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The Action of Mexidol on the State of Conditioned Reflex Activity after Traumatic Brain Lesions

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It is demonstrated that even partial damage to the hippocampus is accompanied by impairments to counting time intervals lasting several months (1200–1500 presentations) without alteration of other complex conditioned reflex responses. After treatment with mexidol, which has a wide spectrum of actions, particularly antioxidant, antihypoxic, and antistress, rats showed a normal process of acquisition of a conditioned reflex to time. Unlike animals of the control group, there was no prolonged period during which there was complete impairment of the mechanism for counting time intervals. Thanks to significant improvements in autonomic processes and emotional-motivational responses, experiments could use a large number of conditioned stimuli. For example, after brain trauma mexidol strengthened compensatory-restorative processes: animals showed accelerated recovery of impaired functions, with decreases in the rate of retrograde degeneration of brain areas directly connected to the damaged parts, phenomena such as Monakow diaschisis were not observed, and so on.

One of the most important questions in any cerebral pathology is the possibility of increasing the brain's reserve capacities. A variety of methods are used for this purpose: 1) prolonged training (mental and physical), which allows non-functioning nerve cells and conducting pathways to be "triggered;" 2) pharmacological correction of changes, particularly with antioxidants, which have recently found ever wider use. Our long-term complex studies have addressed the effects of mexidol on the process of aging and a variety of stress states [14]. These experiments demonstrated that mexidol can also be used for the prophylaxis of structural-functional changes and for increasing the reserve capacities of the brain at the integral, organ, cellular, and subcellular levels.

Recent years have seen significant increases in the incidence of craniocerebral trauma. Contemporary medicine has few effective substances for treating these conditions, particularly in the acute and rehabilitation periods. Mexidol, which was synthesized by Smirnov and Dyumaev [19], has a wide spectrum of actions, particularly antioxidant and antihypoxic. It inhibits the processes of lipid peroxidation, induces a complex of membrane-stabilizing effects, alters the permeability and phospholipid composition of biomembranes, etc. At the same time, data have been obtained showed that important components of the pathogenesis of brain trauma include activation of lipid peroxidation, disruption of neuron biomembranes, derangements in the systems regulating free-radical processes leading to significant levels of free radical release, as well as acute impairment of cerebral circulation, hypoxia, etc. [1, 2, 5–9, 15–18, 20].

The aim of the present work was to seek possible enhancements in compensatory-restorative processes in the body after traumatic brain lesions. Aims were: 1) to study the effects of hippocampal damage on the acquisition, dynamics, and state of conditioned reflex activity over long periods of time; 2) to study the effects of mexidol on the state of higher nervous activity after hippocampal lesions.

METHODS

Experiments were performed using Wistar rats using a food-related motor method. Conditioned reflexes (CR)

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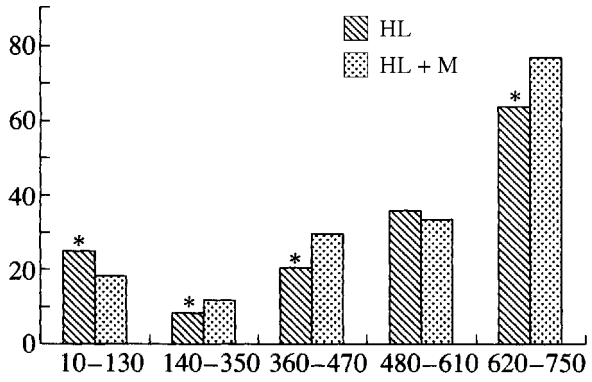

Fig. 1. Stages in the acquisition of the CR to time in two groups of rats: animals with hippocampal lesions and treated with mexidol (HL + M) and untreated lesioned animals (HL). The horizontal axis shows the number of CR presentations in five stages; the vertical axis shows the mean number of correct responses, %. *$p < 0.05$.

were developed in all animals to actual and trace stimuli: CR to time (counting of time intervals) and temporospatial differentiation, where, apart from orientation in space, the rats also had to count the duration of the conditioned signal. As shown by our previous data, CR to time and temporospatial differentiation are associated with different brain structures [11, 12].

