SUPPRESSION OF MEMORY IN RATS BY BRAIN ANTIBODIES OF TRAINED RATS

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If γ globulin obtained from the serum of rabbits immunized with the brain homogenate of rats trained to make defensive movements to escape from or avoid an electric current, and then exhausted with the brain homogenate of intact rats, is injected into other rats it significantly depresses the formation and maintenance of the corresponding skilled defensive movements by comparison with control animals receiving exhausted γ globulin from the serum of rabbits immunized with brain homogenate from untrained rats.

Recent investigations [2-6, 10] have shown that the genetic apparatus of differentiated cells, especially cells of the nervous system of higher animals and man, plays an important role in the mechanism of function of these cells. If information reaches cells of the nervous system, the synthesis of protein and RNA in the cells is activated. However, it is not yet known whether new types of RNA and proteins, not detectable in the cells before training either because of their total absence, or because of the extremely low intensity of their synthesis, appear in the cells or whether the synthesis of existing types of RNA and proteins is increased. Recent investigations have confirmed the hypothesis that the spectrum of synthesized RNA is modified by training [8, 12].

The object of the present investigation was to discover whether during training of animals in a certain skill, there is a change in the antigenic spectrum of the brain, which could indicate a change in the spectrum of proteins synthesized in the brain and the appearance of certain new proteins specific for that particular skill.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200 g. The rats were trained to carry out skilled defensive movements in a maze. Before the experiment began, the animal was placed in the entrance chamber. Opening the door of the chamber for 5 sec was accompanied by the ringing of an electric bell, and immediately after this stopped, an electric current was passed along a wire running along the floor of the maze. Depending on the experimental conditions the rat could escape from the action of the current only by running along the safe path through the maze. After several compulsory runs to escape from the current, the animal learned how to avoid it, so that,when the rat was placed in the entrance chamber of the maze, switching on the current instantly caused the rat to run along the safe path. After the animal had been trained to escape from the current, it was then trained to avoid it, so that merely ringing the bell caused the rat to run along the safe path.

Training was carried out daily with the rat making 30 runs at intervals of 2 min. The number of escape responses, the number of avoidance responses, and the duration of each run were recorded. The latter depended, as a rule, on the degree of deviation of the rat from the safe (correct) path, for there was very little difference between the speed of movement of the different groups of rats.

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Two series of experiments were carried out. The animals used in each series were divided into two groups: experimental and control. The effect of γ globulin obtained from rabbits immunized with brain homogenate of the rats on the ability of rats to learn to escape from and to avoid the current was studied in series I, and its effect on the maintenance of these skills in rats was studied in series II.

Rats trained as described above for 7 days, and also control rats (i.e., untrained), were sacrificed in the cold, the brain was perfused with physiological saline, and its higher levels (above the pons) were homogenized and lyophilized. The rabbits were injected with 1 ml of 15% brain homogenate mixed with Freund's adjuvant in the ratio of 1:1. Altogether 5 injections were given at intervals of 3 days. One month after the beginning of immunization blood was taken from the rabbits and serum obtained from it. The sera of 4 rabbits immunized with identical homogenates were pooled. Parallel with these procedures, a brain homogenate was obtained from untrained rats, and the serum was exhausted by mixing it with the homogenate thus obtained (2 parts of serum to 1 part of homogenate). The resulting mixture was incubated for 2 h at 37°C and then centrifuged for 15 min at 1000 rpm. The γ globulin was isolated from the supernatant by Kendall's method [11] and dialyzed in the cold. The reaction with barium chloride, carried out to determine the completeness of dialysis, revealed no difference between all the dialysates used. The dialysate obtained from the serum of rabbits immunized with the brain of trained rats was used for injection into the animals of the experimental group, and that from animals immunized with brain of untrained rats was injected into the animals of the control group. The dialysate, equal to 20% of the initial volume of the serum, was injected in a dose of 0.05 ml into the lateral ventricle of the rat through a metal cannula previously implanted by a stereotaxic technique.

