pyramidal neurons in the prefrontal cortex is depressed presynaptically by dopamine acting via D1 receptors.[82] However, the excitability of pyramidal neurons is modulated by effects of not only inhibitory, but also excitatory interneurons. Inhibitory interneurons in the cerebral cortex differ not only structurally, depending on the character of their dendrite and axon branches, but also in the specific features of background pulse activity, the responses to a depolarization current surge. It was mentioned before that in terms of functions several interneuron groups are identified in the cortex. Neurons with fast pulses predominantly innervate the soma or the initial segment of the pyramidal cell axon, which allows controlling the initiation of the action potential. Other interneurons primarily regulate excitation of dendrites and the efficiency of excitatory inputs. Therefore, it is important to evaluate correctly the possible dopamine effect not only directly on pyramidal neurons, but on excitatory and inhibitory interneurons in neocortex too. It is known that dopamine may exert a significant effect on excitatory and inhibitory responses of individual neurons, *inter alia*, on various groups of interneurons in the sensomotor cortex. Possessing different properties and associations, these interneuron groups, affected by dopamine, manifest different types of responses.[83] The task of our studies was not only to evaluate the affects of this substance on specific neuron groups, but also to try and identify specific features of its modulating impact on neuron groups, which are caused by glutamate and GABA agonists and antagonists in conditions close to the real brain function.

The alleviating effect of dopamine on the background and induced activities of neurons recorded in our experiments, predominantly pyramidal neurons, can be explained by their direct activation as well as activation of the excitatory association interneurons. The inhibitory effect of dopamine can be manifested in

the blocking of dopaminergic synapses, in particular, in artificial experimental conditions, using sulpiride and SCH.

Dopaminergic synapses are formed both on pyramidal neurons and on interneurons. It is known that the reversal excitatory transmission between pyramidal neurons, for example, in the prefrontal cortex, is presynaptically depressed by dopamine, acting via D1 receptors.[82] However, as discussed in the previous chapter, excitability of pyramidal neurons is modulated by inhibitory interneurons too.

The previous sources devoted to experimental evaluation of the dopamine effects on the neuron activity in the neocortex include very broad analysis of neuron activity in the rats' premotor cortex, since this particular cortical region has developed dopaminergic associations and is always involved into the working memory and targeted behavior processes in animals. Solution of problems related to the working memory showed that the prefrontal cortex neurons manifest a stable increase of activity, supporting targeted information and preparation for future actions. Dopamine modulates these two processes via D1 receptors.

However, Durstewitz et al.[10] assume that the dopamine function in the period on delayed activity and the underlying neuron mechanisms are investigated insufficiently and, hence, are not quite clear.

In order to check the effect of dopamine on modulation of ion current that can lead to a more stable involvement of neurons in a specific function, the authors developed a special model for analyzing the prefrontal cortex neuron activity. Usually, dopaminergic effects are studied in *in vitro* models. They tried to reproduce effects of significant increase of dopamine concentrations on direct responses caused by an external signal, on the delayed type of activity and the spontaneous activity in model nets. It is known that in such case the force of afferent stimulation, required to disturbed the delayed type of activity, increases parallel to the increase of the dopamine-caused shift on the network parameters, thus ensuring a more stable current activity of Na^+- and NMDA conduction. Conduction stability may increase with a decrease of AMPA.

The researchers wanted to check also the role of the increased GABA(A) conduction, which is manifested after stimulation of dopaminergic synapses D1 receptors. Durstewitz et al.[10] paid attention to the fact that physiological effects of dopamine, obtained by different researchers, may significantly vary in different brain regions: in hippocampus, striatum, and prefrontal cortex. Therefore, in studying a specific structure, the obtained results can, and should be, compared only with data, obtained on the same object and in the same or, at least, close experimental conditions. For example, it is believed that researchers studying the working memory in behavioral responses of animals must concentrate their attention basically on D1, but not on D2 receptor agonists and antagonists. It is assumed that short-term D2 effects, possibly, perform other functions, not related directly to retention of traces in the working memory.

From reference sources, it stems that dopamine:

- shifts the sodium current activation threshold towards a greater hyperpolarization of potentials and slow inactivation processes of these currents;
- reduces the slow inactivating potassium current in prefrontal cortex pyramidal neurons;
- reduces the duration and amplitude of dendrite calcium spikes;
- increases NMDA-type synaptic currents in the prefrontal cortex via D1 receptors; and
- reduces AMPA-type currents in the frontal cortex and striatum simultaneously. The total effect of combined changes in NMDA and AMPA currents is the reduction of the EPSP amplitude and increasing of its duration.

It should be noted that the opinions of scientists about the character of dopamine effect on neocortex neurons are not always similar. The results of initial studies of activation of dopaminergic fibers, beginning from the ventral segmental nucleus,[84] showed that dopamine effects decrease the spontaneous and induced activities of prefrontal cortex neurons in a wakeful animal. These authors verified the effects of high dopamine concentrations on passive and active properties of pyramidal neuron membranes in the prefrontal

cortex. For intracellular lead in an acute experiment, they measured cellular excitability in response to a 1-sec depolarizing current surge in the control and after application of dopamine (0.05–30.0 μM). This caused a decrease of the number of action potentials compared to the previous control. In some experiments, following dopamine washing, a short-term excitation increase was observed.

Pharmacological analysis using D1 receptor agonists and antagonists (SKF38393, SKF81297, SCH 23390), as well as D2 receptor agonists and antagonists, quinpirole and sulpiride, suggested that the reduction of the pulse activity and input resistance of neocortex neurons was mediated by activation of D2 receptors. The obtained results were used to draw a conclusion that dopamine in high concentrations exerts inhibitory effects on pyramidal neurons of layer V of the prefrontal cortex in rats through activation of D2 receptors.

This conclusion was confirmed, to some extent, by other researchers, for example, in the recently published work by Awenovicz et al.,[85] where the authors used experimental material to prove that dopamine inhibits activity of pyramidal path neurons in the premotor cortex in rodents. These authors studied changes in the background pulse activity of neurons in the cerebral premotor cortex in rats after a local application of dopamine. The study was made in an acute experiment on identified pyramidal path neurons in rats, using multichannel microelectrodes, which allowed also recording neuron activity and making, when required, ionophoresis of several synaptically active substances.

However, these experiments included a too protracted, in our view, application of dopamine for 30 sec, which caused a progressive nonlinear decrease of the background spontaneous discharge frequency almost in all identified pyramidal tract neurons. Of course, such unnaturally long ionophoretic application of dopamine is perceived as a strange and hardly justified technique. Nevertheless, it is interesting to note that this decrease was manifested and reached 71% of the initial background level only about 20 and 30 sec of ionophoresis. When dopamine antagonists, D1 selective (SCH 23390) and D2 selective (eticlopride), were

applied, both turned to be effective in terms of blocking the dopamine-induced inhibition of the background activity of almost all of the studied pyramidal neurons. The mean background level was the same during joint application of dopamine and SCH 23390, within 3% of the initial level, and for dopamine and eticlopride, within 11% of the background level. The background frequency of pyramidal tract neuron discharges significantly increased during glutamate ionophoresis, up to 141% of the initial level. This increase was eliminated for all pyramidal neurons (the frequency reached 98% of the initial level) after joint application of glutamate and dopamine, which indicates the character of dopaminergic interaction with glutamate transmission.

In our opinion, this study, in addition to the duration of applications, had another disadvantage, i.e., it included only investigation of the background activity of neurons. Actually, the authors attempted to study an artificial situation, not correspondent to the physiological reality, rather than the natural process. Also, by emphasizing their attention on pyramidal neurons, they seemed to forget that dopamine could exert and, undoubtedly, exerted effect on the system of association cortical neurons. It should be taken into account that some researchers include SCH 23390 also into antagonists of central serotonin receptors. Therefore, it could not be ruled out that serotonin-sensitive structures participated in the produced effect. It was found, for example, that such effect does not exceed 50–60% of the effect when selective 5-HT–cinanserine, methysergide, and ketanserin blockers were applied simultaneously.

In view of this, of significant interest for proper understanding of interneuron relations in the neocortex and the role in their implementation of dopamine are investigations aimed at analyzing the mechanisms of dopaminergic activation of fast-discharge interneurons that exert inhibition in the cortex. In the earlier experiments, Grobin and Deutch[11] paid attention to the fact that dopaminergic axons in the prefrontal cortex formed synapses not only with pyramidal neurons, but also with interneurons. It ensues from electrophysiological studies that dopamine polarizes some interneurons, containing GABA. The authors studied dopaminergic

regulation of the extracellular GABA level *in vivo*, using microdialysis. It turned out that systemic application of apomorphine, a common D1 and D2 receptor agonist, increased its level in the prefrontal cortex, but did not change the level of glycine. The apomorphine-induced increase of GABA was blocked by TTX infusion. Local dialysis infusion of quinpirole, a D1 receptor agonist, led to dose-dependent increase of the intracellular GABA. And, vice versa, application of D1 receptor agonist SKF38393 did not change its level. The capacity of systemic apomorphine to increase extracellular GABA level in the prefrontal cortex was blocked by local application of D2 receptor antagonist sulpiride, but did not decrease notably during local application of D1 receptor antagonist SCH 23390. Similarly, local application of D2 receptor agonist quinpirole increased the extracellular GABA level, but not the level of SCH 23390. In the opinion of the authors, these facts testify that dopamine agonists increase the release of GABA in the prefrontal cortex through stimulation of D2 receptors. Given the above experimental data, the authors assume that changes in the GABA function on the cortex in schizophrenia, for example, are the changes in the dopaminergic cortex function.

The mechanism underlying the inhibitory effects of dopamine on the excitability of pyramidal neurons of layer V in the prelimbic region of the medial prefrontal cortex in rats was studied by other authors. In particular, Gulledge and Jaffe[84] showed that in control conditions it depressed generation of action potentials and input resistance. The presence of GABA(A) receptors antagonists blocked the dopamine-induced depression of action potential generation and eliminated the delayed increase of excitation up to 20 min after its washing. Unlike the generation of pulses, disinhibition did not affect the temporary depression of input resistance, caused by dopamine, which indicated the independent effect of this substance on the input resistance and pulse generation.

According to the hypothesis stating that dopamine influences the effects of the pyramidal cell through GABAergic mechanism, it increases the frequency of spontaneous inhibitory postsynaptic currents both in the presence and absence of TTX. Moreover, focal application of GABA in the perisomatic region simulates the

inhibitory effects when it is injected into the neuron medium without affecting the neuron resistance. Focal application of bicuculline in the same site prevents the inhibitory effect of dopamine on the pulse generation, despite the fact that it does not make any effect on the input resistance. Depression of the input resistance caused by dopamine terminates and is assimilated by the blocker of TTX sodium channels. According to the authors, these results show that the presence of this substance reduces excitability of pyramidal neurons by means of two independent mechanisms. At the same time, dopamine initiates the delayed and protracted increase of excitability, which is partially masked by synaptic inhibition.

More recent studies by Gorelova et al.[13] showed that dopamine affects pyramidal neurons in the cortex not only directly, but can affect their activity indirectly through a system of modulating effects by interneurons. Experiments *in vitro* on neurons in layers V–VI of the prefrontal cortex in rats showed that dopamine substantially increased the frequency of pulse activity of spontaneous GABA-mediating neurons which innervate the same pyramidal neurons. Among the above-mentioned four classes of interneurons, the FS neuron pulse duration makes less than 1 msec. In the case of a strong intracellular depolarizing surge, these neurons are discharged into a series of non-adaptive pulses. They have a low input resistance and a more positive membrane potential, compared to other interneurons.

Such interneurons are described in layers II–II and V of the frontal cortex in rats and monkeys. Dopamine causes their depolarization. Morphologically, these are multipolar cells with clear dendrites and a developed local axonal tree; therefore, they resemble typical basket cells. Dopamine application into a bath, depending on its concentration, causes a reverse depolarization of the FS neuron membrane by 2–6 mV, for a period of 5–9 min. Adding of TTX to the perfusate for blocking sodium channels and CdCl for blocking synaptic transmission does not affect depolarization, induced by dopamine. It is assumed that it directly affects FS neurons and this depolarization does not depend on sodium channels.

During depolarization of FS neurons, caused by dopamine, the neuron excitability increases. The depolarization surge, which before the application was below the threshold so as to cause generation of pulses or which caused only a few pulses, after the application of this substance induced a protracted series of non-adaptive neuron discharges. As a result, it was concluded that dopamine acting via D1/D5 receptors (but not via D2/D4-receptors) directly depolarizes interneurons. SCH 23390, D1 receptor antagonist, significantly reduced depolarization, caused by application of this agonist.

Issues of modulation of perisomatic and peridendritic inhibition in the prefrontal cortex and the role of dopamine are discussed in other works, in particular, by Gao et al.[80] They paid attention to two basic types of local microchains in the prefrontal cortex in rats: association of the pyramidal cell with other pyramidal cells and association of the pyramidal cell with non-pyramidal cells. It is assumed that monosynaptic associations between two or several pyramidal neurons provide the basis for the reverse excitability in the cortex and represent a key mechanism, underlying the stable activity of prefrontal cortex neurons. Associations between pyramidal and non-pyramidal neurons play an important role in the establishment of the neuron specificity and temporal integration among other functionally significant actions. The authors used coupled recording of activity of two neurons in order to study the modulating effect of dopamine on the excitatory synaptic transmission between pyramidal neurons and FS interneurons and to compare these effects with the effect of the re-entrant excitation via coupled recording in the neuron chains of layer V of the prefrontal cortex. It was found that the dopamine effect more depends on the site in the chain where it operates; it is not the same for all excited synapses. This action provides for further increase and establishment of a balance between excitation and inhibition in the layer at cortex output.

Effects of dopamine and glutamate interaction on the excitability of pyramidal neurons of the prefrontal cortex in rats were investigated by other authors who arrived at similar results. For example, Tseng and O'Donnell[86] in the experiments on sections

in the prefrontal cortex of rats, using "patch clamp" recording, demonstrated that application of NMDA, AMPA and SKF38393, D1 agonist, facilitated the increase of concentration-dependent excitation. While application of D2 receptor agonist, quinpirole, caused a concentration-dependent decrease of excitability, the NMDA-mediated response was potentiated by SKF38393. On the one hand, NMDA and D1 agonist synergism depended on the postsynaptic intracellular calcium and protein kinase A, but did not depend on the membrane depolarization. On the other hand, the excitatory effect of NMDA and AMPA was decreased by the D2 agonist. It was unexpected for the researchers that interaction of D2-NMDA was also blocked by bicuculline and picrotoxin. This pushed the authors of the research to make a conclusion that inhibitory effect of D2 receptors on NMDA-induced responses can be mediated by GABA interneurons. And, vice versa, interaction of D2-AMPA involves inhibition of protein kinase A, activation of phospholipase C-IP and intracellular calcium at the postsynaptic level. The authors came to a conclusion that the modulating effects of D1 and D2 receptors of the prefrontal cortex pyramidal neurons are mediated by multiple intracellular mechanisms and activation of GABA(A) receptors, dependent on the involvement of glutamate receptor subtypes.

Investigations made by Seamans et al.[87] add some details into the above data. The researchers paid attention to the role of dopamine in modulation of D1/D5 excitatory dopaminergic inputs of layer V in the prefrontal cortex and detailed the specific behavior related to the working memory. It turned out that D1/D5 agonists facilitate the increase of the NMDA component of EPSP through a postsynaptic mechanism. And, vice versa, they also cause the decrease of the non-NMDA component due to some reduction of the transmitter release. According to the authors, dopamine and glutamatergic axon terminals form synaptic triads in postsynaptic dendrites of deep-layer pyramidal neurons in the prefrontal cortex. It should be born in mind that dopaminergic synapses are located also on somatic and dendrite regions of the membrane of the pyramidal and non-pyramidal neurons of the prefrontal cortex. Many authors pay attention to the fact that

dopamine exerts alternative and often opposing effect in different brain regions.

Seamans et al.[23] specially underlined the bidirectional character of dopaminergic modulation of GABAergic inhibition of prefrontal cortex pyramidal neurons. Experiments on sections with voltage recording on the target cell showed that in the majority of pyramidal neurons dopamine conditioned a two-phase effect on sIPSP, formed by the initial sharp decrease of the amplitude, which was followed by a delayed increase of IPSP. Using specific subtypes of agonists and antagonists, the authors established that such decrease of the amplitude was mediated by D2 receptors, while the subsequent slow developing increase of the amplitude was mediated by D1 receptors. A linear combination of the two agonist effects could reproduce a two-phase dopaminergic effect. It is known that D1 agonists increase sIPSP, but do not affect mIPSP. Evidently, they cause high sIPSP, increasing the inner excitability of interneurons and their axons. In contrast to this, D2 receptors did not affect the induced responses on sIPSP, but they conditioned a notable decrease of mIPSP, which suggested a reduced probability of GABA release. In addition, D2 agonists decrease the postsynaptic response to the GABA(A) agonist. D1 and D2 receptors regulate GABAergic activity in the opposite way, through various mechanisms in the prefrontal cortex pyramidal neurons. The authors think that such bidirectional (alternating) modulation can be very important for manifesting the properties of active nets in this brain region. In experiments on prefrontal cortex neurons, on sections with the recording of voltage, Trantham-Davidson et al.[15] showed that dopamine concentration is a crucial determinant in activation of D1 or D2 signaling. It was mentioned before that the low dopamine concentration (<500 nM) increases sIPSP via D1 receptors, protein kinase A, and cAMP, while higher concentrations (>1 μM) decrease sIPSP. The blocking of any molecule in the D2-related path opens D1-mediated increase in sIPSP, prompting that D1 effects terminate at higher concentrations via this mediated path. Thus, dopamine concentration, acting via specific signal loops, can define the relative quantity of cortex inhibition and, due to this, can differentially regulate cortical net neurons.

