REVIEWS

Genome of Brain Neurons in Organization of Systemic Mechanisms of Behavior

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Here we review the mechanisms underlying systemic regulation of the genome in brain nerve cells. Expression of early and late behavioral genes is regulated by systemic processes in dominant functional systems. The main elements of this molecular and genetic cascade are similar during learning and development.

Key Words: *genes; brain; behavior; learning; functional systems*

In recent years much progress was achieved in biology. The genome of humans and some animals was determined. However, its role in the behavior of humans and animals is poorly understood. The theory of functional systems offers new possibilities for studying the role of molecular and genetic mechanisms in the systemic organization of behavior [2,6].

Functional systems are dynamic and self-regulating structures that are self-organized from various organs and tissues. The elements of functional systems interact with each other and contribute to attainment of a positive adaptive result. Functional systems modulate parameters of the internal environment and results of behavioral activity, which determines the optimal level of metabolic processes in tissues.

Most functional systems maintaining various parameters of the internal medium at an optimum level are genetically determined. They appeared after long biological evolution, develop during embryogenesis, and are present in all specimens of the certain species. Learning plays little role in the formation of these functional systems. The central and peripheral components in these systems are activated immediately after their ontogenetic maturation.

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Functional systems regulating instinctive activity of animals and humans and acting under ecologically stable conditions are genetically determined. However, many functional systems determining behavioral activity of animals and humans in unstable environment are dynamic structures formed for the first time during the interaction between an organism and environment (*i.e.*, after learning).

P. K. Anokhin described the inner operational architectonics underlying central organization of functional systems [2]. It includes the following consecutive events: afferent synthesis, decision making, anticipation of the result (acceptor of result), efferent synthesis, action, attainment of the result, and comparison of this result with the acceptor of results via back afferentation (Fig. 1).

All components of the central architectonics of functional systems promote attainment of adaptive behavioral results. Functional activity of various functional systems includes isomorphic, discrete, and consecutive systemoquanta. Each systemoquantum is determined by and complies the requirement [6].

Systemoquanta affecting behavioral activity of living organisms trigger the dominant motivation associated with metabolic and social requirements. This motivation interacts with situational and triggering afferentation and mechanisms of memory at the stage of afferent synthesis, organizes central architectonics

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of functional systems, and forms an apparatus for anticipation of the result that would satisfy the initial requirements (acceptor of result). This process determines directed activity of individuals.

The initial requirement can be satisfied immediately or via intermediate positive and negative results. Each stage of effective activity directed toward satisfaction of the requirement is evaluated by the acceptor of results via reverse afferentation from various sense organs and receptors (Fig. 2).

The inner mechanisms of behavioral systemoquanta are evaluated by living organisms via the apparatus of emotions. Coincidence of reverse afferentation with the acceptor of results and satisfaction of the corresponding requirement reinforce directed activity. The behavioral act is completed after attainment of the adaptive result accompanied by positive emotions. In case of discrepancy the orientation and exploratory reaction often produces negative emotions, reorganizes systemic architectonics of the behavioral act, and forms a new behavioral strategy.

Systemoquanta of functional systems determining behavioral and mental activity of organisms under various environmental conditions have relatively small genetic "skeleton" of dominant biological motivations and mechanisms of reinforcement. The mechanisms of learning and memory play a major role in their activity.

Our previous studies showed that expression of immediate early genes in brain neurons is intensified during training in attainment of the result under varying environmental conditions [3,4]. These genes (*e.g.*, *c-fos*) inducible transcriptional factors initiating cascade long-term changes in activity of effector target genes (late genes) that modify functions of neurons.

Experiments with selective blockade of *c-fos* expression in the brain by specific antisense oligonucleotides indicated that this mechanism plays a major role in learning and memory [13,15]. The inhibition of *c-fos* mRNA translation in brain structures impairs long-term, but not short-term memory in various models of learning with different animals.

Similar results were obtained in experiments with other members of the AP-I gene family and immediate early genes of various classes, *i.e.*, zif/268 (NGFI-A, Egr-1, or ZENK in birds) that encodes transcriptional factor from the family of zinc-finger proteins [10].

