International Scientific Journal
Journal of Medical and Biological Sciences
http://bioscience.scientific-journal.com

Morphological and Biochemical Changes in the Hippocampus After the Destruction of Dorsal Amigdalofugal Way

R.M.Bagirova
Azerbaijan State Academy of Physical Culture and Sports, Department "General and sports physiology"
Baku, Azerbaijan Republic
rafiga_bagirova1@mail.ru

Abstract — In chronic experiments on rabbits, we demonstrated that electrolytic destruction of the dorsal amigdalofugal way - *stria terminalis* results in intense depression of the integral electrical activity of the hippocampus. Characteristics of the responses induced by electrical and chemostimulation of the hippocampus under the above conditions allowed us to conclude that a significant drop in the excitability of hippocampal neurons themselves, but not changes in the structure and intensity of afferent impulsation from the extrahippocampal structures, underlies such a depression. Morphological changes in neurons and satellite glial cells, such as lysis of the Nissl substance, hyperchromatosis, and wrinkling of the cells after destruction of the *stria terminalis*, were proof of the correctness of our conclusion. To elucidate the reasons for these changes were produced biochemical research in the dorsal, ventral hippocampus and the medial nucleus of the septum before and after electrolytic destruction of the dorsal amigdalofugal way. It is postulated that one of the main factors underlying the generalized effects observed in our experiments is probably the disturbance of functional characteristics of the hypothalamo-hypophyseal neurosecretory system induced by destruction of the amygdalofugal connections; this disturbance results in significant pathological shifts of metabolism in the hippocampus.

Keywords – dorsal amigdalofugal way ,electrohippocampogram, electrical and chemostimulation, morphological and biochemical changes, Nissl substance, protein fraction.

I. Introduction

The phenomenon of disappearance of the theta rhythm after lesioning of the septum and fornix [7] has in its time stimulated a series of reports describing attempts to understand the roles of various structures in determining the electrical activity of the hippocampus and the functional significance of the theta rhythm [1,3,4,5]. From this point of view, results of studies demonstrating long-term depression of overall hippocampal activity in conditions of lesioning of the dorsal amigdalofugal way (stria terminalis) [2] are of particular interest. In these conditions, chemical and electrical stimulation of one of the main components regulating hippocampal activity – the medial septal area – did not restore the hippocampal EEG. The irreversibility of this effect (the longest observation time was six months) suggests that this phenomenon of long-term depression of the EEG is most probably associated with a decrease in the excitability of most hippocampal neurons to a critical level at which the probability of generating elementary postsynaptic potentials, which underlie the formation of EEG quanta, becomes minimal. The present report describes an experiment to provide experimental grounds for this hypothesis using electrical and chemical stimulation. In our study, we tried using electrical and chemical stimulations, as well as morphological and biochemical investigations, to obtain experimental confirmations of the above statements.

II. Methods

Experiments were carried out on 16 mature rabbits. Through a chemotrode inserted into the CA3 field, 1% strychnine solution, 5 to 50 µg serotonin, 15 to 20 µg noradrenaline, or 0.5 to 1.5 µg carbachol were applied (pressure injections to this region of the dorsal hippocampus; the volume of injections was 5-6 µl). For histological studies, brains of the animals were fixed in Carnoy solution and dehydrated using a series of alcohols of increasing concentrations; the blocks including the structures under study were embedded in paraffin. Frontal 10-µm-thick slices were prepared with a microtome, stained with 0.1% cresyl violet solution, treated with alcohol, cleared in xylene, embedded in balsam, and examined under a light microscope. The objects under study were neurons and glial cells of the hippocampus and septum. Disc electrophoresis of brain proteins was carried out in the apparatus firm «Reanal» (Hungary) in a vertical type apparatus in tubules of 6 mm diameter and 10 cm length by the method of Ornstein and Davis (Davis B.J., 1964) with the use of gels № 1:4%-tion concentrating (pH 6.7) and 7.5% separating (pH 8.9) PAAG. Electrophoresis was performed in Tris-glycine buffer pH 8.3 for 120 minutes at a current intensity 2 mA at the passage macroporous gel and then 3 mA per tube during passage through running gel. Into each tube 0.1 mm was applied gomogenezat brain. Protein spectrum was studied after staining gels amidovogo black 10 B and their destained in 7% acetic acid solution.