Some researchers assign a special role to D3 receptors, in particular, in the process of development of behavioral sensitization, which occurs after a new stimulation.[88] It was found that D3 receptors have 70-time higher affinity to dopamine, compared to D1 and D2 receptors. Such misbalance in the ligand affinity dictates a higher involvement of D3 receptors at regular dopamine concentrations. Sensitization is the result of a partial accommodation of D3 receptors, leading to a progressive locomotor increase after a new stimulus. In opinion of the authors, the specific features of different tolerance of D3 against D1 and D2 receptors can explain the observed development of sensitization after application of cocaine, but not amphetamine. It is assumed that D3 antagonists may precede sensitization.

It should be remembered that the mesocortical dopamine projection, ascending from VTA, terminates, for example, in rats, on pyramidal neurons of the prefrontal cortex layers V–VI. Many of these neurons, in turn, are projected to VTA, thereby providing a path though which the prefrontal cortex can modulate the mesoaccumbens system. Changes of the dopamine function within these systems can condition different neuronal disorders, in particular, schizophrenia and drug addiction. Despite a clear evidence that dopamine modulates the behavioral output from the prefrontal cortex, specific knowledge about mechanisms which change the activity of neurons in this brain region remains limited. Many researchers concentrated their attention on modulation by dopaminergic receptors of potential-dependent potassium currents in experiments on dissociated prefrontal cortex pyramidal neurons. These currents are responsible for the steady-state potential, repolarization and hyperpolarization of the cell and for the shape of the voltage path in case of the subthreshold amplitude. It is assumed that this can help attain a specific target by which dopamine regulates the cognitive function.[89]

Some researchers paid attention to specific effects of dopamine on NMDA receptors in the prefrontal cortex pyramidal neurons and in other brain regions in rats. In particular, one of the studies[90] attempted to check the regulation of NMDA currents by D1 receptors in the prefrontal cortex pyramidal neurons. Applica-

tion of the D1 receptor agonist SKF81297 led to a significant and stable increase of the induced NMDA current in isolated pyramidal neurons. This effect did not depend on protein kinase A or protein phosphatase 1, but was eliminated during neuron incubation in a medium free from calcium. Intracellular application of calcium calmodulin chelator or inhibitor significantly prevented D1 modulation of NMDA receptor currents. Moreover, inhibition of protein kinase C activity or disturbance of protein kinase C association with the binding protein also substantially reduces the D1 receptor effects on the NMDA receptors. This hyper-regulation of NMDA receptors by dopamine D1 receptors, given the previous data on their hyper-regulation, provides cellular mechanisms for reciprocal interaction of D1 and NMDA receptors. In opinion of the authors, such interaction can play an important role in modulation of synaptic plasticity and, hence, in cognitive and emotional processes.

Wirkner et al.[91] attempted to evaluate dopamine-glutamate interaction in the prefrontal cortex in rats, on sections. The authors proved that dopamine (100 μM) potentiates depolarization, induced by application of NMDA (10 mM), but does not affect the response caused by application of AMPA or D2 receptor agonist SKF383393. Such effect was also produced using D1 receptor agonist SCH 23390.

We studied the effects of isolated application of dopamine, its agonists and antagonists, in wakeful animals with a developed conditioned reflex of putting a paw on the support. We also analyzed responses of neurons in the cerebral cortex when animals performed this conditioned reflex. Initially, we made a control investigation of the background neuron response and the pulse response induced by a conditional stimulus and then we applied dopamine, its agonists and antagonists, as well as glutamate transmission agonists and antagonists. In a separate series of experiments, we checked the influence of dopamine on effects of glutamate metabotropic antagonists MCPG and S-4C-PG (Fig. 14).

Figure 14 shows that application of dopamine in the first neuron is accompanied by the increase of background activity from 9.2 to 17.2 pulse/sec, and in the second neuron, from 6 to 9.4 pulse/sec. At the same time, the intensity of the induced response

increases more than 2-fold in both cases. In the subsequent control, the level of background activity in the first neuron even increased, while in the second neuron, it returned to the initial level. The intensity of induced pulse responses decreased, approximating the initial responses in the initial control. Also, in all three series the latency of the conditioned reflex movement of the paw varied within 50–70 msec. When a glutamate metabotropic receptor antagonist MCPG was applied, the animal manifested substantial changed in the background, induced, and motor activities: the background activity of the first neuron reduced 4 times, that of the second neuron 2 times, and the induced pulse responses sharply decreased.

It is important that local application for 4 msec, at 10 nA, caused a sharp increase of the latency period of the motor conditioned reflex response in the animal limb by about 450 sec in both cases. The background and induced neuron activities started to recover very gradually, which can be seen in histograms of the subsequent control. The motor response latency manifested a clear trend to return to the previous level. In joint application of MCPG and dopamine, the levels of background and induced pulse activities increased. The latency of the first neuron movement did not change, but to some extent increased for the second neuron. In any case, it is evident that the inhibitory effect of MCPG on the neuron activity not only decreases, but is fully eliminated in the presence of dopamine. In fact, the background activity of neurons and their response to the conditional stimulus approximated the initial level in the last control series. We can possibly argue that dopamine manifests its stabilizing effect on the background and induced pulse activities in a very expressive way when they deviate from the natural physiological level, conditioned by disturbance of the metabotropic glutamate transmission.

In general, such organization of the experiment helped investigate responses of 21 neurons, which allowed a statistical evaluation of interaction between dopamine and metabotropic glutamate transmission antagonist.

Figure 15 shows that application of dopamine with a high reliability increased the level of the background activity of studied

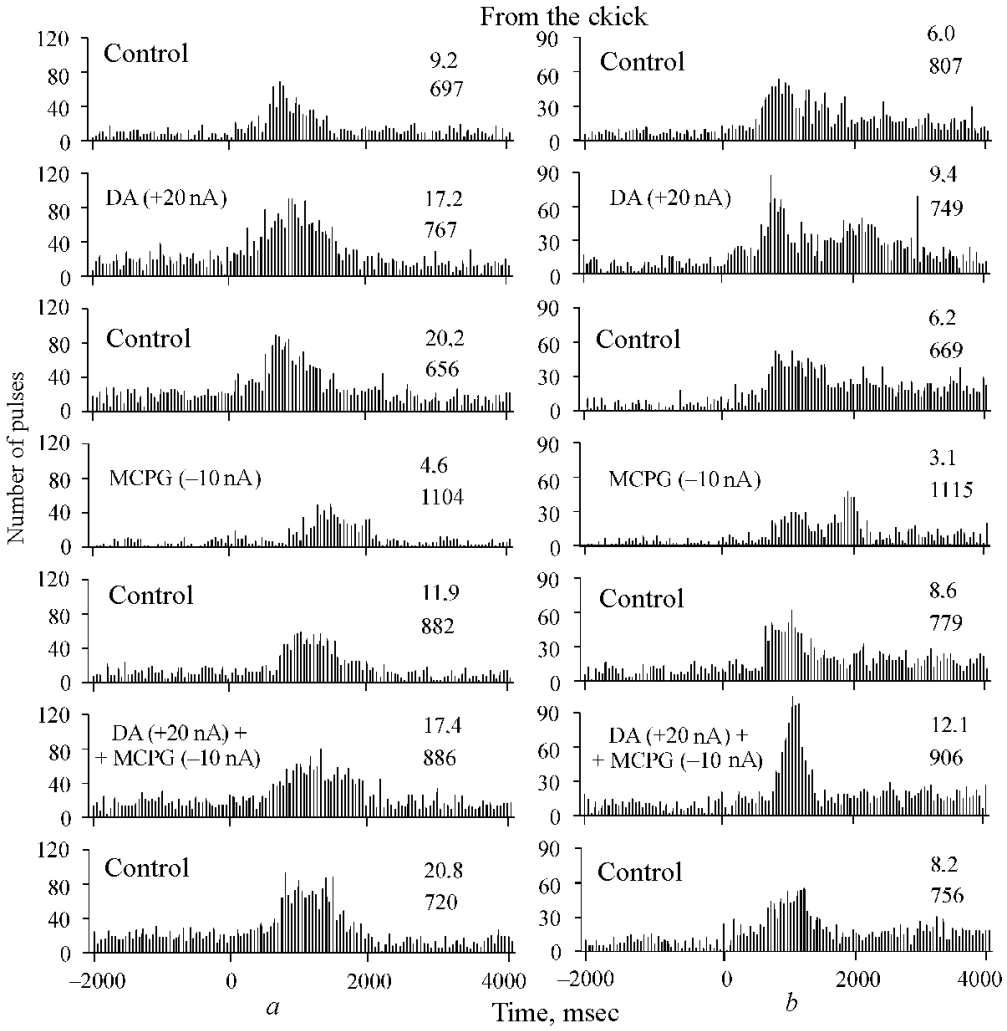


FIGURE 14. Effect of dopamine and metabotropic glutamate receptor antagonist MCPG on the background (figures at the top right, pulse/sec) and induced pulse activities of two neurons (a, b) in the sensomotor cortex and the latency of the conditioned reflex motor response of the limb (figures at the bottom right, msec).

neurons, while application of MCPG, on the contrary, decreased this level. In the case of joint application of dopamine and MCPG, these changes of the background activity were actually leveled. This high-reliability decrease of the induced pulse activity by MCPG after joint application of dopamine and MCPG was replaced by its reliable increase. The reliable increase of the pulse response latency, caused by application of MCPG, was fully leveled when dopamine was present. A sharp increase of the motor response latency period, shown in two histograms of the previous figure, was not manifested in the entire sample of investigated neurons; however, the increase of the pulse response latency, underlying this movement, proved to be statistically reliable. Nevertheless, despite the fact that in some experiments with application of MCPG the motor response latency values were proven to be different from the initial value, for the summary evaluation of the sample as a whole these differences were not statistically reliable. Such differences of the response latency for individual neurons and the entire sample in general, on the one hand, and the conditioned reflex movement latency, on the other, can be explained by the fact that the experimenter cannot always insert the microelectrode correctly into the site of the motor cortex, which is directly associated with the muscle, taking part in the recorded limb movement. In our experiment, the entire radius that allows the microelectrode to "see" the pulsed activity of the neuron, through which the experimenter can influence this activity by ionophoretic application, does not exceed 100–150 μm.

In our experiments, we investigated a sample of 21 neurons by applying another metabotropic glutamate transmission antagonist S-4C-PG (Fig. 16). This is a competitive antagonist of mGluR1 which is also agonist of mGluR2.

Initially, the response of the investigated neuron and its changes after applications of dopamine were expressed weakly. After a series of application of this substance, as shown in histograms in the right column in Figure 16, built from the beginning of the movement, it can be seen that the neuron pulse response leads the movement by 300 msec or more, which indicated its belonging to the pyramidal path neurons. Application of S-4C-PG,

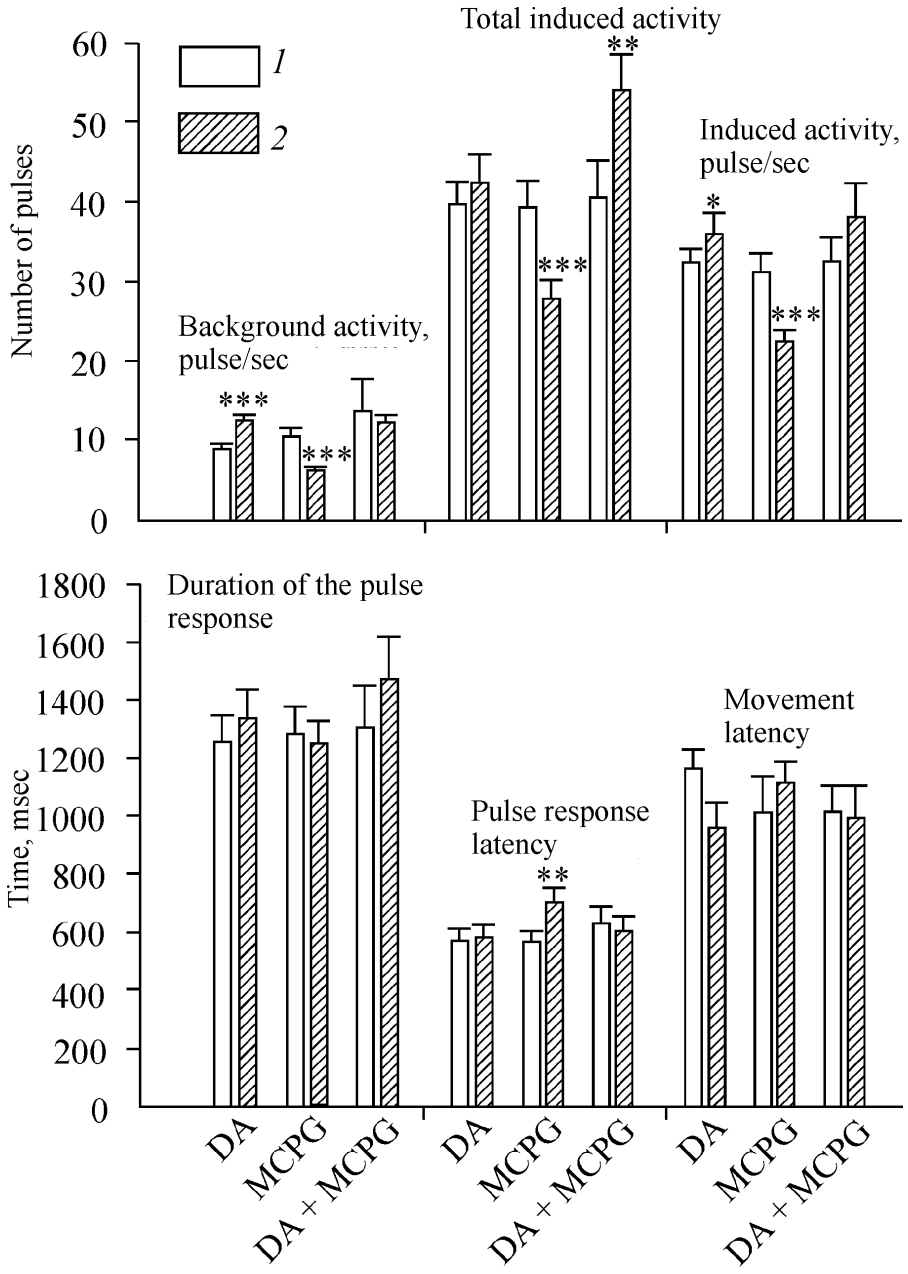


FIGURE 15. Summary statistical estimation of the effect of application of dopamine and metabotropic receptor antagonist MCPG on the activity of neurons in the sensomotor cortex in cats: 1) control, 2) applied substance; asterisks denote the reliability of results: one asterisk — $P < 0.05$, two asterisks — $P < 0.01$, three asterisks — $P < 0.001$.

a competitive antagonist of mGluR1 receptors and, at the same time, an agonist of mGluR2 receptors, increased the neuron background activity. This also, to some extent, increased the neuron pulse response to the stimulus. Despite the fact that the pulse response, leading the beginning of the movement, increased to 450 msec, the conditioned reflex movement latency increased by 300 msec (from 1408 to 1708 msec). In the subsequent control and in the series of experiments with joint application of dopamine and S-4C-PG, the conditioned reflex latency returned to the initial controlled level. At the same time, a significant growth of the background and induced pulse activities was observed. Thus, when dopamine is applied and metabotropic receptors are affected during the experiment, the lead of the specific neuron pulse response increases, as well as the time when the motor response begins. Possibly, the combined effect of these two synaptically active substances facilitates an earlier involvement of the muscle in the conditioned reflex movement.

Given that in natural conditions dopamine affects another, functionally different, group of receptors, i.e., the group of ionotropic glutamate receptors, we attempted to analyze the features and possibilities of dopamine influence on effects of ionotropic NMDA receptors. For analysis, we selected the ionotropic receptor antagonist AP-4, rather than the previously tested antagonists AP-7 or AP-5.

Figure 17 shows the change in the pulse response. Under the effect of AP-4, the latency of the investigated neuron responses sharply increased and the responses themselves were protracted in time. It is interesting to note that this was accompanied by a significant increase of the motor response latency in both cases: it increased by more than 700 msec, compared to the control. Application of dopamine caused a moderate increase of the background and induced activities. In joint application of dopamine and AP-4, the intensity of the pulse activity restored. The background activity level approximated the initial value. Also, the latent periods of conditioned reflex motor responses returned to the initial level. It should be noted that the application of dopamine per se could have induced some increase in the background and

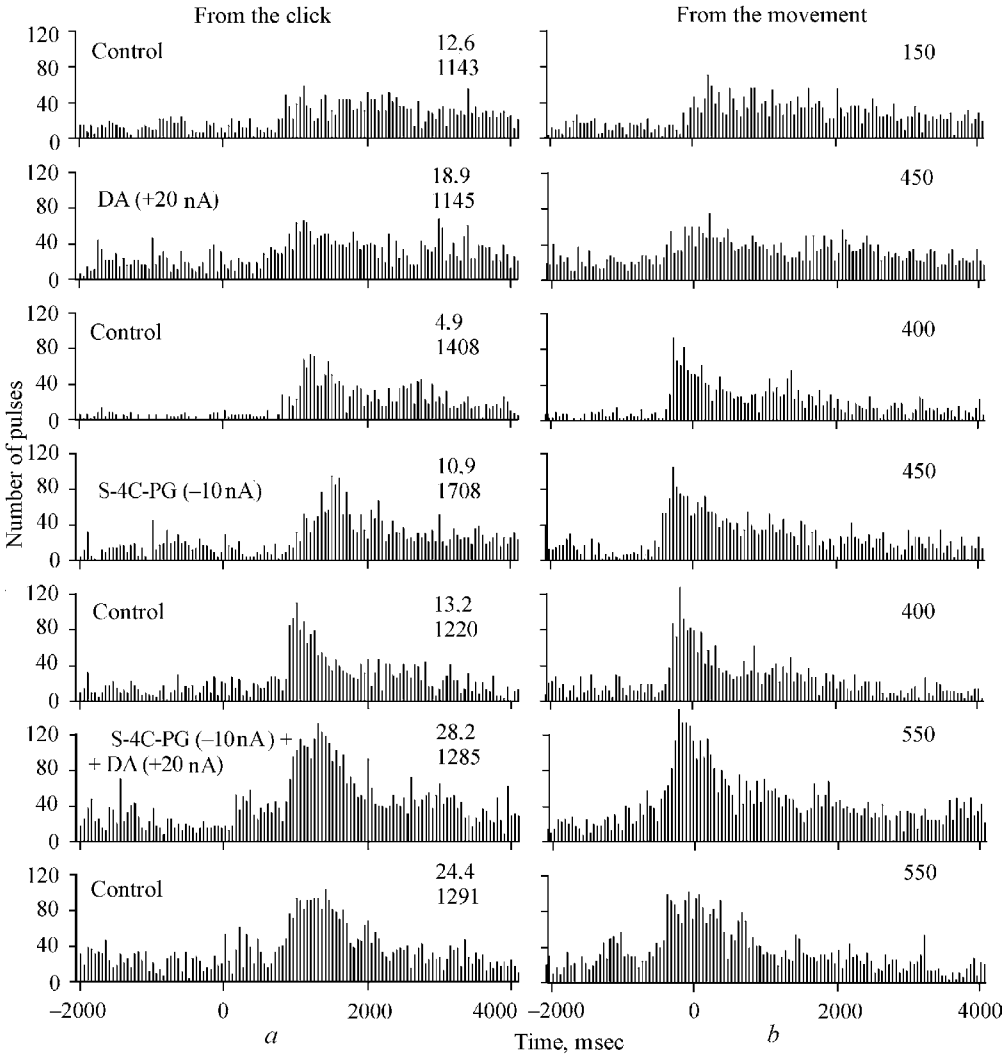


FIGURE 16. The effect of metabotropic combined competitive glutamate transmission agonist and antagonist S-4C-PG on the background and induced pulse activities in the sensomotor cortex pyramidal neuron in cats.

induced activities, but these changes were most often unreliable. Figure 17 shows that application of AP-4 was accompanied by reduction of the amplitude and increase of the duration of the total pulse response as well as by a significant shift in the pulse response to the right, which leads to an increase in the latent period of the motor response of two investigated neurons from 820 and 1056 msec to 1560 and 1833 msec, respectively. This increase of the movement latency was eliminated after joint application of dopamine and AP-4. Interaction of these synaptically active substances was analyzed on 18 neurons (Fig. 18).