Expression of early genes in the brain during learning is a systemic process [1]. Populations of nerve cells expressing early genes during learning are present in various brain regions. Expression topography is determined by the type of treatment and task of learning. These data suggest that activation of genes for inducible transcriptional factors is related to the appearance of new functional systems during learning. We compared expression of c-Fos transcriptional factor and systemic specialization of neuronal activity during training of animals in a new form of directed behavior [5]. A close correlation was found between the count of cells expressing c-Fos during training in attainment of the result and specialization of cortical cells in a new functional system of behavior.

The theory of functional systems raises the question: which is the role of immediate early and late

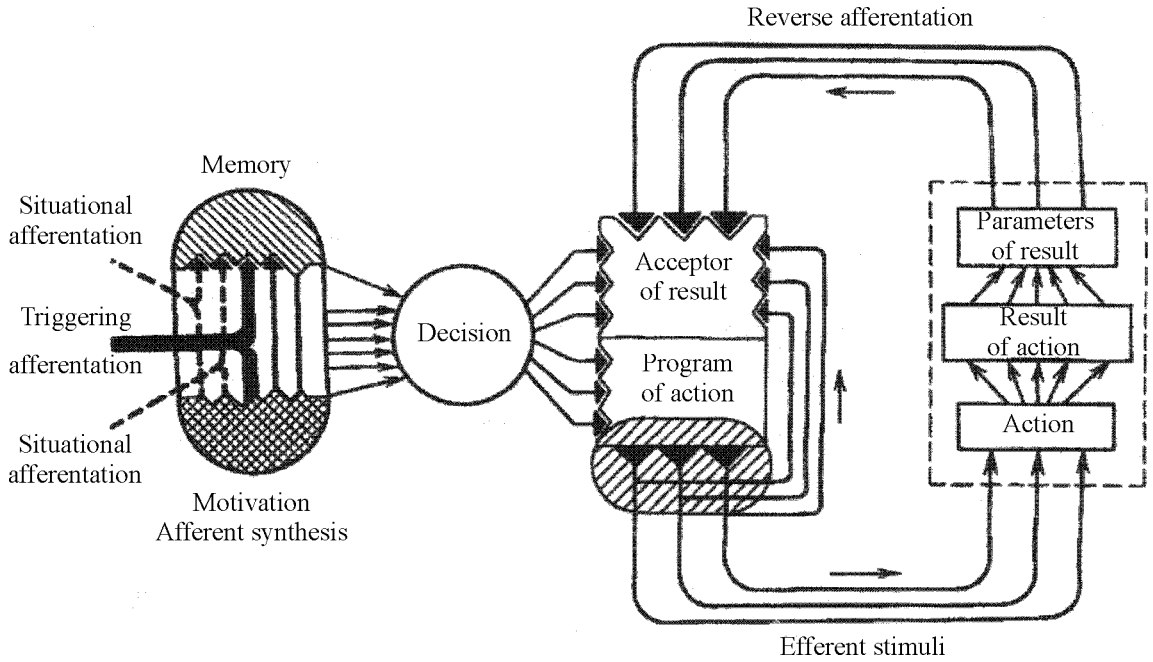

Fig. 1. Central operational architectonics of the functional system for behavior [2].