III. Results and discussion

The results of these experiments showed that the baseline

hippocampal and septal EEG demonstrated irregular activity dominated by oscillations in the range 4–6 Hz. Application of test substances to the dorsal hippocampus before lesioning of the stria terminalis led to ambiguous results. Thus, while serotonin increased the EEG in the region 5–6 Hz, noradrenaline displaced the peak of the frequency characteristic to the region 4–5 Hz. The effects of strychnine and carbachol were rather different. In this experimental situation, carbachol (like strychnine) resulted in generation of high-amplitude, regular θ waves of frequency 6–7.5 Hz at different time points, which with time could transform into epidischarges (Fig. 1). The EEG changes seen after carbachol and strychnine started in all leads simultaneously and were seen for prolonged periods of time (the maximum observation period was 3 h).

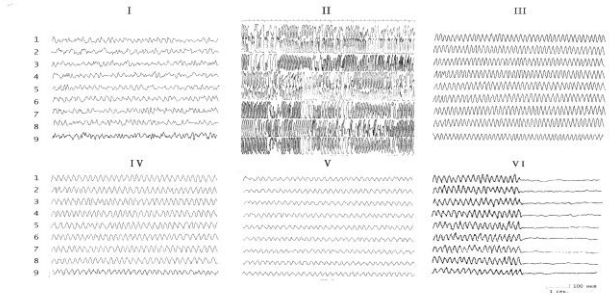

Fig. 1. The influence of electric and chemical stimulation field CA3 dorsal hippocampus on the electrical activity of the hippocampus. I-background; II-instant electrical stimulation; III-after application of carbachol; IV- after the application of serotonin;V-after application of noradrenaline; VI-application of carbachol on the background of the destruction of the dorsal amigdalofugal way. 1,2)Field CA1;3, 4) field CA3 of the ipsi- and contralateral hemispheres; 5,6) ventral hippocampus of the ipsi- and contralateral hemispheres; 7,8) dentate gyrus of the ipsi- and contralateral hemispheres; 9) medial nucleus of the septum.

Destruction of dorsal amyqdalofuqal tract in contrast to ventral one has been found lead to full and irreversible blockade of hippocampal theta-rhythm (Fig. 2). Administration of biogenic monoamines into the hippocampus after lesioning of the stria terminalis did not induce any changes at all.

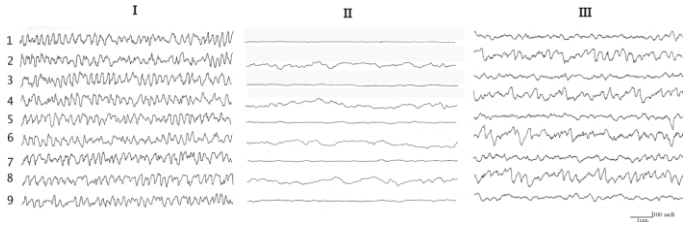

Fig. 2. Changes in hippocampal electrical activity in rabbits under conditions of destruction of the dorsal and ventral amigdalofugal ways . I-background; II- after destruction of the dorsal amigdalofugal way; III-after destruction of the ventral amigdalofugal way. The rest of the notation is the same as in Figure 1.

Recovery of the electrical activity of the hippocampus due to electrical stimulation of various extrahippocampal structures (mRF, hypothalamus, amygdala) did not occur, while stimulation of the hippocampus itself produced only epidischarges when the maximum stimulationcurrent was used. The effects of application of carbachol and strychnine were rather different. In this situation, there was a tendency to recovery of the overall activity of the hippocampus and septum, with some features consisting of short-lived (20–30 sec) periodically repeated generation of regular rhythmic activity in the range 6–7.5 Hz. Attention is drawn to the fact that, on the one hand, recovery of electrical activity in the hippocampus and septum occurred spontaneously in all leads, while on the other, there was marked synchronicity in the generation of electrical activity. The effects of carbachol and strychnine were largely similar and were long-lasting.

Examination of the slices of the hippocampus and septum of experimental animals after coagulation of the *stria terminalis* demonstrated that profound morphological changes were detected in both neurons and glial cells of these regions (Fig. 3).

