Characterization of Formation and Trace Rhythm Reproduction by Rabbit Hippocampal Neurons during Early and Late Ontogeny

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The formation and reproduction of memory traces by hippocampal neurons were studied and a relationship between the number of presented series of periodical electrocutaneous stimulation and degree of trace acquisition of the rhythm, on the one hand, and rabbit age (6-30 days, 1, 4-5, and 7 years), on the other, was detected. The hippocampus of 6-7-day and 7-year-old rabbits is characterized by low neuron activity and inability to trace acquisition of rhythm. The pulse frequency and trace acquisition of the rhythm in animals aged 8-14 days and 4-5 years (middle age) formed slower than in adult animals (after 2-4 stimulation series on days 2-4 of experiment) and could not be reproduced on the next day without reminding. In rabbits aged 25-30 days and 1 year the basal activity reached the optimum level and trace acquisition of rhythm was observed after 1-2 series on days 1-2 of experiment and was reproduced without reminding on the next day. The detected physiological stages are in good correlation with the morphochemical organization of the rabbit hippocampus at the stages of early and late ontogeny.

Key Words: *hippocampus; pulse activity; training; ontogeny; aging*

The hippocampus is essential memory functions as one of the components in the organization of adaptive reactions [2-6]. The memory function undergoes several stages of development in the ontogeny and fades durig aging [3,5,9,11-13]. However, some aspects in the neuron function and mechanisms of memory trace formation at different age remain little studied. Study of the processes of rhythm acquisition by neurons after cessation of periodical stimulation (analog of conditioned reactions to time) is a physiologically adequate model for understanding of cell mechanisms of trace processes, representing the time organization of memory; due to this model, the efficiency of training process can be evaluated [3]. Study of the neuronal pulse reactions of the hippocampus in the course trace acquisition of the rhythm in animals of different age will detect not only age-specific involvement of neurons in adaptive behavior, but also the components associated with the formation of memory and adaptive function of the brain.

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MATERIALS AND METHODS

Experiments were carried out on awake (without anesthesia) partially restricted rabbits aged 6-30 days, 1, 4-5, and 7 years. Pulsed activity of hippocampal neurons was recorded extracellularly via glass microelectrodes. An analog of conditioned time reflex (trace acquisition of rhythm, TAR) was developed by periodical 10-20-min stimulation (1-2 Hz) of the forepaw. The animal was presented 1-4 series of stimulation (as a rule, of the same frequency) during 1 day. Pulsed activity of neurons was recorded during 1 h before and after periodical

stimulation on day 1 of the experiment and during the next 3-4 days in order to detect the length of memory trace retention. Experimental protocols and number of rhythmic stimulation series were the same for rabbits of all age groups [2,4].

The characteristics of pulsed activity were evaluated by spectral analysis of autocorrelation function [14]. The neuron was considered to develop TAR, if the peak of the respective frequency on the spectral density histogram (SDH) after rhythmic stimulation surpassed the level of white noise with 5% confidence.

Rabbit brain was fixed after the experiment and the position of the microelectrode tip was histologically verified on serial sections.

RESULTS

The hippocampus of 6-7-day- and 7-year-old rabbits contained an appreciable number of "silent" neurons. About 80-90% basal active cells exhibited low spike activity (0.4-5.0 pulse/sec; Figs. 1; 2, *b*). Presentation of a series of rhythmic stimulation virtually did not increase firing frequency; no acquisition of the time parameters of the stimuli was observed in any of the 2-3 days of experiments (Fig. 2, *b*).

The number of cells exhibiting basal activity increased in the hippocampus of 8-14-day- and 4-5-year-old rabbits; more than 60% of these neurons were discharged at a frequency of 2-10 pulse/sec (Fig. 1). Stimulation led to a nonspecific increase in firing activity, the number of these cells increasing from 60 to 78%; neurons with a frequency of 11-30 pulse/sec were detected. The formation and reproduction of rhythmic trace activity were observed mainly on days 2-3 of the experiment after 2-4 series of rhythmic stimulation of the limb, and were observed in 17% of hippocampal neurons of young rabbits and in 19% neurons of 5-year-old rabbits (Fig. 2, *c*, *e*). However, the attained TAR was not reproduced on the next day without repeated "reminding" stimulation.

