PHYSIOLOGY

Influence of Hemorrhagic Shock on Learning Activity in the Late Posthemorrhagic Period

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Anxiety and learning activity (acquisition of the conditioned behavior reaction trained with a bonus) were investigated in rats during 4 weeks after hemorrhagic shock. Some phasic behavioral alterations have been found in animals undergone hemorrhagic shock.

Key words: *hemorrhagic shock, arterial hypertension, anxiety, learning*

Notable alterations develop in animal behavior in the early posthemorrhagic period, manifested as worse memorization of experimental situation [2].

Severe disturbances of ultrastructure and biochemical parameters in the cerebral cortex neurons occur in the late posthemorrhagic period [4]. They may bring about some impairments of the behavioral reactions in animals.

We studied the process of learning in rats at different stages of the recovery period after hemorrhagic shock (HS).

MATERIALS AND METHODS

Experiments were performed on 91 outbred male albino rats (280-340 g). The animals, 6 to 7 in a cage, were housed in standard vivarium conditions with natural day-lighting. Arterial hypotension down to the mean pressure of 40 mmHg was produced in anesthetized rats (Nembutal, 40 mg/kg) by letting of the blood via a catheter inserted into the tail artery. Arterial pressure (AP) was maintained at this level during 1 hour, then the drained blood was reinfused.

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Prior to learning, 5-min trials on anxiety in the elevated-plus maze (EPM) [5] were carried out before HS and after HS. The following parameters were recorded: number of entries into arms of EPM, time spent in the open arms, number of rearings, grooming movements, and number of defecations. These tests were performed in order to provide rats in the test and control groups having the same level of anxiety, which modifies their abilities to learning [3]. Anxiety level was evaluated either by time spent in open arms of EPM or by number of dips. Changes in anxiety in the posthemorrhagic period were calculated as the difference of the values obtained before HS and before learning.

Learning consisted in acquisition of the food search learning task in T-maze [1] with arm length of 30 cm each. On day 1, deprived of food rats were placed for 1 h into the maze, where balls of bread were scattered, with the aim of adaptation and fatigue of orienting-exploratory activity. During the next 4 days animals were trained. Each day they were placed for 3 min or less into the maze 5 times, successively. As a bonus were used bread balls. The following parameters were recorded: the latency (period between placing into and leaving starting compartment); the reaction time (the time needed for reaching and taking food); the number of mistakes

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(the number of enters into the arm, which is opposite to that containing food); the number of realized reactions (the number of cases, when animal reached the food for 3 min). Rats were given food once a day just after the trial. The performance of learned response took place a week after the last training.

Three groups of animals were compared: intact, control (sham-hemorrhagic, operated without bloodletting), and test rats, undergone 1-h arterial hypotension.

Learning trials in the T-maze were carried out after 1, 2 and 4 weeks since HS. A separate group of animals was used for tests at each stage of the posthemorrhagic period in order to exclude mistakes associated with repetitive tests on the same animals.

The data were statistically analyzed using Statgraphics package. Non-parametric criteria (Wilcoxon—Mann—Whitney *U*-test and Fisher method) were used for comparison between groups.

RESULTS

Studying of acquisition and performance of the food search learning task in the T-maze in rats given the task when 1 week passed since HS showed that the latency of reactions and the latency of realized reactions in the test group were significantly increased ($p<0.05$) in comparison with the control group as measured for the performance of trained reaction after 1 week of learning (Table 1). In addition, test rats showed increased number of mistakes ($p<0.05$), indicating a weakening of memorization in the test group. However, the dynamics of learning process (changes in the parameters during first 4 days) remained the same.

No statistically significant differences were found between the test and control group, when 2 weeks passed since HS before learning. The latency of reactions, the time of reaction, the number of mistakes, and the time of realized reactions were the same. Therefore, no memory impairments associated with 1-h arterial hypotension were observed, and the dynamics of learning conditioned reaction was unchanged (Table 1).