Rats were placed in a chamber, which contained a reinforcement platform. On presentation of the conditioned stimulus, rats climbed onto the platform and received food from an aperture in the chamber wall. A single actual stimulus (a tone) was used, with a strict temporal stereotype: the duration of the conditioned signal was 5–10 sec in different animals, and the interval between stimuli was 60 sec; each experiment included 10–15 combinations. After fixation of the CR to the actual stimulus (the tone), sequential omission of one, two, three, and more signals was started. Rats gradually started to operate in the "pure" time regime, i.e., without use of actual stimuli in the experiment – they acquired a CR to time. This CR was regarded as correct when there was no more than one intersignal excursion over a period of 60 sec; the error tolerance for responses to time was ±10 sec.

During acquisition of temporospatial differentiation, rats were placed in the start sector of a T- or Y-shaped maze. On presentation of a tone for 10 sec (T_{10}), animals received food in the right-hand sector, while presentation of a tone lasting 3 sec (T_3) was associated with food in the left-hand sector. Signals were presented in random order. Processing of experimental data included determination of the numbers of correct and incorrect responses (separately for each stimulus and combined for both stimuli) , along with null reactions (refusals to decide), and responses with instant correction of errors.

Experiments were conducted over 12–15 months; experimental animals were divided into three groups. Rats of group 1 were used for investigations of the acquisition and state of the CR over prolonged periods of time without surgical intervention ($n = 30$), animals of group 2 were used for assessment of conditioned reflex activity after lesioning of the hippocampus ($n = 13$). The state of the CR in group 3 rats was evaluated after lesioning of the hippocampus on a background of mexidol treatment ($n = 11$). Rats of this group received daily doses of water-soluble mexidol (100 mg/kg) during drinking, presented as three one-month courses with 30-day intervals between courses. Animals of the two latter groups were trained to the CR to the tone, used as a strict temporal stereotype, before surgery.

Hippocampal lesions were produced by I. V. Viktorov using an excitatory amino acid, i.e., quinolinic acid, injected into the hippocampus with a Hamilton microsyringe [13]. The authors would like to express their thanks for this assistance.

Lesions were assessed morphologically by embedding rat brains in paraffin. Frontal sections were stained by the Nissl method.

The dynamics of the state of the CR to time was analyzed statistically in the first two groups of rats using the *t* test for independent signs.

RESULTS

In intact rats, the CR to the tone developed over 10–30 combinations; the number of correct response reached 90–100% and in most animals remained at this level throughout the study period. The CR to the tone was regarded as fixed when the proportion of correct responses was at least 80% during 20 presentations.

Mathematical analysis of the results obtained from studies of the acquisition of the CR to the tone and the CR to time was based not only on the proportion of correct responses, but also the relative accuracy of counting the time interval (m_N), which is a measure of the displacement of intersignal responses to the end of the interval. The parameter was calculated as [3]

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

where n_N is the total number of responses during the intersignal period of duration t after the Nth combination, Δt_i is the size of the interval between the start of the stimulus and the appearance of intersignal reactions at the end of this time interval.

At the beginning of CR acquisition, there were many intersignal reactions distributed throughout the whole of the interstimulus interval; m_N was 0.40–0.60. By tone presentation 40–50 and sometimes by presentation 60–80, intersig-

