

Neuropsychological Disorders Indicative of Postresuscitation Encephalopathy in Rats

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The aim of this research was to study the effect of 12-minute clinical death on innate and acquired behavior, biogenic amine concentration, and the composition and quantity of neural populations in specific brain regions of white rats. The study shows that in animals during the postresuscitation period with formal restoration of neurological status, there are changes in emotional reactivity, orientation-exploration reactions, impairment of learning and memory, decrease in exercise tolerance and pain sensitivity. These processes are accompanied by alterations in serotonin and norepinephrine levels in the frontal cerebral cortex, dopamine and serotonin levels in the striatum, certain biochemical indices in blood plasma and neural loss in the CA1 sector of the hippocampus and lateral portions of the cerebellum.

Keywords: neuropsychological disorders, clinical death, postresuscitation encephalopathy, emotional reactivity, orientation-exploration reaction, biogenic amines, neuronal population

El propósito de este estudio fue examinar el efecto de una muerte clínica de 12 minutos de duración sobre el comportamiento innato y adquirido, la concentración amino biogénica, y la composición y cantidad de las poblaciones neuronales en regiones específicas en ratas blancas. El estudio muestra que durante el período con restauración formal del estatus neurológico, hay cambios en los animales en la reactividad emocional, las reacciones de orientación-exploración, trastornos de aprendizaje y memoria, reducción de la tolerancia al ejercicio y la sensibilidad al dolor. Estos procesos se acompañan de alteraciones en los niveles de serotonina y norepinefrina en la corteza cerebral frontal, en los niveles de dopamina y serotonina en el cuerpo estriado, ciertos índices bioquímicos en el plasma sanguíneo y pérdida neuronal en el sector CA1 del hipocampo y en porciones laterales del cerebelo.

Palabras clave: trastornos neuropsicológicos, muerte clínica, encefalopatía post-reanimación, reactividad emocional, reacción orientación-exploración, aminas biogénicas, población neuronal

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Cerebral ischemia and consequent reperfusion have severe structural and functional sequelae that affect the function of all systems in the organism and define its subsequent vital activity. Initial neurological status restoration is by no means evidence of complete recuperation, as demonstrated by numerous data on tardive development of psychoneurological impairment in patients surviving terminal states (Alekseeva, 1979; Gerhardt & Boast, 1988; Gurvitch, Alekseeva, & Semchenko, 1996; Negovsky, Gurvitch, & Zolotokrylina, 1987). Without doubt, it is of importance to carry out multifaceted investigation of the pathophysiology of the postresuscitation process, including the mechanisms of neuropsychological disturbance, as the highest form of organism adaptation to environmental changes, in order to develop pathogenetically based therapy of postresuscitation disease.

The aim of the present study was to carry out experimental investigation of the phenomenology and mechanisms of postresuscitation disturbance of cerebral integration functions by a comprehensive study of innate and acquired forms of behavior, pain sensitivity, exercise tolerance, neuromorphological, neurochemical, and biochemical changes in male rats after a 12-min cardiac arrest and apparent restoration of neurological status.

Method

Animal Model

Experiments were carried out on 70 male nonlinear white rats with initial body weight of 200-250g. Circulatory arrest of 12 min duration in ether-anaesthetized rats was evoked by intrathoracic clamping of the cardiac vascular bundle (Korpachev, Lysenkov, & Tel, 1982). Anesthesia was assessed by "side fall" and "softening" of the bodies of the rats (the animal becomes flaccid and there is often spontaneous defecation). As it was necessary for further research for there to be a normal functional state, a facilitated method of ether application and visual assessment of the animal's state was used. In total, narcosis lasted 2-3 min. In order to operate, the animal is then fastened to a special mount by its four paws and upper jaw. It is important to note that, prior to the commencement of the operation, the rats were deprived of food for 24 hours, which, according to results of our previous study, positively influences survival of clinical death in rats (Gurvich, Mutuskina, & Mirotvornaia, 1986).

The consecutive resuscitation measures included external cardiac massage in combination with artificial pulmonary ventilation and intratracheal administration of adrenalin at a dose of 0.1 mg/kg. Before commencement of resuscitation, the animals were orally intubated and the transtracheal catheter was removed. Ventilation with 100% O₂ was then continued using a volume-controlled ventilator (UIDG-1, Russia) set at a rate of 60 breaths/min, and a tidal volume

of 10 ml/kg, 1:1 inspiratory/expiratory ratio for 30 min, until the appearance of spontaneous breathing and corneal reflexes. O₂ was delivered at 250 ml/min. External cardiac massage was performed for 1-1.5 min, until the appearance of the first spontaneous heart beat.