Figure 18 shows that application of the glutamate transmission antagonist AP-4 was proved to decrease the background activity level and the pulse frequency during the response; however, because of the definitely increased duration of the response the number of pulses in the response was proved to increase. Besides, it was also statistically proven that application of AP-4 increased the pulse response latency and the conditioned reflex movement latency. It is interesting to note that in the case of joint application of dopamine and AP-4, the pulse response latency and the conditioned reflex movement latency return to the initial level, despite the fact that the pulse response duration definitely remains increased in the presence of dopamine.

The dopamine and glutamate interaction in the prefrontal cortex in rats was analyzed on sections by Wirkner et al.[91] The authors proved that application of dopamine (100 μm) potentiated depolarization caused by application of NMDA (10 mM), but did not affect the response caused by application of AMPA or the D2 receptor agonist SKF38393. Probably, this means that in our case the effect is reached by activation of NMDA and D1 receptors.

After familiarization with the effects of dopamine on metabotropic and ionotropic glutamate transmission antagonists in order to better evaluate the mechanisms and their effects on agonists and antagonists of the dopaminergic transmission, it was interesting to identify the degree of selective effect of dopamine on various subtypes of dopaminergic receptors. In particular, it was interesting to learn, whether sulpiride, a recognized D2 receptor antagonist, influenced only the group of D2–D3–D4 receptors or

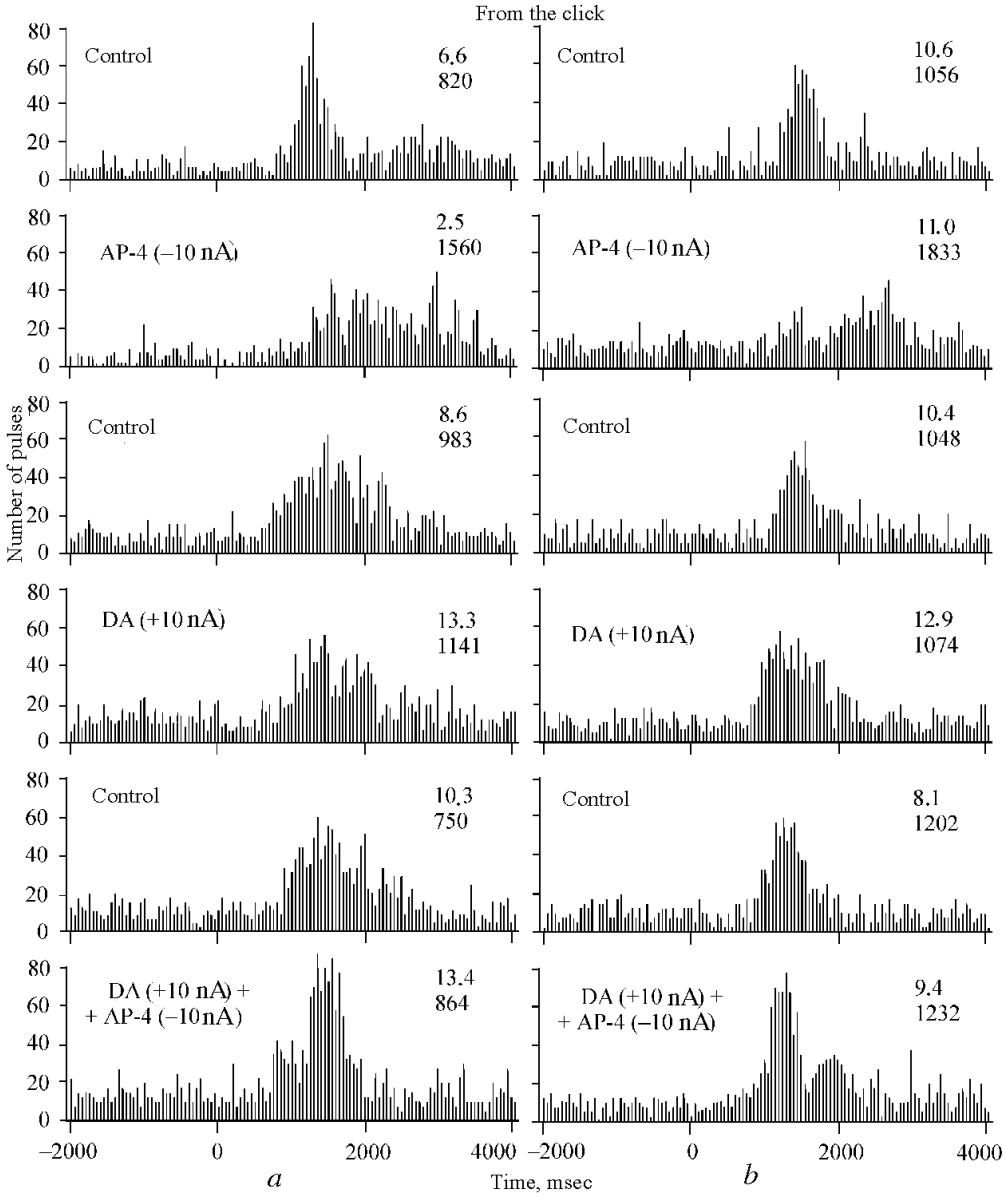


FIGURE 17. Stabilizing effect of dopamine (DA) on the background (figures at the top right, pulse/sec) and induced pulse activities of two neurons (a, b) and the latency of the conditioned reflex motor response of the limb (figures at the bottom right, msec), caused by application of AP-4, an antagonist of NMDA receptors.

whether it could influence the effects caused by activation of the group of D1/D5 receptors.

Figure 19 shows the activity of two neurons, recorded in different animals. Based on the data in Figure 19, it can be seen that application of quinpirole, a dopamine agonist influencing D2/D3 receptors, increases the background and induced pulse activities. On the contrary, application of sulpiride, an antagonist of D2 receptors, inhibits the pulse activity and increases the neuron response latency; when these two substances are applied together, the background and induced activities do not substantially differ from the control level. Figure 19b shows that the depressive action of sulpiride on the background and induced neuron activities under the effect of the antagonist SKF38393, influencing D1 receptors, is not manifested, while the level of pulse activity even increases. Nevertheless, the depressive effect of sulpiride is manifested later. In the final control, the background and induced activities in the last histogram are close to the activity shown in the second histogram (Fig. 19b).

Application of sulpiride, after application of quinpirole (Fig. 19a), caused in the first neuron an insignificant decrease of the background activity and an expressed decrease of the induced pulse activity, compared to the previous background level. As regards another neuron with an increased initial background activity, application of this substance led to a sharp decrease of the background activity almost 2-fold (from 14.4 to 7.6 pulse/sec) and significantly decreased the induced pulse activity. As expected, the impact of dopamine agonists, quinpirole (on D2 receptors) and SKF38393 (on D1 receptors), was accompanied by an increase of the background and induced pulse activities. In this case, the pulse response latency, the response duration, and the motor response latency did not change significantly. Combined application of sulpiride and quinpirole facilitated the restoration of the background and induced neuron activities. It is interesting to learn, though so far not quite clearly, how joint application of the D1 receptor agonist, SKF38393, and the blocker of D2 receptors, sulpiride, eliminated the effect of the latter: the intensity of the induced response remained the same as in application of SKF38393; the level

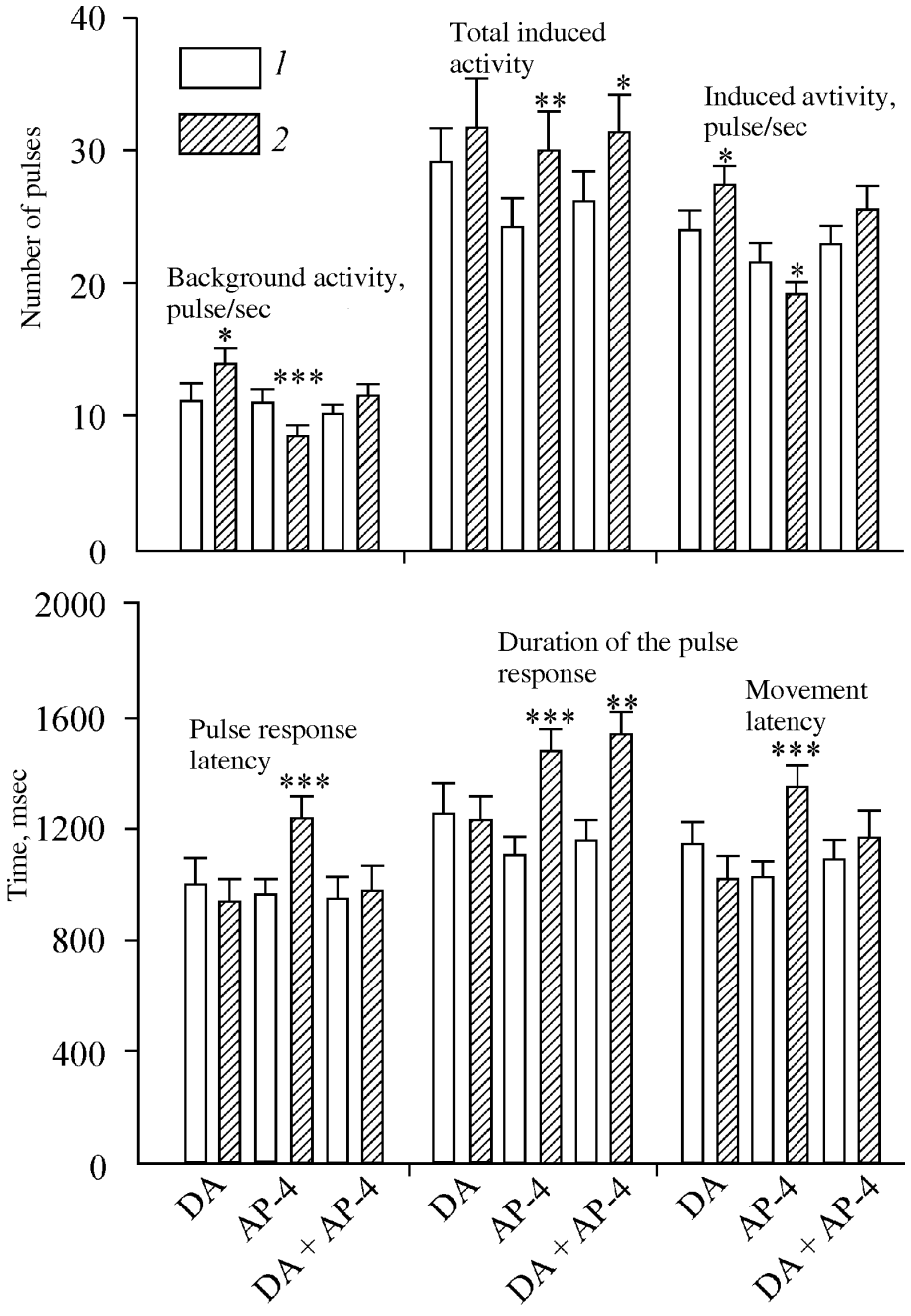


FIGURE 18. Statistical estimation of dopamine and AP-4 effect on the pulse activity of neurons in the sensomotor cortex. For symbols see Figure 15.

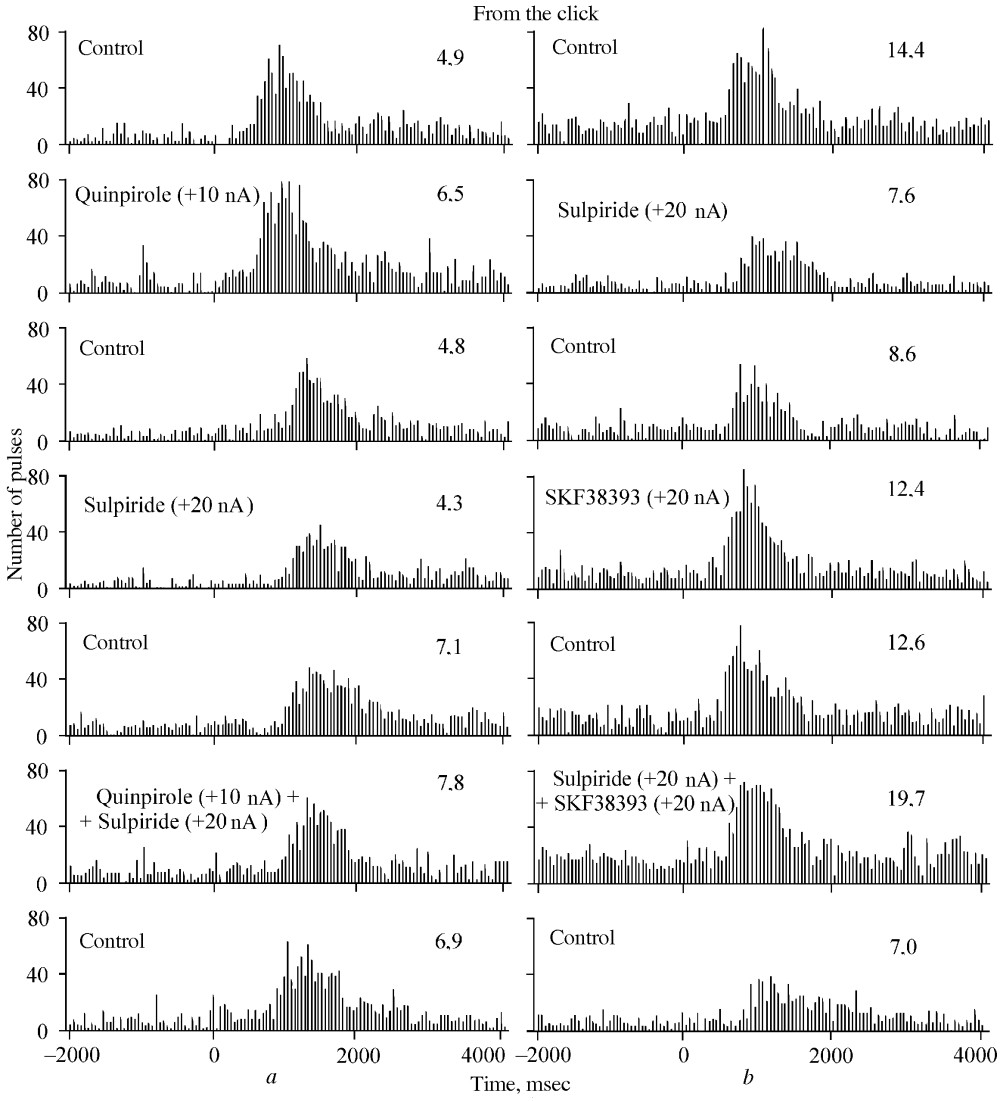


FIGURE 19. Effect of dopamine agonists and antagonists on the background (figures at the top right, pulse/sec) and induced activities of two neurons (a, b) in the sensomotor cortex of a wakeful cat during a conditioned reflex of putting a paw on the support.

of background activity was actually equal to the summary background activity when sulpiride and SKF38393 were applied separately (19.7 pulse/sec compared to 7.6 and 12.4 pulse/sec).

As shown in the previous figures, application of dopamine and its agonists was accompanied by a moderate increase of the background and induced activities; however, no changes in the pulse response duration, latency of pulse responses or the conditioned reflex movement latency were observed.

Of interest are also the experiments when we analyzed the effect of SCH 23390, a D1 receptor antagonist. It turned out that local application of this dopamine agonist is sufficient to cause some depression of the background activity as well as the intensity of the induced response and, most important, to significantly increase the latent period of the conditioned reflex motor response (Fig. 20).