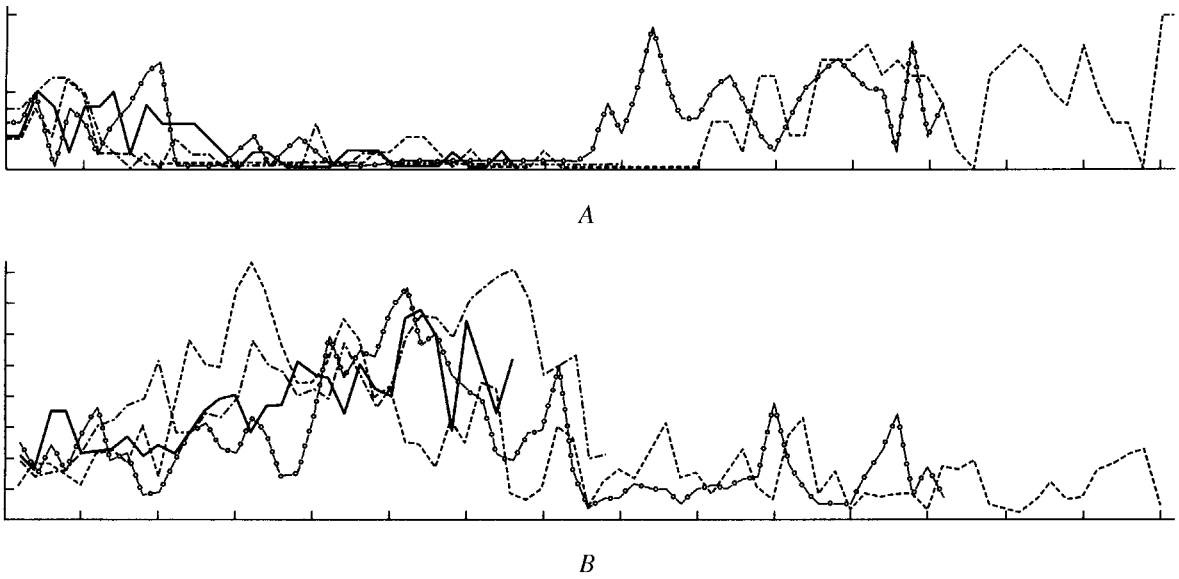

Fig. 2. The dynamics of acquisition of the CR to time in four rats with hippocampal lesions. *A*) The abscissa shows the number of CR presentations; the ordinate shows the number of correct responses, %; *B*) the abscissa shows the number of CR presentations; the ordinate shows the number of intersignal excursions, %.

TABLE 1. Stages in the Acquisition of the Conditioned Reflex to Time in Rats with Hippocampal Lesions without Treatment with Mexidol (HL) and with Mexidol Treatment (HL + M)

Number of presentations	Group of rats	Mean number of correct responses, %	Standard error	
10–130	HL	24.69*	2.81	$p < 0.042$
	HL + M	17.62	1.73	
140–350	HL	7.73*	0.83	$p < 0.002$
	HL + M	11.5	0.81	
360–470	HL	20.08*	2.79	$p < 0.012$
	HL + M	29.66	2.12	
480–610	HL	35.28	2.85	$p < 0.500$
	HL + M	32.78	2.3	
620–750	HL	63.29*	4.43	$p < 0.016$
	HL + M	76.57	2.63	

* Statistically significant differences between the HL group and the HL + M group.

nal reactions shifted to 40 sec, i.e., m_N increased to 0.70. By combination 70–120, m_N was 0.75 or greater, which corresponded to the appearance of intersignal reactions at 45–48 sec. After 100 performances of the CR to the tone, intersignal reactions appeared rarely, sometimes two or three during one experiment. Thus, the CR to time in intact rats was acquired by a mean of 90 ± 10 combinations of the actual stimulus with a constant time interval and then remained at a quite high level (75–100%) throughout the observation period. From the first omissions of the actual stimulus (the tone), the proportion of correct CR responses to time amounted to 70–100%. The acquired CR to time was stable (on average, 95%).

Acquisition of temporospatial differentiation in intact rats occurred slowly. Decisions were regarded as correct when the proportion of correct responses was greater than 50%.

Rats with hippocampal lesions also experienced difficulty with temporospatial differentiation, which is based not only on trace reflexes, but also on reflexes to signal duration ratios. The average proportion of correct responses was 65%, compared with 11% for incorrect responses; refusals amounted to 24%.