Procedure

Daily, for two weeks after clinical death, the general state and neurological status of the rats were assessed (Lysenkov, Korpachev, & Tel, 1982). Two weeks following clinical death, against a background of apparently restored neurological status, the rats underwent behavioral testing. Testing was repeated after three months.

Behavior Testing

Anxiety Tests. Comprehensive assessment of the rats' anxiety-phobic state was carried out according to the method proposed by Rodina (Rodina, Krupina, Kryzhanovskii, & Oknina, 1993) and with the aid of the Elevated Plus Maze test. The Rodina method is made up of 9 tests: Measurement of the latent period of descent, passage through an aperture, exit from "home," exit from the center of an open field, rating of the occurrence of the backward movement, hiding behavior, vocalization, and ear-pressing reactions in response to the action of the experimenter's hands. The named animal behavioral reactions are assessed on a point-scale form. Characterization of the animals' anxiety-phobic state as a whole was assessed by adding up the points. The Elevated Plus Maze consists of two open illuminated and two closed dark intersecting tubes (100 × 150 × 10 cm). During testing, the animal is placed at the center of the maze and for 5 min, the following indices are recorded visually: the amount of entrances into the light and dark portions of the maze, vertical activity (number of stands—rises on hind paws), number of grooming reactions, amount of defecation (number of boluses), the number of peeping into open compartments, the number of lowerings, and the time spent in open illuminated compartment. The presence of the animal at the center of the intersection of the tubes was assessed as location in an illuminated compartment.

Open Field Without Stress. The animals' orientation-exploration reactions were studied in stress-free conditions in the RODEO-2 test and in stressful conditions in the open-field test. The RODEO-2 apparatus permits the automatic separate recording of vertical and horizontal components of movement according to the amount of intersected dual infrared rays, as well as the number of investigated apertures in the floor and roof of the compartment (lower and upper burrows). The size of the compartment was 47 × 47 × 27 cm, with 16 apertures. The recording of activity (in apparatus units) was carried out in silence and darkness every 30 s for 2 min.

Open Field With Stress. The Open Field is a round arena 80 cm in diameter with a wooden floor, divided into sectors by eight diameters and two concentric circles at equal distances from each other. The arena is surrounded by a 40 cm-high wall. During testing, the animal is placed at the center of the arena and visual assessment is made of vertical and horizontal motor activity, the number of retreats from the wall and entrance into the center of the arena, grooming, and the number of defecations. Testing was carried out for 5 min. Three minutes after placing the animal in the field, the ordinary incandescent lamp (150 W) was substituted by a red lamp (15 W) for 1 min, with consecutive return to initial illumination.

T-Maze. The development of conditioned feeding reflexes was assessed, in conditions of 23-hour food deprivation, in a standard T-maze (compartment length 30 cm) for 4 days. The animal was placed daily in the maze five times for a period not exceeding 3 min. Bread balls were used as reinforcement. During the experiment, the following indices were registered: the latent period (time taken from placement to exit from the start compartment); reaction time (time required for the animal to reach the necessary compartment of the maze and take the food); the amount of reactions (the number of times the animal locates the reinforcement during the 3-min test period); the number of mistakes (number of entries into the unrewarded compartment).

Step-up Active Avoidance Task. The development of active avoidance conditioned reflexes was assessed in a special chamber made up of an electrified metallic grating floor and a plastic shelf in one corner at a height of 15 cm. The aim of the experiment was to teach the animal to avoid shocks by jumping onto the plastic shelf after hearing a conditioned signal. Four sessions were carried out at 24-hour intervals with 10 combinations of conditioned and unconditioned stimuli in each session. A bell ringing for 3 s was used as the conditioned stimulus. The unconditioned stimulus was a current switched on 2 s after the sounding of the conditioned stimulus (the current magnitude was selected individually by visual assessment of the behavioral reaction to pain stimulation). The interstimulus interval was 15-25 s. During the experiment, the following indices were registered: the amount of reactions (the number of times the animal jumped onto the shelf after the conditioned stimulus and before the unconditioned stimulus); the number of interstimulus reactions (the number of jumps onto the shelf between conditioned and unconditioned stimuli); and short latency reactions (the number of cases in which the animal jumped onto the shelf within 1-2 s following unconditioned stimulation).

Pain Sensitivity. The animals' level of pain sensitivity was assessed using the Tail Flick and Hot Plate tests. In the Tail Flick test, pain stimulation was carried out by immersing the tail to a depth of 5 cm into 56°C warm water.

The time taken to get rid of pain stimulation (time from the moment of immersion to the moment of tail flick) was recorded for 1 hour at 10-min intervals; reaction time was taken to be the mean recorded result. In the Hot Plate test, the animal is placed on a metal surface heated to 53°C. Reaction time was registered: the time from the moment of placement to the first lick of the hind paws.

