Variations in the Content of Some Trace Elements and Macroelements in the Hippocampus of Rats during Learning and Memory Retrieval after Destruction of the Entorhinal Cortex

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We studied variations in the concentration of Na, K, Ca, and Mg in the hippocampus of rats with damaged entorhinal cortex. The concentration of Mg, Na, K, and Ca remained practically unchanged, while the content of Zn and Cu increased during learning and memory retrieval in animals with damaged entorhinal cortex. Our results indicate that Zn and Cu, constituents of metalloproteins and metalloenzymes, play an important role in hippocampal function.

Key Words: *hippocampus; entorhinal cortex; learning and memory; trace elements*

Close functional relationship between limbic structures forms the basis for not only emotional and motivational states, but also any conditioned response (*e.g.*, learning and memory). The stage of formation and storage of information is characterized by increased synthesis of specific macromolecules requiring primarily Ca and Mg ions, as well as Zn and Cu ions [2-5]. For evaluation of the role of the major afferent fibers in the regulation of metabolic activity of the hippocampus during learning and memory formation we used rats with damaged entorhinal cortex.

MATERIALS AND METHODS

Experiments were performed on adult outbred albino rats weighing 180-200 g. The animals were divided into 3 groups (6 rats per group). Intact animals (group 1) were trained in a maze to achieve the state of automatism (usually for 4 days); maze performance was tested after 1 week. Group 2 rats were trained to the state of automatism; the entorhinal cortex in these animals was destructed on day 5. They were placed in a maze on day 8 after surgery. Group 3 rats were trained for 5 days starting from day 8 after surgery.

The entorhinal cortex in hexenal-narcotized animals was bilaterally destructed using electrodes and a thermocautery device (2-mA anode current, 15-25 sec). Destruction was performed according to stereotaxic coordinates for the medial septal nucleus: AP=0.5; H=5.5; L=±0.2 (Fifkova and Marsala).

The rats were trained in a maze. The maze consisted of bridges elevated above the experimental area. The animals were placed near the entrance. The rats were helped to find the optimal path to the nest box during the 1st training session. In the follow-up period, the animals found this path by themselves. Three sessions were performed in each day. Learning capacity was evaluated by the number of errors and time of passing the maze.

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TABLE 1. Concentration of Na, K, Ca, and Mg in Rat Hippocampus after Destruction of the Entorhinal Cortex

Group	Na	K	Ca	Mg
1	766±3	2267.0±1.5	29.6±1.2	136.0±2.1
2	847.0±0.1	2387.0±1.1	41±1	141.0±1.2
3	830.0±1.8	2229.0±1.2	33.0±1.9	144.0±1.8

The concentration of metals (Na, K, Ca, and Mg) was measured by the atomic absorption method. The animals were decapitated on days 13 and 15. The hippocampus was removed, dried, weighted, and treated with HNO_3. The measurements were performed on a Bekam-495 double-beam atomic absorption spectrometer. Metal concentration was calculated as follows:

$$C=\frac{A_X}{A_{ST}}\times\frac{V}{M}\times C_{ST},$$

where C is measured concentration (μg/g dry tissue); C_{ST} is concentration of the standard (μg/ml); A_{ST} is signal of the standard (rel. units); A_X is signal of the studied sample (rel. units); V is total volume of the sample (ml); and m is dry weight of the sample (g).

The results were analyzed by Student's test and Fischer's test.

RESULTS

Intact animals were successfully trained, which was seen from decreased number of errors and shortened time of passing the maze. The state of automatism was achieved by the 4th day of learning: the time required to cross the maze and reach the nest box did not exceed 10 sec. Group 2 animals were characterized by high anxiety and could not find the optimum path to the nest box, which attested to impaired maze performance. In group 3 animals, severe disturbances in associative learning were revealed even after the 8th day of learning. No statistically significant increase in the concentration of Na and K was found (Table 1).

TABLE 2. Concentration of Zn and Cu in Rat Hippocampus after Destruction of the Entorhinal Cortex

Group	Zn	Cu
1	64.9±1.8	12.6±1.2
2	80.2±1.7	26.9±3.8
3	70.0±0.6	14.2±0.2

The concentration of Ca, Mg (Table 1), Zn, and Cu (Table 2) significantly increased in group 2 and 3 animals.

Published data show that the perforant pathway originating from the entorhinal cortex is glutamatergic. Afferent fibers from the entorhinal cortex extend to the molecular layer of the dentate fascia, while fibers from the dentate fascia terminate in neurons of the CA_3 field. Afferent fibers from the entorhinal cortex probably modulate activity of mossy fibers. The concentration of Zn is high in endings of these fibers. Damage to the entorhinal cortex probably shifts the balance between glutamatergic, monoaminergic, and GABAergic innervation, which can affect metabolic activity of various enzyme systems, including metal-containing enzymes. The decrease in learning capacity, consolidation, and memory retrieval supports this hypothesis [6-8]. Despite the development of severe behavioral disorders, the increase in Zn concentration can be associated with metabolic changes in Zn-containing proteins in not only mossy fibers, but also in other cells (*e.g.*, glial cells) characterized by high level of metal-containing enzymes. Enhanced metabolic activity of metalloproteins in hippocampal cells after damage to one of the major regions in the parahippocampal area is probably associated with compensatory reserves of glial cells relative to deafferented neurons in the dentate fascia. Despite the antagonistic effects of Ca, Zn, and Cu ions, their concentration underwent similar changes, probably because the concentration of these ions was measured by the atomic absorption method, which did not take into account variations in the ratio in cellular and subcellular structures.

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