It is noteworthy that the dynamics of acquisition of the learning task altered in animals that underwent 1-h arterial hypotension. On day 2 of learning in the T-maze, the time of reaction was significantly reduced ($p<0.05$). In addition, substantially increased number of realized reactions in comparison with the control ($p<0.01$) was observed on day 1 of learning. The performance of conditioned reaction by rats trained

Table 1. Changes in Behavioral Parameters of Rats Learned in T-Maze at Different Stages of Posthemorrhagic Period

Stage, days of learning		Latency of reactions, sec			Latency of realized reactions, sec			Reaction time, sec			Reaction net time, sec			Number of mistakes			Number of realized tasks		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1 week after HS	1st	21.2	31.4	33.8	12.5	16.6	20.7	34.4	50.0	51.2	21.9	33.3	30.4	0.7	1.0	0.9	1.5	2.1	1.9
(n=11)	2nd	48.5	26.5	38.1	41.0	24.5	22.2	79.4	44.3	49.0	38.4	19.7	26.7	0.5	0.3	0.5	1.9	2.5	2.6
	3rd	29.2	18.9	34.3	20.3	19.7	14.3	33.3	37.8	30.0	13.0	13.2	15.7	0.2	0.1	0.2	2.7	3.5	2.9
	4th	68.2	23.5	52.3	16.1	15.3	17.3	32.1	30.3	35.5	16.1	15.0	18.1	0.1	0.2	0.2	3.2	4.6	3.5
	performance	17.2	4.2	19.1*	11.0	4.2	19.1*	23.4	16.9	31.9	12.4	12.7	12.8	0.3	0.0	0.5	4.1	4.7	4.8*
2 week after HS	1st	26.9	46.2	26.8	12.1	40.8	12.1	54.5	69.7	60.5	42.5	28.9	48.4	1.4	1.8	2.0	1.6	1.6	3.2
(n=8)	2nd	23.8	22.1	29.7	20.6	16.6	19.9	75.3	56.8	40.0	54.7	40.3	20.1	0.8	1.4	0.9	2.1	3.5	3.8
	3rd	27.2	8.2	23.8	15.0	8.2	11.8	41.6	22.5	26.2	26.6	14.4	14.5	0.8	0.6	1.0	2.8	4.3	4.2
	4th	27.4	5.9	11.3	21.4	5.9	8.4	29.7	15.3	17.1	8.3	9.4	8.7	0.3	0.6	0.6	3.0	4.9	4.9
	performance	19.4	2.5	5.8	16.7	2.5	5.8	28.8	7.9	12.8	12.1	5.5	6.9	0.4	0.4	0.2	4.0	4.8	5.0
4 week after HS	1st	18.6	15.2	20.2	13.9	9.7	17.0	40.8	59.5	58.4	27.4	49.8	41.7	1.5	1.4	1.1	2.2	1.5	3.1**
(n=11)	2nd	40.8	79.2	46.8	12.6	12.4	15.9	49.4	49.1	32.0*	36.8	36.7	16.1**	0.9	0.6	0.8	2.0	1.3	2.8
	3rd	22.3	45.1	21.9	14.4	23.3	14.2	32.9	58.9	40.9	18.5	35.7	26.7	0.6	0.7	0.6	3.1	3.6	3.8
	4th	12.9	21.9	11.4	11.1	21.8	7.9	24.5	30.7	15.7	13.4	8.9	7.9	0.9	0.6	0.7	4.3	3.8	4.5
	performance	12.6	19.3	11.8	12.1	13.2	7.3	21.6	26.4	14.1	9.6	13.2	6.9	0.6	0.1	0.4	4.5	4.0	4.5

Note. Rats: 1) intact; 2) control (undergone operation); 3) test. *$p<0.05$, **$p<0.01$ compared with control.