Exercise Tolerance. Assessment of the animals' exercise tolerance was carried out using the "vertical net" test. During testing, the animal is placed on wire netting (area 30 × 60 with mesh dimension 1.5 × 1.5 mm) and the time from the moment of placement to falling is recorded. Measurement was carried out three times in a row with analysis of the maximum time of position maintenance.

Morphology

At the end of experimentation (4-5 months after clinical death), the animals underwent ether anesthesia morphometric and neurochemical analysis of brain structures, as well as biochemical blood analysis.

Using the method of differential morphometric analysis (Avrushchenko, 1994) the Purkinje cells of the lateral regions of the cerebellum, neurons of the V stratum of the sensorimotor cortex, pyramidal cells of the CA1 and CA4 sectors of the hippocampus of 10 control and 10 resuscitated animals were studied. The above-mentioned regions of the rat brain were chosen for morphometric analysis based on their high sensitivity to ischemia. Cell count was carried out under magnification of X400 of slices tained in the Nissl method or (for the identification of glial cells) with acidic fuchsine. Cell distribution density per area unit or per stratum millimeter of Purkinje cells and pyramidal hippocampal neurons was measured. Normal cells were identified as "light"; morphologically altered cells were "dark." The term dark was used for morphologically unaltered neurons with unusually dark tained nuclei and cytoplasm. Morphologically altered neurons included cells with varying pathology. Free neurons and neurons with satellite glia were considered separately.

Neurochemistry

Using the method of fluid chromatography under high pressure with electrochemical detection (Shilova, Kovalev, Lilp, Korochkin, & Poletaeva, 1998), the concentration of monoamines (norepinephrine, dopamine, and serotonin) was determined in the frontal cortex and striatum in 7 control and 7 resuscitated rats.

Biochemistry

Using the biochemical analyzer Cobas Mira Plus Roche, the concentration of serum alkaline phosphatase, alanine

aminotransferase, aspartate aminotransferase, bilirubin, creatinine, urea, cholesterol, triglycerides, glucose, total protein, calcium, and iron was determined in 13 control and 10 resuscitated rats.

Data Analysis

In order to process the data, mean and standard deviation values were calculated for most of the data. Group comparison was carried out with parametric (Student) and non-parametric (Mann-Whitney, Fisher) criteria.

Results

During the two weeks following the 12-min clinical death, 32.5% of the rats died. Post mortem study revealed that the causes of death were: congenital pneumonia (the most common cause), nonrestoration of cardiac function without obvious pulmonary pathology, and retarded restoration of cardiac or respiratory function leading to death several hours after successful resuscitation (in comparison with mean indices of these parameters). The input of each of the mentioned pathologies was not calculated as percentages, as the pathomorphological and pathological anatomic analysis of postresuscitation states was not included in the research task.

The external disappearance of neurological deficits in the surviving rats was noted towards the 7-10th day following resuscitation.

Further comprehensive study of various behavior forms consisted of six tests that were carried out during the following 4 weeks. Testing commenced on the 3rd week and was repeated 3 months after initial testing.

Assessment of rat anxiety-phobic status in conditions of no alternative (Rodina et al., 1993) revealed significant decrease in the anxiety index of resuscitated rats in comparison to controls during the entire observation period (31 and 33%, $p < .05$, after 2 weeks and 3 months, respectively). On the other hand, testing in conditions of choice of behavior strategy in the Elevated Plus Maze revealed a statistically significant decrease in the number of entries and the time spent in illuminated sectors (27 and 26%), as well as an increase in the amount of defecation, in comparison to controls ($p < .05$), which is typical of high anxiety levels.

During the first testing in stressful conditions of the Open Field, an increase was revealed in the vertical component of motor activity in resuscitated rats in comparison to controls in the period of testing with bright illumination (the first 3 min) and at the moment of illumination change-over at the 4th min (see Figure 1).

Simultaneously, there was an increase in the number of retreats from the wall and exits to the center of the arena (2.2 and 3.3 times, $p < .05$, respectively) and a decrease in the amount of defecation (1.4 times, $p < .05$), as well as a significant increase in horizontal and vertical motor activity

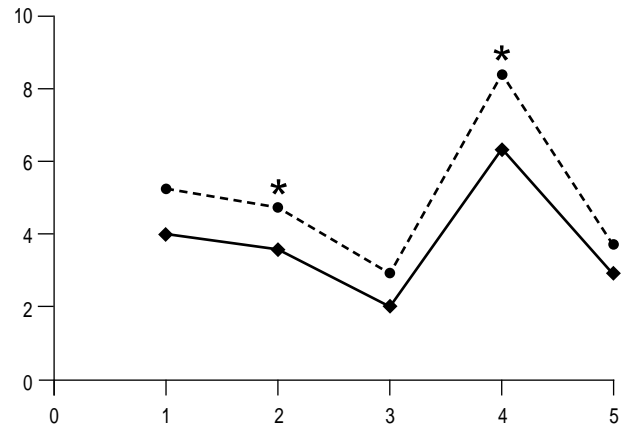


Figure 1. The influence of 12-min duration clinical death on the behavior of white rats in an open field.