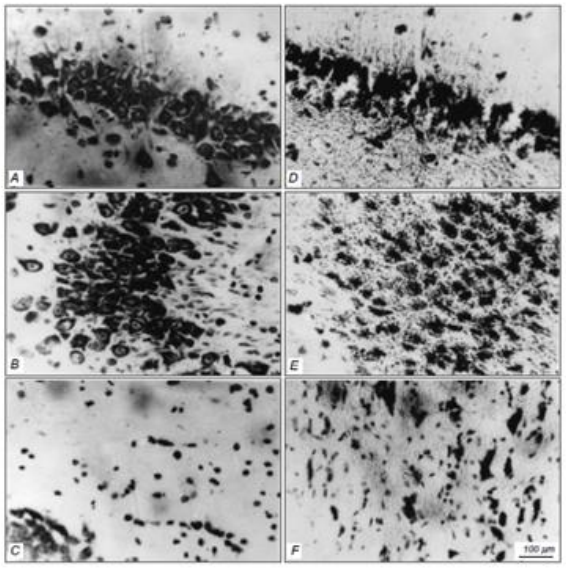

Fig. 3. State of the Nissl substance in neurons and glia of the hippocampus and medial septal nucleus before and after destruction of the dorsal amygdalofugal pathway. A-F) CA1 and CA 3 fields and the medial nucleus of the septum before (A-C) and after destruction (D-F).

In particular, sections of the neuronal somata appear clear, i.e., lysis of the chromophilic matter (Nissl substance) was observed. A clearly pronounced edema of the apical dendrites was observed. In some neurons, hyperchromatosis of the nucleus and decrease in the volume of the latter were typical findings. In this case, basket-like cells within the CA1 and CA3 fields, as compared with the pyramidal neurons, underwent less pronounced morphological modifications, whereas the intensity of staining of the tigroid matter was preserved to a significant extent. In the medial septum, clearly pronounced hydropic changes were also observed; they were especially expressed in

the multipolar neurons. The cross sections of many cells looked like transparent contours, where the tigroid almost disappeared. Cellular borders of the individual neurons were smeared, and the nuclei were distorted and compressed. In the cytoplasm, numerous vacuoles and manifestations of edema of the somata, nuclei, and cell processes were observed. In glial cells, clearness and swelling of the somata was observed. In thecytoplasm of some glial cells, light vacuoles were seen. In the karyoplasm of glial cells, light zones were observed. We also observed clearness of the cytoplasm around the nuclei due to redistribution of the tigroid substance and its displacement toward the periphery.

Studies have shown that up to destruction of dorsal amigdalofugal way in the dorsal hippocampus allocated 8 fractions, 7 fractions in the ventral and medial nucleus of the septum - 9 fractions. Revealed that before the destruction of dorsal amigdalofugal way in the dorsal hippocampus albumins constitute 8.58%, in the ventral hippocampal region - 6.91%, and in the medial nucleus of the septum albumins constitute 32.6%.. Prealbumin in the dorsal hippocampus up - 1.42%, ventral his department - 5.06%, and in the medial nucleus of the septum - 3.52%. Later fractions neyroglobulins. It was found that in the normal state neyroglobuliny are: in the dorsal hippocampus α- neyroglobulins – 38.1%, β- neyroglobulins - 37.34%, γ-neyroglobulins up 14.6%, in the ventral hippocampus α - neyroglobulins – 24.28%, β-neyroglobuliny – 34,95%, γ-neyroglobuliny – 28.97%:, and in the media in the medial nucleus of the septum α -neyroglobuliny - 19.78%, β-neyroglobuliny - 39,4% up - γ-neyroglobuliny 4,8% of total proteins. Biochemical studies have shown that the destruction of dorsal amigdalofugal way leads to irreversible changes in the protein spectra of dorsal, ventral hippocampus and the medial nucleus of the septum: in none of the investigated areas were not received any protein fraction produced after the destruction of dorsal amigdalofugal way (Fig. 4).