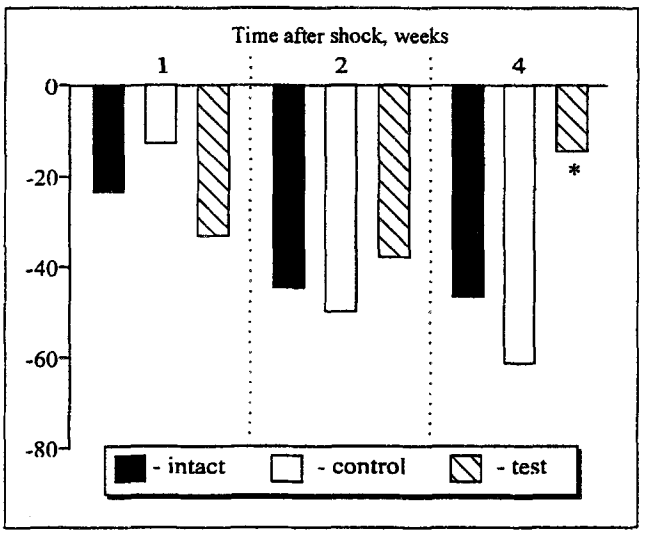

Fig. 1. Changes in the time spent in the bright arm of the elevated-plus maze at different stages of posthemorrhagic period. Ordinate: the difference between the time intervals spent in the bright arm after hemorrhagic shock prior to learning and those before hemorrhagic shock. *$p<0.05$, compared with control.

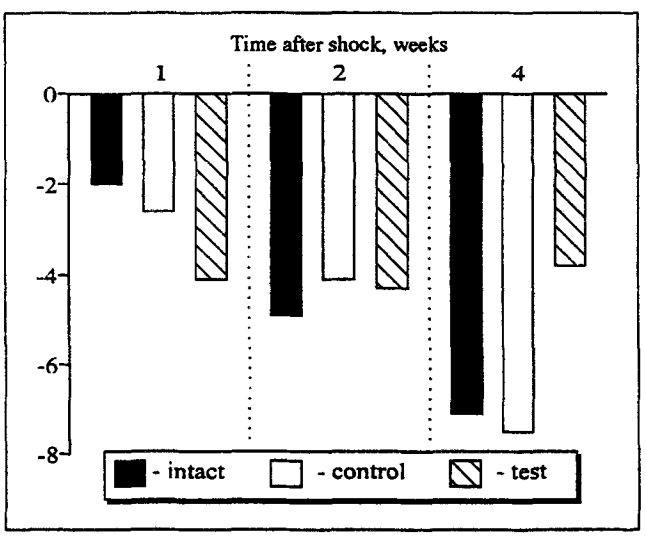

Fig. 2. Changes in the number of dips in the elevated-plus maze at different stages of posthemorrhagic period. Ordinate: the difference of the number of dips after hemorrhagic shock prior to learning and that before hemorrhagic shock.

after 4 weeks since HS did not show any differences between the test and the control groups. Though it was characteristic of intact and control animals that the number of realized reactions gradually increased from day 1 through day 4 of learning, this was not the case as regards the test group. It can be suggested that consolidation of memory trace in disturbed in rats of the test group.

The data obtained in experiments with T-maze are comparable to those obtained for these rats in EPM (Figs. 1 and 2).

The testing of rats in EPM before learning 1 week after HS did not reveal statistically significant differences between the control and test groups. However, the number of dips and time spent in the bright arm tended to decrease. As these parameters vary inversely with the level of anxiety, rats undergone HS had a greater level of anxiety than in the control group.

No differences in the number of dips and time spent in the bright arm were observed between control and test groups 2 weeks after HS.

Four weeks after HS, increased time spent in the bright arm of EPM (Fig. 1) and a tendency toward an increase in the number of dips (Fig. 2) were observed in the test group as compared with the control. This is indicative of reduced anxiety in animals 4 weeks after 1-h arterial hypotension. This may point to changes in fear motivation, as the number of defecations in the bright arm of the maze decreased in comparison with that in animals tested after 2 weeks ($p<0.05$). One can assume that a disbalance between the fear motivation, which is an adaptive reaction, and the orienting-exploring reaction is responsible for reduced anxiety in the late posthemorrhagic period.

The rapidity of reaction found after 4 weeks presumably relates to reduced anxiety in rats during the late posthemorrhagic period. Such abnormalities may happen due to disbalance between excitatory and inhibitory systems.

Thus, some phasic behavior alterations occur during the recovery period in animals undergone 1-h hemorrhagic shock. At the early stage (after 1 week), a weakening of memorization occurs, which is similar to that found in other HS model experiments [2]. At the intermediate stage (after 2 weeks), behavioral parameters tend to recover, while during the late period (after 4 weeks) behavior impairments typical of the early stage disappear. It has been noted that the level of anxiety and time parameters obtained in the T-maze decreased, presumably bing anxiety-dependent. In addition, consolidation of memory trace seems to be disturbed in the late period.

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