PHARMACOLOGY AND TOXICOLOGY

Effect of Amiridine on Conditioned Reflex in Rats with Damaged Hippocampus

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 2, pp. 173-175, February, 1997
Original article submitted September 20, 1995

It is shown that even after partial damage to the hippocampus in male rats by quinolinic acid, which causes selective degeneration of hippocampal neurons, their conditioned reflex to time remains impaired for a period of several months. Treatment with amiridine improves the learning of time-interval estimation by such rats and promotes compensatory/restorative processes in the brain. The brain contains a great diversity of connections, and their stimulation may offer an opportunity for enhancing the operation of compensatory/restorative mechanisms in it.

Key Words: *amiridine; quinolinic acid; conditioned reflex to time; spatiotemporal differentiations; hippocampal damage*

In this study the effect of the drug amiridine on learning was assessed in rats with damaged hippocampus in order to gain a better understanding of the compensatory/restorative processes occurring in the brain after injury. Amiridine was developed at the Research Center for Safety of Biologically Active Substances and used in the treatment of dementias of various origins. This drug exerts an antiamnestic effect in animals with memory disorders, which is accompanied by normalization of lipid fluidity in brain synaptosomes [2,3,6].

MATERIALS AND METHODS

The study was carried out for 12-15 months on 90 male Wistar rats using the alimentary motor methodology. All rats were kept at 21-22°C on a 12-h lighting schedule in cages with an area of 2145 cm^2, 10 animals per cage. They had free access to food and water.

The rats were divided into two groups. Rats of group 1 learned alimentary conditioned reflexes before the hippocampus damage with quinolinic acid (QA), an excitatory amino acid, while group 2 rats learned these reflexes after hippocampal damage with QA.

The hippocampus was damaged as follows: QA was dissolved in Hanks' balanced salt solution (pH adjusted to 7.2 with 1 N NaOH [5]), after which 60 nmol of QA was injected in a volume of 1 μl with Hamilton's microsyringe both directly into the hippocampus and into the lateral ventricles. In both groups, amiridine was administered *per os* in a daily dose of 0.1 mg/kg for 3 months.

For the learning of conditioned reflexes, rats were placed in a chamber with a platform, where they received reinforcement from a window in the chamber wall upon delivery of a conditioned stimulus (signal). The conditioned stimulus was a tone lasting 5-10 sec depending on the animal's response; the interval between the unconditioned and conditioned

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0007-4888/97/0002-0147$18.00

stimuli was 60 sec, and up to 15 their combinations were presented per test. After the conditioned response to the stimulus was established, 1, 2, 3 or more signals were omitted sequentially. Gradually the rats started working in a "pure" time mode (without the presentation of stimuli, i.e., they had elaborated a conditioned reflex to time). This response was considered to be correct if there was no more than one run by the rat to take food during a 60-sec intersignal interval; the permissible error in the responses to time was ±10 sec.

Mathematical processing of the results obtained during the elaboration of conditioned reflexes to tone and time involved calculation of the percentages of correct responses and the relative accuracy of time-interval estimation by the animals (m_N), which indicated the degree of shift in intersignal responses by the end of the interval; the following formula was used:

$$m_N=\frac{\sum_{i=1}^{n_N}\Delta t_i/n_N}{t}\quad(i=1, 2, 3, \ldots n),$$

where n_N is the total number of responses in the intersignal interval of duration t after the Nth combination and Δt_i is the interval between the stimulus presentation and the occurrence of intersignal response. The higher the m_N, the greater the shift of intersignal responses by the end of the time interval.

For the elaboration of spatiotemporal differentiation, rat was placed in the starting compartment of a T maze and received food in its right arm upon presentation of a tone lasting 10 sec (T_{10}) and in its left arm upon presentation of a tone lasting 3 sec (T_3). These signals were alternated in a random manner. In processing the results (separately for each tone and the overall result for both tones), correct, incorrect, and zero (refusal to make decision) responses were considered, as were responses with an immediate correction of errors.

RESULTS

Intact rats were able to develop a conditioned reflex to tone in 1 or 2 tests (10-20 stimulus combinations). The number of correct responses reached 90-100% and remained at this level throughout the observation period. The conditioned reflex to tone was considered as established if the proportion of correct responses after 20 stimulus presentations was at least 80%. At the initial stage of learning the conditioned reflex to tone, numerous intersignal responses were observed throughout the intersignal interval (m_N=0); by the 40th-60th (or sometimes by the 80th) presentation, intersignal responses shifted to the 40th sec, i.e., the m_N increased to 0.7 or a higher value. By the 100th presentation, intersignal responses were rarely observed. Starting from the first few stimulus omissions, the proportion of correct responses ranged from 70% to 100%. The acquired conditioned reflex to time was characterized by a high degree of stability (95% of correct responses on average). In the tests used to develop spatiotemporal differentiations, the average proportion of correct responses was 73% (these differentiations were considered to have been learned if the proportion of correct responses was >50%).