X-axis – Time in minutes; Y-axis – vertical activity. Solid line – control rats ($n = 29$), dotted line – resuscitated rats ($n = 27$). * – significant distinction from control rats ($p < .05$).

at the 4th min at the moment of illumination change in comparison to the first 3 minutes of testing ($p < .05$). The described increase in rat exploration and motor activity following cardiac arrest was maintained at consecutive testing.

Testing of the animals in stress-free conditions (RODEO-2) showed that the total magnitude of the vertical component of motor activity and the number of investigated apertures in the floor of the compartment over the 3-min testing period were significantly higher in the resuscitated rat group (14 and 19%, respectively, $p = .05$). Consecutive testing 3 months later revealed a difference in the rate of decline of motor activity in the studied rat groups. In controls, there was a significant decrease in horizontal activity during second testing in comparison to the first (43%, $p < .05$), whereas in the resuscitated rat group, the decrease in this index was nonsignificant (11%, $p = .1$).

The above-mentioned results demonstrate alterations in emotional-motivational states and increased reactivity to new conditions and in particular, to stress factors in resuscitated rats.

The results of the development of conditioned feeding reflexes in the T-maze reveals an acceleration of learning processes in resuscitated rats in comparison to controls: A significant increase in the number of reactions, decrease in latent periods of exit from the starting compartment during the 4-day learning period (see Figure 2), and decrease in reaction time on the 4th day of learning (55%, $p < .05$) were demonstrated.

Consecutive testing 3 months later revealed that the developed skills in controls were well retained. Meanwhile, in resuscitated rats, there was deterioration in the learning parameters in comparison with initial testing: a decrease in the number of performed reactions (1.2-fold, $p < .05$), increase in reaction time and number of errors (3.9-fold and 2.0-fold, $p < .05$, respectively) were demonstrated.

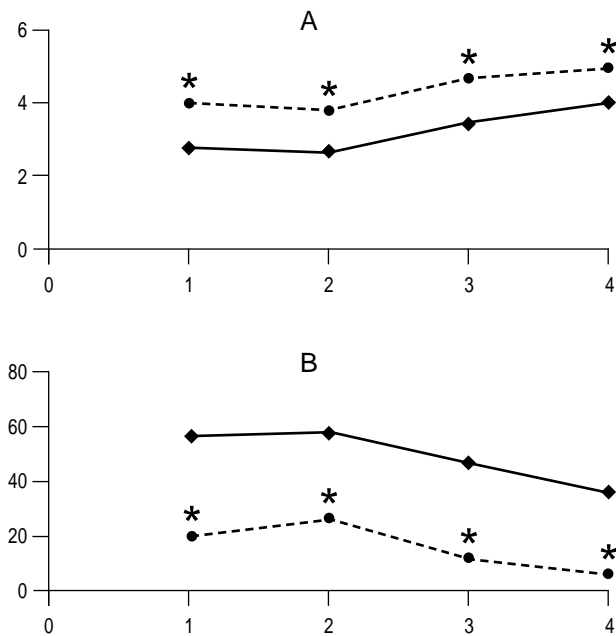


Figure 2. The influence of 12-min clinical death on the behavior of white rats in the T-maze.

X-axis – days of learning; Y-axis: A – number of executed reactions; B – latent period. Solid line – Control rats ($n = 29$), dotted line – resuscitated rats ($n = 27$). * – significant distinction from control rats ($p < .05$).

Throughout the active avoidance conditioned reflex development period in resuscitated rats, a significantly smaller number of reactions (1.8-1.5-fold, $p = .05$) with an increase was revealed in the number of short latency reactions (1.6-3.6-fold, $p = .05$) on the 1st, 3rd, and 4th days of testing, in comparison to controls. The number of intersignal reactions did not differ between groups. Reproduction of acquired skills of active avoidance 1 month later revealed a significant decrease in the amount of executed reactions and an increase in the number of short latency reactions in the resuscitated rat group in comparison to controls ($p < .05$), as well as a tendency towards a decrease in the number of intersignal reactions ($p = .08$). The study of cognitive function after resuscitation revealed contrary changes of learning abilities depending on the modality of the conditioned signal and impairment of the recovery of memory traces.

