Active and Passive Behavior of Animals during the Postresuscitation Period

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Behavioral reactions (open-field test, elevated plus-maze, pain stress, and feeding behavior) were studied in various periods after clinical death caused by circulatory arrest for 10 or 15 min. We revealed two different phases of behavioral changes: active behavior directed at attaining a specific goal and passive behavior directed towards isolation of the organism from external signals and functional minimization. Active behavior determined by pathological excitation in the central nervous system increased the severity of structural damage to hippocampal CA1 neurons during the postresuscitation period. By contrast, passive behavior and minimization of functions preserved structural integrity in these neurons.

Key Words: *postresuscitation period; active and passive behavior; structural integrity of hippocampal CA1 neurons*

There are two strategies for functioning of biological systems under extreme conditions. Active strategy is associated with hyperfunction and increased consumption of substrates and energy. This strategy is directed towards achievement of a beneficial result and maintenance of homeostasis. The passive strategy is related to minimization of functions and economy of plastic and energy reserves and directed at preservation of structural integrity in the system. These types of activity play an adaptive role, but active strategy is provided by widening of regulatory relationships, while passive strategy is determined by narrowing of relationships and isolation of the organism, systems, organs, and cells from external signals. These types of activity occur at various evolutionary levels. In complex organisms they proceed under normal (dominating and non-dominating systems, functional heterogeneity) and pathological conditions (severe hypoxia, ischemia, and fever) [5]. It is poorly understood, whether these forms of adaptation are modified during the postresuscitation period. Moreover, their role in the process of recovery remains unclear.

Here we studied behavioral characteristics of rats after clinical death.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 180-230 g. Systemic circulatory arrest (CA) was modeled by intrathoracic ligation of coronary vessels for 10 or 15 min under ether anesthesia [3] and followed by closed-chest cardiac massage and artificial ventilation. The control group included intact rats.

Behavioral reactions in the open field (OF) and elevated plus-maze were studied during the postresuscitation period. Acquisition of the feeding response was tested. The OF behavior was automatically recorded on a RODEO-2 device. We recorded horizontal locomotor activity, count of rearing postures accompanied and not accompanied by exploration of top holes, and number of explored floor holes. Total behavioral activity (TBA) was calculated as the sum of para-

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meters recorded over 3 min. The response to acute stress (electrocutaneous painful stimulation of limbs, 30 V, 5 sec) was determined by inhibition of OF behavior 3-5 min after pain treatment. The time of horizontal locomotor activity (sec) and numbers of rearings, grooming reactions, transitions between closed arms, entries into open arms, and overhangs in the elevated plus-maze were estimated visually over 5 min.

A 4-level feeding response was elicited in a free-choice paradigm using a multialternative sectored maze to study conditioned-response activity of rats [4]. Twenty 13-min training sessions were performed at 48-h intervals (the rats were deprived of food for 22 h before each session). Experiments were conducted on treated and control animals.

The rats were sacrificed under ether anesthesia. The brains were taken for morphological examination of neuronal populations in the hippocampal CA1 region. This area is most sensitive to ischemia and plays an important role in learning. Neuronal density, number and ratio of light, dark, and morphologically unchanged pyramidal cells, and count of cells with the satellite glia were estimated [1].

The results were analyzed by Wilcoxon—Mann—Whitney *U* test, Fischer's angular transformation (φ test), and Student's *t* test.

RESULTS

Orientation and exploratory activity of rats in the OF test 7-10 days after CA for 10 or 15 min depended on the severity of ischemic brain injury. TBA of animals exposed to 10-min CA increased to 117% of the control ($p \leq 0.05$). After increasing the duration of clinical death to 15 min behavioral activity decreased to 75.2% of the control ($p \leq 0.05$). Reactivity of the central nervous system (CNS) to external factors decreased with increasing the severity of ischemic brain injury, which was manifested also after exposure to pain stress. We found that 7-10 days after 10-min clinical death TBA of stressed rats in the OF test decreased more significantly compared to control animals (Fig. 1). These results illustrate high reactivity of the brain to stress.