The comparison in Figure 20 between the effects on the background and induced activities of two neurons in the sensomotor cortex shows that application of SCH 23390 causes their moderate decrease and, at the same time, facilitates a significant increase in the pulse response latency and prolongation of the conditioned reflex motor response latency. The movement latency increases from 1329 to 2154 msec in one experiment and from 1237 to 1848 msec in another. Application of bicuculline, probably, causes an increase of its background and induced activities, by eliminating inhibitory GABAergic effect of interneurons on the recorded pyramidal neuron. Therefore, the depressive or inhibitory effects of SCH 23390 in conditions of joint application with bicuculline are eliminated. The pulse response intensity and its latent period as well as the motor response latency are proven not to be different from the initial control values. This allowed us to assert that in the case of interaction of sensomotor cortex neurons in natural conditions the effect of insufficient influence of dopamine on D1 receptors is eliminated when the inhibitory effect is reduced.

Some statistical data on the character of changes in the neuron activity and the conditioned reflex motor response under the effect of D1 receptor antagonist SCH 23390 and bicuculline, produced in

experiments on 21 neurons, are shown in Figure 21. From these data it follows that application of SCH 23390 definitively decreases the background activity and the frequency of induced pulse activity, which leads, with a high reliability, to increase of the pulse response duration and latency, as well as to a definite increase in the conditioned reflex motor response latency. Blocking of inhibitory effects of SCH 23390 on the cortical neurons is achieved by bicuculline. In this case, the effects of application of SCH 23390 on the pulse response latency, duration of pulse response, and movement latency are eliminated. This fact, probably, indicates that the effect reached by application of SCH 23390 depends on the involvement of inhibitory interneurons in the response. When bicuculline exerts its depressive effect on the inhibitory neurons, all of the effects caused by application of SCH 23390 are fully eliminated. Some results of this part of the investigations are given in Table 5.

Statistical data given in Table 5 indicate that in the steady state the ionophoretic application of dopamine is manifested only by a true increase of the background activity level. A similar effect is exerted by application of the D2 receptor agonist, quinpirole, and the D1 receptor agonist, SKF39393: as in the case of application of dopamine, the above substances lead to a highly reliable increase of the background activity. However, the duration, intensity, and latency of responses in this case do not change substantially. The blocking of D2 receptors by sulpiride or D1 receptors by SCH 23390 exerts a more effective effect on the performance of neurons in the sensomotor cortex. Sulpiride facilitates a sharp increase of the latency of pulse responses as well as their duration and increase of latent periods of conditioned reflex responses. SCH 23390, a D1 receptor antagonist, causes a highly reliable decrease of the background pulse activity of neocortex neurons, increase of the pulse response latency and duration, as well as increase of the conditioned reflex response latency. The significance and efficiency of dopamine and its agonists, quinpirole and SKF39393, are manifested only in the case of an artificial (or, possibly, pathological) decrease of the effect on the cortical neuron activity.

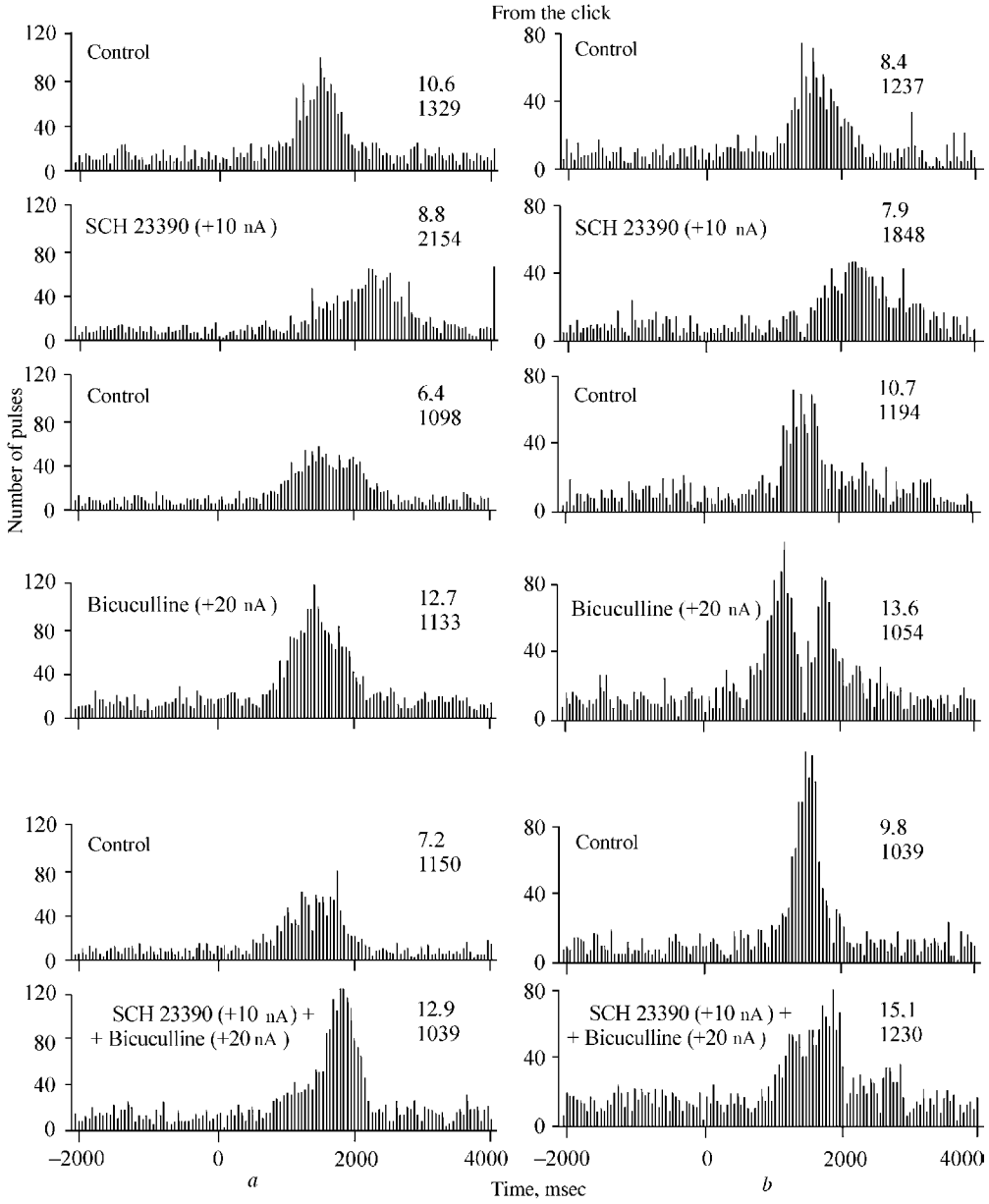


FIGURE 20. Restorative effect of bicuculline on the background (on the right, pulse/sec) and induced pulse activities of two neurons (a, b) in the sensomotor cortex and on the conditioned reflex motor response latency (on the bottom, msec), caused by the D1 receptor antagonist SCH 23390.

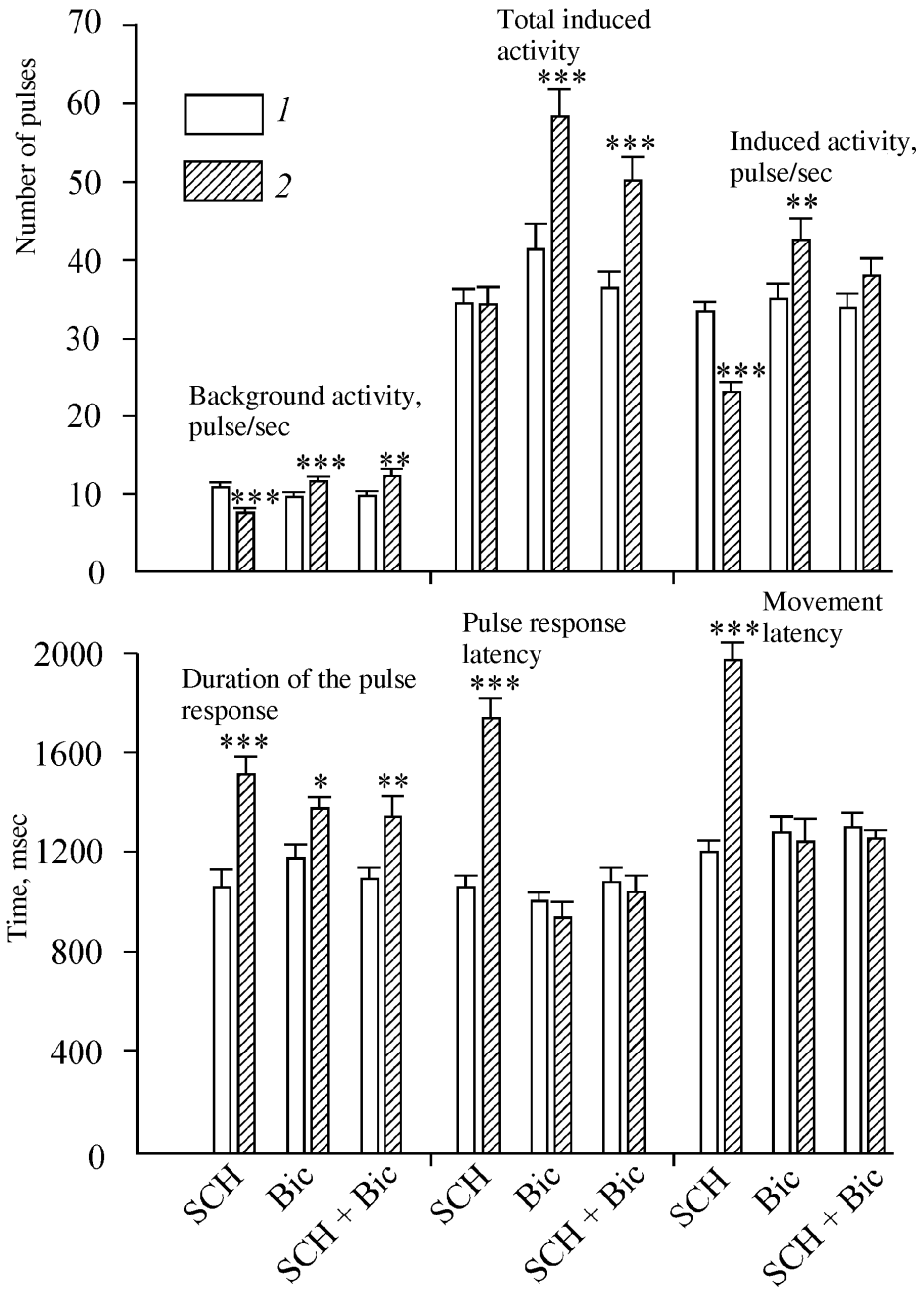


FIGURE 21. Statistical estimation of bicuculline and SCH 23390 effect on the pulse activity of neurons in the sensomotor cortex and on the conditioned reflex motor response latency. For symbols see Figure 15.

It should be once again emphasized that the effects of dopamine strongly depend on the state of the inhibitory GABAergic cerebral cortex system. This was well demonstrated by the figures correspondent to the data in Figure 21 and in Table 6. Table 6 compares the effects of application of SCH 23390, the selective D1 receptor antagonist, with the effects of its joint application with the GABAergic transmission antagonist, bicuculline. Application of SCH 23390 decreases, with a high reliability, the background activity and the frequency of the induced pulse activity of neurons and increases the neuron response latency and increases its duration, as well as motor response latency. In joint application of SCH 23390 and bicuculline the effects, caused by this dopamine antagonist, are leveled. This gives the ground to assert that the effects conditioned by the blocking of D1 receptors by SCH 23390 mostly depend on the system of inhibitory neurons in the investigated sensomotor cortex region. The blocking by bicuculline of the effects of inhibitory interneurons on the pyramidal neurons whose activity we record eliminates the effect caused by application of the dopamine blocker.

The depressive impact of SCH 23390 on the pulse response latency, the background activity, and the pulse frequency during the response, as well as the movement latency in the case of joint application of SCH 23390 and bicuculline is replaced by a reliable increase of the background and induced activities their intensity and duration. The pulse response latency and the movement latency return to the initial values.

It should be noted that the demonstrated, probably favorable, effect reached by application of bicuculline and SCH 23390, is also accompanied by specific changes in ionotropic and metabotropic glutamate synaptic transmission. These "accompanying changes" are presented in Table 4.

Thus, in the case of joint application of dopamine of the metabotropic glutamate transmission antagonist MCPG and bicuculline, the background activity does not decrease, as in the case of application of MCPG, but, on the contrary, increases. The pulse response latency decreases rather than increases, while its intensity increases. The motor response latency neither decreases, as in the

TABLE 5. Changes in neuron and motor activities after ionophoretic application of dopamine, its agonists and antagonists

Substance	Number of neurons	Background activity, pulse/sec		Pulse response latency, msec		Pulse response duration, msec		Movement latency, msec	
		C	A	C	A	C	A	C	A
DA	22	12.0 ± 0.7	12.0 ± 0.9***	565.9 ± 37.4	570.8 ± 32.7	1154.0 ± 82.3	1215.0 ± 89.2	761.0 ± 41.2	782.0 ± 45.4
Quinpirole	40	11.5 ± 0.7	14.0 ± 0.6***	647 ± 19.7	640.0 ± 26.6	882.5 ± 54.6	1106.0 ± 75.4	754.0 ± 39.3	803.0 ± 44.4
Sulpiride	23	11.1 ± 0.8	10.2 ± 1.0	647.8 ± 27.3	776.0 ± 31.0***	741.3 ± 36.0	873.0 ± 47.0**	836.0 ± 62.4	1067.0±68.0***
SKF38393	24	12.6 ± 1.2	16.2 ± 2.0***	702.0 ± 27.9	693.7 ± 25.5	797.9 ± 35.4	866.0 ± 47.6*	845.0 ± 62.6	869.0 ± 60.3
SKF83566	32	8.6 ± 0.7	10.3 ± 1.3*	568.7 ± 37.2	612.5 ± 31.9	1287.0 ± 92.1	1273.0 ± 89.5	817.0 ± 40.0	934.0 ± 47.0**
SCH 23390	21	11.1 ± 0.6	7.9 ± 0.7***	1071.4 ± 42.1	1745.0 ± 74.4***	1070.0 ± 42.2	1517.0 ±68.8***	1207.8 ± 42.4	1979.2±74.5***

Note. DA — dopamine. Here and in Tables 6 and 7: C — control, A — application; asterisks designate the reliability level, calculated by the Student *t*-criterion: one asterisk $P < 0.05$; two asterisks $P < 0.01$, three asterisks $P < 0.001$.

TABLE 6. Comparative statistical assessment of effects of D1 receptor antagonist, SCH 23390 and bicuculline, as well as their joint application on the intensity of the background activity and neurons of the sensomotor cortex in cats

Substance	Number of neurons	Background activity, pulse/sec		Total induced activity		Induced activity, pulse/sec		Pulse response duration, msec		Pulse response latency, msec		Movement latency, msec	
		C	A	C	A	C	A	C	A	C	A	C	A
Bicuculline	21	10.1 ± 0.5	11.9 ± 0.6***	41.3 ± 3.3	58.1 ± 3.4***	34.9 ± 1.9	42.5 ± 2.5*	1178.1 ± 56.6	1378.1 ± 54.8*	1014.7 ± 29.6	943.7 ± 59.1	1287.5 ± 59.4	1253.1 ± 91.2
SCH 23390	21	11.1 ± 0.6	7.8 ± 0.4***	34.5 ± 1.6	34.2 ± 2.1	33.3 ± 1.6	23.2 ± 1.3***	1070.5 ± 42.2	1517.5 ± 68.8***	1071.3 ± 42.2	1745.0 ± 74.4***	1207.8 ± 42.4	1979.1 ± 74.4***
Bicuculline + SCH 23390	21	10.0 ± 0.4	12.6 ± 0.7**	36.5 ± 1.9	50.1 ± 2.9***	33.8 ± 1.6	37.9 ± 2.1	1095.7 ± 57.5	1350.0 ± 78.8**	1090.1 ± 83.7	1045.4 ± 65.4	1305.0 ± 61.2	1265.3 ± 30.8

case of dopamine, but nor increases as in application of MCPG. Thus, we can believe that the dopamine influence on the effects caused by MCPG is also mediated, to some extent, due to depression of the inhibitory effects on the recorded pyramidal neuron. The ionophoretic application of MCPG, the antagonist of group I–II of metabotropic glutamatergic receptors, led to a decrease of the background and induced activities of neurons (46 out of 72 investigated neurons). In such case, the duration and intensity of the induced pulse responses decreased for 37 neurons. However, the statistically reliable increase of the pulse response latency was recorded (see Table 4).

Thus, the alleviating effect of bicuculline application on the depression, caused by application of SCH 23390 (Table 6), was significantly conditioned by its simultaneous influence on the glutamatergic system of neocortex neurons.

Dopaminergic synaptic terminals on the bodies and apical dendrites, belonging to neurons of the ventral tegmental complex and, partially, to the nigrostriatal complex, form predominantly symmetrical synapses (87%) on the neocortical pyramidal neurons. In the human cortex, about 60% of dopaminergic terminals contact with dendrite aciculae and 40% directly with dendrite stems. Dopaminergic synapses are, as a rule, symmetrical (type II according to Grey) and only 13% of them are asymmetrical. They are described not only on pyramidal neurons, but also on interneurons. Unlike the dominant [3H]raclopride binding in layer V, the two-layer [3H]SCH 23390 binding was observed in most cytoarchitectonic fields with the highest concentrations in layers I–III and V–VI. On the one hand, it was shown that D1 receptors localize not only on pyramidal cell dendrites, but also on parvalbumin-containing and calretinin-containing interneurons. On the other hand, it is known that, for example, in the medial prefrontal cortex in rats, dopaminergic receptors mostly concentrate in layers V and VI on the nerve cell bodies. Joint localization of D1 and D2 receptors on the same interneuron can be observed only in 25% of neurons. Cells with D1 receptors only normally represent non-pyramidal neurons. Cells with only D2 receptors represent large interneurons and small pyramidal neurons. Electrical stimula-

tion of the black matter and VTA causes EPSP–IPSP sequence in the investigated neurons. About 50% of the investigated intracellular frontal cortex neurons respond to application of dopamine.[30]

To explain the facts observed in our experiments, we can assume that the responding structure is composed of two interneurons, cause excitation or inhibition of inhibitory interneurons and direct inhibition of pyramidal neurons. Bicuculline, blocking GABA effect, eliminates depressive effects of inhibitory neurons. If, in turn, these interneurons are activated by excitatory metabotropic synapses, then their effect can be blocked by application of MCPG. However, it is more realistic to assume that the few excitatory glutamate receptors on the pyramidal neuron bodies represent metabotropic receptors, sensitive to application of MCPG. In natural conditions, they can cause intensive excitatory effect on neurons, despite their small number. Bicuculline, eliminating local inhibitory effects on the neuron even after switching off of excitatory metabotropic glutamate effects on the neuron soma, supports the excitatory effects of dendrites to restore neuron pulse responses. If dopamine depresses the activity of inhibitory neurons, then the decrease of their activity leads to the same decrease of the inhibitory effect on pyramidal neurons, as it is done by bicuculline. Probably, such combination of ionophoretic applications of bicuculline with the receptor blocker SCH 23390 shows that the blocking of GABA receptors can have an effective impact on the depressive effect on the D1 receptor antagonist SCH 23390. The significance of the produced results is in that they demonstrate the importance of metabotropic receptors in the organization and implementation of conditioned reflex responses. Unlike bicuculline, dopamine is the real natural participant of interneuron synaptic bonds.