Rats with bilaterally damaged hippocampus rapidly developed conditioned reflex to the presented stimulus (after 10-30 combinations), and once the

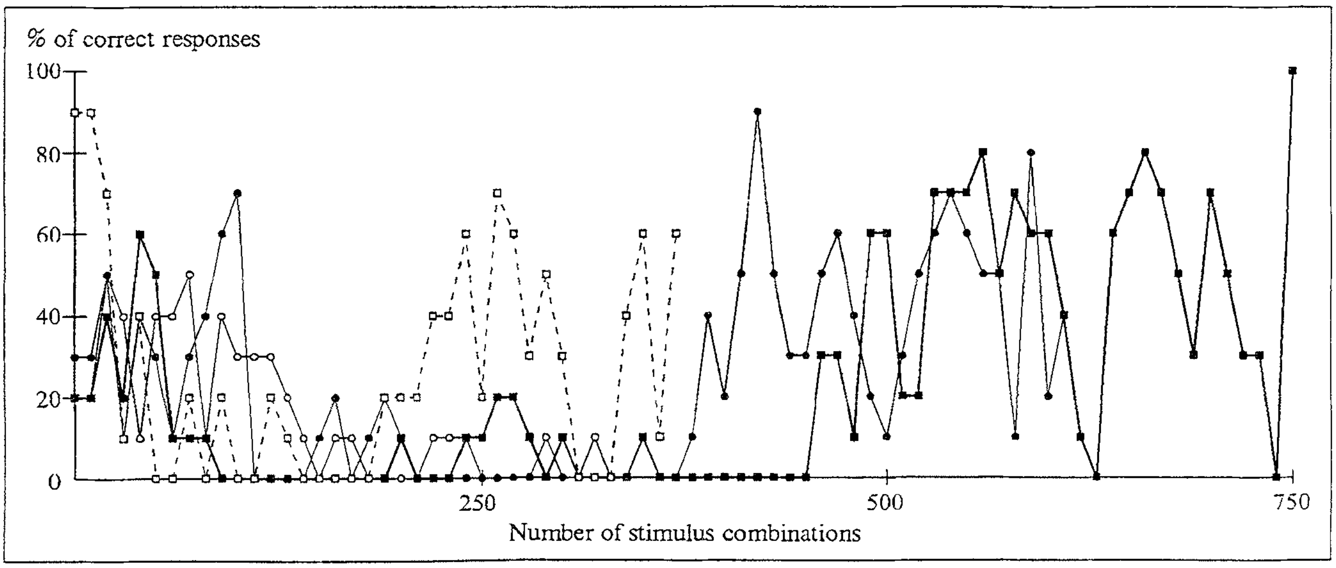

Fig. 1. Learning of the conditioned reflex to time by four rats with damaged hippocampus.

