ISSN 1819-7124, Neurochemical Journal, 2009, Vol. 3, No. 1, pp. 29–34. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © V.P. Nikitin, V.V. Sherstnev, 2009, published in Neirokhimiya, 2009, Vol. 26, No. 1, pp. 35–41.

THEORETICAL ARTICLES

The Concept of the Integrative Activities of Neurons and Mechanisms of Neuroplasticity

V. P. Nikitin[1] and V. V. Sherstnev

Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow, Russia

Received October 13, 2008

Abstract—The current state of the problem of the integrative activities of neurons is considered on the basis of literature data and system analysis of results obtained in the studies of mechanisms of a simple form of learning in the edible snail. We describe postsynaptic mechanisms of mediation, excitatory inputs that converge to a neuron, and their specific molecular transformations and integration inside the cytoplasm and nucleus. We propose a hypothetical scheme of intracellular integration of excitatory inputs in a neuron during learning as a basis for long-term postsynaptic plastic changes.

Key words: synapse, plasticity, learning, integration, genes

DOI: 10.1134/S1819712409010048

INTRODUCTION

The problem of the integrative activities of neurons was formulated at the beginning of the 20th century by Sherington, who believed that each neuron in the CNS takes into account all incoming signals and on their basis it creates its impulsive "message" with a new assignment. P. Anokhin analyzed with problem in the framework of the theory of functional systems and proposed consideration of neurons as "conceptual bridges" that connect processes at a molecular level with the integral functioning of the brain. A key point of the integrative activity of neurons, in this case, is intraneuronal molecular genetic transformations of different chemically heterogeneous excitations that converge on the nerve cell. This hypothesis is referred to as the "chemical" hypothesis of the integrative activity of neurons. It has been supported by the results of numerous studies that indicate that the neurochemical mechanisms of mediation and intracellular "processing" of neuronal excitatory inputs, including the activation of secondary intracellular messengers, protein kinases, phosphorylation and the synthesis of proteins, and activation of gene transcription, are very specific [1–5].

Current concepts of the integrative functions of nerve cells are based on the hypothesis of neuroplasticity, which postulates that nerve cells have both bioelectric and intracellular metabolic mechanisms of integration of converging excitatory inputs. A result of integration of an electrical signal is some firing pattern of a nerve cell, which may provide only short-term processes of neuroplasticity and, in the majority of cases, the duration of these processes does not exceed milliseconds/seconds. Intracellular molecular genetic integration of excitation results in morpho-functional changes in nerve cells and some synaptic connections of neurons (a process referred to as synapse-specific plasticity) [4, 5]. The gene-dependent remodeling of synapses is of larger duration and may underlie the formation of long-term memory [4, 5].

Note that, despite some advances in the studies of the mechanisms of the integrative activities of neurons, this problem is poorly studied. The problem of the molecular specificity of postsynaptic and intranuclear processes that underlie the integration of excitations and the induction of long-term synapse-specific plasticity is of special interest.

In this work, we considered the following basic statement of the chemical hypothesis of the integrative functioning of neurons on the basis of our experimental data: the convergence of excitatory inputs of different sensory and biological modalities on a neuron and the mediation of these inputs inside the cell by specific neurochemical processes, the specific molecular mechanisms of integration of excitatory inputs in postsynaptic and nuclear regions, along with the neurochemical integration of excitatory inputs, results in selective morphofunctional changes in some synaptic connections of a neuron (synapse-specific plasticity). The best studied mechanisms of synapse-specific neuroplasticity are discussed in the frameworks of the integrative activities of neurons.