The presented data show the possibility of the natural influence of dopamine on the real function of cortical neurons and possible corrective role of dopaminergic effects with a decrease of the effects of glutamate via metabotropic receptors. In our opinion, the results obtained are of interest since they demonstrate the importance of metabotropic receptors in organization and implementation of conditioned reflex responses.

In general, the investigations showed that dopamine and its agonists, quinpirole and SKF facilitate the increase of the background and induced pulse responses by the cortical neurons without substantial alteration of the conditioned reflex activities of the animal.

Dopamine antagonists, sulpiride and SCH 23390, on the contrary, decreased the background and induced pulse activities of cortical neurons, which was accompanied not only by a reliable increase in the pulse response latent period, but also by a growth of the application of dopamine. Joint application of dopamine and its antagonists definitely eliminated the effects of the latter. Such stabilizing effect of dopamine manifested itself also when depression of the background and induced activities and increase of the pulse response latent period and the conditioned reflex movement latency were caused by application of antagonists of ionotropic and metabotropic glutamate receptors AP-4 and MCPG. Since the effects of the above antagonists are also eliminated by the blocker of GABAergic transmission bicuculline, it can be assumed that dopamine implements its stabilizing effect primarily through the influence on the association inhibitory interneurons. Ultimately, this leads to an increase of the sIPSP frequency and amplitude. In the presence of TTX dopamine does not influence the mIPSP frequency, amplitude, and kinetics and the excitatory postsynaptic current in the inhibitory interneurons or pyramidal neurons. Such results, probably, show that dopamine can directly excite cortical interneurons.

The investigations of modulation of the membrane and synaptic properties of cerebral cortical interneurons in rats[21] showed that dopamine directly excited inhibitory neurons and that this led to reduction of excitation of pyramidal cortical neurons. It is known that dopaminergic innervation of the sensomotor cortex, coming from VTA, forms dopaminergic synapses both on pyramidal neurons and interneurons. Also, it is known that the re-entrant excitatory transmission between pyramidal neurons, for example, in prefrontal cortex, is presynaptically depressed by dopamine acting via D1 receptors.[80] Excitation of pyramidal neurons is also modulated by inhibitory interneurons. It has been mentioned

above that inhibitory interneurons in the cortex differ, for example, in their pulse activity in response to the depolarization current surge and the character of their dendrite and axon branches.[92] It was found that neurons with fast pulses predominantly innervate the soma and the initial segment of the pyramidal cell axon, which allows them to control the initiation of the action potential. At the same time, other interneurons primarily regulate excitation of dendrites and efficiency of excitatory inputs. Hence, it is important to evaluate the possible effect of dopamine on the inhibitory interneurons. It can exert a significant influence on the excitatory and inhibitory neuron responses, *inter alia*, on various groups of sensomotor cortex interneurons. It is shown that these groups of interneurons, possessing different properties and associations, may cause different types of responses when affected by dopamine.[83]

The results presented indicate the capacity of the natural effect of dopamine on the real function of cortical neurons and the possible corrective role of dopaminergic effects in reducing glutamate effects via metabotropic receptors.

Summing up the data presented in this section, we can draw the following conclusions:

1. Local application of dopamine moderately increases the background and induced pulse activities of pyramidal neurons in a wakeful animal during the conditioned reflex movement, without changing the pulse response latency and the conditioned reflex movement latency.
2. Blocking of glutamate metabotropic receptors (mGluR1 and mGluR2), caused by ionophoretic application of their antagonist MCPG, leads to depression of the background and induced pulse activities of sensomotor cortex pyramidal neurons. In such case, the pulse response and the associated conditioned reflex movement latency statistically increase.
3. After joint application of dopamine and MCPG, the depressive effects of MCPG on the background and induced activities are eliminated and the pulse response and the conditioned reflex movement latency return to the initial level.
4. It is assumed that dopamine minimizes the decrease of effects of metabotropic glutamate receptors on the cortical

neurons in a wakeful animal. The effect of dopamine on the real function of cortical neurons represents corrective and stabilizing impacts on the neuron activity with decreased effects of glutamate via metabotropic receptors. This could be used to eliminate some pathological disturbances of glutamate transmission.

VI. EFFECT OF AMANTADINE ON ACTIVITY OF SENSOMOTOR CORTICAL NEURONS

An important task in analyzing the involvement of dopamine in the operation of neocortical neurons of a wakeful animal is the approximation of experiments to the real-life conditions of dopamine release in order to be sure that the character and intensity of the experimental dopaminergic effect are close to the effects experienced by neurons in natural conditions. A question arises: to which extent does the experiment reproduce the real-life conditions in terms of the localization, intensity, and duration of dopamine effect? In our view, one of the possible approaches to achieving this goal could be the use of a preparation that ensures dopamine release in such doses and sites that are close to its natural doses and localization, instead of the use of dopamine and its separate antagonists. Also in our opinion, a possible version of this preparation could be a dopamine releaser, i.e., amantadine. One of the earlier works drawing attention to this substance as dopamine releaser was the investigations by Scatton et al.,[93] who demonstrated that its impact increases the synthesis and release of dopamine in rat's striatum. It is known that amantadine facilitates dopamine release from dopaminergic terminals and varicosity in the sites and, probably, in the doses in which they can be released in real life when dopaminergic fibers are activated. Other authors[94] also assumed that it acts as a central stimulant increasing the release of endogenous dopamine. Therefore, we initially presumed that analysis of dopamine effects on neocortex processes could be approximated to real-life conditions, if ionophoresis of dopamine is replaced by ionophoresis of amantadine. However, now it is known that, together with its effect on the dopaminergic system leading to dopa-

mine release, amantadine facilitates the process of accelerated closing of NMDA-activated glutamate channels. This occurs due to the binding of sites where it can be accumulated after the closing of the channel and release of the agonist.[95]

Unlike many other channel-forming molecules, amantadine causes a faster closing of NMDA receptor gates, and according to some researchers, this leads to its depressive effect on responses caused by glutamate, which is used for treatment of Parkinson's disease. It is also believed that application of amantadine and its derivates improves the chronic pain condition, helps in rehabilitation of post-injury brain damage, and positively affects the cognitive processes in patients with multiple sclerosis, Huntington's and Alzheimer diseases and patients with depression.

However, it is quite obvious that such properties of amantadine are related namely to its role of dopamine releaser: in the central nervous system, it possesses also dopamine-modulating activity, enhancing the release and inhibiting the reverse cell trapping of dopamine. Besides, it is known that it also changes the function of acetylcholine nicotine receptors in muscles and has a weak antagonistic effect in hippocampus nicotine acetylcholine receptors.[96] Earlier, it was demonstrated on hippocampus neurons that amantadine, indeed, inhibited the function of the above receptors.[97]

It is interesting to note that the initial effect of Parkinson's disease treatment by amantadine was explained by the opinion that it affected the dopaminergic system, and only later a conclusion was made that curative effect of this substance was, primarily, the result of inhibition of NMDA responses.[98] In comparatively recent researches, the molecular mechanism of amantadine operation was evaluated and described in more detail.[99] The authors confirmed that it not only affected the dopaminergic system, but, indeed, blocked the NMDA receptor channels. Now it is assumed that curative effects of amantadine are, above all, the result of its blocking effect on the NMDA receptor ion channels.

Also, it was demonstrated that amantadine behaves as antagonist of sigma 1 receptors, which involves them into modulation of dopamine receptors.[100] According to these authors,

the idea that the pharmacological effect of amantadine on the dopaminergic transmission is the result of noncompetitive antagonism to glutamate NMDA receptors is not fully correct. Affecting sigma 1 receptors, it potentiates bradykinin-induced mobilization of intracellular potassium, simulating the effect of sigma 1 receptor agonist, i.e., PRE-084. In general, there exists a huge literature material, including hundreds of works, in which amantadine is viewed, above all, not as a dopamine releaser, but as a nonspecific antagonist of NMDA receptors.

However, despite all doubts about the role of this substance as a dopamine releaser, amantadine and its derivates, memantine and ammonium adamant, have been used for treatment of Parkinson's disease, its akinetic rigid version, and other dopamine-deficient disorders of the nervous system for about 30 years. Especially in akinetic crises, amantadine in combination with L-Dopa effectively eliminates most of the Parkinson's disease symptoms, even when L-Dopa immunotherapy becomes ineffective. Besides, when L-Dopa treatment was initially ineffective, the use of amantadine provided a positive effect on the patients.[101] Attempts to investigate the mechanisms by which this substance increases the quantity of extracellular dopamine were made at the level of striatum by a group of Japanese authors.[102]

Thus, beginning experimental investigations of amantadine effects on the dopaminergic activity of neocortical neurons and given the above opinions of the researchers, it should be remembered that it is not only a dopamine releaser, but also a compound that can accelerate specific effects on the glutamate transmission through an accelerated closing of glutamate ionotropic NMDA channels as well as on other types of synaptic transmission.

Given the depressive effect of amantadine on the activity of NMDA receptors, we first tried to clarify its impact on the glutamate transmission in the cortex, when NMDA receptors were activated. Of course, we did not study synaptic processes directly and, therefore, can evaluate its impact only indirectly, by the character of changes of the background and induced pulse activities of neurons during a conditioned reflex.

Figure 22 shows an example of an experiment with application of amantadine and NMDA. As a rule, application of NMDA, sharply increases the background and induced activities of the neuron, without a noticeable effect on the animal's motor response latency. However in the control, after cancellation of application of NMDA, the background and, especially, the induced pulse activities become sharply weakened, i.e., NMDA significantly depresses both background and induced activities. This depression retains for many minutes. The subsequent (10 min later) application of amantadine together with NMDA also causes a significant increase of the background and induced neuron activities. However, this increase is somewhat less effective, compared to application of NMDA alone. Therefore, we can argue that amantadine, indeed, reduces, to some extent, the effects caused by application of NMDA.

However, it is interesting to note that in the subsequent control a sharp decrease of the background and induced neuron activities, compared to the initial control response, was not recorded. It is obvious that application of amantadine together with NMDA, although causing a less intensive increase of the response, leaves a less expressed depression trace at the level of pulse activity, compared to application of NMDA alone. Application of amantadine alone, presented at the end of the series, is accompanied by increased background and induced neuron activities which are almost the same as the activity of NMDA applied alone. If we compare histograms in the right columns where the same implementations of pulse responses are built from the movement, then it becomes evident that the response to amantadine differs insignificantly by the intensity from responses when NMDA is applied alone. The question is: where is the depressive effect of amantadine on the glutamate transmission?

However, it should be noted that responses caused by amantadine are more intensive and expressive, compared to the regular response, caused by application of dopamine. These responses were demonstrated in the previous section.

We also tried to verify the effect of amantadine application on variations of responses caused by application of NMDA receptor

antagonist AP-7. In the section devoted to analysis of the effects of glutamate and GABA on the pulse activity of neurons we paid attention to somewhat unexpected effect of AP-7 application, namely: the intensity of the neuron pulse response after application of this NMDA receptor antagonist was not depressed, as could be expected, but, on the contrary, clearly enhanced.

As shown in Figure 23, amantadine causes, in both neurons, an increase in the background activity, compared to the previous control, from 10 to 21.2 and from 7.1 to 10.9 pulse/sec. The intensity of the caused pulse responses also moderately increases. Application of the glutamate transmission antagonist AP-7 is accompanied by a more significant increase of the background and induced activities, compared to the previous control (Fig. 23a). In any case, the depressive effect of AP-7 on the background and induced neuron activities was not recorded, but, on the contrary, the intensity of both neurons significantly enhanced, although the intensity of response in the second neuron had a little difference from the response in the previous control. After joint application of amantadine and AP-7, with an enhanced initial background activity level, the intensity of the response was hardly lower than the intensity of the response when amantadine was applied alone. In this case, the background activity increased from 9 to 18.4 pulse/sec (Fig. 23a) and from 11.9 to 14.8 pulse/sec (Fig. 23b), compared to the previous control. However, when these two substances were applied together, the induced pulse response was somewhat decreased. No substantial effect on the motor response latency value was found when amantadine was applied. Thus, we can argue that although amantadine affects the character of the background and induced pulse activities through the system of NMDA receptors, as stated by many researchers, nevertheless, it does not reduce the pulse response intensity and the conditioned reflex movement latency.

After the preliminary introduction of the character of possible amantadine effect on the NMDA glutamate transmission, we evaluated its impact on the system of dopaminergic agonists and antagonists, as well the GABAergic system. Above all, we shall analyze the character of relations between amantadine and sulpiride, a dopamine blocker.

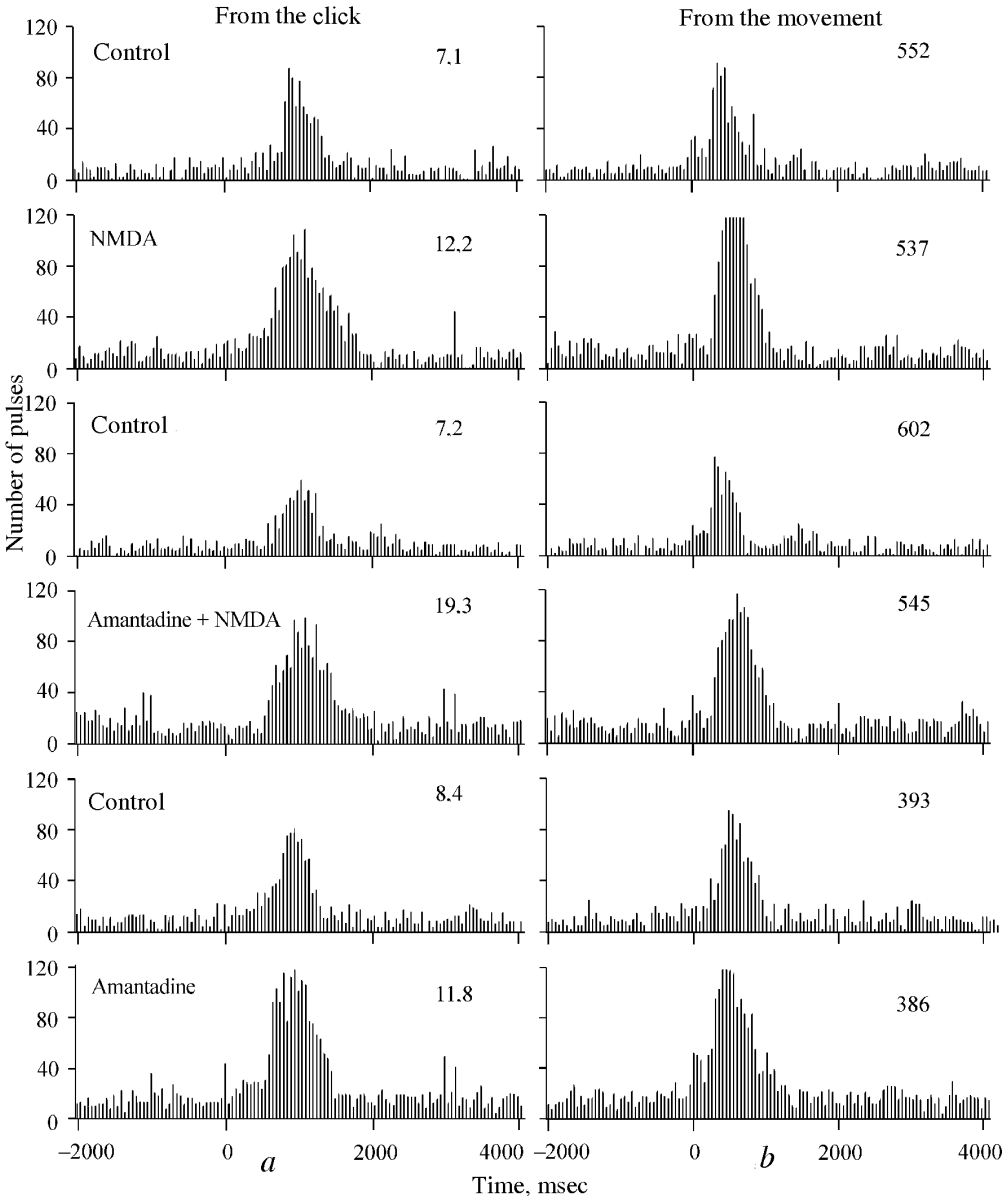


FIGURE 22. Effect of amantadine and NMDA on the background (figures on the left, pulse/sec) and induced activities of the neuron and the conditioned reflex motor response latency (figures on the right, msec). The current is +20 nA.