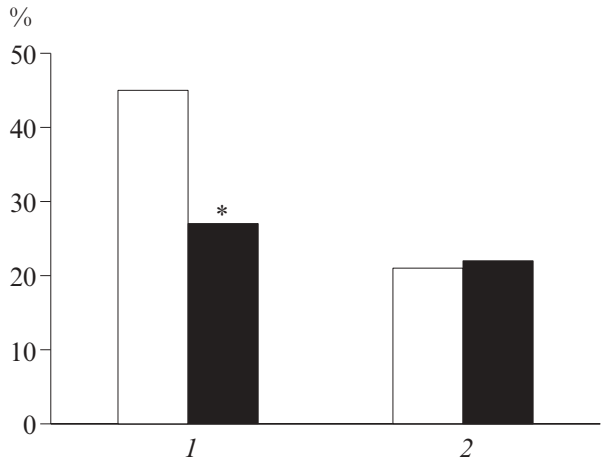

Fig. 1. Total behavioral activity of control (light bars) and resuscitated rats (dark bars) in the open-field test after acute stress. Clinical death for 10 (*1*) and 15 min (*2*). *$p \leq 0.05$ compared to the corresponding control group.

After 15-min CA the OF behavior of stressed rats did not differ from the control (Fig. 1). Therefore, these rats were characterized by lower reactivity to acute stress than animals exposed to 10-min CA.

It should be emphasized that the rats tended to avoid additional behavioral reactions even after 10-min clinical death. In elevated plus maze (in contrast to OF) the animals decrease the influence of conditioned stimuli by staying in dark closed arms. After 10-min clinical death behavioral activity of rats in the elevated plus maze was lower than in control animals. For example, resuscitated rats displayed a lower number of entries into illuminated open arms (by 3 times, Table 1).

Orientation and exploratory activity underwent phasic changes during the resuscitation period. Feeding behavior of animals exposed to 15-min CA was recorded 12-14 days after resuscitation. The study of orientation and exploratory behavior during the first training session showed that horizontal and vertical activities in resuscitated rats (n=10) were higher than in control animals (n=10). Resuscitated and control rats crossed 214.3±26.6 and 130.5±21.0 sectors of the maze, respectively. The numbers of vertical postures

TABLE 1. Elevated Plus-Maze Behavior of Rats Exposed to 10-min Circulatory Arrest 7 Days after Resuscitation ($M \pm m$)

Group	Number of entries into open arms of the maze	Number of transitions between closed arms of the maze	Number of overhangs	Number of vertical rearing postures	Time of horizontal locomotor activity, sec
Control (n=32)	3.3±0.5	3.5±0.5	2.6±0.5	15.8±1.5	24.0±2.3
Resuscitated (n=35)	1.1±0.3*	0.8±0.2*	0.7±0.2*	10.6±1.0*	10.5±1.6*

Note. *$p \leq 0.01$ compared to the control.

TABLE 2. Behavior of Rats during the First Training Session in Acquisition of the Feeding Response (*M*±*m*)

Group	Number of crossed sectors	Number of vertical rearing postures
Control trained (*n*=15)	156.2±23.4	29.0±3.6
Control untrained (*n*=15)	116.8±24.3	19.9±3.6*
Resuscitated trained (*n*=21)	198.0±15.1	40.9±2.8
Resuscitated untrained (*n*=8)	137.5±17.3*	30.9±3.8*+

Note. *$p<0.05$ compared to trained control and resuscitated rats; +$p\leq0.05$ compared to untrained control rats.

for resuscitated and control animals were 31.8±3.8 and 19.5±3.4, respectively ($p\leq0.05$).