CONVERGENCE OF EXCITATORY INPUTS OF DIFFERENT MODALITIES TO A NEURON AND THEIR MEDIATION INSIDE THE CELL BY SPECIFIC NEUROCHEMICAL PROCESSES

Our studies were performed with two of the nine known command neurons of defense behavior, LPl1 and PPl1. These cells play an important role in the per-

[1] Corresponding author; address: ul. Mokhovaya 11, building 4, Moscow, 125009 Russia; phone: +7 (495) 601-2130; e-mail: nikitin.vp@mail.ru

formance of inherited and acquired forms of snail behavior. The receptive fields of the LPl1 and PPl1 neurons include all skin surfaces of the body; however, there is a specific zone located on a vitally important area of the snail, the head of the snail, whose sensory stimulation induces more pronounced responses of cells than stimulations of nonspecific zones, for example, the snail's foot [6].

The command neurons of defense behavior receive inputs of different biological and sensory modalities [6]. These cells respond to tactile, chemical, vestibular, thermal, and light stimulations. It has been shown that the activity of command neurons depends on the state of different functional systems, such as defensive, food-procuring, and sexual systems. The effects of these systems are mediated by different biologically active substances: serotonin, glutamate, acetylcholine, opiates, neuropeptide FMRFamides, gastrin, cholecystokinin etc. [6–8].

We studied the integrative functions of these cells during elaboration of one of the simplest forms of learning, nociceptive sensitization. Elaboration of sensitization was performed by application of a concentrated solution of quinine on the snail head. To test the synaptic effects of sensitization in the LPl1 and PPl1 neurons we used sensory stimulations with different modalities and locations of their application on the snail body. Chemical sensory stimulators (weak solution of quinine) and tactile stimulation were applied to the head or middle area of the foot [9].

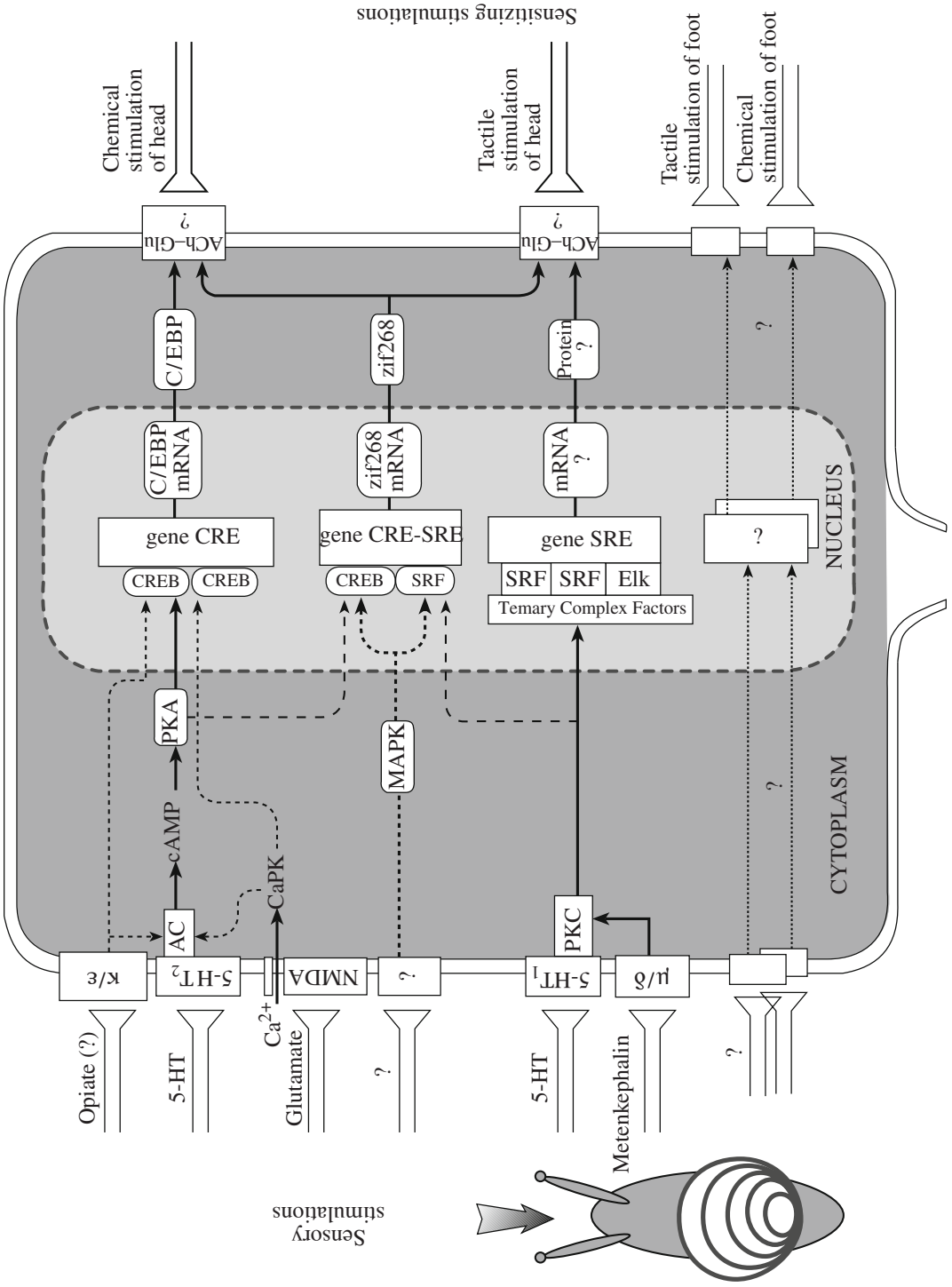
We have found [Nikitin, 1991–2006] that elaboration of nociceptive sensitization in edible snails was accompanied by the long-term more than for 24 h facilitation of the efficacy of synaptic transmission of the LPl1 and PPl1 neurons with sensory inputs from the snail head. Synaptic facilitation was developed in 1.5 hours after sensitization, which suggests that its mechanisms involve different cascades of molecular events, including the genetic apparatus of neurons.

