ROLE OF NUCLEIC ACID SYNTHESIS IN THE MECHANISM OF THE STABILIZATION OF CONDITIONED REFLEXES AND MEMORY

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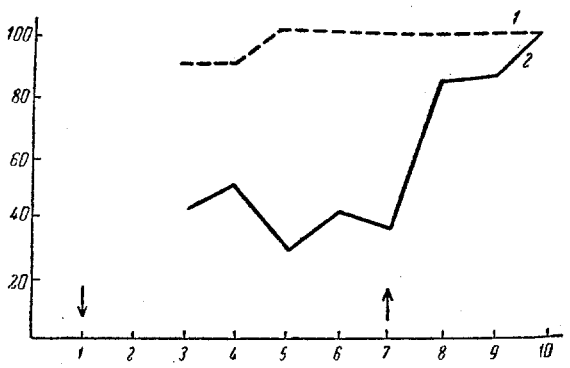
Investigations conducted in recent years have indicated that the activity of the brain, which provides for the formation of conditioned reflexes and the establishment of elementary forms of memory, based on the conditioned reflex mechanism, depends to a high degree on the nucleic acid and the protein metabolism [5,10-12,15,16]. The facts obtained in these investigations may be evaluated either from the standpoint of the hypothesis of the role of nucleic acids and proteins in the intracellular coding of stimuli of the external environment [15], or on the basis of the representation that nucleic acid and protein synthesis is essential for the growth of the processes of the neurons and the establishment of new interneuron synaptic connections. These hypotheses include a definite general factor, consisting of the fact that in the formation of a conditioned reflex and strong recall, the increased function of the neurons of a definite functional constellation activates the genetic apparatus of these neurons, and this activation is expressed in the synthesis of a certain amount of RNA. Actually, independent of whether RNA synthesis is necessary for the growth and establishment of interneuron connections, or whether it is needed for the newly synthesized RNA to be able to code the frequency characteristics of the incoming impulses, the process of formation of new portions of RNA on the existing DNA itself should be a vital link in the mechanism of the formation of conditioned reflexes and strong recall, if one of the above mentioned hypotheses is correct.

On the basis of these considerations, in this work we studied the influence of a factor that selectively blocks the DNA-dependent synthesis of RNA, actinomycin 2703, on the process of formation of new conditioned reflexes and on the stabilization of strong, previously developed conditioned reflexes.

INVESTIGATION PROCEDURE

The experiments were conducted according to a modified motor-food method [4] on male rats of the Wistar line, weighing about 200 g. A total of four series of experiments were conducted (five rats in each). In series I, a conditioned reflex to light was preliminarily developed in the rats, and against a background of injections of actinomycin, a reflex to the tone 400 cps was developed. In the animals of series II, a conditioned reflex to the tone 400 cps was preliminarily developed, and against a background of injections of actinomycin, a conditioned reflex to red light was developed. In series III, the influence of actinomycin on the development of conditioned reflexes was investigated in rats in which conditioned reflexes had not been developed before this. The animals of series IV, which received injections only of the solvent (aqueous alcohol mixture), served as the control.

In the study of the process of establishment of a conditioned reflex, we determined two indices: the appearance of the reflex and its stabilization. The appearance of the reflex was considered to be the appearance of a positive reaction to the conditioned stimulus, while stabilization was considered to be the appearance of the reflex ten times in a row. In the experiments, we used the Soviet preparation, actinomycin 2703, produced at the



Dynamics of the formation of a conditioned reflex to sound in control animals (1) and in animals that received actinomycin 2703 (2). Along Y axis, presence of conditioned reflex reaction (in percent of total number of presentations of the conditioned stimulus); along X axis, time from the beginning of the experiment (in days). The arrows indicate the beginning and cessation of administration of the preparation.

Laboratory of Natural Compounds of the Institute of Experimental Oncology, Academy of Medical Sciences USSR. This preparation represents a mixture of actinomycin D and other actinomycins. Special biochemical investigations indicated that in vitro it selectively and entirely inhibits RNA synthesis on the structural genes of the DNA of animal cells [1,2,9], while in vivo it inhibits the activation of nucleic acid synthesis, growth and division of differentiated cells of the internal organs of mammals, with compensatory hyperfunction of these cells [8]. Actinomycin 2703 was administered subcutaneously in aqueous alcohol solution, in a dose of 10 μg of the preparation per 100 g of weight of the animal. Injections of actinomycin 2703 were administered daily at the same time over a period of 7 days. The development of a conditioned reflex in nonirradiated rats (series III) and of a new conditioned reflex in animals with a developed conditioned reflex (series I and II) was begun after three injections of the preparation i.e., 72 h after the beginning of its administration.

RESULTS OF THE INVESTIGATION AND THEIR DISCUSSION

The sum total of our experiments consists of the fact that actinomycin 2703 does not exert any significant effect upon the previously developed motor-food conditioned reflexes to sound and light at the doses used and indicated duration of its administration, and at the same time sharply inhibits the development of new motor-food conditioned reflexes to these stimuli, entirely eliminating their stabilization.

The most indicative are the results of experimental series I, in which a conditioned reflex to sound was developed in the animals with previously developed strong conditioned reflexes to light against the background of administration of actinomycin 2703 (see table).