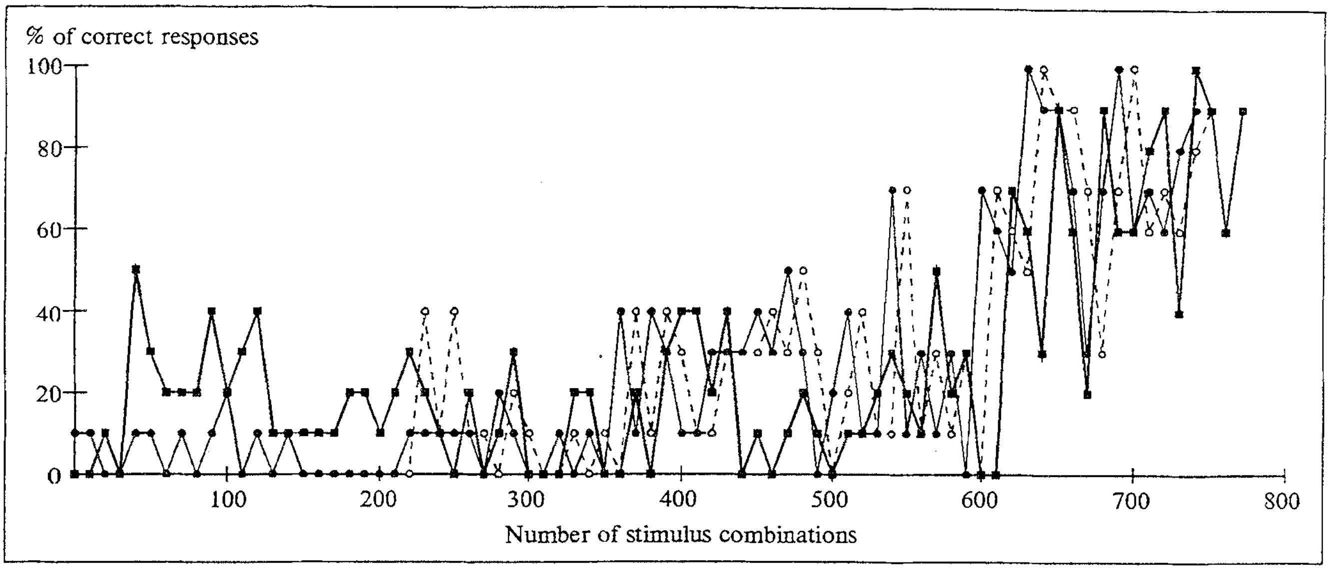

Fig. 2. Learning of the conditioned reflex to time by three operated rats treated with amiridine.

stimulus (after 10-30 combinations), and once the reflex had been learned the proportion of correct responses remained high (95% on average). Rats with damaged hippocampus also coped with the difficult spatiotemporal differentiation at the basis of which lie not only trace reflexes but also relations between time intervals. The average proportions of their correct, incorrect, and zero responses were 65%, 11%, and 24%, respectively.

On the other hand, partial bilateral damage to the hippocampus considerably delayed and worsened the time-interval learning. Rats with hippocampal damage learned the conditioned reflex 5-6 times more slowly than intact rats. The slow acquisition of the reflex and the low proportion of correct responses (20-30%) during the first 350-450 presentations could be explained by the large number of intersignal responses. We have identified three periods in all operated rats (Fig. 1), the first period encompassing the range from 0 to 100-130 combinations (the average proportion of correct responses was 37%), the second, the range from 130 to 550 combinations (2.75%), and the third, the range from 550 to 1200 combinations (50%). The average proportion of correct response for the three periods was 30%.

Amiridine-treated rats learned the conditioned reflex to time slowly (Fig. 2) but, unlike the control rats with damaged hippocampus, they did not show a prolonged second period during which the mechanism of time-interval estimation was disrupted almost completely. The learning process was continuous, and the average proportion of correct responses calculated for the entire observation period was 31.3%. The process of learning the conditioned response was characterized by undulation with some worsening of the learning between combinations 210—360 and 500—590, indicating that the rats were acquiring the reflex with difficulty. The proportion of correct responses was 21% (600 combinations) before the conditioned reflex was established and 72.6% (150 combinations) after its establishment.

A control morphological study showed that QA caused selective damage to hippocampal cells, predominantly in areas Ca1, CA3, and CA4; middle portions of the dentate fascia were also partly damaged in some rats. The ventral hippocampus was less damaged in most rats than the dorsal.

The question of whether, when, and to what extent impaired bodily functions can be restored is closely linked to that of their location and organization. The application of appropriate investigative techniques enables the preferential involvement of particular brain structures in mediating various bodily functions to be demonstrated. When such structures are damaged, mechanisms responsible for the localization and organization of brain functions remain impaired for prolonged periods (sometimes without subsequent restitution). A parallelism between the volume of destruction and the volume and rate of recovery is not always observed in such cases. We showed earlier that the hippocampus and associated entities constitute the structural basis for time-interval estimation (complete electrolytic destruction of the hippocampus eliminated the conditioned reflex to time [4,5]).

In the present study the conditioned reflex to time remained impaired for several months even after partial damage to hippocampal neurons by QA. Amiridine exerted a well-defined beneficial effect on the learning of time-interval estimation, as evidenced by the absence of a second period in time-interval estimation (in control rats this period was observed between presentations 100 and 390) and by a higher proportion of correct responses (72.6%) than in control rats (50%) during the learning of the conditioned reflex to time.

Amiridine promoted compensatory/restorative processes, apparently by stimulating undamaged areas of the hippocampus predominantly in its ventral part and by involving in these processes those brain structures which are directly associated with the hippocampus.

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