Studies of the neurochemical mechanisms of the excitations induced by sensitizing stimulations showed that they are mediated by some postsynaptic receptors of LPl1 and PPl1 neurons. Using specific agonists and antagonists, we identified glutamate NMDA receptors, serotonin 5-HT_2-receptors, and opioid κ/ε-receptors. Inhibition of any of these receptors during elaboration of sensitization resulted in selective suppression of synaptic facilitation in sensory "inputs" from chemoreceptors of the snail head, whereas facilitation in sensory "inputs" from mechanoreceptors of the snail foot and head developed as in the neurons of control animals [7]. Regulation of another sensory input of the neuron LPl1, from mechanoreceptors of head, involves the 5-HT_1-receptors of serotonin and opioid σ/δ-receptors. In this case, inhibition of serotonin receptors during elaboration of sensitization resulted in suppression of synaptic facilitation in the responses induced by tactile stimulations of the head. A similar depressive effect on synaptic facilitation was caused by the activation of opioid μ/δ-receptors by met-enkephalin (figure).

Thus, we have found that sensitizing stimulations initiate heterochemical excitations that converge to some postsynaptic receptors of the LPl1 and PPl1 command neurons and induce selective changes in the efficacy of sensory synaptic "inputs." The aim of successive studies was to elucidate the mechanisms of intracellular mediation of excitatory inputs that converge to neurons and their integration in the postsynaptic area.

One of the most widespread and well-known intracellular mediators of excitatory inputs is cyclic AMP. We have found that injection of cAMP into the LPl1 and PPl1 neurons of non-sensitized snails resulted in synaptic facilitation only in synaptic inputs from chemoreceptors of the snail head, whereas responses to tactile stimulation of the head and foot did not change. Biochemical and neurophysiological studies have shown that an increase in the level of cAMP in the snail cells may result from activation of serotonin-dependent adenylate cyclase (the enzyme responsible for cAMP synthesis) [10]. In addition to serotonin, cAMP may mediate the effects of activation of opioid receptors [11]. It is also known that activation of NMDA-receptors results in opening of their channel complex and influx of calcium ions into the cell [5]. Calcium ions can modulate the activity of Ca^{2+}/calmodulin-dependent adenylate cyclase. In addition, it has been found that κ- and NMDA-receptors interact in mollusk neurons [11]. Thus, stimulation of serotonin 5-HT_2-receptors and NMDA- and κ/ε-receptors may result in changes in the cAMP level in command neurons. However, it is highly probable that any of the mentioned receptors has its effect via its "own" intracellular mediators.