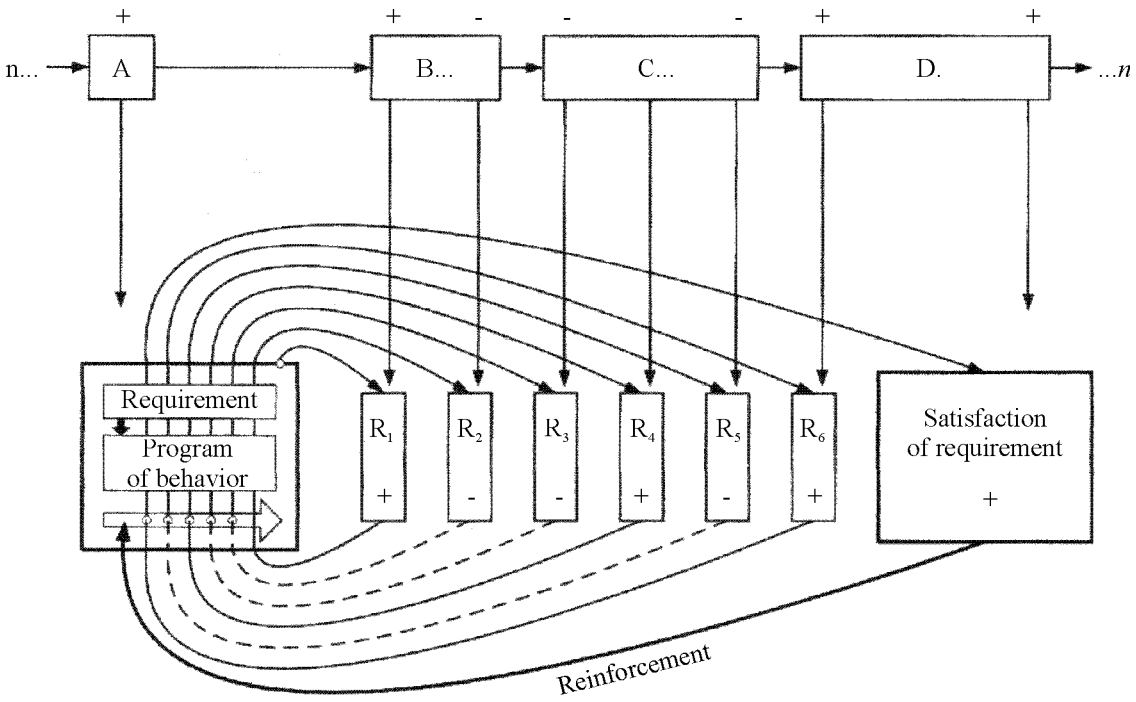

Fig. 2. Systemoquantum of behavior as a unit of systemic activity in the organism. *n*→A→B→C→D→*n*: environmental events. R_1-R_6: intermediate results of behavior promoting (+) or hindering (-)attainment of the initial requirement [6].

gene expression in the formation of individual components in systemoquanta of congenital and acquired forms of behavior and mechanisms of interaction between the main components in the central architectonics of behavioral acts (dominant motivation and reinforcement)?

Our experiments showed that early gene expression in the brain of animals is induced in a novel situation [3]. Under these conditions the animals meet with difficulties in achieving the positive result and/or display pronounced orientation and exploratory reactions. The intensity of early gene expression increases during the formation of biological motivations difficult to be satisfied (*e.g.*, feeling of hunger, fear, and sexual desire). Expression of early genes is not initiated, if this dominant motivation is easily realized [7]. The usual behavioral activity of animals, performance of automatized tasks, influence of common signals and events, and maintenance of animals under normal and stable conditions without learning do not cause expression of immediate early genes in the nervous system.

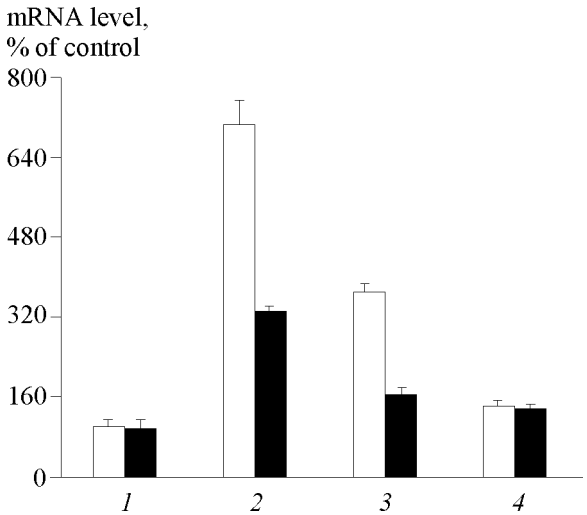

Fig. 3. Expression of immediate early genes *c-fos* and *c-jun* in mouse brain cortex during training of avoidance of electrocutaneous stimulation. Ordinate: content of *c-fos* (light bars) and *c-jun* (dark bars) mRNA in the cerebral cortex 30 min after training. *1*) passive control (without electrical stimulation), *2*) learning, *3*) active control (electrical stimulation), *4*) task performance.