Figure 24 shows that ionophoretic application of amantadine increases the background and induced pulse activities and, in addition, can affect the motor response latency, depending on the initial background level. When amantadine was applied, the motor response latency increased by more than 150 msec during the first neuron response, while it decreased by more than 600 msec during the second neuron response. After application of sulpiride, this indicator changed in the same direction for both neurons: it increased from 1080 msec to 2065 msec and from 1120 msec to 2440 msec, i.e., by 985 and 1320 msec, respectively, compared to the previous control. It is interesting to note that the time of a specific neuron inclusion into the conditioned reflex motor response changed too. At the start of the experiment and in the control, the second neuron pulse response leads the beginning of the movement by 250 msec, whereas at application of amantadine, only by 150 msec. The value of the lead (advance) testifies to the neuron belonging to pyramidal tract neurons. When sulpiride is applied, the lead of the pulse response and movement increases to 350 msec. In the subsequent control, the start of the neuron pulse response led the start of the movement by 50 msec only, which, according to general conceptions, should testify to that the studied neuron in not a pyramidal tract neuron. However, during joint application of amantadine and sulpiride the start of the pulse response for this neuron led the start of the movement by 400 msec.

Application of sulpiride changes little the neuron background activity in relation to the control (from 8.9 to 5.8 msec for the first neuron) or not at all (from 15.5 to 15.4 msec for the second neuron), but decreases the intensity of the response itself and, at the same time, increases the pulse response latency as well as the conditioned reflex motor response latency more than 2-fold, compared to the previous control. Application of sulpiride increased the movement latency for the first neuron from 1080 to 2065 msec and returned to 1090 msec in the subsequent control. In the second neuron, the motor response latency in the initial control made 1120 msec, after application of sulpiride 2440 msec, and in the subsequent control reduced to 1170 msec. Also of interest is the

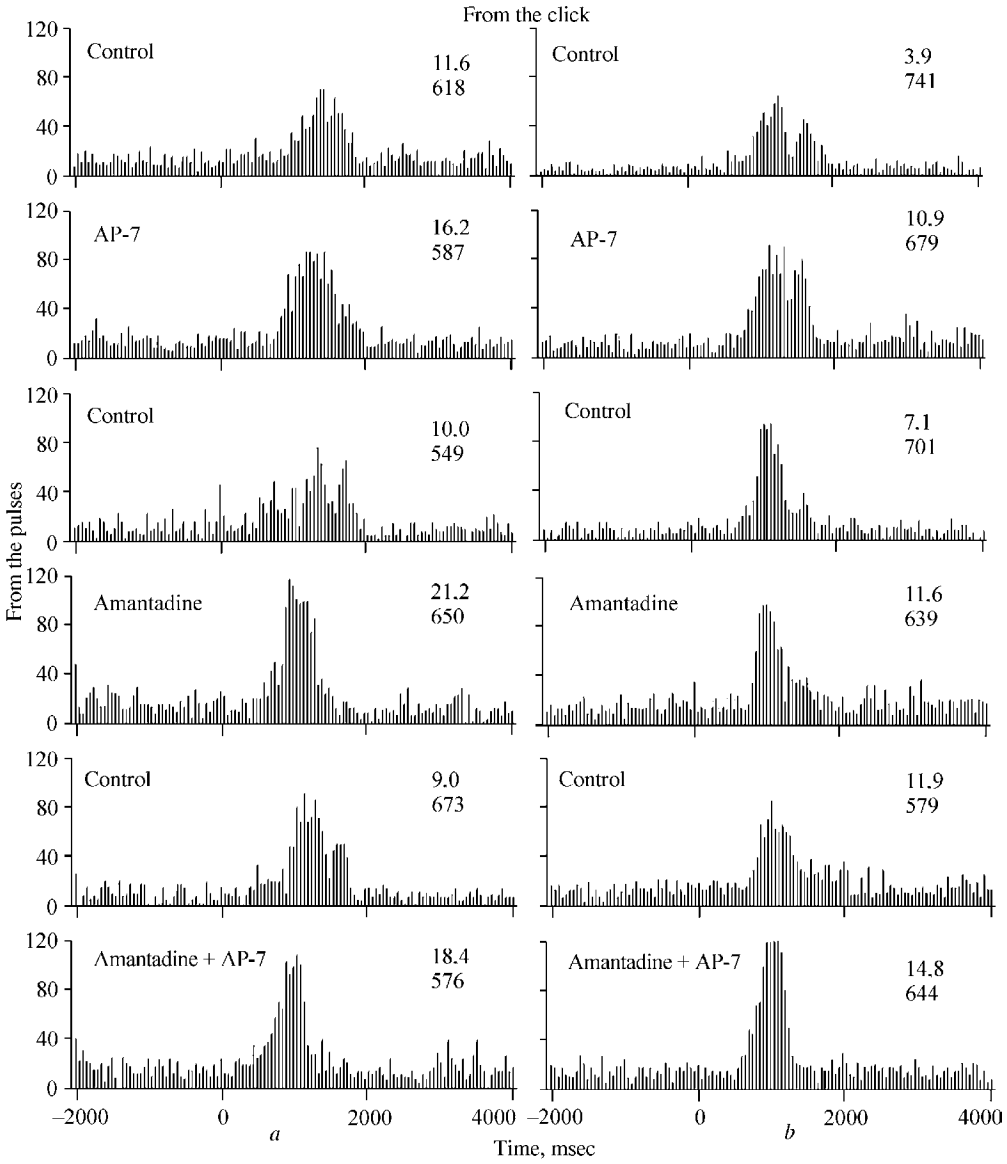


FIGURE 23. Effect of amantadine and AP-7 on the background (figures on the top, pulse/sec) and induced activities of two neurons (a, b) and the conditioned reflex motor response latency (figures on the bottom, msec). The current is +20 nA.

character of variation of the pulse response lead in relation to the beginning of the movement in the second neuron. The initial lead was 250 msec, which confirmed that this neuron belonged to pyramidal tract neurons, involved into the initiation of the investigated motor response. After application of amantadine this lead decreased to 150 msec, and after application of sulpiride it increased to 350 msec, whereas, in the subsequent control the lead was only 50 msec. However, in the case of joint application of both synaptically active substances it reached 400 msec by the end of the experiment. This testifies to the fact that the actual involvement of a specific pyramidal tract neuron in the organization of occasional movement varies significantly and depends on the current modulating effect of dopamine, in particular. The effective enhancing effect of amantadine on the neuron pulse response, which was previously depressed by application of sulpiride, is the best evidence that the preparation effect is similar to dopamine and fully eliminates the effect of the D2 receptor blocking when sulpiride is applied. If a negative effect of amantadine on the activity of NMDA receptors occurs, then it is comparatively weakly expressed in the general response.

Statistical assessment of changes, caused by application of amantadine and sulpiride in a group of investigated neurons, is shown in Figure 25. It can be seen that application of amantadine was accompanied by a statistically reliable increase of the background and induced activities. It was proved that application of sulpiride decreased the background activity and the frequency of the induced pulse activity of the neuron. As a result, the neuron pulse response latency and the conditioned reflex motor response latency were increased by sulpiride with a high reliability. Amantadine, definitely increasing the level of background and induced pulse activities, did not change either the pulse response latency or its latency or the conditioned reflex movement latency. However, after joint application, amantadine fully eliminated the effects of sulpiride: the levels of background and induced pulse activities not only returned to the initial value, but also exceeded this level with statistical reliability. Also, the pulse response latency and the conditioned reflex movement latency returned to the basic values.

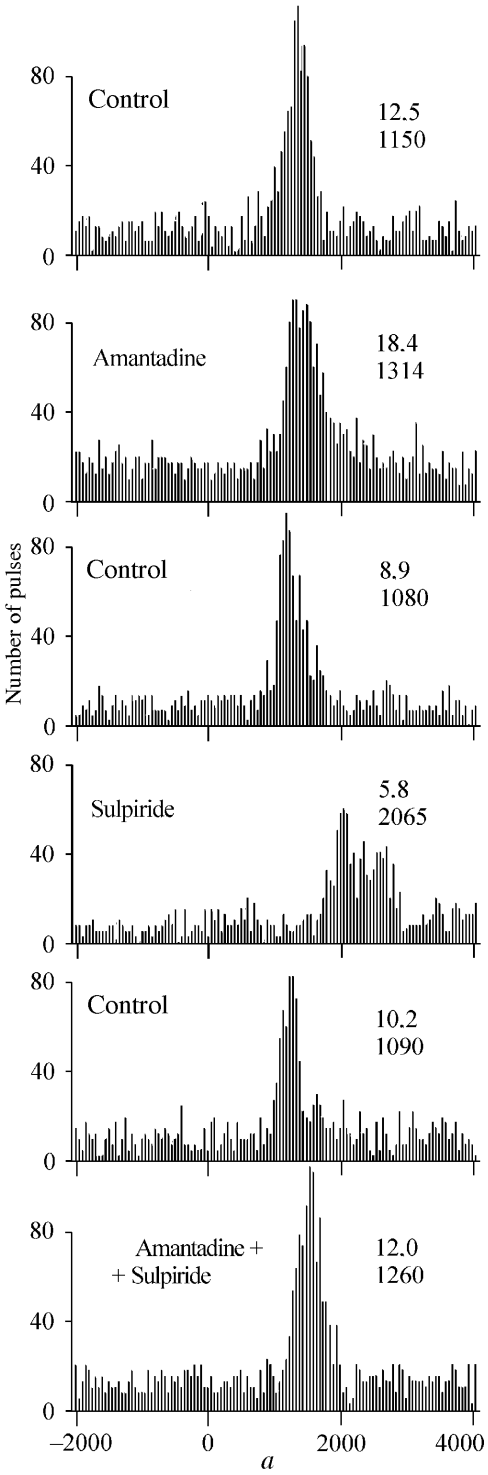
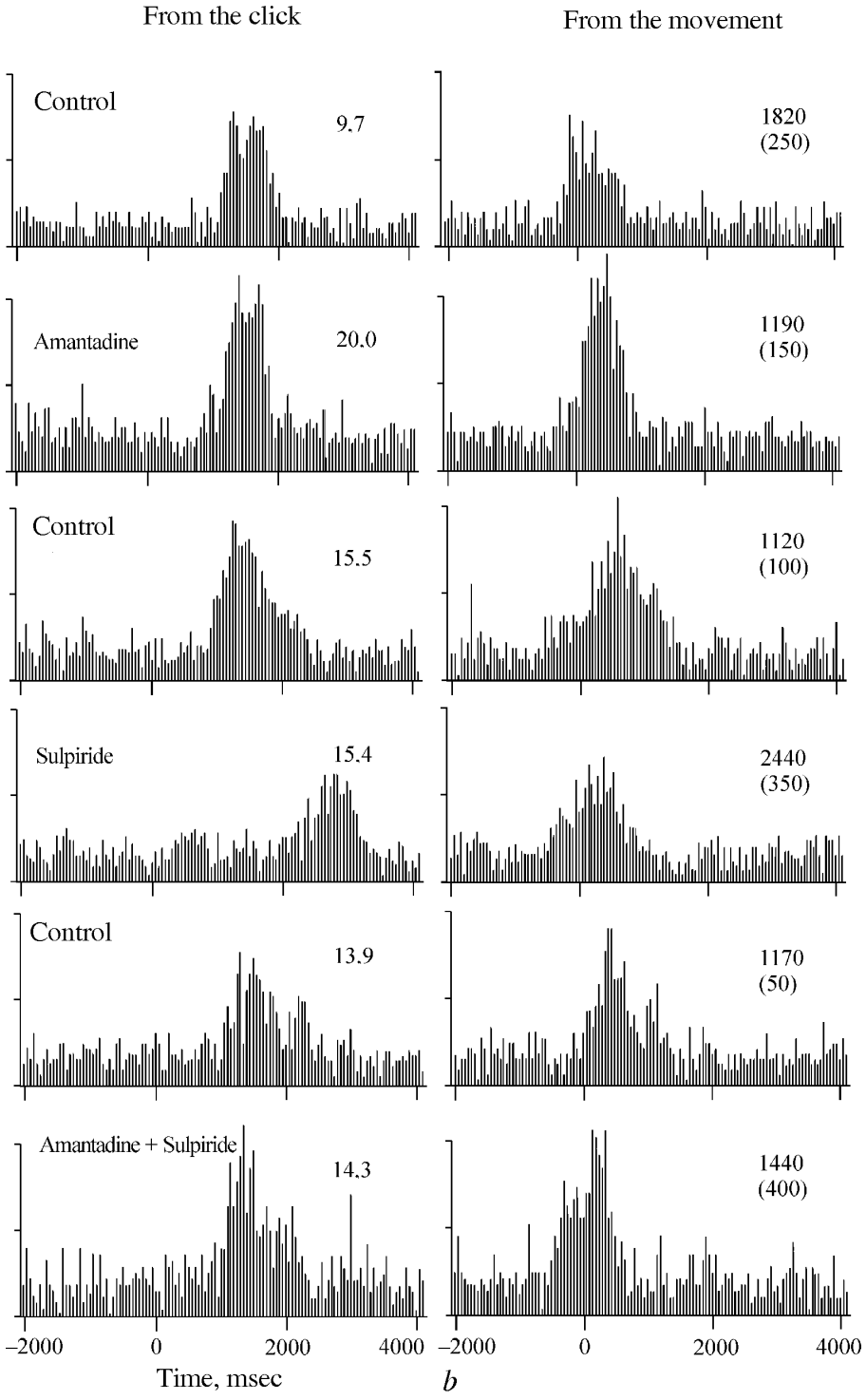


FIGURE 24. Effects of amantadine and sulpiride on the background and induced activities of two neurons (a, b) in the sensomotor cortex. Figures in the left column: at the top right: the frequency of background pulse activity (pulse/sec), at the bottom right: the conditioned reflex motor response latency (pulse/sec). Zero in histograms corresponds to the time of sound application. Figures in the right column: at the top right: conditioned reflex motor response latency (msec); at the bottom right (in brackets): time (msec) for which the started pulse neuron response leads the start the motor response. The current is +20 nA.


From the click
From the movement
Control
9.7
1820
(250)
Amantadine
20.0
1190
(150)
Control
15.5
1120
(100)
Sulpiride
15.4
2440
(350)
Control
13.9
1170
(50)
Amantadine + Sulpiride
14.3
1440
(400)
–2000
0
2000
4000
–2000
0
2000
4000
Time, msec

b

It is obvious that this specific character of amantadine effect represents not only theoretical, but a significant clinical interest. It is worthwhile paying attention to the fact that application of amantadine did not cause any negative effects related to the disturbance of the neuron glutamate activation.

We considered it important to evaluate the interaction of amantadine not only with glutamate synaptic associations, but also with the system of associations of GABAergic neurons. To this end, we investigated its impacts on the effects caused by application of GABA and bicuculline.

Figure 26 gives examples of responses of two neurons in sensomotor cortex after separate and joint application of amantadine and GABA. It can be seen that application of amantadine caused higher background and induced pulse activities in both neurons and was accompanied by slight changes in the movement latency. In the subsequent control, the level of background activity decreased and the level of latency to some extent increased for both neurons. In both cases, application of GABA caused a reliable decrease in the background and induced pulse activities and significantly delayed the beginning of the pulse response. At the same time, GABA substantially increased the movement latency, from 955 to 1819 msec and from 915 to 1581 msec. During the first neuron response, the response latency returned to the values slightly different from the previous control. After joint application of GABA and amantadine, the depressive effect of GABA was not manifested at all. This testifies to a high dependence of the motor responses and, especially, their latent periods on the efficiency of the neocortical dopaminergic system. Thus, we can argue that amantadine can fully eliminate the effects caused by an increase in the GABA concentration in the neuron environment. This, probably, means that it can overcome active inhibitory effects on the cortical neurons.

The differences between the effects, caused by application of amantadine and bicuculline, are less expressive (Fig. 27). Figure 27a shows that amantadine sharply increases the intensity of background and induced pulse activities of the neuron, while bicuculline, applied after the control, in fact, did not affect the intensity in the first

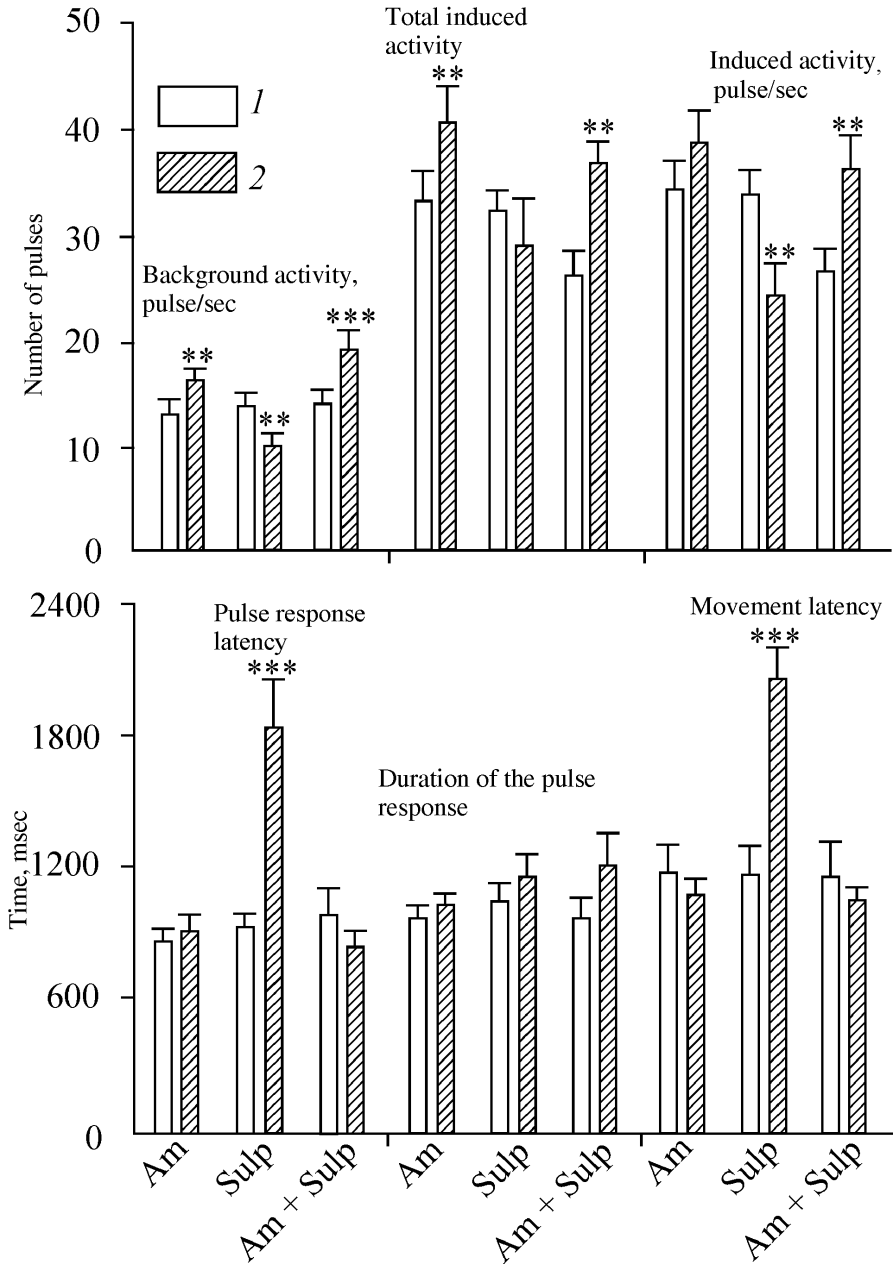


FIGURE 25. Statistical estimation of the effect of amantadine and sulpiride on the pulse activity of neurons as well as the latency of pulse and conditioned reflex motor responses of 15 sensomotor cortex neurons: 1) control, 2) applied substance; asterisks denote the reliability of results: one asterisk — $P < 0.05$, two asterisks — $P < 0.01$, three asterisks — $P < 0.001$.

neuron and enhanced the intensity in the second neuron. Joint application of amantadine and bicuculline caused a less expressive response than in the previous control. Figure 27b shows the responses recorded for the second neuron for which application of amantadine caused changes that are little different from the response in the previous control. Bicuculline clearly enhances the background and, especially, the induced responses. However, joint application of these substances caused a response not higher than the response after the application of bicuculline alone. It should be noted that in both experiments joint application of the substances was accompanied by a substantial and proven decrease of the conditioned reflex movement latency, compared to the previous control: from 449 to 311 msec for the first neuron and from 925 to 650 msec for the second.