Our studies have shown that in the sensitized snails, the synaptic facilitation in another synaptic "input" of command neurons, from mechanoreceptors of the head, was selectively suppressed during intracellular injections of inhibitors of protein kinase C [7]. It is possible that in command neurons the activity of protein kinase C increases after stimulation of 5-HT_1-like receptors of serotonin; however, it is also possible that there are some other neuromediator "inputs" that regulate this kinase (figure). In addition, we have found that facilitation of responses induced by tactile stimulations of the head of sensitized snails was selectively suppressed by inhibitors of a cystein-containing protease, caspase-3. Studies of different organs and tissues of animals have shown that caspase-3 is involved in both apoptotic and neuroplastic processes in nerve tissues and one of its substrates is protein kinase C [12]. A limited proteolytic cleavage by caspase-3 results in the activation of this kinase. It was mentioned above that the mechanisms of regulation of sensory inputs from mechanoreceptors of the snail head involve opioid μ/δ-receptors. Activation of these receptors by met-enkephalin during the process of sensitization resulted in the suppression of synaptic transmission in this sen-



Hypothetical scheme of molecular integration of excitatory inputs in the LPl1 neuron during the process of nociceptive sensitization in edible snail. For details, see text.

sory "input," presumably due to the inhibition of the activity of protein kinase C.

On the basis of our data, it is possible to select the following mechanisms of postsynaptic integration of excitations in command neurons of defensive behavior LPl1 and PPl1.

1. Sensitizing stimulations induce heterochemical excitations that converge to postsynaptic receptors, which are specifically associated with some systems of intracellular mediators.

2. Synaptic facilitation in the sensory inputs of the neurons studied requires the combined activation of several postsynaptic receptors, which serve as a target for converging sensitizing excitations. Inhibition of any of one of three identified neuromediator receptors (glutamate NMDA, serotonin 5-HT_2, and opioid κ/ε), which regulate sensory inputs from the head chemoreceptors, results in complete suppression of synaptic facilitation in these inputs. On the other hand, the selective activation of one of these receptors or secondary messengers induced only partial imitation of the effects of sensitization. For example, the application of serotonin to the command neurons or intracellular injection of cAMP induced synaptic facilitation, which was maintained for 2–4 hours and was less pronounced than the facilitation induced by elaboration of sensitization [7].

SPECIFIC INTEGRATION OF EXCITATORY INPUTS IN THE NUCLEAR AREA OF NEURONS

We have found that the process of sensitization in the presence of inhibitors of synthesis of protein or RNA suppressed the facilitation of responses in all sensory inputs of the command neurons [7]. These results, as well as data on the selective involvement of different cascades of signal pathways in the induction of synaptic facilitation in command neurons, served as the basis for the identification of several genes and transcription factors involved in the mechanisms of synaptic plasticity.

One of the genes involved in learning, whose expression is induced by extracellular stimuli and cAMP-dependent regulator of transcription, is the early gene C/EBP (CCAAT enhancer binding protein) [4]. Our experiments revealed that the process of sensitization during intracellular injection of oligonucleotides that specifically inhibit mRNA or C/EBP proteins resulted in the suppression of facilitation of the LPl1 neuron in response to chemical sensory stimulations of the snail head [7]. However, facilitation of responses to tactile stimulations of the head and foot remained unchanged under these conditions.