However, ineffectiveness of preformed behavioral acts, influence of new or unexpected environmental factors, and disappearance of common and expectable factors (particularly during emotional stress) are followed by rapid activation of inducible early genes in the nervous system.

Learning is accompanied by similar changes. Expression of early genes in the nervous system is activated in this situation. Gene transcription decreases after reinforcement, when the animal repeatedly satisfies its dominant requirement (Fig. 3). The expression of early genes progressively decreases with adaptation of animals to novel environmental conditions and repeated exposure to exogenous factors [4].

The analysis of these data from the viewpoint of the theory of functional systems indicates that discrepancy between reverse, situational, or triggering afferentation and preformed mechanisms of congenital or acquired functional systems is the main cause for

activation of early gene expression in brain cells of awake animals. In other words, genetic reactions of brain cells in animals displaying a certain form of behavior are determined by a factor of novelty in comparing environmental afferentation, behavior, and experience stored in memory.

Probably, central architectonics of the functional system includes two elements responsible for the regulation of genetic reactions in nerve cells.

A comparison of situational and triggering afferentation with individual and species experience proceeds during afferent synthesis. Early gene expression in the brain of animals that meet with changes in the environment, influence of new agents, and disappearance of common factors is associated with discrepancy in systemic organization of behavior. At the behavioral level, these changes are manifested in the generalized orientation and exploratory reaction that initiates learning.

The acceptor of result is another key apparatus for verification in functional systems. Difficulties in satisfaction of dominant motivations and discrepancy between the acceptor of results and reverse afferentation from the result of behavior initiate axtivation of early genes.

These data show that genetic behavioral reactions of nerve cells can appear in the initial (formation of dominant motivation) and late stages of construction of systemoquanta determining the adaptive behavior (evaluation of the achieved result).

The question arises: which processes block early gene expression after repeated reinforcement during learning?

Our previous experiments showed that inhibitors of protein synthesis (cycloheximide and puromycin) and RNA synthesis (8-azaguanine and actinomycin D) administered into the lateral cerebral ventricle practically do not block biological motivations of hunger and fear in rabbits having no experience in their satis-