Analysis of the static components of exercise tolerance of the rats revealed that, 2 weeks after clinical death, the resuscitated rats did not differ from controls in the time of position maintenance on the vertical netting. However, after 3 months, the resuscitated rats showed significant decrease of this index in comparison to controls (44%, $p < .05$), which may be interpreted as evidence of the development of myopathy.

The determination of the level of pain sensitivity was carried out using two tests: Tail Flick and Hot Plate. There was no difference in the time of tail flick between the resuscitated rats and controls during the whole period of

observation. However, in the Hot Plate test, a marked increase in reaction time in the resuscitated rat group in comparison to controls was revealed both in the first and second testing (67 and 65%, respectively, $p < .05$).

At the termination of the experiments after 4.5 months following clinical death, neurochemical and morphometric analysis of the rat brain, and biochemical blood analysis were carried out.

Assessment of the concentration of biogenic amines in the frontal cortex of the rat brain revealed significant depletion of serotonin of 49% ($p < .05$) and a tendency towards a decrease in concentration of norepinephrine of 23% ($.05 < p < .1$) in resuscitated rats in comparison to controls (see Figure 3).

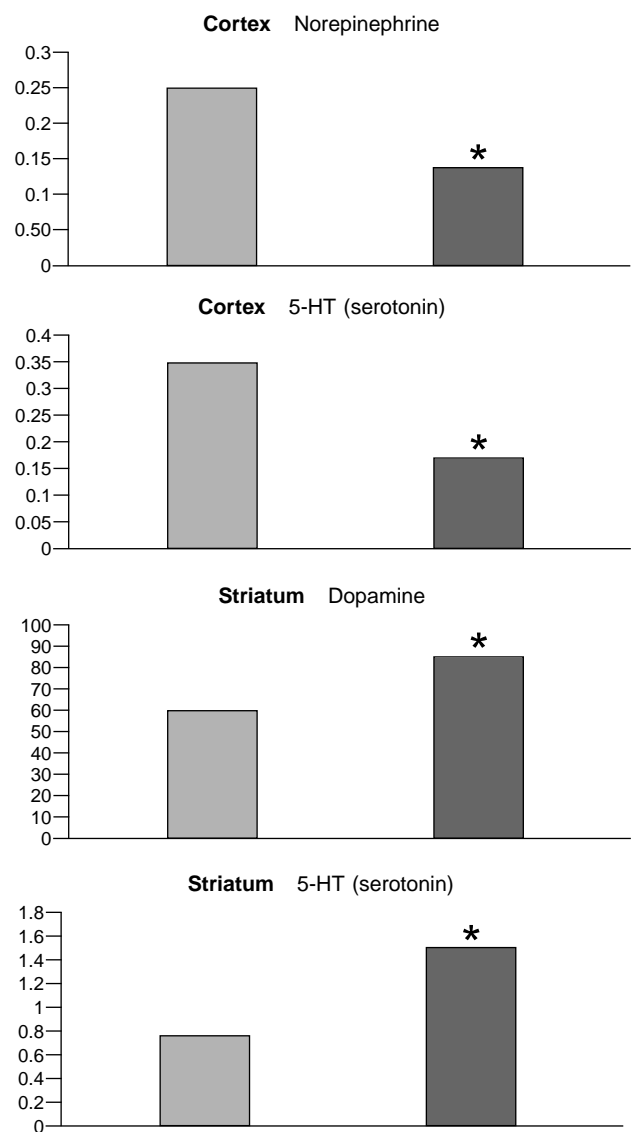


Figure 3. Monoamine concentration (vg/g of tissue) in rat brain cortex and striatum after 12-min clinical death.

X-axis – monoamine concentration in vg/g of tissue. Light columns – control rats ($n = 10$), dark bars – ischemic rats ($n = 10$). * – significant distinction from controls ($p < .05$).

Furthermore, in the striatum of the rats following clinical death, an increase in the concentration of dopamine and, in particular, serotonin was revealed (48 and 105%, respectively, $p < .05$).

Morphometric analysis of the pyramidal neurons of the CA4r sector of the hippocampus and the cells of the fourth stratum of the sensorimotor cortex did not reveal any difference between controls and resuscitated rats in terms of total density and composition of these neuronal populations. Nevertheless, there was significant distinction in the neuronal population of the CA1 hippocampal sector and in the cerebellum. In comparison to controls, resuscitated rats showed a decrease in the total density of the population of pyramidal neurons of the CA1 sector of the hippocampus (11%, $p = .05$), resulting from a decrease in the quantity of light cells (20%, $p = .05$) due to a decrease in the amount of both free

and satellite glia neurons. In the lateral regions of the cerebellum of resuscitated rats in comparison to controls, there was a decrease in the total density of Purkinje cells of 12.2% ($p < .05$) as the result of the decrease in the quantity of normal dark cells and morphologically altered neurons (14.3 and 26.3%, respectively, $p = .05$) due to a decrease in both free and satellite glia neurons (see Figure 4).