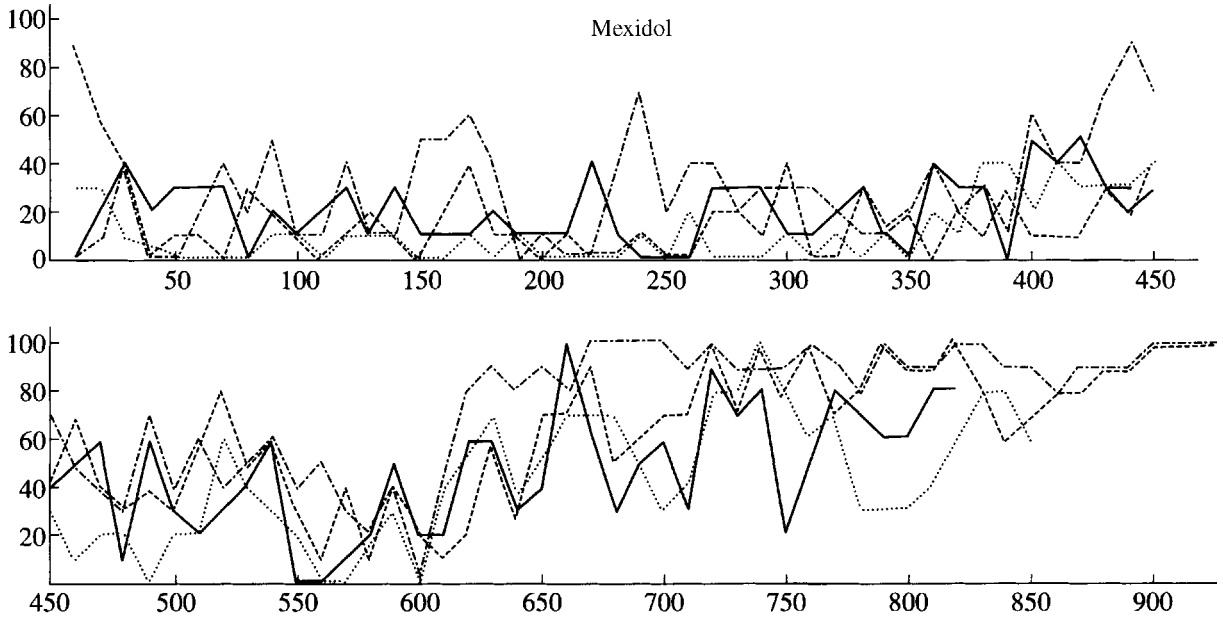

Fig. 3. Dynamics of acquisition of the CR to time in four rats with hippocampal lesions and treated with mexidol. For further details see caption to Fig. 2, *A*.

At the same time, even partial lesioning of the hippocampus significantly slowed and degraded acquisition of the ability to count time intervals. Acquisition of the CR to time in rats with bilateral hippocampal lesions was slowed by a factor of 5–6. Analysis of conditioned reflexes to counting time intervals in all operated rats revealed five periods (Fig. 1; Table 1). In three of the five periods, rats with hippocampal lesions and given mexidol showed statistically significantly better acquisition of the CR to time as compared with rats with hippocampal lesions and no treatment.

Figure 2 shows examples of the dynamics of acquisition of the CR to time in rats with hippocampal lesions not treated with mexidol: the fist period, from 0 to 100–130 combinations, showed a mean proportion of correct responses of 37%; the second (140–400 combinations) included an average of 2.75% correct responses, and the third period (from 410 combinations to the end of the experiment) gave a mean of 50% correct responses. The slow acquisition of the CR to time and the low proportion of correct responses (20–30%) during the first 350–400 combinations were due to large numbers of intersignal reactions. The mean proportion of correct responses throughout the observation period was 30%. Animals with hippocampal lesions showed stress reactions and grooming, with worsening of emotional-motivational reactions.

In rats with hippocampal lesions and treated with mexidol (Fig. 3), acquisition of the CR to time occurred just as slowly as in operated animals not treated with mexidol. However, unlike untreated animals with hippocampal lesions, they showed a less clearly defined prolonged second period during which there was almost complete loss of the mechanism of counting time intervals. Animals treated with mexidol showed a continuous learning process. Acquisition of the CR to time showed some waves in its dynamics, which was evidence for their difficulty with acquisition. The proportion of correct responses before fixation of the CR to time was 22.8% (from presentation 1 to presentation 600), compared with 72% after acquisition (from presentation 600 to 920 over two months). The average proportion of correct responses over the whole observation period was 37.6%. Use of mexidol was accompanied by improvement in autonomic and emotional-motivational reactions.

One important point was observed during studies with operated animals: it was clearly demonstrated that rats treated with mexidol showed increases in lifetime. In particular, 10 of 11 animals in the group of rats with quinolinic acid hippocampal lesions and treated with mexidol were still alive by combination 750, compared with four of 13 of those not given mexidol (Fig. 4).

Morphological monitoring showed that quinolinic acid induced selective damage to hippocampal cells mainly in fields CA1, CA3, and CA4, sometimes also causing damage to the middle parts of the dentate fascia; the dorsal parts of the hippocampus were generally damaged more than the ventral parts.