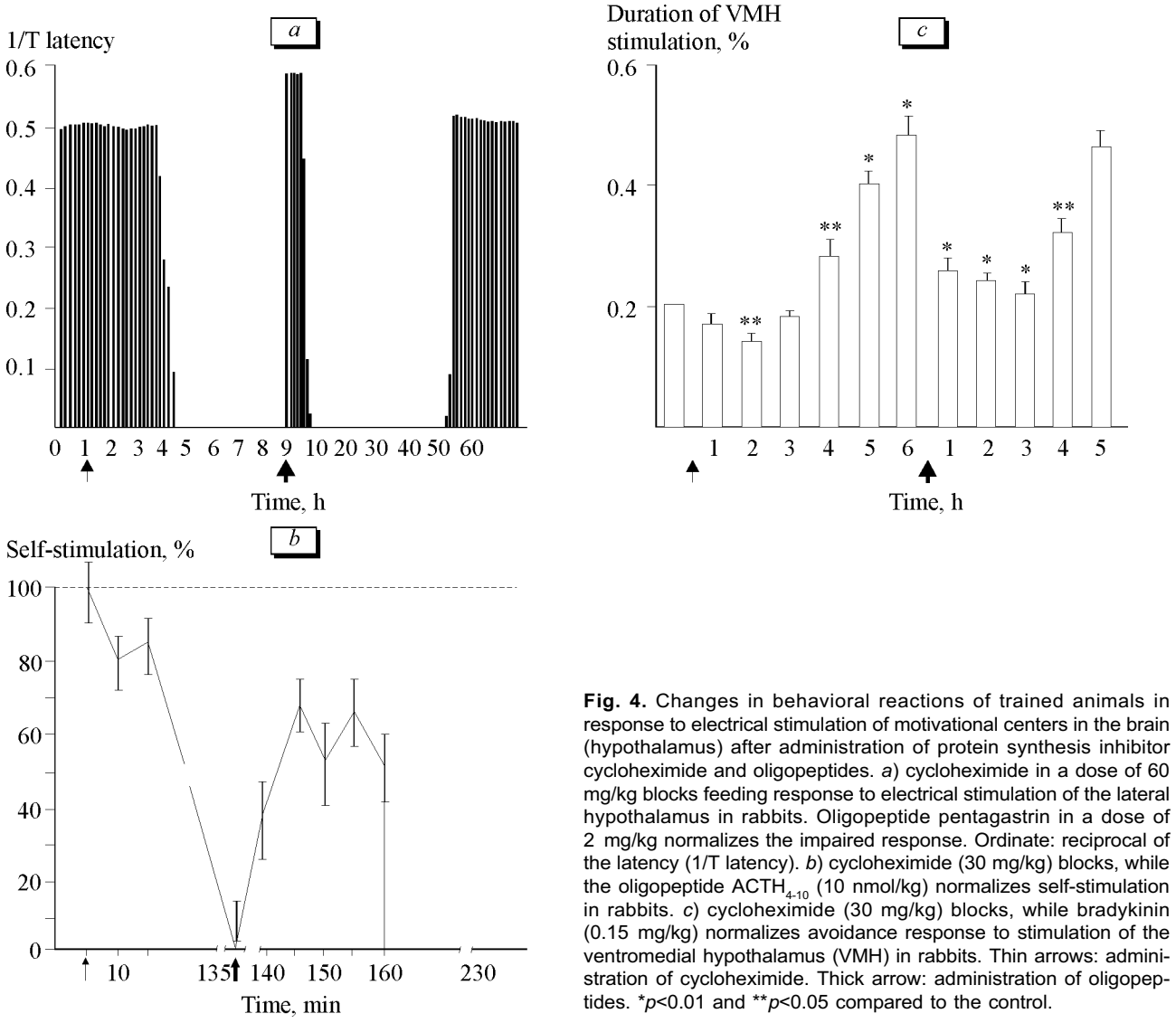

Fig. 4. Changes in behavioral reactions of trained animals in response to electrical stimulation of motivational centers in the brain (hypothalamus) after administration of protein synthesis inhibitor cycloheximide and oligopeptides. *a*) cycloheximide in a dose of 60 mg/kg blocks feeding response to electrical stimulation of the lateral hypothalamus in rabbits. Oligopeptide pentagastrin in a dose of 2 mg/kg normalizes the impaired response. Ordinate: reciprocal of the latency (1/T latency). *b*) cycloheximide (30 mg/kg) blocks, while the oligopeptide $ACTH_{4-10}$ (10 nmol/kg) normalizes self-stimulation in rabbits. *c*) cycloheximide (30 mg/kg) blocks, while bradykinin (0.15 mg/kg) normalizes avoidance response to stimulation of the ventromedial hypothalamus (VMH) in rabbits. Thin arrows: administration of cycloheximide. Thick arrow: administration of oligopeptides. *$p<0.01$ and **$p<0.05$ compared to the control.

faction in the experimental chamber [18]. After reinforcement (satisfaction of motivations caused by electrical stimulation of the lateral and ventromedial hypothalamic nuclei) protein synthesis blockers produced an inhibitory effect on biological motivations.

If food was given to animals after starvation or electrical stimulation of feeding centers in the lateral hypothalamus, they gained experience in satisfying this motivation. Protein synthesis blockers cycloheximide and puromycin temporally suppressed feeding behavior in these animals [18]. Similar results were obtained in experiments with animals that could avoid electrical stimulation of the fear centers in the ventromedial hypothalamus: protein synthesis blockers abolished the avoidance response.