The illustrated character of changes in the neuron activity can be a definite proof that the effects, caused by these synaptically active substances, are conditioned by their impact, ultimately, on the same paths and structures, namely, on the association inhibitory neurons. Bicuculline eliminates their inhibitory effects on the pyramidal cells, finally increasing the activities of the latter. Amantadine also enhances the activity of pyramidal neurons, but it also affects them directly and, possibly, via the system of association excitatory neurons.

Statistical differences in the effects, caused by application of amantadine in combination with GABA and bicuculline, are shown in histograms in Figure 28. Amantadine eliminates the proven decrease of the background and induced pulse activities, related to activation of the inhibitory neocortical system and returns the pulse response latency and the conditioned reflex response latency to the initial level.

The summarized results for 16 neurons, recorded after joint application of amantadine and GABA, and for 18 neurons, recorded for joint application of amantadine and bicuculline, show that GABA did not eliminate the effect of amantadine, whose application exceeded the background and induced pulse activities of the neuron.

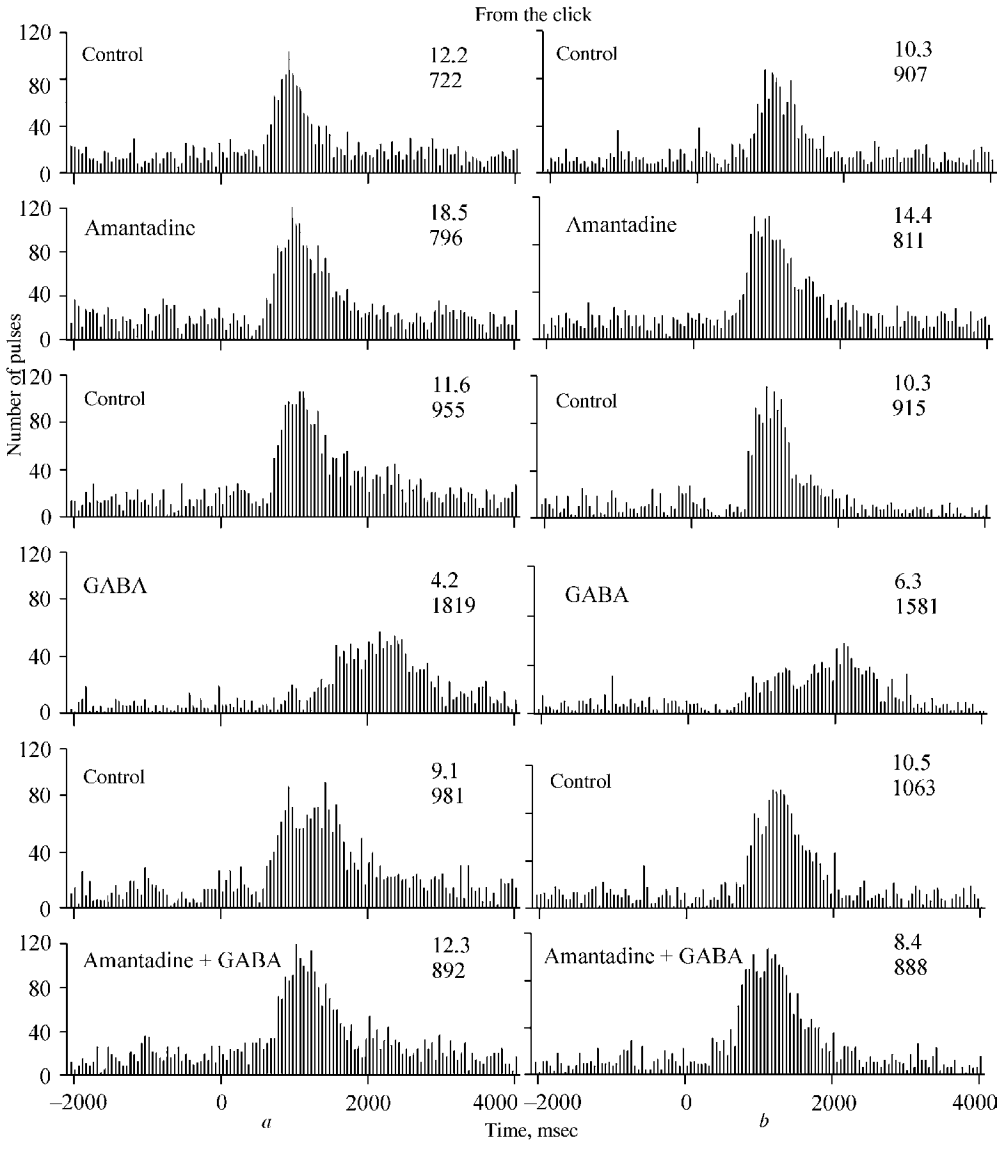


FIGURE 26. Recovery impact of amantadine on the background (figures at the top, pulse/sec) and induced pulse activities of two neurons (a, b) on the senso-motor cortex, conditioned by application of GABA. Current is +20 nA. Figures at the bottom: the conditioned reflex motor response latency, msec.

However, amantadine did not totally eliminate the effect of GABA on the increase of the pulse response latency, response duration and the value of the conditioned reflex movement latency. Application of bicuculline, blocking GABA, is accompanied by some increase in the induced activity and an increase in the background activity, but does not substantially affects the neuron responses and does not facilitate definite changes in the pulse response latency and the motor latency.

Other authors demonstrated earlier that the intensity of combined dopamine receptors with G protein was functionally increased on the striatum neuron membrane, obtained from rats after application of amantadine. For us, a convincing evidence of the fact that amantadine, above all, affects specifically the dopaminergic system as a dopamine releaser, is its impact on the effects caused by application of sulpiride. These effects almost completely coincide with the effects, caused by application of dopamine: the depressive effects of such application are easily eliminated by the blocking of D2 receptors. This effect could hardly be produced, if this application were substituted by application of the best optional blocker of NMDA receptors.

It is more feasible to evaluate the impact of amantadine on the effects of some synaptically active substances when they are observed in real conditions close to natural conditions. To this end, we made additionally three small series of experiments to evaluate the role of amantadine together with sulpiride, GABA, and bicuculline (Table 7).

We saw above that amantadine definitely enhances the background and induced pulse activities of neurons, but there is no statistical evidence that it affects other indicators. This is confirmed by all three series of experiments (Table 7). However, after joint application with synaptically active substances, such as sulpiride and GABA, which exert a significant effect on a number of features of the neuron pulse response and the conditioned reflex response latency, the activity of amantadine is manifested very effectively and uniquely. As follows from the data in Table 7, it definitely increases the level of background and induced pulse activities. However, no definite changes in the motor response

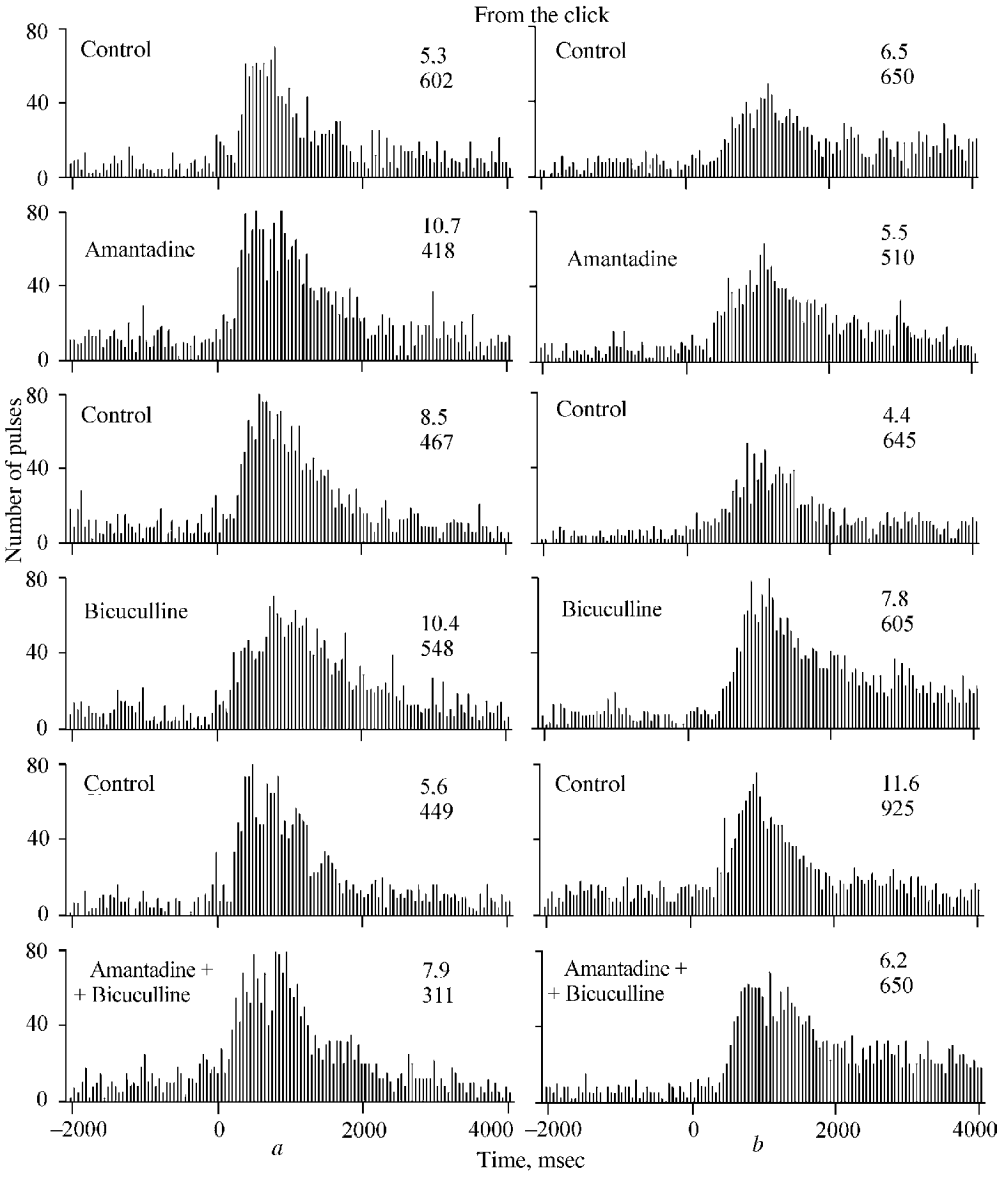


FIGURE 27. Effect of amantadine and bicuculline on the background (figures at the top, pulse/sec) and induced pulse activities of two neurons (a, b) in the sensomotor cortex. Figures at the bottom, the latency of the conditioned reflex motor response, msec.

latency and the conditioned reflex motor response latency occurred. Application of sulpiride increases the neuron response latency and the conditioned reflex movement latency with a high degree of reliability. After joint application, amantadine fully eliminates the effects caused by sulpiride. Similar results were obtained in the experiment with combined application of amantadine and GABA. Application of GABA decreased the background and induced pulse activities. On the contrary, after combined application of amantadine and GABA, accompanied by a definite increase in the background and induced activities, GABA effect was fully eliminated. Besides, it fully eliminated the truly reliable increase of the pulse response latency and the conditioned reflex motor response latency, caused by application of GABA. Thus, it is obvious that amantadine influences similar effects conditioned by application of sulpiride and GABA, almost in the same way, i.e., it levels these effects.

A comparatively recent work[99] attempted to summarize the ideas about the character of amantadine interaction with NMDA receptors, confirming the fact of accelerated closure of the channel during the channel blocking. According to the authors, unlike many other described channel-blocking molecules, amantadine and memantine caused accelerated closure of NMDA receptor channel gates, thereby, slowing down and inhibiting the current passage through the receptor channel. It is important that the channel closure stabilizes.

Summing up some results of analysis of the amantadine effects on the activity of sensomotor cortical neurons, we can argue that it is, above all, a dopamine releaser, and not only a preparation affecting NMDA receptors, and that in this capacity it can significantly change the character of relations between neurons in the neocortex.[93,103] The effect of amantadine on dopaminergic neurons has been studied by many researchers. For example, Toide[104] demonstrated that intraperitoneal infusion of this substance increased L-Dopa metabolism by 24%. This allowed the author to assume that amantadine accelerated dopaminergic transmission, increasing the dopamine release in the frontal cortex.

However, other points of view can be found. In one of the earlier works, Brown and Redfern[105] argued that amantadine did not

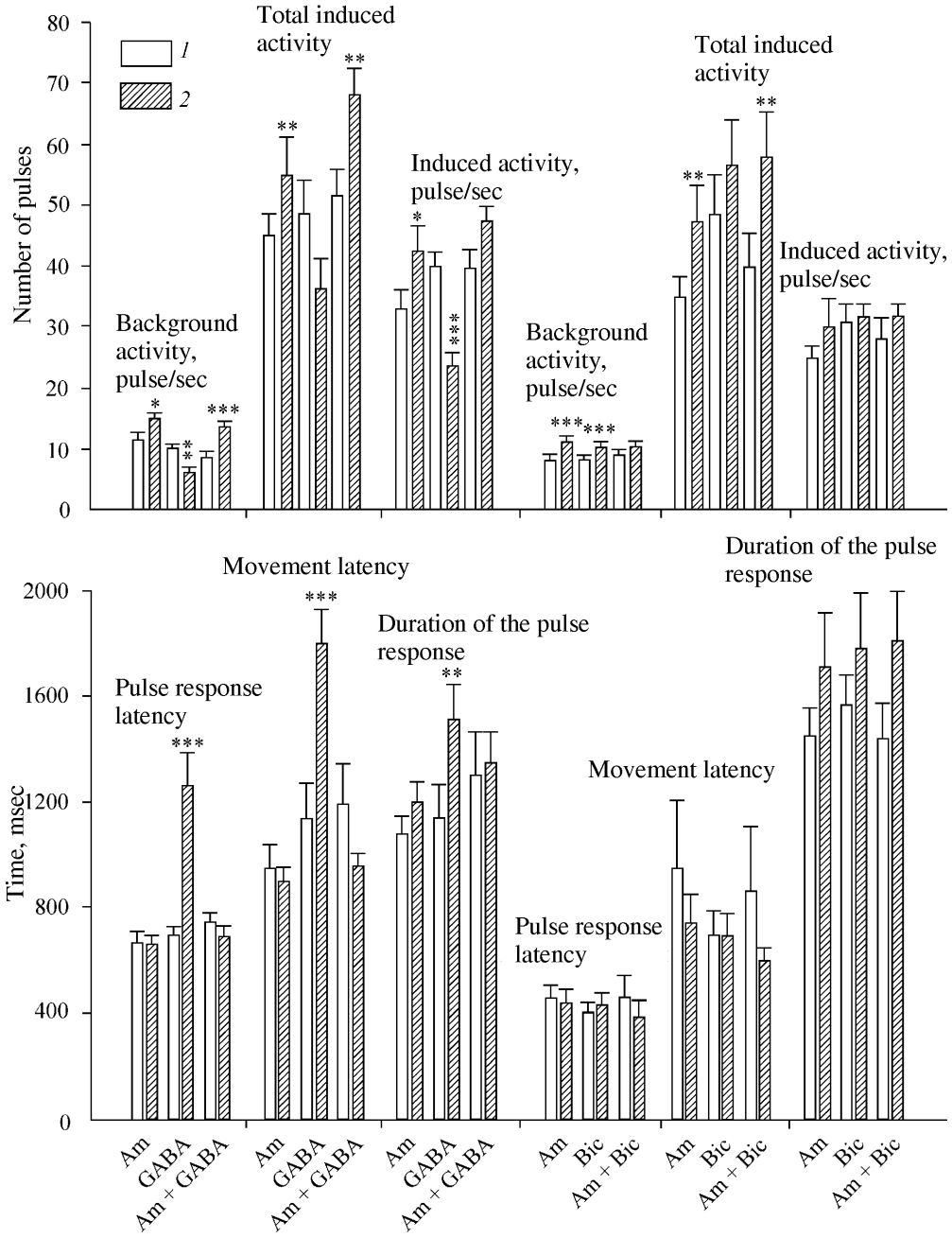


FIGURE 28. Statistical estimation of the role of amantadine in the regulation of GABAergic system of the cerebral sensomotor cortex. For symbols see Figure 25.

