# CHANGES IN CONTENT OF SPECIFIC AND NONSPECIFIC NERVE TISSUE PROTEINS IN RAT BRAIN STRUCTURES DURING TWO-WAY DEFENSIVE AVOIDANCE CONDITIONING

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Learning in animals is accompanied by changes in protein metabolism in various brain structures [1-3, 8-10]. The dynamics of the content of these proteins is determined by the time elapsing after learning [1, 2]. The consolidation process taking place in this case is linked with synthesis of brain-specific proteins such as, for example, S-100 and 14-13-2 [2]. Conditioned reflex formation in goldfish was found to be accompanied by changes not only in the content of nerve-specific proteins, but also in the level of proteins nonspecific for the brain [9, 10].

The object of this investigation was to study the dynamics of the content of proteins specific and nonspecific for nerve tissue in certain formations of the CNS in rats during learning.

## EXPERIMENTAL METHOD

Male Wistar rats weighing 130-150 g were used. A two-way defensive conditioned avoidance reflex (DCAR) was formed in a shuttle box [5]. The animals of the active control (six rats) received the same number of uncombined photic and electrodermal stimuli as the rats to be conditioned (six animals).

Changes in the content of nonspecific protein $P_1$ and brain-specific protein $P_2$ were determined immunologically by the length of the precipitation arcs formed by them immediately after DCAR formation and presentation of uncombined conditioned and unconditioned stimuli, and also 1 and 7 days after the procedures. The passive control group contained 12 intact rats.

The rats were decapitated, the brain removed, and the hippocampus, caudate nucleus, brain stem (region of the gigantocellular nucleus of the reticular formation), and motor and visual areas of the cortex were separated in the cold. Each structure was investigated in two rats, and paired formations were removed on both sides. The tissue was homogenized in 1.5 volumes of cold 0.005 M Na,K-phosphate buffer, pH 7.4. Homogenates of rat brain structures were analyzed by analytical immunoelectrophoresis in accordance with the scheme described previously [6]. The immunoelectrophoretic gels were developed by means of a standard mixture of 20 individual rabbit sera obtained after several reimmunizations. Differences in the $P_1$ and $P_2$ content in the rat brain structures were assessed by dispersion analysis, using Fisher's criterion [4]. Differences were considered to be significant at the $P \leqslant 0.05$ level.

## EXPERIMENTAL RESULTS

It was shown previously that protein antigens $P_1$ and $P_2$ have identical mobility on immunoelectrophoresis, corresponding to that of $\beta_1$-globulins, they do not contain any carbohydrate, polysaccharide, or lipid impurities, and they are found consistently in all structures of the CNS tested; $P_2$ is specific for the brain, whereas $P_1$ is immunologically identical with proteins of other rat organs [7]. During DCAR formation the trend of changes in

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TABLE 1. Content of Protein Antigens $P_1$ and $P_2$ in Some Structures of the Rat Brain Immediately, 24 h and 1 Week after Formation of DCAR and Uncombined Presentation of Conditioned and Unconditioned Stimuli (in % of passive control)

| Structure | Time of investigation after procedure | $P_1$ | | | | $P_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | active control | P | trained animals | P | active control | P | trained animals | P |
| Hippocampus | Immediately | 79,7 | Not significant | 72,9 | <0,05 | 93,2 | Not significant | 53,4 | Not significant |
| | 24h | 57,4 | <0,01 | 71,8 | <0,05 | 75,4 | <0,01 | 69,5 | <0,01 |
| | 7 days | 68,6 | <0,05 | 84,0 | Not significant | 71,2 | <0,01 | 92,4 | Not significant |
| Caudate nucleus | Immediately | 64,3 | <0,01 | 103,0 | Not significant | 78,6 | <0,01 | 84,6 | <0,05 |
| | 1 h | 63,1 | <0,01 | 94,6 | The same | 73,5 | <0,01 | 100,0 | Not significant |
| | 7 days | 64,3 | <0,05 | 78,0 | " " | 73,5 | <0,01 | 81,2 | The same |
| Motor cortex | Immediately | 67,8 | <0,05 | 71,7 | <0,05 | 81,4 | <0,05 | 93,2 | Not significant |
| | 1 h | 81,7 | Not significant | 125,7 | <0,05 | 94,1 | Not significant | 112,7 | The same |
| | 7 days | 85,7 | The same | 122,2 | <0,05 | 101,7 | The same | 115,3 | " " |
| Visual cortex | Immediately | 64,8 | <0,01 | 102,8 | Not significant | 81,4 | <0,05 | 100,9 | Not significant |
| | 1 h | 100,0 | Not significant | 85,5 | The same | 97,3 | Not significant | 93,8 | Not significant |
| | 7 days | 83,2 | The same | 108,4 | The same | 94,7 | The same | 98,2 | The same |
| Brain stem | Immediately | 66,1 | <0,05 | 82,8 | Not significant | 70,0 | <0,05 | 84,1 | <0,05 |
| | 1 h | 86,2 | <0,05 | 121,3 | The same | 85,8 | Not significant | 97,3 | Not significant |
| | 7 days | 79,3 | Not significant | 101,1 | " " | 92,0 | The same | 94,7 | The same |