Administration of pentagastrin into the lateral cerebral ventricle normalized the feeding response that was abolished during blockade of protein synthesis with cycloheximide (Fig. 4). After repeated reinforcement of feeding motivation, feeding behavior depended on the effect of pentagastrin. Intracerebroventricular administration of the antiserum against gastrin abolished feeding behavior in these animals [19]. Defensive reactions blocked with cycloheximide returned to normal after intracerebroventricular administration of bradykinin.

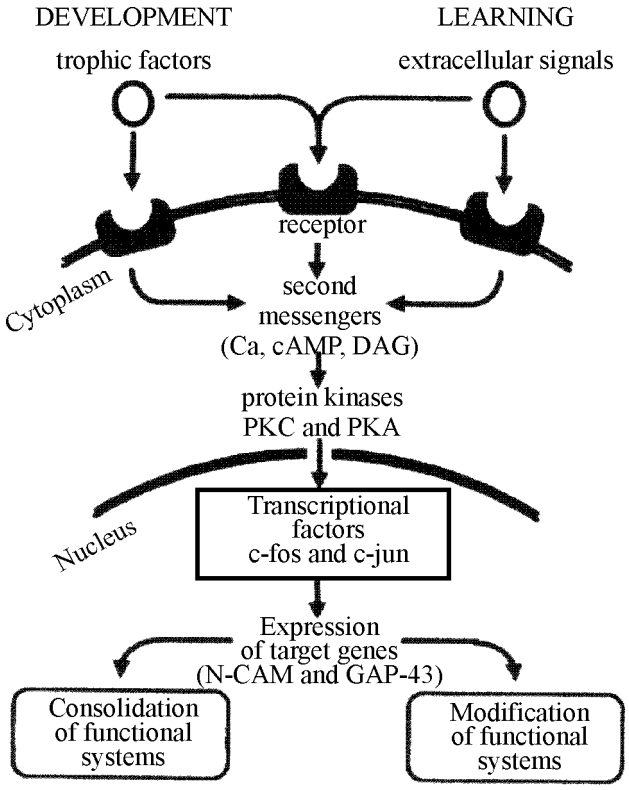

Fig. 5. Similarity of molecular processes underlying the formation of nervous relationships in a developing organism (left) and their changes in an adult organism during learning (right).

These data illustrate that the genome of individual brain neurons in animals gains new functional properties after reinforcement of directed motivational activity. Under the influence of dominant motivation, these cells express effector protein molecules that organize a certain type of behavior.

The question arises: which mechanisms underlay these long-term changes in gene expression after repeated reinforcement of dominant motivation?

Previous studies showed that activation of gene expression during learning (*e.g.*, *c-fos* gene expression) is followed by transcription and synthesis of protein substances in the brain of animals (after 4-8 h) [4]. Our experiments demonstrated that administration of the protein synthesis inhibitor anisomycin 3-5 h after learning impairs consolidation of long-term memory [20]. However, genes for transcriptional factors *c-fos* and *c-jun* were not expressed during the second phase of RNA synthesis [4].

We hypothesized that the genomic response of nerve cells to learning proceeds in two stages and is similar to the reaction of other cells to growth factors. Activation of early gene expression is followed by expression of regulated target genes [8].

Which effector genes are activated in nerve cells under the influence of induced transcriptional factors during learning? Probably, genes encoding precursors of oligopeptides pentagastrin and bradykinin modulate expression during reinforcement of feeding and defensive reactions. However, the mechanisms regulating expression of these genes by transcriptional factors activated during learning remain unknown. Some other genes regulated by transcriptional factors Fos and Jun were extensively studied in this respect [16]. c-fos and c-jun proteins are components of transcriptional complexes regulating genes involved in cell proliferation and differentiation. The regulation of transcription by this complex is realized via its interaction with specific cis-elements of DNA containing the TGACTCA sequence (AP-I element).

The AP-I element is present in promoter regions of various genes that are activated is response to the influence of extreme factors [12]. Genes for pre-proenkephalin, S-100, neurofilaments, tyrosine hydroxylase, and N-CAM (neural cell adhesion molecule) have the AP-I site [17].