It is known that the effect of cAMP on the C/EBP gene is mediated by protein kinase A, which phosphorylates the transcription factor CREB (cAMP-response element-binding protein), which binds with the CRE sequence (cAMP response element) in the promoter of the C/EBP gene [4]. In addition, CREB is controlled by other kinases, including Ca^{2+}/calmodulin kinases [5]. It is possible to hypothesize that, in the command neurons, CREB, in addition to protein kinase A, is regulated by calmodulin kinases activated by calcium ions, which entered the cells through NMDA receptors, because cAMP injected intracellularly induced only partial reproduction of the effects of sensitization [5]. It is also possible that the CREB activity depends on the opioid κ/ε-receptors. In addition, it has been shown that activation of the C/EBP gene depends on the interaction the CRE sequence with different subtypes of CREB, activator, inhibitor, and modulator which, in turn, may be regulated by different kinases [4, 5] (figure).

Thus, interaction of CREB subtypes and the effect of different protein kinases on transcription factors of CREB are molecular mechanisms of the intranuclear neurochemical integration of excitatory inputs converging to neurons; this integration yields selective regulation of synaptic inputs from the head chemoreceptors.

As was noted above, protein kinase C and caspase-3 are key factors in the induction of long-term genetic regulation of command neuron sensory input from head mechanoreceptors. One of transcription factors whose phosphorylation and activity depends on protein kinase C and caspase-3 is serum response factor (SRF) [13]. We found that injection of oligonucleotides inhibiting SRF protein into the LPl1 neuron during the process of sensitization results in the suppression of facilitation of responses induced by tactile stimulations of the snail head. However, facilitation of responses induced by chemical stimulations of the head or tactile stimulations of the foot developed as in the neurons of control sensitized animals.

The promoters of some genes, including immediate early genes such as c-fos, fosB, junB, and zif268, have an SRF-binding DNA sequence, a serum response element (SRE), which serves as a location for the assembly of multiprotein complexes, including SRF dimers and proteins related to the ternary complex factor family [13]. This mechanism integrates different molecular regulators of SRF-dependent genes and serves as an example of nuclear integration of excitations involved in specific synaptic regulation.

We have studied the role of the early gene zif268 (the zinc-finger protein 268, also referred to as egr-1, TIS8, NGFI-A, or krox24) in the mechanisms of synaptic plasticity. It has been found that injection of antisense nucleotides to zif268 mRNA into neurons during the process of sensitization in snails results in the selective suppression of the facilitation of responses in the sensory inputs from chemo- and mechanoreceptors of the head [7]. The facilitation of responses induced by tactile stimulations of the foot was not suppressed under these conditions.

The promoter of the zif268gene contains SRE and CRE sequences, therefore, it is possible to hypothesize that transcription of the zif268 gene in the LPl1 neuron depends on the activity of the SRF and CREB transcription factors. An alternative mechanism of regulation of

transcription of the zif268 gene includes MAPK kinases (mitogen activated protein kinase) [5]. In its turn, MAPK activity may be regulated by both protein kinases C and A and by mechanisms independent of them, for example, tyrosine kinase receptors, glutamate receptors, calcium ions, etc. [4, 5].

Thus, the mechanisms of the second stage of integration of excitatory inputs that converge to command neurons during the process of sensitization are located in the nuclear area. These mechanisms include regulation of transcription factors by different kinases and interaction of different transcription factors with gene promoters (figure).

SELECTIVE CHANGES IN THE EFFICACY OF SEVERAL SYNAPTIC LINKS OF NEURON ARE A RESULT OF THE INTRACELLULAR INTEGRATION OF EXCITATORY INPUTS

A principal issue of the problem discussed is how the "products" of transcription/translation synthesized during sensitization affect synaptic plasticity. The mRNAs of several early genes were found in dendrites and, presumably, they are involved in the regulation of synaptic transmission [5]. Relatively short latent periods of the development of synaptic facilitation in command neurons of sensitized snails and their dependence on the activity of early genes suggest that the "products" of transcription/translation of the genes studied are directly involved in the mechanisms of regulation of synaptic plasticity.

