Some Reasons for Modifications of Rat's Conditioned Behavior after Resuscitation

Yu. V. Zarzhetskii, M. Sh. Avruschenko, E. A. Mutuskina, and I. E. Trubina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 132, No. 11, pp. 506-509, November, 2001
Original article submitted August 8, 2001

In rats survived systemic circulatory arrest and resuscitation, pathological changes such as increased excitability of the central nervous system and decreased volume of simultaneously acquired information considerably modulate conditioned activity. The interaction between these factors facilitates learning after formation of targeted behavioral pattern.

Key Words: *behavior; circulatory arrest; postresuscitation changes in neurons*

Behavioral tests with both positive and negative reinforcement revealed more rapid learning in rats survived resuscitation compared to intact animals [3,4]. Irrespective of stimulus modality, learning was accelerated at the final stages of training, *i.e.* after acquiring some information about the task and reducing situational uncertainty. Resuscitated rats were characterized by higher excitability compared to intact animals and, therefore, by higher behavioral activity, which is important for successful learning [6,9]. However, it remained unclear why this factor does not manifest itself at the initial stages of learning and what factors accelerate acquisition at later stages. It was hypothesized that the volume of simultaneously acquired information in resuscitated animals is reduced compared to intact animals. This hypothesis can be verified by testing learning capacities in paradigms not associated with accumulation of information about the proposed task. Another approach is evaluation of the relationships between functional and structural characteristics of the brain. Here we evaluated recognition of simultaneously presented images and analyzed structural changes in the brain after learning with positive and negative reinforcement in resuscitated rats.

Institute of General Reanimatology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** niiorramn@mediann.ru. Zarzhetskii Yu. V.

MATERIALS AND METHODS

The data were obtained from 73 male albino rats weighing 180-200 g. Circulatory arrest (CA, 35 animals) was modeled by 10-12-min intrathoracal ligation of the cardiac vascular bundle [5]. Resuscitation was performed by closed chest massage and jet ventilation. Intact rats (n=38) served as the control.

Discrimination between two simultaneously presented stimuli (black and white horizontal and vertical strips) was studied in 16 rats survived 10-min CA and 18 intact rats [2]. Prior to discrimination learning the animals were trained to avoid electroshock by running to a safe compartment. Learning was started 14-16 days after resuscitation and included 3 sessions with 130 trials in total.

Training with positive reinforcement (food) was performed in a complex maze [4] and started 10-12 days after resuscitation. The animal enters the maze, takes a seed first from one and then from the other of the two feeding racks, goes out of the maze, and then enters it again for the next reinforcement. Learning parameters were analyzed in 14 resuscitated and 15 intact animals. The brains of 5 animals from each group were taken for morphological examination.

Active avoidance in a shuttle box was used as a model of conditioning with negative reinforcement [3]. Control animals and rats survived 12-min CA were trained 14 days after resuscitation. As in the previous

0007-4888/01/1325-1045$25.00

test, the brains of 5 good learners from the experimental and control groups were taken for morphological examination.

Various brain structures were analyzed using morphometrical methods [1]. The density and composition of the following neuronal populations were evaluated: pyramidal cells of the hippocampal CA1 and CA4 areas, Purkinje cells of the lateral cerebellum and pyramidal cells of the layer V of the sensorimotor cortex.

The data were analyzed statistically using Student's *t* test.

RESULTS

The percentage of successful learners in the discrimination test among resuscitated and intact animals did not differ. The electrical stimuli (current) were also similar in these groups. However, the number of presentations before attaining learning criterion was higher in resuscitated animals compared to intact animals (Table 1). These results support the idea on reduced volume of simultaneously acquired information in resuscitated animals. This factor is probably responsible for delayed adaptation of resuscitated rats to novel environment in the open field test [7] and for impaired learning in water maze after global cerebral ischemia of different duration. J. A. Nunn et al. [10] explained the latter phenomenon by spatial memory impairment. It should be noted that the changes in discrimination learning observed in our study can not be explained by spatial memory impairment, since positive stimulus was randomly positioned to the left or to the right from the animal.

The morphological study of the brain revealed similar structural changes in resuscitated animals, which did not depend on experimental paradigm and reinforcement. In the cerebellum, the density of Purkinje cell population in resuscitated animals decreased by 14.2% (maze test, $p<0.025$) and 16.1% (shuttle-box test, $0.05<p<0.1$), compared to the control. In the CA4 area of the hippocampus, the density of neuronal populations in animals trained in different learning schedules was similar, but the number of morphologically changed neurons increased by 31.3% (maze test, $0.05<p<0.1$) and 51.7% (shuttle-box test, $p<0.05$) compared to intact controls. The total density of neuronal populations in the cortex and hippocampal CA1 area of resuscitated animals did not differ from the control, but after maze training (but not shuttle-box training) the percentage of morphologically changed neurons in the cortex and CA1 area increased by 31.0% ($0.05<p<0.1$) and 33.9% ($p<0.01$), respectively. Thus, resuscitated animals were characterized by pathological changes in the brain at the level of neuronal populations. Furthermore, we previously found that resuscitated rats demonstrating accelerated maze learning had more pronounced pathological changes in the hippocampal CA1 area compared slow learners [3]. Taking into account that dendrites and synapses are more sensitive to hypoxia than somas [8], the observed changes in the neuronal populations are most likely a part of postresuscitation damage to brain structures. These changes reduced the number of functional elements and contacts in the neuronal network and, therefore, the number of degrees of freedom in the brain as in a complex system. This reduces the volume of simultaneously acquired information.