N-CAM genes belonging to the family of genes for cell adhesion molecules are of particular interest in studying of learning and memory. Cell adhesion molecules, or morphoregulatory molecules, are expressed on the surface of cell membranes and regulate aggregation and disaggregation of cells during the development [11]. Blockade of expression or binding of cell adhesion molecules impairs morphogenetic patterns of the development.

The N-CAM gene is expressed in embryonic and adult brains. Mouse with directed mutation of the N-CAM gene are characterized by abnormal morphology of the brain, behavioral changes, and deficit of learning [22]. New N-CAM proteins are synthesized only during the second phase, while the synthesis of LI and Ng-CAM proteins (neural and glial cell adhesion molecules) occurs during both phases of protein synthe-sis [14].

Antibodies to cell adhesion molecules produce amnesia in animals only over the fixed critical period after learning (6-8 h in rats [9] and 4-6 h in chickens [14]). This process coincides with expression of late genes (target genes for products of immediate early genes) after learning. Promoters of N-CAM genes carry AP-I elements binding Fos/Jun transcriptional factors. It can be suggested that during learning N-CAM genes are involved in cascade molecular events initiated by immediate early genes [4].

Our previous studies showed that antibodies to LI molecules administered immediately before learning (critical period 1), 5-6 h after learning (critical period 2), and 14-17 h after learning (critical period 3) impair memory [21]. Experiments with protein synthesis inhibitor anisomycin demonstrated that the third critical period of memory consolidation is not associated with the synthesis of new proteins [20]. Probably, membrane glycoproteins (*e.g.*, LI) are posttranscriptionally glycosylated at this stage. The specific inhibitor of glycosylation 2-deoxygalactose impairs memory consolidation at this stage. Protein glycosylation critical for memory consolidation proceeds in 2 stages. The first stage coincides with learning. The second stage starts 4-5 h after learning and lasts 3-17 h (depending on the type of learning) [20]. Glycosylation of membrane glycoproteins, including cell adhesion molecules, converts them into the active form and provides intercellular interaction, which results in selection and stabilization of synapses maintaining the long-term memory.

We believe that learning initiates the following molecular and genetic processes in nerve cells. Novelty and discrepancy between the current situation, experience of animals, and acceptor of the result in dominant functional systems trigger cascade receptor and cytoplasmic processes and activate immediate early genes in neurons. Some immediate early genes encode transcriptional factors. These proteins are transported into the cell nucleus and initiate the second phase of protein synthesis several hours after the exposure. Late effector genes activated during this process include genes for cell adhesion molecules playing a key role in morphogenesis during embryogenesis. After reactivation of morphoregulatory molecules in the adult brain during learning, nerve cells gain the ability to reconstruct synaptic relationships in the composition of modified or newly formed functional systems. The main elements and the stages of this molecular cascade process are similar during learning and development [4]. These data suggest that molecular and genetic processes accompanying learning and development of the nervous system are similar at the level of regulation of gene expression (Fig. 5). It should be emphasized that activity of genes in brain cells of adult animals is regulated by systemic processes in the dominant functional system.

We revealed 3 major factors responsible for systemic regulation of genetic neuronal reactions in behavior. Expression of immediate early genes in the brain is potentiated by the requirement or motivation and inhibited after satisfaction. Expression of immediate early gene is intensified when the animal meets with difficulties in achieving the result, which produces a stress situation. Brain neurons express new protein molecules after reinforcement and attainment of the adaptive result. This process does not proceed in animals with unsatisfied requirements.

It remains unclear, whether expression is typical of cells in the functional system having discrepant information or this process reflects learning and proceeds in new neurons that did play a role in satisfaction of the requirement and are involved in attainment of the result for the first time. Undoubtedly, new behavioral reactions and situations affect long-term memory realized via protein synthesis in the brain and modulate activation of immediate early and late genes. These changes do not depend on reinforcement (attainment of the adaptive result). How the elements of positive (reinforced) and negative experience (nonreinforced) are differentiated in memory? Whether these differences are related to the specific molecular effects on the genome? These problems require further investigations.

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