The food-procurring response was conditioned in 50% control rats over 20 training sessions. The number of trained animals tended to increase after 15-min CA (78%, $p=0.06$). High-efficiency acquisition of the feeding response in resuscitated rats was probably related to their increased excitability. It was manifested in increased orientation and exploratory activity. Even during the first session, trained rats differed from untrained animals in greater behavioral activity (Table 2). However, the "value" of learning was different in control and resuscitated rats. During acquisition of the feeding response, weight gain in treated rats was much lower than in control animals (36.2±5.8 and 56.8±5.7 g, respectively, $p\leq0.05$).

Morphological examination of hippocampal CA1 neurons evaluated the role and structural characteristics of changes in animal learning after resuscitation. In the control group the total density of neurons in good learners was 12.4% higher than in poor learners ($p\leq0.05$). In the postresuscitation state this index did not differ in good and poor learners. The study of hippocampal CA1 neurons showed that good learners characterized by high behavioral activity differed from poor learners demonstrating low activity by low number and ratio of free light neurons, which is typical of the postresuscitation period (Table 3) [2].

Similar relationships between behavioral activity and efficiency of learning in the food-procuring paradigm in control and resuscitated rats suggest that behavioral reactions depend not only on the conditions of death and duration of the postresuscitation period, but also on typological characteristics of higher nervous activity (HNA). A special series was conducted to evaluate the role of HNA in the postresuscitation changes. The animals exposed to 10-min CA were kept in a vivarium under conditions similar to those for control animals. Two months after resuscitation the rats were divided into high-activity and low-activity groups depending on their OF and elevated plus-maze behavior. Morphological examination showed that the total density of hippocampal CA1 neurons in highly active rats was higher than in low active animals by 9.6% ($p\leq0.05$). The density of light and dark cells was similar in these rats. However, the number of morphologically unchanged cells in highly active rats surpassed that in low active animals by 44.2% ($p\leq0.05$). After clinical death the total neuronal density in highly active and low active rats was lower than in control animals by 44.4 and 9.3%, respectively ($p\leq0.05$). In highly active rats the number of light, dark, and morphologically unchanged neurons decreased by 44.5, 43.9, and 45.6%, respectively ($p\leq0.05$). In low active animals the number of light cells decreased by 11.1% ($p\leq0.05$), while the density of other cells did not differ from the control. Our findings indicate that during the postresuscitation period, destruction and death of neurons in highly active rats are less pronounced than in low active animals. Only light cells constituting the population of most reactive neurons undergo destruction in low active rats [2]. By contrast, destruction of various neurons occurs in highly active rats.

These results show that the postresuscitation period is characterized by the existence of active and passive types of behavior. The type of behavior (*i.e.*, directed activity with various systemic interrelations, functional minimization, and isolation of CNS from external signals) depends on the severity of ischemic brain injury, periodic changes in functional activity of brain structures during the postresuscitation period,

TABLE 3. Population of Hippocampal CA1 Pyramidal Neurons in Rats Exposed to 15-min CA and Differing in the Time of Feeding Response Acquisition (*M*±*m*)

Group	Light nonglial neurons		Morphologically unchanged neurons		Total density of population
	number	ratio	number	ratio	
Rapidly training (*n*=4)	100.6±7.8*	0.393±0.012*	45.4±2.5	0.178±0.002	255.3±15.0
Slowly training (*n*=3)	132.4±1.0	0.446±0.004	45.6±3.5	0.154±0.013	297.1±4.6

Note. *$p\leq0.05$ compared to slowly training rats.

and typological characteristics of HNA. An abnormal increase in excitability of CNS is observed in a certain stage after resuscitation and determines the pattern of active behavior. However, this strategy of behavior is biologically inexpedient. Hyperreactivity to pain stress leads to the decrease in poststress activity. Under these conditions the feeding response is not accompanied by an adequate increase in body weight. Highly active rats are characterized by the development of severe morphological damage to hippocampal CA1 neurons. Passive behavior suggests functional minimization and isolation of neurons from excessive afferentation, which contributes to preservation of structural integrity during the postresuscitation period.

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