The rats of series I received an injection of dialysate daily for 3 days; after each injection they were placed in a maze where they were trained as described above. The animals of series II were trained for the first 3 days by the same method, and during the next 3 days they received the dialysate. On the 7th day, maintenance of the skills was tested in these rats. The test consisted of 30 applications of the conditions stimulus (the bell), of which the first 10 were not reinforced by the electric current. The animal was placed in the entrance chamber and 5 sec later the bell was switched on. If the rat had not gone from the chamber 10 sec after the beginning of ringing the bell, the result of absence of running was recorded. In the next 20 applications, the electric current was switched on 5 sec after the bell if the animal was still in the entrance chamber. As soon as the rat left the chamber the current was switched off until the end of the run. The same indices were recorded as during training. In addition, the index of correct preservation (ICP) of the skill was calculated as the ratio, in percent, between the number of correct runs during the test of skill preservation and the number of correct runs on the last (3rd) day of training.

EXPERIMENTAL RESULTS AND DISCUSSION

The results given in Table 1 show that on the 2nd-3rd day of training the animals of the control group showed significantly more correct responses of avoiding the current than the rats of the experimental group, while the number of responses of escaping from the current and the duration of each run were greater for the animals of the experimental group than for those of the control group, although the difference was not always statistically significant. Injection of dialysate into the animals of the experimental group significantly impaired the preservation of the skill by comparison with the control group of animals. For instance, in the animals of the experimental group, ICP was 28±5%, compared with 55±5% in the controls ($P < 0.001$), the number of escape responses was 8±2 and 16±2 respectively ($P < 0.001$), and the duration of each run was 45±2 and 15±2 sec ($P < 0.001$) respectively.

Recent investigations [9, 13] have established the antigenic specificity of the brain and of its various structures, and also the depressant effect of brain antibodies on the function and electrical activity of nerve cells. However, possible changes in the antigenic spectrum of the brain during training in a specific skill has never been investigated.

The considerable impairment in training and memorizing of skilled defensive movements of escaping from and avoiding an electric current observed in the present experiments in rats receiving γ globulin, isolated from the serum of rabbits immunized with brain homogenates from trained animals, indicates that during training there is a change in the antigenic spectrum of the brain, and a change in the spectrum of the proteins or substances connected with them, synthesized in the brain, specific for that particular skill.

TABLE 1. Effect of γ Globulin Obtained against Skilled Defensive Movements of Escaping from and Avoiding an Electric Current on Training (M±m)

Group of rats	No. correct responses avoiding current			No. responses of escaping from current			Duration of a single run (in sec)		
	1	2	3	1	2	3	1	2	3
Control (19 rats)	15,37±1,4	25,1±0,3	27,84±0,17	8,47±1,6	16,47±2,2	20,42±2,2	33,26±4,8	9,64±1,1	9,01±0,85
Experimental (20 rats)	15,25±1,7	17,75±1,9	21,25±2,1	8,0±1,9	12,9±2,7	15,65±2,4	27,35±3,3	22,65±5,1	16,35±3,8
P	>0,05	<0,01	<0,01	>0,05	>0,05	>0,05	>0,05	<0,05	>0,05

Note. 1, 2, 3: Days of experiment.

The function of these new types of protein molecules remains unknown. They can participate in the synthesis of mediators responsible for the formation of functional connections in neuron chains, or they may be components of the receptor groups of synapses forming these connections. On the basis of the facts described above and of other evidence which has been obtained a hypothesis can be put forward which can be called the "neuronal selection" hypothesis by analogy with the clonal selection theory of immunogenesis. According to this hypothesis, macromolecular heterogeneity of neurons (or groups of neurons) arises during embryogenesis when anatomical connections formed between them. In these different groups of neurons, different segments of DNA free from histone blocking are correspondingly responsible for synthesis of their specific proteins. Taken as a whole, they constitute the phylogenetic or genetic memory. Coding of the ontogenetic information reaching the brain (individual experience, ontogenetic memory) takes place by activation of some of these anatomically pre-existing connections. Since each of these connections is maintained by its own specific proteins, the synthesis of which is sharply increased at this period, the antigenic spectrum of the brain is altered. This is what was found in the present experiments.

According to the writers' earlier hypothesis [1], synthesis of specific proteins in each group of neurons is activated by a mechanism similar to that described by Jacob and Monod [7], within a segment of DNA free from histone blocking.

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