TABLE 7. Statistical assessment of the impact of amantadine on the neuron pulse activity, the conditioned reflex response and the changes, caused by application of sulpiride, GABA and bicuculline

Substance	Number of neurons	Background activity, pulse/sec		Total induced activity		Induced activity, pulse/sec		Induced activity, pulse/sec	Pulse response duration, msec		Pulse response latency, msec		Movement latency, msec	
		C	A	C	A	C	A	C	C	A	C	A	C	A
Amantadine	16	13.1 ± 1.4	16.5 ± 0.9**	33.3 ± 2.5	40.8 ± 3.4**	34.3 ± 2.9	38.8 ± 2.9	34.3 ± 2.9	973.3 ± 58.1	1043.0 ± 40.5	866.7 ± 50.4	903 ± 78.2	1190.0 ± 128.0	1086.0 ± 74.4
Sulpiride	15	14.0 ± 1.1	10.1 ± 1.2**	32.6 ± 1.6	29.2 ± 4.1	33.9 ± 2.2	24.3 ± 2.9**	33.9 ± 2.2	1057.0 ± 72.7	1169.0 ± 98.1	934.4 ± 59.4	1842.0 ± 211.3***	1180.0 ± 129.0	2073.0 ± 144.6***
Amantadine + Sulpiride	15	14.1 ± 1.2	19.4 ± 1.6***	26.2 ± 2.6	36.9 ± 2.4**	26.6 ± 2.5	36.0 ± 3.3*	26.6 ± 2.5	972.3 ± 107.1	1222.0 ± 142.0	991.2 ± 118.0	845.5 ± 64.1	1174.0 ± 155.0	1059.0 ± 59.3
Amantadine	16	11.5 ± 1.3	14.9 ± 0.8*	44.9 ± 7.9	54.6 ± 6.4	32.8 ± 3.4	42.3 ± 4.1*	32.8 ± 3.4	1072.2 ± 89.1	1188.8 ± 80.2	661.1 ± 45.4	655.5 ± 38.1	944.4 ± 92.9	894.3 ± 56.1
GABA	16	10.0 ± 0.8	6.1 ± 0.6**	48.4 ± 5.6	36.1 ± 4.9	39.7 ± 2.3	23.4 ± 2.1***	39.7 ± 2.3	1131.0 ± 7.8	1506.0 ± 133.0**	693.7 ± 31.9	1256.2 ± 128.0***	1131.0 ± 134.0	1793.7 ± 130.4***
Amantadine + GABA	16	8.7 ± 0.8	13.6 ± 0.9**	51.4 ± 4.4	68.1 ± 4.3**	39.5 ± 3.0	47.0 ± 2.5*	39.5 ± 3.0	1295.0 ± 163.0	1340.0 ± 118.0	740.0 ± 39.2	680.0 ± 47.8	1185.0 ± 153.0	950.2 ± 51.3
Amantadine	18	7.9 ± 0.8	11.3 ± 1.0***	34.5 ± 3.2	46.9 ± 5.9**	24.4 ± 1.9	29.4 ± 4.8	24.4 ± 1.9	1438.0 ± 109.0	1700.0 ± 207.0	450.0 ± 47.1	433.0 ± 49.3	938.0 ± 258.8	733.3 ± 106.3
Bicuculline	18	10.9 ± 0.9	8.8 ± 0.8	48.1 ± 6.4	56.1 ± 7.6	30.0 ± 2.8	31.2 ± 2.1	30.0 ± 2.8	1555.0 ± 115.0	1772.0 ± 210.0	394.0 ± 38.2	422.0 ± 47.2	683.0 ± 93.1	677.7 ± 91.7
Amantadine + Bicuculline	18	8.8 ± 0.8	10.1 ± 0.9	39.5 ± 3.4	57.5 ± 6.5***	27.6 ± 3.4	31.3 ± 1.9	27.6 ± 3.4	1427.0 ± 135.0	1800.0 ± 205.5**	450.0 ± 83.7	377.7 ± 62.4	850.0 ± 248.2	583.0 ± 51.2

cause any noticeable effect on the dopamine concentration even when it was daily infused for 9 days. In *in vitro* experiments amantadine inhibits the trapping of dopamine by synaptosomes and causes its slight release from synaptosomes only at high concentrations. Therefore, the authors believed that the effect of amantadine in Parkinson's disease treatment should not be related to the dopaminergic mechanism. Later, an original version of interaction of this substance with the dopaminergic system was proposed by Japanese researchers.[102] According to their arguments, amantadine increases the extracellular dopamine in striatum by inhibiting its reverse trapping and/or blocking the NMDA receptor channel, which opposes its function. As mentioned before, some researchers[106] presented proofs that the effects of amantadine application are related to its impact on the sigma 1 receptors. In experiments with homogenate of the neuroblastoma cells, these authors demonstrated that amantadine and memantine potentiate the bradykinin-induced mobilization of intracellular calcium, simulating the effect of the sigma 1 receptor agonist PRE-084. It was found that in the striatum neurons membrane, obtained from rats after amantadine treatment, the functional coupling of dopamine receptors with G proteins increases.

The general conclusions about the character of amantadine effects on the sensomotor cortical neuron activity in a wakeful animal, performing conditioned reflex motor responses, can be reduced to the following statements:

1. Amantadine increases the background and induced activities of neocortical neurons.
2. An insignificant variation of amantadine effect, recorded after joint application with NMDA, indeed, testifies to a partial competition between these two preparations.
3. Amantadine, as in the case of dopamine, eliminates the depressive effect of the dopaminergic transmission blocker, sulpiride, on the background and induced pulse activities of cortical neurons.
4. Amantadine eliminates the inhibitory effect of GABA, manifested in the decreased background and induced pulse activities, increased neuron pulse response latency in the senso- motor cortex, and increased conditioned reflex movement latency.

VII. CONCLUSIONS

This work includes the data on the activity of sensomotor cortical neurons in a wakeful cat performing a conditioned reflex by putting a paw on the support in the control experiment and after application of synaptically active substances, which can affect both the character of the background and induced activities of neurons in the cerebral cortex and changes in the latency of the conditioned reflex response of the animal, i.e., its behavioral response. It allows not only evaluating the role of synaptically active substances in the organization of pulse responses, but also in the performance of behavioral conditioned reflex responses, as well as obtaining data on possible ways of their adjustment by means of pharmacological impacts on the response of some neurons.

Nowadays, analysis of effects of specific synaptically active substances on the neuronal structures has been properly developed, which allows the experimenter to investigate and evaluate changes occurring at the level of an individual structure, a group of cells, a membrane of specific cell types, their receptors, individual channels and at the molecular and submolecular levels. Nevertheless, an important link of such analysis is the evaluation of the complex impacts of synaptic effects on the real physiological function. In this respect, none of the analytical methods can, probably, substitute an experiment identifying specific impacts of certain synaptically active substances on the real and natural motor function of a wakeful animal. This is because evaluation of any pharmacological impact should include data on the character of functional changes in performing a real-life function.

Therefore, special attention should be paid to experiments with depression of glutamate transmission after application of a metabotropic glutamate transmission antagonist, MCPG, and experiments with application of an ionotropic glutamate transmission antagonist, AP-7. As could be expected, application of MCPG led to alleviation of the background and induced neuron activities, while application of AP-7 was accompanied not by depression, but by an unexpected significant increase of the background activity

and the induced pulse response of the neuron. We believe that this phenomenon can be explained in the following way.

Depression of the synaptic transmission after application of MCPG indicates that metabotropic synapses are located predominantly on the pyramidal neuron bodies and their nearest dendrites and, possibly, on the excitatory interneurons, which facilitate pulse activity of the investigated large pyramidal neuron in natural activation conditions. Therefore, the blocking of these synapses by means of metabotropic glutamate transmission antagonists is accompanied by depression of pulse activity of recorded large pyramidal neurons. The alleviating impact of ionotropic glutamate transmission antagonists is, probably, explained by the fact that ionotropic glutamate receptors are dominant and mostly concentrated in the association inhibitory interneurons. This class of receptors causes activation of inhibitory neurons. Ultimately, this leads to depression of the background and induced pulse activities of the recorded pyramidal neurons. The blocking of these ionotropic excitatory inputs to inhibitory interneurons by application of ionotropic glutamate transmission antagonists reduces the control by inhibitory interneurons over investigated sensomotor cortex pyramidal neurons and leads to a significant alleviation of the pulse activity of pyramidal neurons.

Of special interest are, of course, the results of experiments including application of dopamine and amantadine, on the one hand, and dopaminergic transmission blockers, on the other. As demonstrated by a series of experiments with dopamine application, in regular conditions it causes only a moderate increase of the background and induces pulse activities. However, when attempts are made to block dopaminergic transmission by means of sulpiride or SCH 23390, the duration of the latent periods of neuron pulse responses sharply increases, while their background and induced activities decrease and the latency of the conditioned reflex movement significantly increases. In these conditions, the true role of dopamine is clearly manifested, since its application eliminates all of the above shifts.

An interesting addition to such data can be the observations made while comparing separate and joint application of SCH 23390

and bicuculline. Application of SCH 23390 is accompanied by a reliable depression of the background and induced pulse activities, as well as by a truly proven increase of the pulse response duration, the neuron response latency, and the conditioned reflex movement latency. However, application of bicuculline alone is accompanied by increased background and induced pulse activities. In this case, the neuron response latency and the conditioned reflex movement latency do not change significantly. After joint application of bicuculline and SCH 23390, the increase of the background and induced pulse activities is observed, which is typical of the first substance, while the increase of pulse response latency and the movement latency, caused by application of SCH 23390, is fully eliminated. Elimination of these effects for a specific neuron with simultaneous recovery of the initial level of motor response latency after joint application with these dopamine antagonists or amantadine demonstrated exclusive capacities of dopamine to maintain and adjust optical functions relations of neurons in the neocortex and, at the same time, at the level of behavioral responses, after adjustment of the newly developed conditioned reflex response. In fact, this indicates that the role of dopamine is fully manifested only in emergency circumstances when unexpected outside factors begin to disturb the real functions.

This does not mean the denial of the huge input made by researchers of neuron structures using dopamine and a number of dopaminergic preparation in understanding the functional synaptic organization of both individual neocortical neurons and their complicated structural ensembles. We believe that a comprehensive approach to analysis, using data about the functional changes in individual neurons, caused by specific synaptically active substances, and changes of the function at the level of behavioral response, can give important additional materials useful for evaluating investigated phenomena in the cerebral cortex and as well as specific prerequisites for adjustment of functional motor disturbances.

The methods we used do not allow the experimenter to see many details. However, recording the activity of large, most often, pyramidal neurons, located at the output of the sensomotor cortex,

and changing the effects of this activity by means of synaptically active substances, we can identify some features and additional details of the real neuron functional organization of the investigated neocortex region, which is not always possible to do and analyze in an acute experiment (for an isolated preparation). Despite the fact that in our experiment we recorded only pulse activity of individual neurons at the deep levels of the neocortex and also recorded the conditioned reflex motor response, application of synaptically active substances allowed us to make a summary evaluation of pharmacological effects on the synaptic associations of neurons and their functional role. The results of application of substances, connected directly with the glutamate and GABAergic activation of synapses in the course of the experiment, could be substantially varied due to modulating effect of dopamine. It was mentioned above, that by recording the pulse activity of one and the same neuron, located at a depth of 1.5–2.0 mm in the cortex, and investigating its response for six-seven series in 10 implementations per series and 5–7 min intervals between the series (totally 70–90 min), we were convinced that we recorded the activity of large pyramidal neurons with a great external electrical field. This assumption was confirmed, in particular, by the fact that in about one–third of neurons the response to the conditional sound irritation led the beginning of the conditioned reflex movement of the limb by 150 msec or more, which proved that these neurons belonged to the pyramidal tract neurons. Of course, using methods of application of dopamine and its agonists and antagonists and recording only the pulse activity of large neurons at the system output (in our case, they were predominantly neurons inside the cortex layers), we could investigate and evaluate all possible changes in intracortical, interneuron and synaptic relations, developed between neurons in a specific cortical region when a concrete function was performed. However, we observed the resultant activity of neurons, located at the system output, and the motor response of the animal, which, due to a change in the conditioned reflex motor response latency, allowed us to make an indirect evaluation of interneuron events in the specific neocortical region. From the sources mentioned in this work, it is known that

application of dopamine as well as of other synaptically active substances can affect the investigated neuron both indirectly and via a system of association neurons. Dopamine application exerts a direct excitatory effect on the investigated neuron through activation of the system of D1/D5 receptors and a depressive or inhibitory effect through the group of D2–D4 receptors. Besides, dopamine exerts excitatory and inhibitory effects on the investigated pyramidal neurons in deep cortical layers, even in conditions of local application, also through the system of association excitatory and inhibitory interneurons. It was demonstrated before that activation by dopamine of excitatory and inhibitory interneurons, just as dopamine activation of excitatory and inhibitory receptors directly on pyramidal neurons, depends in particular on the dopamine concentration too. As a result, due to variability of the ways dopamine transmits its effects on the pyramidal neuron, as well as the dependence of the activation of dopaminergic receptors on the pyramidal neuron on the dopamine concentration, the modulating effect of dopamine, i.e., its result, can also be variable.

Based on the obtained results, it is quite obvious that during application of dopamine, its agonists and antagonists, significant changes occur at synaptic outputs not only of large pyramidal neurons, but also of association, excitatory, and inhibitory interneurons in the investigated cortex region. These changes facilitate the formation of alternative directional pulse responses of cortical pyramidal neurons to a conditional stimulus, as well as a specific conditioned reflex motor response latency.

It was noted in the introduction that systemic application of amphetamine, a D1/D2 receptors antagonist, by microdialysis leads to an increase of the extracellular GABA in prefrontal cortex. A similar effect was caused by quinpirole, a D2 receptor agonist. Sulpiride blocked this effect. Application of the D1 receptor agonist, SKF38393, did not change the level of GABA. Such observation gave the right to conclude that dopamine increases the GABA release in the prefrontal cortex through D2 receptors.[11] As can be seen, our experiments allowed us to draw somewhat different conclusions: application of sulpiride is accompanied by depression of the background and induced pulse activities of neurons and

leads to a significant delay in the neuron pulse response latency and the correspondent conditioned reflex movement latency. These effects are eliminated by application of dopamine and quinpirole. The fact that application of dopamine causes depolarization of the membrane and facilitates increase of excitability of interneurons with fast pulses (association inhibitory interneurons) once again confirms the feasibility of inhibitory effect on this synaptically active substance on the neocortical neurons. The D1 receptor-induced increase of interneuron excitability increases GABAergic transmission to pyramidal neurons, which ultimately can be manifested in the depression of the stable pulse activity of pyramidal neurons. It is known that the D1 receptor density in all cortical layers is higher than the density of D2 receptors. Given literature data and the results of our observations, it is obvious that, besides direct effect on the pyramidal neuron activity, dopamine affects them by switching of fast-discharging interneurons, causing inhibition, i.e., its modulating effect can be exerted in two ways.

In the discussed work, we attempted to evaluate the real involvement of the cerebral sensomotor cortex dopaminergic system in responses of glutamate and GABAergic neurons systems when animals performed the motor response developed through instrumental training. It was found that direct application of dopamine causes, at first look, very insignificant changes in the background and induced neurons responses, as well as in the behavioral response in general, for example, in the developed conditioned reflex response of putting a paw on the support. The role and the character of dopamine involvement in neuron responses clearly increase and manifest when dopamine antagonists are locally applied in the investigated sensomotor cortex area. Thus, it was shown that application of the D2 receptor antagonist, sulpiride, simulating actually one of serious pathological deviations in the operation of the dopaminergic system, causes a significant delay of pulse neuron responses, which is also reflected by a sharp increase of the conditioned reflex motor response latency. In such conditions, the role of dopamine in the organization of the motor function in the cortex becomes evident. Application of dopamine

together with sulpiride immediately eliminates the effect of sulpiride. It is known that application of amantadine also influences the effect of the blocking of dopaminergic transmission in the same way as dopamine. Moreover, sometimes it is possible to get a close result by applying bicuculline, a GABA blocker. A substantial difference is that in real conditions neither amantadine nor bicuculline exist in the neocortex, while dopamine is always present in normal conditions. It is also important that effects, caused by dopamine and amantadine, have the same direction. These effects, probably, significantly change the activation of inhibitory interneurons. Only afterwards, they affect glutamate, excitatory associations between sensomotor cortex neurons. For example, Table 7 shows that amantadine eliminates the depressive effect of sulpiride on the background and induced pulse activities of neurons and modulates a statistically reliable increase of conditioned reflex neuron response latency and the associated movement latency. Amantadine exerts a similar effect on the responses caused by application of GABA. Joint application of amantadine and GABA eliminates the reduction of the background and induced pulse activities, the proven increase of the pulse response latency, and the conditioned reflex movement latency, induced by a conditional stimulus when GABA is applied.

We believe that not only the expressive effect on dopamine and amantadine on the conditioned reflex movement latency deserves special attention; their convincing effect on the lead of the pulse response in the assumed pyramidal neurons against the beginning of the motor response is also important, which was demonstrated, in particular, in experiments with application of amantadine (Fig. 24).

Experimental facts, which show that the intensity of activity changes in the specific neurons of the local deep neocortex regions is accompanied by substantial functional disturbances of the animal behavioral responses, above all, of the conditioned reflex movement latency, should also be taken into consideration. It is difficult to imagine that local application of MCPG, a metabotropic glutamate transmission antagonist, or AP-4, an ionotropic transmission antagonist, or dopamine receptor antagonists, sulpiride and SCH23390, can significantly — sometimes, by a factor of two —

increase the latency of initiation of the conditioned reflex movement.

We hope that analysis of the effects of dopaminergic transmission agonists and antagonists in the neocortex of a wakeful animal performing a real function will help correctly evaluate the role of specific dopaminergic preparations in the adjustment of functional interneuron relations in the cerebral sensomotor cortex.

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