Biochemical analysis of 12 serum indices 4.5 months after resuscitation did not reveal any overall significant difference with those of the age-matched control group, indicating adequate compensation of the states of the rats. However, the resuscitated rats showed an increase in the concentration of serum creatinine, cholesterol, and a decrease in the concentration of iron of 34, 30, and 49%, respectively ($p = .05$), as well as a tendency towards a rise in glucose content and total serum calcium (see Table 1).

Tardive postresuscitation decrease in exercise tolerance to static exertion, coupled with an increase in the concentration of creatinine confirms the hypothesis of the development of myopathy. Simultaneous increase in cholesterol and glucose level together with previously revealed results of postresuscitation disturbances of the regulation of blood pressure and a tendency towards hypertension (16) indicates the formation of cardiovascular risk factors.

Discussion

The data acquired through experimentation on animals provides evidence of the influence of clinical death on the emotional-motivational aspects of rat behavior. A decrease in the levels of anxiety and extreme enhancement of behavioral activity was demonstrated during the 4-month post-resuscitation period, on the basis of which one can assume significant changes in the emotional state of resuscitated rats for a protracted duration. Furthermore, it has been shown that these changes presented varying intensities depending on experimental conditions. In conditions of stress, there is a decrease in the level of anxiety, whereas in the presence of alternatives (Elevated Plus Maze), the resuscitated rats preferred to remain in safer conditions. According to literature (Hattori, Lee, Hurn, & Crain, 2000; Kato et al., 1997), after temporary bilateral clamping of the carotid arteries in conjunction with systemic hypotension, changes in behavior were depressive in nature.

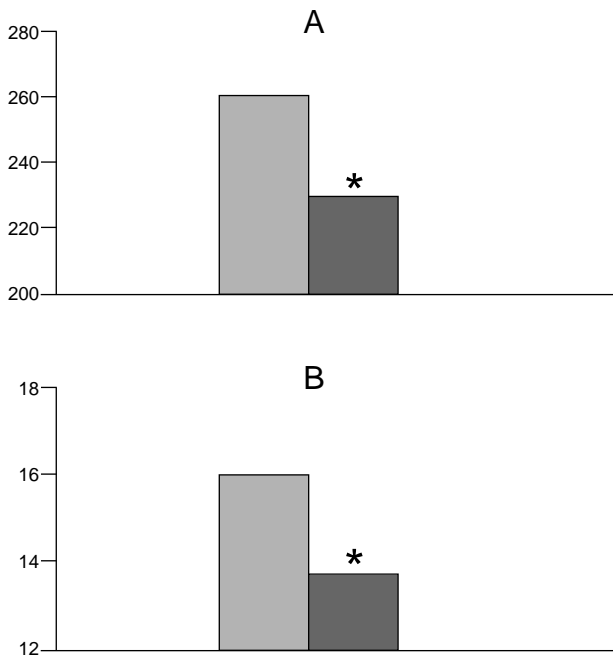


Figure 4. Total population density of pyramidal neurons in the CA1 sector of the hippocampus (A) and Purkinje cells in the lateral region of the cerebellum (B) of the rat brain after 12-min clinical death.

X-axis – number of neurons per unit length. Light columns– control rats ($n = 10$), dark columns – resuscitated rats ($n = 10$). * – significant distinction from control rats ($p < .05$).

Table 1

Biochemical Changes in the Deferred Postresuscitation Period in Rats after 12-min Clinical Death

Plasma content	Cholesterol	Sucrose	Ca	Fe	Creatinine	Urea
% of control	30%↑ *	↑	↑	49%↓ *	34%↑ *	=

Note. Control group – $n = 13$, ischemic group – $n = 10$.

* - significant differences with control ($p < .05$).

Testing of the rats 2 weeks after clinical death in stress-free conditions (RODEO-2) revealed an increase in orientation-exploration reactions. In addition to this, in stressful conditions (Open Field) during the same period, changes in behavior were most distinct. It is considered that the behavior of an animal placed in unfamiliar conditions is determined first and foremost by the relationship between two antagonistic motivations: exploratory and defensive (Buresch, Bureschova, & Huston, 1991; Markel, 1981). The acquired data allows us to suggest the suppression of passive defensive reactions in resuscitated rats, most likely resulting in a shift of general motivation towards the activation of orientation-exploration behavior. Increase in animal behavioral activity in novel conditions is considered a typical sequel to endured cerebral ischemia of any origin and is associated with insufficiency of adaptive processes in the CNS (Gerhardt & Boast, 1988; Hara, Sukamoto, & Kogure, 1993; Mileson & Schwartz 1991) and imbalance of processes of excitation and inhibition. Furthermore, it is evident that when provoking fright by abrupt change of illumination (during the 4th minute of testing in the Open Field), motor activity of the resuscitated rats is greater than that of the intact rats, suggesting the formers' inability to assess the experimental conditions adequately. A similar reaction was noted in adult rats that had survived early postnatal hypoxia, in which case, the provocation factor was a change in background sounds (Buwalda, Nyakas, Vosselman, & Luiten, 1995).