The possibility for modulation of the electrical activity of the hippocampus, and especially of the EHipG theta rhythm, because of changes in the functional state of extrahippocampal structures of the brain (including the hypothalamus and amygdala) was demonstrated earlier [5-7]. A basic aspect of the data we obtained in our experiments is that we demonstrated deep and longlasting depressive modulation of the mass electrical activity of the hippocampus under conditions of coagulation of the *stria terminalis*. Our data allow us to believe that the above depression is related to changes in the functional state of hippocampal neurons themselves, but not to *stria terminalis* destruction determined modification of the pattern and intensity of afferent impulsation coming from extrahippocampal structures. The characteristic effects of electrical stimulation applied to the septum of experimental animals are indicative of a significant decrease in the excitability of the hippocampal neurons. The effects of chemostimulation of this structure also prove the existence of the above phenomenon. Histological and biochemical observations, according to which deep changes occur in the structure of hippocampal neurons and satellite glia after coagulation of the *stria terminalis*, also confirm the conclusion

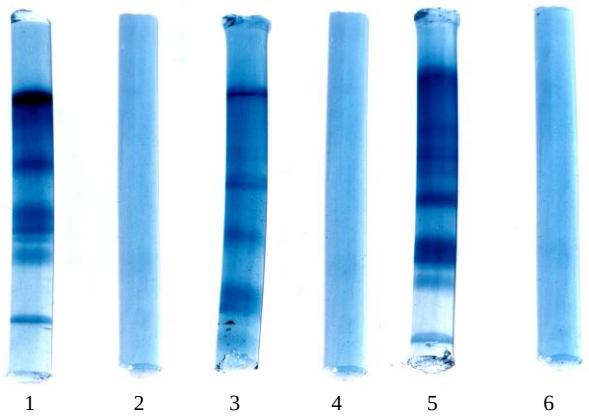

Fig.4. Electrophoregram of dorsal, ventral hippocampus and the medial nucleus of the septum before and after the destruction of the DAW. 1,3,5 - dorsal, ventral hippocampus and medial nucleus of the dorsal amigdalofugal way.

of decreased excitability of the neurons in the septo-hippocampal system.

Recovery of electrical activity in the form of regular oscillations only in response to factors inducing stable neuron membrane depolarization may be evidence primarily of profound metabolic derangements occurring simultaneously in all hippocampal fields and indicating the diffuse, neurohumoral nature of changes resulting from lesioning of the amygdalofugal connections. It can be suggested that the long-term depression of the hippocampal EEG observed here results from disturbance to the normal functioning of the hypothalamo-hypophyseal neurosecretory system due to lesioning of the stria terminalis, which ultimately leads to long-lasting changes in the excitability of hippocampal neurons. However, whatever the mechanism of EEG depression in this experimental situation, it is clear that the integrity of the dorsal amygdalofugal pathway is one of the key factors in regulating neuron excitability in the septohippocampal system.

References

[1]. E. S. Brazhnik, O. S. Vinogradova, and A. M. Karanov, “Regulation of theta activity of neurons in the septum by cortical and stem structures,” *Zh. Vyssh. Nerv. Deyat.*, 34, No. 1, 71–78 (1984).

[2]. G. G. Gasanov, A. É. Kasimov, and R. M. Bagirova, “Contributions of the amygdala and hypothalamus to the formation of hippocampal theta activity,” *Biol. Nauki.*, 3, 51–53 (1989).

[3]. V. F. Kichigina, T. A. Kudina, K. I. Zenchenko, and O. S. Vinogradova, “Background activity of rabbit hippocampal neurons after exclusion of structures regulating the theta rhythm,” *Zh. Vyssh. Nerv. Deyat.*, 48, No. 3, 505–509 (1998).

[4]. V. M. Shaban, “Electrophysiology of the hippocampus,” *Usp. Fiziol. Nauk.*, 7, No. 3, 57–68 (1976).

[5]. H. Anchel and D. B. Lindsley, “Differentiation of two reticulo-hypothalamic systems regulating hippocampal activity,” *EEG Clin. Neurophysiol.*, 32, No. 3, 209–213 (1972).

[6]. B. Fujimori, T. Yokota, Y. Ishibashi, and T. Takei, “Analysis of the electro- encephalogram by a histogram method,” *EEG. Clin. Neurophysiol.*, **10**, No. 2, 241–252 (1958).

[7]. J. D. Green and A. A. Arduini, “Hippocampal electrical activity in arousal,” *J. Neurophysiol.*, 17, No. 6, 533–535 (1954).