From the table it is evident that changes in the developed conditioned reflexes to light were observed only after five to six injections of the preparation. After the injections of actinomycin 2703 was stopped, a rapid restoration of the original quantitative characteristics of the indicated reflex set in.

In the rats of this series, a new conditioned reflex (to sound) arose at the second to third combination, but it was not stabilized against a background of administration of actinomycin 2703, in spite of 30-35 combinations. Strengthening of the reflex was noted only after 10 to 15 combinations after administration of the preparation had ceased. In the control animals, which did not receive the preparation, stabilization of this reflex usually occurred after five to eight combinations (see figure).

Thus, the administration of actinomycin 2703, while not exerting any significant influence on previously developed conditioned reflexes to light, excluded stabilization of a newly developed conditioned reflex to a stronger stimulus, sound.

Analogous results were obtained in the animals of series II; in the animals of series III, which had no previously developed conditioned reflexes, the new conditioned reflex also was not developed; in series IV (control), the administration of the solvent to the animals exerted no effect upon the existing and newly developed reflexes.

The data that we obtained show that the disturbance of the process of formation of conditioned reflexes induced by actinomycin 2703 cannot be explained by phenomena of general intoxication and by a corresponding decrease in the motor activity and food excitability. More evidence against such an explanation is the conservation of previously developed conditioned reflexes, the substantial magnitude of the reactions between signals, the distinct expression of the orientative reflex, and the relative conservation of appetite in animals that received actinomycin.

Since actinomycin blocks the DNA-dependent RNA synthesis, the results obtained mean that RNA synthesis on the DNA templates is vital to the process of stabilization of new conditioned reflexes; old, previously formed

Changes in Previously Developed Conditioned Reflexes to Light After Administration of Actinomycin 2703

Index	Before injection of actinomycin	Against a background of injection of actinomycin							After cessation of administration of actinomycin	
		First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day
% of samples with presence of reflexes	100	100	100	100	100	95	80	80	80	100
Average value of conditioned reflex (in arbitrary units)	35,8 ± 1,42	36,4 ± 1,78	3,00 ± 1,16	31,9 ± 2,21	30,6 ± 3,14	33,2 ± 3,26	25 ± 2,80	25 ± 3,70	30,6 ± 3,68	36,3 ± 2,68
Average latent period (in sec)	1,31 ± 0,07	1,38 ± 0,09	1,28 ± 0,08	1,31 ± 0,12	1,24 ± 0,15	1,57 ± 1,16	1,92 ± 0,24	1,23 ± 0,16	1,51 ± 0,17	1,42 ± 0,11
Average (during experiment) value of reactions between signals (in arbitrary units)	746	536	336	772	706	734	646	480	980	1126

conditioned reflexes, on the contrary, can function "uninterruptedly" for a rather long time when the DNA-dependent RNA synthesis is inhibited. This fact, as was indicated above, may be satisfactorily explained from the standpoint of the two basic hypotheses on the role of the synthesis of nucleic acids and proteins in the mechanism of conditioned reflexes and memory. However, these hypotheses themselves are far from equally substantiated, since there are a number of facts contradicting the hypothesis of intracellular coding of the impulses arising to the neuron in the nucleic acid molecules and indicating that the growth and change of interneuron connections are a more probable basis for the stabilization of conditioned reflexes and memory. In view of this, it should be kept in mind that the neuron, just like any other differentiated cell, is characterized by a bilateral relationship between the function and the genetic apparatus. The direct relationship consists of the fact that the genetic apparatus based on scheme DNA → RNA → protein guarantees the appearance, and then the constant renewal of all the cellular structures, in particular structures that perform a specific physiological function i.e., the generation and transmission of excitation. Feedback represents information directed from the cytoplasm to the nucleus and indicating the level of physiological function of the cell. Regulating the activity of the genetic apparatus in accord with the level of function, this line of communication prevents the wearing out of cellular structures.

Cytophysiological investigations [15] have shown that the essentially physiological hyperfunction of the intact neuron very rapidly results in a considerable increase in the decomposition and synthesis of RNA and protein; if hyperfunction is sufficiently prolonged and intense, such activation of the genetic apparatus becomes the basis of growth of the neuron and its processes [3,8, 14]. This means that an important feature of the organization of the neuron under the conditions of the intact organism is the prompt and intensive realization of feedback, existing between its function and the genetic apparatus, the possibility of rapid and extensive mobilization of the genetic apparatus when function is increased. Since the process of development of a conditioned reflex is provided for by intensive functioning of a definite combination of neurons, it may be assumed that, occurring originally in the form of a circulation of excitation in the reverberating network or in any other form, such physiological hyperfunction activates the genetic apparatus and leads to growth or reorganization of the processes of the neurons and synaptic structures. As a result of this, the primarily functional process receives a plastic, structural supply and becomes a resistant and economical basis for the establishment of conditioned reflexes and memory.

Leaving aside a number of aspects of this premise, let us emphasize its main factor: that the realization of the intraneuron interrelationship between the functions and genetic apparatus of the cell plays the basic role in the formation of interneuron synaptic connections and the formation of multineuron systems comprising the structural basis of the stabilization of conditioned reflexes and memory.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