It was mentioned above that the C/EBP gene is specifically involved in mechanisms of synaptic facilitation in the sensory input from chemoreceptors of the snail head to command neurons. In addition, this sensory input is regulated by zif268. Note that induction of synaptic facilitation critically depends on transcripts of both genes, because inhibition of any of them yields complete suppression of facilitation of responses to the stimulation of chemoreceptors. We believe that the synaptic effects of the products of transcription/translation of the C/EBP and zif268 genes occur due to their interaction in the postsynaptic areas of sensory inputs from chemoreceptors of the snail head to command neurons. Sensory inputs from head mechanoreceptors to the LPl1 neuron also seem to have a mechanism for the double regulation of these inputs by transcripts of the zif268 gene and some unidentified gene activated by protein kinase C/SRF. Mechanisms of interaction of transcription "products" of different genes during regulation of the same synaptic links require further studies. However, it is possible to hypothesize that one of the specific mechanisms of the development of the effects of the genes studied may be the regulation of the local synthesis of proteins in the postsynaptic area, and the functions of these proteins may be directly associated with modification of the morphofunctional features of synapses [4, 5].

Thus, the postsynaptic mechanisms of regulation of sensory inputs from chemo- and mechanoreceptors of the head are the third intraneuronal stage of integration of excitation that converges to command neurons during the sensitization of snails.

Note that the mechanisms of plasticity of sensory inputs from receptor zones nonspecific for the LPl1 and RPl1 neurons (in the snail foot) seems to considerably differ from the mechanisms found in the inputs from specific zones located in the snail head. The process of sensitization resulted in the facilitation of synaptic inputs from the animal's foot, which was suppressed by calcium chelators, inhibitors of calmodulin, and blockers of translation. However, we have not yet found neurochemical factors that have a selective effect on the plasticity of these inputs.

CONCLUSIONS

On the basis of our results, it is possible to separate three "major points" of the neurochemical integration of excitation in command neurons of defensive behavior, which provide specific regulation of sensory synaptic links of the neuron.

1. Integration of excitations induced by sensitizing stimulations in the postsynaptic area of activated synapses is based on the specific involvement of certain postsynaptic receptors and the interaction of secondary intracellular mediators.

2. Integration of excitations in the nuclear area is based on regulation of the activity of transcription factors by different secondary messengers and interaction of different transcription factors in the promoter of some neuronal genes.

3. Integration of excitations in the postsynaptic area of some sensory inputs of neuron is based on the selective regulation of morphofunctional remodeling of synapses by the "products" of transcription/translation of early genes, which is a specific mechanism of synapse–specific plasticity.

Thus, our data suggest that neurons possess a mechanism for specific synaptic plasticity based on the specificity of molecular processes involved in this mechanism; sensitizing stimulations converge to certain postsynaptic receptors and activation of different secondary messengers, which trigger different transcription factors and genes. This results in the synthesis of synapse-specific RNA and proteins that selectively regulate certain synaptic links of neurons. This mechanism of the integrative functions of neurons is typical of cells that presumably have multimodal convergent features, as command neurons of defensive behavior do.

Another molecular mechanism that provides synapse-specific plasticity was found in studies performed with the nerve cells of the mammalian hippocampus and the mollusk Aplysia. It has been found that some molecular processes develop in synapses stimulated during modeling of learning (activations of different

kinases, local synthesis of proteins etc.); these processes are referred to as mechanisms of synaptic tagging [4, 5]. "Tagged" synapses acquired the capacity to selectively co-opt mRNA and proteins involved in long-term morphofunctional remodeling of these synapses. It is possible that this mechanism of synapse-specific plasticity is typical of functionally different types of nerve cells, including the command neurons for the defensive behavior of snails.