TABLE 1. Parameters of Discrimination Learning in Intact and Resuscitated Rats at Simultaneous Presentation of Visual Patterns ($M\pm m$)

Index	Intact (*n*=18)	Resuscitated (*n*=16)
Number of successful learners, %	50	56
Number of presentations necessary for conditioning	91.6±8.2	116.10±3.82*
Current, mA	0.230±0.019	0.190±0.012

Note. *$p\leq0.05$ compared to intact rats.

TABLE 2. Parameters of Maze Learning with Food Reinforcement in Intact and Resuscitated Rats ($M\pm m$)

The number of reinforcements	Intact (*n*=15)	Resuscitated (*n*=14)
For rack localization	11.40±1.44	10.30±1.46
For discovery of association between racks	32.80±3.04	28.40±2.72
For completion of task solution	78.40±7.44	45.50±5.76*
Complete learning	35.7±2.6	33.6±3.2
Session 3 after complete learning	42.2±5.3	58.80±4.64**

Note. *$p\leq0.01$, **$p\leq0.05$ compared to intact animals.

Our findings in combination with published data allow us to understand the mechanisms underlying changes in conditioned activity during the first 2 months after resuscitation.

It was shown that at the exploratory and orienting stages of maze learning (session 1) behavioral activity in resuscitated rats was higher than in intact animals [4]. Both groups needed approximately equal number of reinforcements to determine the location of feeding racks with respect to the entrance and to discover association between them (Table 2). However, resuscitated animals needed fewer reinforcements to complete acquisition than intact animals (Table 2). At the stage of consolidation (2 session after acquisition), the number of reinforcements per session in resuscitated rats considerably exceeded that in intact animals (Table 2). This suggests that resuscitated rats had higher food motivation. It should be noted that special assessment of food motivation by measuring the amount of dry food consumed for 5 min after 24-h food deprivation revealed no difference between intact and resuscitated animals at a given period postresuscitation [4].

Increased brain excitability and decreased volume of simultaneously acquired information after resuscitation make it possible to explain the difference in learning parameters between resuscitated and intact rats in the following way. At the early stage of learning with its high situational uncertainty the low volume of simultaneously acquired information makes it difficult to cope with experimental environment and to isolate the factors significant for successful learning. In resuscitated animals, this deficit is compensated by high brain excitability. After discovering the functional association between feeding racks in the course of purposeful behavior formation, the low volume of simultaneously acquired information contribute to learning process by cutting off information not related to task solution and thus enhancing food motivation. In resuscitated animals, this enhancement is also supported by higher brain excitability.

In conclusion, the present data indicate that post-resuscitation changes in brain condition resulting in higher brain excitability and lower volume of simultaneously acquired information exert significant effects on conditioned activity in resuscitated animals.

This study was supported by Russian Foundation for Basic Research (grant No. 99-04-48504).

REFERENCES

1. M. Sh. Avruschenko, *Anest. Reanimatol.*, No. 5, 41-44 (1994).
2. J. Buresh and Olga Bureshova, *Techniques and Basic Experiments for the Study of Brain and Behavior* [Russian Translation], Moscow (1991), pp. 230-235.
3. Yu. V. Zarzhetskii, M. Sh. Avruschenko, E, A. Mutuskina, and I. E. Trubina, *Byull. Eksp. Biol. Med.*, **129**, Suppl. 2, 9-11 (2000).
4. Yu. V. Zarzhetskii, E. A. Mutuskina, and I. E. Trubina, *Ibid.*, 21-24.
5. V. G. Korpachev, S. P. Lysenkov, and L. Z. Tel', *Pat. Fiziol.,* No. 3, 78-80 (1982).
6. N. G. Lopatina and V. V. Ponomarenko, in *Physiology of Behavior: Neurobiological Regularities* [in Russian], Leningrad (1987), pp. 9-59.
7. I. V. Nazarenko, A. A. Kamenskii, T. A. Gudasheva*, et al., Byull. Eksp. Biol. Med.*, **125**, No. 1, 34-37 (1998).
8. V. V. Semchenko, S. S. Stepanov, and G. V. Alekseeva, *Postanoxic Encephalopathy* [in Russian], Omsk (1999).
9. G. Sheperd, *Neurobiology* [in Russian Translation], Moscow (1987), Vol. 2, 299-301.
10. J. A. Nunn, E. Le Peillet, C. A. Netto, *et al., Behav. Brain Res.*, **62**, No. 1, 41-54 (1994).