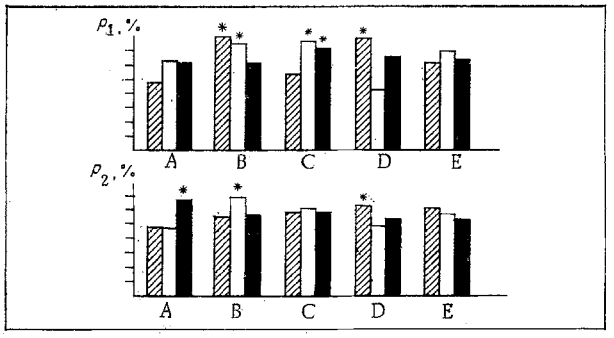


Fig. 1. Content of protein antigens $P_1$ (above) and $P_2$ (below) in some rat brain structures immediately (obliquely shaded columns), 24 h (unshaded columns), and 7 days (black columns) after DCAR formation. Ordinate, effect of combining conditioned and unconditioned stimuli on $P_1$ and $P_2$ levels (% of active control). A) Hippocampus; B) caudate nucleus; C) motor cortex; D) visual cortex; E) brain stem (region of gigantocellular nucleus of reticular formation). *P < 0.05 compared with active control.

the content of brain-specific and brain-nonspecific proteins differed. Immediately after learning a decrease in the $P_1$ concentration was observed in the hippocampus and motor cortex, but after uncombined presentation of the conditioned and unconditioned stimuli this decrease was observed in all regions of the rat brain tested except the hippocampus (Table 1). The $P_2$ level fell immediately after formation of the DCAR in the caudate nucleus and brain stem, but in animals of the active control, like the $P_1$ level, it fell in all CNS structures tested except the hippocampus.

As Table 1 shows, 24 h after conditioning, just as immediately after training, the $P_1$ content differed from that in rats of the passive control in the hippocampus and motor cortex, but the changes were in opposite directions: The $P_1$ level in the hippocampus was lower but in the motor cortex higher than in intact animals. In response to uncombined presentation of photic and electrodermal stimuli a fall in the $P_1$ level was observed in the hippocampus, caudate nucleus, and brain stem 24 h after conditioning, but not in the two areas of cortex tested.

The $P_1$ level 7 days after formation of the DCAR was raised only in the motor cortex, but the $P_2$ content was unchanged in all parts of the CNS tested. In animals of the active control the $P_1$ and $P_2$ levels were lowered in the hippocampus and caudate nucleus.

Data on the effect of conditioning on changes in the $P_1$ and $P_2$ content (as percentages of the active control) are given in Fig. 1. No significant difference in the $P_1$ content in the hippocampus could be observed throughout the period of investigation, but there was a tendency for the level of this protein to rise 1 and 7 days after DCAR formation, and the $P_2$ content rose significantly 7 days after training. In the caudate nucleus the $P_1$ content was increased immediately after conditioning, remained at this level 24 h after learning, and returned to normal 7 days after the end of the experiment. The $P_2$ content was increased 24 h after combined presentation of photic and electrodermal stimuli, but returned to its level in animals of the active control 7 days after training. Accumulation of $P_1$ in the motor cortex was observed 1 and 7 days after conditioning, but the $P_2$ content was unchanged. The $P_1$ and $P_2$ content in the visual cortex rose immediately after training, and 24 h after conditioning they reached values obtained in animals of the active control. In the brain stem no significant changes were found in the $P_1$ and $P_2$ levels, although 24 h after conditioning the $P_1$ content showed a tendency to rise.

The content of the nonspecific protein $P_1$ was increased immediately after DCAR formation in the caudate nucleus and visual cortex, 24 h after conditioning in the caudate nucleus and motor cortex, and 7 days after conditioning in the motor cortex only. The level of the brain-specific protein $P_2$, on the other hand, was higher than in animals of the active control immediately after training in the visual cortex, 24 h after training in the caudate nucleus, and 7 days after training in the hippocampus.

These results suggest that brain-specific and brain-nonspecific proteins participate at different stages of the process of DCAR formation, and their functions ($P_1$ and $P_2$) are different, although it may be that both proteins are concerned in processes of consolidation and storage of information.

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