RESULTS

Our long-term morphophysiological experiments using adequate experimental models have studied the state of higher nervous activity after lesioning of very different

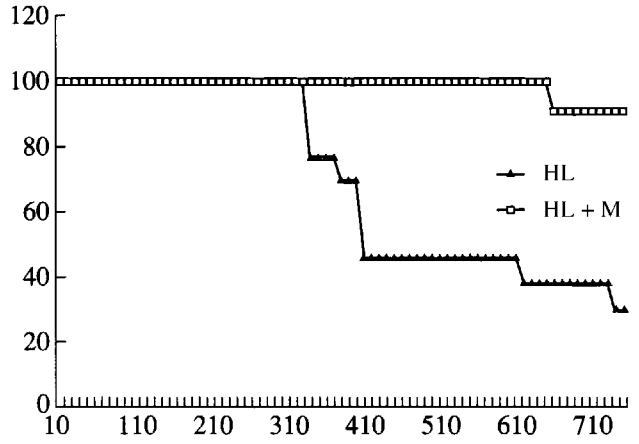

Fig. 4. Survival of rats with hippocampal lesions not treated with mexidol (group HL) and treated with mexidol (group HL + M) during acquisition of the CR to time. The abscissa shows the number of CR presentations; the ordinate shows the number of live rats, %.

parts of the brain, including the hippocampus and caudate nuclei [10–12]. For example, the hippocampus and formations directly connected with it have been shown to provide the structural basis for counting time intervals. Lesioning of the hippocampus or damage to the hippocampus has long (sometimes without subsequent recovery) leads to impairment of conditioned reflexes to time. Lesions to the caudate nuclei are accompanied by significant worsening of temporospatial differentiation. Our data show that the rate of recovery or improvement in the impaired functions depends on a number of principles: the position of the lesion, the set of connections, stabilization of particular types of inhibitory processes, complex rearrangements of interactions between centers, etc. Parallelism is not always seen between lesion volume and the rate of recovery of impaired functions.

The present experiments showed that even partial lesions of the hippocampus are accompanied by impairment to counting of time intervals lasting several months (1200–1500 presentations) with persistence of other complex conditioned reflex responses associated with non-lesioned brain structures (temporospatial differentiation).

Courses of mexidol had clear positive effects on the ability to count time intervals. In operated rats treated with mexidol, there was a slow but continuous process of acquisition; these rats showed a poorly marked prolonged second period, which was seen in rats of group 2 (hippocampal lesions without mexidol), during which there was complete loss of the mechanism for counting time intervals. It can be suggested that rats of group 2, unlike those treated with mexidol, were unable to activate the brain's reserve capacities. This sharp worsening evidently coincided with the onset of retrograde degeneration of structures directly connected to the hippocampus. Rats given mexidol also showed a greater proportion of correct responses in the acquired CR to time (72%, compared with 50% in group 2). Thanks to the significant improvement in autonomic processes and emotional-motivational reactions, these experiments could include larger numbers of conditioned stimuli. Our previous complex studies [14] showed that aging and stress states primarily involve damage to mitochondria (cellular energy metabolism), the protein-synthesizing apparatus, and synapses. After mexidol, there were larger numbers of dark mitochondria with clear cristae (enhanced cellular energetics and, apparently, suppression of free radical formation), mitochondrial and endoplasmic reticulum membranes were stabilized, the numbers of various synaptic contacts were increased, the numbers of synapses with longer active zones was increased, and so on. Thus, the antioxidant mexidol, which has a wide spectrum of actions, particularly antihypoxic and antistress actions, facilitates the appearance of compensatory-restorative processes, stimulating undamaged parts of the hippocampus, involving brain structures directly connected to the hippocampus, and preventing retrograde degeneration of these structures. Operated rats showed also showed no functional changes of the Monakow diaschisis type. It is suggested that the sudden interruption of the usual incoming stimulation is the cause of diaschisis. Functional exhaustion of subcortical formations has been shown to be faster and more profound when brain trauma also affects projection pathways [4, 21].

It should be noted that both in these data and in the results of our previous experiments [13, 14], prolonged mental training plays a great role in strengthening the reserve capacities of the brain.

CONCLUSIONS

1. The antioxidant mexidol, which has a wide spectrum of actions, especially antihypoxic and antistress actions, yielded a less marked prolonged period of complete impairment of counting time intervals and gave a slow but constant acquisition of a conditioned reflex to time in rats with traumatic lesions of the hippocampus.

2. Mexidol given after brain trauma enhanced compensatory-restorative processes. Animals showed improvements in the recovery of impaired functions and showed no manifestations of the Monakow diaschisis type; there were increases in the active duration of life.

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