The spike activity increased in the hippocampus of 15-25-day- and 1-year-old (adult) rabbits. More than 60% neurons discharged at a frequency of 11-30 pulse/sec (Fig. 1; 2, *a*). The number of low-active neurons decreased to 10%. The capacity of neurons to TAR formation increased (Fig. 2, *d*, *f*), which was noted for 16% neurons after 1-2 sessions of periodical stimulation on day 1 and was retained on the next day before stimulation in 12% cases. Repeated rhythmic stimulation on day 2 of experiment led to the formation of TAR in 21% hippocampal neurons in little rabbits. By the age of

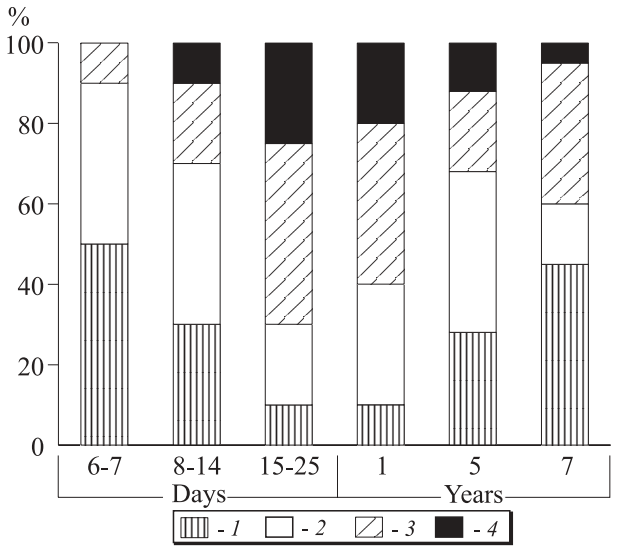

Fig. 1. Distribution of the hippocampal neuron basal activity frequencies in rabbits aged 6-7, 8-14, 15-25 days and 1, 5, and 7 years. Bars: ordinate: percentage of cells with respective counts per sec. *1*) 0.4-1.0 pulse/sec; *2*) 2-5 pulse/sec; *3*) 6-10 pulse/sec; *4*) 11-30 pulse/sec.

30 days pulsed activity of neurons was characterized by high values, the trace activity acquired the features characteristic of the adult rabbit hippocampal neurons (rapid acquisition and reproduction of traces, consolidating after repeated stimulation and reproduced on the next day).

If we consider basal activity as an expression of a certain level of neuron function, these physiological results clearly correlate with the age-associated stages in the morphochemical organization of animal brain at the stages of early and late ontogeny. Increase in the number of basal active cells and level of their spontaneous activity observed in little rabbits was due to the development of afferentation and metabolic processes, improvement of transmitter support, *etc.*, which promoted the development of adaptive behavior in a growing animal [1,2,5,7,8].

Delayed development and lower capacity to reproduction of the time bond analog in middle-aged animals (4-5 years) were paralleled by destructive processes involving the nerve and glia elements. Protein synthesis processes in the hippocampal neurons are decelerated with age, which is seen from decreased nuclear area and protein content and concentration in it [3]. The fact that repeated rhythmic stimulation created conditions under which aging animals remembered and reproduced the rhythm preset by stimulation, although slower than young adult rabbits, is in good agreement with morphological data on changes performing the compensatory function at this age [4].