The mechanisms of synapse-specific plasticity studies in sensory neurons of Aplysia and in our experiments are one of the "simplest," with relatively low selectivity, because they involve certain groups of synapses rather than single synaptic links. However, it is possible to hypothesize that the process of some forms of learning requires higher specificity of the plastic remodeling of synaptic links. For example, it is highly probable that the mechanisms of elaboration, maintenance, and reproduction of classic conditioned reflex skills involve more limited groups or even single synaptic links of one neuron. These links may mediate excitations induced by a conditioned stimulus with high sensory specificity. Note that current experimental data on the molecular mechanisms of induction and maintenance of long-term associated plasticity are very poor. The results of modern molecular biological studies suggest that metabolism of neuron-specific protein molecules may underlie this highly specific interaction of genome and synapses. Our studies, performed with the command neurons for the defensive behavior of the edible snail, showed that the neuron-specific non-histone protein of chromatin, Np 3.5, is involved in the regulation of neuronal synaptic inputs that mediate excitation induced only by certain conditioned stimulation and does not affect the responses of neurons induced by other conditioning stimuli [14].

The above facts suggest that there are different types of integrative processes in neurons and they differ in their cell and molecular mechanisms. Depending on the type of skill produced, the stage of its formation, the functions of the neuron, and its convergent features, there are several mechanisms that provide the integrative activity of a neuron. These mechanisms are characterized by some specificity of the regulation of morphofunctional changes in synaptic links of neurons during learning and the formation of memory.

We believe that the problems of the integrative activities of neurons and neuroplasticity, after some development, have led to a general understanding of genome-mediated integrative processes, which provide specific morphofunctional remodeling of certain synaptic inputs of nerve cells.

REFERENCES

1. Anokhin, K.V. and Sudakov, K.V., *Usp. Fiziol. Nauk*, 1993, vol. 24, no. 3, pp. 53–70.
2. Anokhin, P.K., *Usp. Fiziol. Nauk*, 1974, vol. 5, no. 2, pp. 5–92.
3. Sokolov, E.N. and Nezlina, N.I., *Zh. Vyssh. Nervn. Deyat.*, 2007, vol. 57, no. 1, pp. 5–22.
4. Hawkins, R.D., Kandel, E.R., and Bailey, C.H., *Biol. Bull.*, 2006, vol. 210, no. 3, pp. 174–191.
5. Lynch, M.F., *Physiol. Rev.,* 2004, vol. 84, no. 1, pp. 87–136.
6. Balaban, P.M., *Neurosci. Behav. Rev.*, 2002, vol. 26, no. 5, pp. 597–630.
7. Nikitin, V.P., *Ros. Fiziol. Zh. Im. I.M. Sechenova*, 2006, vol. 92, no. 4, pp. 402–419.
8. Pivovarov, A.S., *Zh. Vyssh. Nervn. Deyat.*, 1992, vol. 42, no. 6, pp. 1271–1286.
9. Nikitin, V.P. and Kozyrev, S.A., *Zhurn. Vyssh. Nervn. Deyat.*, 1991, vol. 41, no. 3, pp. 478–489.
10. Grinkevich, L.N., *Zh. Vyssh. Nervn. Deyat.*, 2001, vol. 51, no. 1, pp. 81–88.
11. Kavaliers, M., Choleris, E., and Saucier, D.M., *Peptides*, 1997, vol. 18, pp. 943–947.
12. Gulyaeva, N.V., *Biokhimiya*, 2003, vol. 68, no. 11, pp. 1459–1470.
13. Buchwalter, G., Gross, C., and Wasylyk, B., *Gene*, 2004, vol. 324, no. 1, pp. 1–14.
14. Kozyrev, S.A., Nikitin, V.P., Goncharuk, V.D., and Sherstnev, V.V., *Neirofiziologiya*, 1995, vol. 27, no. 3, pp. 171–182.