Repeated testing of the rats 3 months after cardiac arrest revealed a decrease in motor activity of both experimental groups in both stressful and stress-free conditions. It is most likely that this is associated with extinction of exploration due to memory consolidation of the conditions in which the animals were repetitively placed (Titov & Kamensky, 1980). However, in the resuscitated rat group, this process was significantly less pronounced, reflecting a decrease in the ability of the rats to memorize specific experimental conditions as a result of the endured clinical death. The result of this study confirms the known evidence of the disturbance of habituation to novel conditions in animals following cerebral ischemia or clinical death (Amano, Hasegawa, Hasegawa, & Nabeshima, 1993).

A decrease in the level of anxiety in resuscitated rats exerts significant influence over the process of the development of food-procuring skills in the T-maze. It is known that the formation of a conditioned reflex with food reinforcement is determined by the relationship between food-procuring and passive defense motivation with domination of the former as a prerogative for the triggering of the respective behavioral act (Maklakova, Dubynin, Nazarenko, Nezavibatko, & Alfeeva, 1995). Assessment of the level of rat feeding motivation did not reveal a distinction between the intact controls and resuscitated rats. Yet, rats following cardiac arrest showed a marked decrease in the latent period of exit from the starting compartment in the

T-maze, which is interpreted most often as a suppression of passive defense motivation (Ashmarin, Levitskaya, Antonova, & Nezavibatko, 1994). Thus, in these conditions, as a result of a shift in motivation balance, there is a prevalence of food-procuring behavior leading to an acceleration of skill development, namely an increase in the number of reactions. However, the acquired data in no manner are evidence of improvement of cognitive function in the post-resuscitation period. Due to increased orientation-exploration reactions, the rat moving aimlessly within the relatively small maze can inadvertently reach the reinforcement compartment and thus complete the reaction. In favor of this point of view is the lack of distinction between the resuscitated rats and controls during the first 2 days of the experiment and the deterioration of reproduction of the previously acquired skill in repeated testing after 3 months. Attenuation of cognitive function and deterioration of the ability to consolidate and retrieve memory traces are considered typical sequelae of endured cerebral ischemia irrespective of etiology (Hattori et al., 2000; Mori, Togashi, Ueno, Matsumoto, & Yoshioka, 2001; Sauve, Doolittle, Walker, Paul, & Scheinman, 1996).

Training in the test with negative reinforcement demonstrated the opposite: Resuscitated animals had significantly poorer development of active avoidance conditioned reflex. In the development of the active avoidance skills, there was a predominance of passive defense motivation. Its suppression in resuscitated rats led to a reduction of learning ability in this test. Furthermore, the decrease in the number of executed reactions and the increase in the number of short latency reactions is evidence of the fact that the rats learned to escape the shock but not to avoid it in response to the conditioned signal; that is, after resuscitation, the rats' ability to extract the conditioned signal and link it with negative reinforcement decreased.

Thus, in the postresuscitation period, changes in emotional state and motivation balance and increased reactivity to stress factors exert significant influence over the formation of conditioned reflex behavior. In particular, a decrease in the anxiety-phobic state of resuscitated rats leads to a decrease in the capability to develop conditioned reflexes in cases when the principal component of the formation of the skills is defensive motivation and the need to clearly detect target signals. This, however, facilitates the development of conditioned reflexes with positive reinforcement, the basis of which is food-procuring behavior and the trigger of which are the conditions of the maze. In favor of this hypothesis are previous data (Zarzhetsky, 2004) on the decrease in the volume of assimilated information in rats following 10-min clinical death.

The investigation of pain sensitivity in resuscitated rats did not reveal any distinction between groups in the Tail Flick test, whereas this index was markedly reduced in the Hot Plate test. However, it is important to note that, according to the data of Kharchenko (1996), in the Tail Flick test in resuscitated animals, there was an increase in reaction

time with testing at early stages of recuperation. It can be assumed that by the 6th week of the post-resuscitation period, there is partial recovery of certain animal reflex actions, developed predominantly not on the central level of the nervous system. It is a known fact that paw licking is a complex behavioral reaction performed through higher brain centers, as opposed to the tail flick reflex, which is formed at the level of the spinal cord (Ashmarin et al., 1994; Borszer, Liehtman, & Hughes, 1990). Resuscitated animals most probably have impaired subjective assessment of the significance of the pain stimulus at higher levels of the central nervous system.