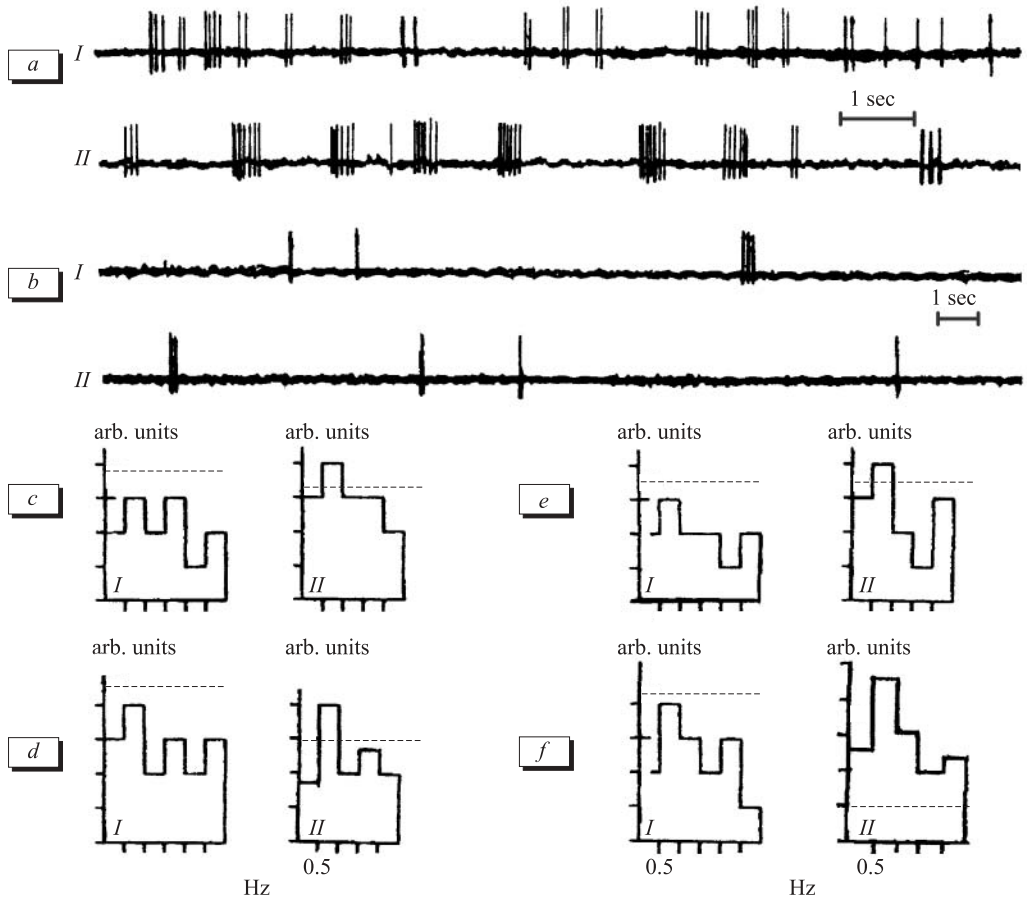

Fig. 2. Changes in the rabbit hippocampal neuron pulsation in different age groups and under the effect of rhythmic stimulation. Neuronogram of the hippocampus of rabbits aged 1 year (*a*), 6 days (*b*, upper line), and 7 years (*b*, lower line) before (*I*) and after (*II*) a series of stimulation at 1 Hz frequency. Hippocampal neuron SDH in rabbits aged 14 (*c*), 25 days (*d*), 5 (*e*) and 1 year (*f*) before (*I*) and after (*II*) stimulation at 1 Hz frequency. SDH: ordinate: spectral density (arb. units); abscissa: number of Hz. Dotted line: confidence level ($p<0.05$).

Fading of plastic characteristics of the hippocampal neurons, errors in evaluation of the time intervals, detected at later stages of ontogeny (very old rabbits, 7 years) seems to indicate the initial stages of the development of disorders in mnestic processes. A drastic deterioration of training capacity is paralleled by the development of destructive processes in the hippocampus (death of neurons and neuronal connections) and involves structural (size of nuclei and cytoplasm), regulatory (protein concentration in the nucleus and cytoplasm), and functional (protein content in nucleus and cytoplasm) levels of hippocampal neurons [3,4,9-13]. Studies of the neuronal mechanisms of adaptive reactions in different age groups showed the functional similarity of deviations from the optimum (reached in mature age) in young and late ontogeny. On the other hand, the time periods, corresponding to stages of this process, are different: the development of a young organism is more rapid than degradation of mnestic functions during aging. This indicates the specific changes during development and loss of functions. Intense formation of numerous neuronal links are observed during the early ontogeny, while during the late ontogeny the priority process is presumably fading of functions of nonspecific systems of the brain, providing more or less normal functioning of already formed contacts.

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