The present multifaceted investigation of the behavior of resuscitated rats over a 5-month period revealed certain general patterns and features of the impairment of integrative activity of the brain. It has been demonstrated that the emotional motivation state is the most susceptible to the effects of global ischemia, with varying behavioral changes depending on experimental conditions. The general pattern is the increase in reactivity to stress and emotional stimuli, and inadequate perception of unfamiliar conditions. In conditions of unavoidable stress, most prominent are the hyperactivation of motor and orientation-exploration reactions and a decrease of anxiety-phobic states of the animal. On the contrary, in stress-free conditions and in particular in the presence of an alternative, the profile of behavioral reactions in resuscitated rats is different and may even be characterized by an increase in passive defensive reactions. There is also a difference in the changes in memory functions of resuscitated rats depending on the modality of reinforcement and the presence of stress components during the learning period. The impairment of this function is more prominent in cases where the principal component of skill formation is defensive motivation and a need to identify target signals.

It is significant that similar changes in cognitive function have been noted in postresuscitation periods in patients following clinical death. Neuropsychological testing of individuals surviving cardiac arrest outside medical institutions demonstrated that 50% of patients presented with impairment of attention and orientation, abrupt mood swings, with a maintenance of memory impairment for a duration of up to 6 months of the postresuscitation period (Drysdale, Grubb, Fox, & O'Carroll, 2000; Grubb, O'Carroll, Cobbe, Sirel, & Fox, 1996; Sauve et al., 1996).

The described changes of the profile of behavioral reactions and cognitive function in animals following clinical death indicates protracted impairment of mental integration-initiation activity in the form of changes in emotional reactivity, excitation, perception and assessment of the environment, the basis of which are complex, alternating in time, interactions of pathologic and adaptive processes in the brain and the organism as a whole. These functional anomalies are coupled with changes in the concentration of biogenic amines and neurodegenerative disturbances in specific structures of the brain.

According to the data of several studies (Bazian, 2001; Kato et al., 1997; Kulagin & Bolondinskii, 1986), increased activity of the serotonergic system diminishes anxiety levels and leads to an increase in rat emotionality. The authors cite data on high brain concentrations of norepinephrine with a decrease in the animal's emotional tension. As mentioned above, resuscitated rats are characterized by an increase in the concentration of serotonin and dopamine in the striatum and a decrease in the levels of serotonin and norepinephrine in the brain cortex. It is important to note, that the striatum play the role of coordinator in processes of the formation of purposeful behavior. On the whole, during the distant postresuscitation period, there are pronounced changes in the activity of the brain neurotransmitter monoaminergic systems.

In this period, there is a decrease in the total density and changes in the composition of the population of pyramidal neurons of the CA1 sector of the hippocampus and Purkinje cells of the lateral regions of the cerebellum. Significantly, there are no alterations in the sensorimotor cortex. Possibly, postresuscitation disturbances of integrative function are primarily caused by neurochemical and structural changes of subcortical structures controlling motor function, emotional state and sensory afferentation, as well as participating in the formation of directed purposeful behavior. At the same time, general systemic disturbances develop in the form of myopathy and the occurrence of three cardiovascular risk factors (atherogenesis, diabetes mellitus, and hypertension).

Overall, the data acquired in this study demonstrate that postresuscitation structural and functional disturbances in the CNS and organism are the result of a complex interaction of adaptive and pathologic processes and reflect the extent and forms of compensation and subcompensation. In these conditions, there is increased vulnerability of the brain, and inadequate or pathogenic impact may be the cause of decompensation and lead to the development of tardive psychoneurological complications.

Conclusions

During the postresuscitation period in animals following protracted clinical death, emotional-motivational aspects of behavior are the most vulnerable, presenting increased reactivity of the organism to novel conditions and in particular to stress factors.

With apparent recovery of the neurological status in animals, there is a decrease in anxiety level, an intensification of exploration activity, accelerated development of food-procuring reflexes, impairment of the development of active avoidance conditioned reflexes, impaired memory recall of previously acquired skills, and decrease in pain sensitivity and exercise tolerance.

Four to five months after clinical death, there is an increase in the concentration of dopamine and serotonin in

the striatum and a decrease in the levels of serotonin and norepinephrine in the cerebral cortex, and a loss of pyramidal neurons in the CA1 sector of the hippocampus and Purkinje cells in the lateral regions of the cerebellum.

Simultaneously, myopathy develops along with the formation of cardiovascular risk factors.

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