SENSIBILIDADE DOS NEURÔNIOS SENSORIMOTORES DO CORTEX DO CÉREBRO DE RATOS A MEDIADORES NO DERRAME HEMORRÁGICO

SENSITIVITY OF RAT BRAIN SENSORIMOTOR CORTEX NEURONS TO MEDIATORS IN HEMORRHAGIC STROKE

ЧУВСТВИТЕЛЬНОСТЬ НЕЙРОНОВ СЕНСОМОТОРНОЙ КОРЫ МОЗГА КРЫС К МЕДИАТОРАМ НА ФОНЕ ГЕМОРАГИЧЕСКОГО ИНСУЛЬТА

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RESUMO

O problema dos distúrbios da circulação cerebral pós-estresse, o mais grave dos quais é hemorragia cerebral ou derrame hemorrágico, é extremamente urgente. Em relação à taxa de sobrevivência, estudos anteriores mostraram que, no mesmo tipo de situações de conflito, indivíduos resistentes e predispostos a influências estressantes emocionais são claramente distinguidos. No entanto, os mecanismos neurais que fornecem regulação neuromediadora das respostas aos efeitos estressantes ainda são pouco estudados. A análise da sensibilidade neuroquímica à acetilcolina e norepinefrina dos neurônios do córtex sensorio-motor de ratos com atividades diferentes de acordo com os parâmetros de comportamento, ou seja, resistência ao estresse emocional influencia antes e após o acidente hemorrágico experimental (AHE). O processo de recuperação após um AHE devido à atividade neural em ratos prognósticos estáveis (VA) e predispostos (NA) a situações estressantes possui características individuais. A influência do estresse agudo antes do desenvolvimento do AHE altera a natureza da atividade dos neurônios do córtex sensorio-motor em ratos VA e NA no terceiro e sétimo dia após o AHE. O EHS leva a alterações específicas na sensibilidade dos neurônios à acetilcolina e norepinefrina em ratos VA e NA. A microionoforese da acetilcolina causa a ativação da atividade do impulso neuronal em ratos VA e NA, e essas alterações são as mesmas após o EHS. A influência do estresse agudo antes do desenvolvimento do AHE não altera a natureza das respostas dos neurônios à microionoforese de noradrenalina em animais com AV. Pelo contrário, em ratos NA observou-se perda da sensibilidade dos neurônios à noradrenalina durante o desenvolvimento da AHE contra o estresse. É possível que a influência aguda do estresse antes do desenvolvimento da AHE possa alterar a atividade do sistema noradrenérgico cerebral em ratos NA, que são predispostos prognosticamente às influências do estresse. Os dados obtidos enfatizam a plasticidade da estrutura nervosa central no desenvolvimento da resposta a influências estressantes. (O número do protocolo de aprovação da comissão de ética é 18-15 de 13 de março de 2018.).

Palavras-chave: *Estresse, acidente vascular cerebral hemorrágico, ratos, neurônios, noradrenalina.*

ABSTRACT

The problem of post-stress disorders of cerebral circulation, the most severe of which is cerebral hemorrhage, or hemorrhagic stroke, is extremely urgent. Concerning the survival rate, previous studies have shown that in the same type of conflict situations, individuals resistant and predisposed to emotional stressful influences are clearly distinguished. However, the neural mechanisms providing neuromediatory regulation of responses to stressful effects are still little studied. The analysis of neurochemical sensitivity to acetylcholine and norepinephrine of the sensorimotor cortex neurons of rats having different activity according to behavior parameters, i.e., resistance to emotional stress influences before and after experimental hemorrhagic stroke (EHS) was carried out. The recovery process after an EHS due to neural activity in prognostically stable (VA) and predisposed (NA) to stressful situation rats has individual characteristics. Acute stress influence before EHS

development changes the nature of neurons activity of the sensorimotor cortex in VA and NA rats on the third and seventh day after EHS. EHS leads to specific changes in the sensitivity of neurons to acetylcholine and norepinephrine in VA and NA rats. Acetylcholine microiontophoresis causes activation of the neuron impulse activity in VA and NA rats, and these changes are the same after EHS. Acute stress influence before EHS development does not change nature of neuron responses to norepinephrine microiontophoresis in VA animals. On the contrary, in NA rats loss of neuron sensitivity to norepinephrine during EHS development against stress was noted. It is possible that acute stress influence before EHS development can change brain noradrenergic system activity in NA rats, which are prognostically predisposed to stress influences. The data obtained stress the plasticity of the central nervous structure in development of response to stressful influences. (The ethics commission approval protocol number is 18-15 from 13th of Marth 2018.)

Keywords: *stress, hemorrhagic stroke, rats, neurons, norepinephrine.*

АННОТАЦИЯ

Проблема развития постстрессорных нарушений мозгового кровообращения, самое тяжелое из которых внутри мозговое кровоизлияние, или геморрагический инсульт, является крайне актуальной. Проведенные ранее исследования продемонстрировали, что в однотипных конфликтных ситуациях, по показателю выживаемости, отчетливо выявляются индивиды устойчивые и предрасположенные к эмоциональным стрессорным воздействиям. Однако нейронные механизмы, обеспечивающие нейромедиаторную регуляцию ответов на стрессорные воздействия, мало исследованы. В работе проведен анализ нейрхимической чувствительности к ацетилхолину и норадреналину нейронов сенсомоторной коры головного мозга крыс, имеющих различную активность по параметрам поведения до и после геморрагического инсульта. Процесс восстановления после экспериментального постстрессорного инсульта по показателям нейронной активности у прогностически устойчивых (ВА) и предрасположенных (НА) к стрессорным воздействиям крыс различен. Геморрагический инсульт приводит к специфическим изменениям чувствительности нейронов сенсомоторной коры к норадреналину у ВА и НА крыс. Острое стрессорное воздействие до формирования инсульта не изменяет характера ответов нейронов на микроионофорез медиатора у ВА животных. У НА крыс, напротив, отмечена потеря чувствительности нервных клеток к норадреналину при формировании геморрагического инсульта на фоне стресса. Можно полагать, что острое стрессорное воздействие до формирования геморрагического инсульта может изменять активность норадренергической системы нейронов сенсомоторной коры НА крыс - прогностически предрасположенных к стрессорным воздействиям. Полученные данные еще раз подчеркивают пластичность центральных нервных структур при формировании ответа на стрессорные воздействия. (Протокол этического комитета №18-15 от 13 марта 2018 г.)

Ключевые слова: *стресс, геморрагический инсульт, крысы, нейроны, норадреналин.*

1. INTRODUCTION

The problem of post-stress disorders of cerebral circulation, the most severe of which is cerebral hemorrhage, or hemorrhagic stroke, is extremely urgent (Yumatov, 2001; Lee *et al.*, 2016; Rainforth *et al.*, 2007; Smith and Eskey, 2011). Despite the high amount of works devoted to the study of the mechanisms of the development of post-stroke changes, a number of questions concerning the development of cerebral circulatory disorders in mammals with different prognostic resistance to similar stress loads remain unsolved.

Concerning the survival rate, previous studies have shown that in the same type of conflict situations, individuals resistant and predisposed to emotional stressful influences are clearly distinguished (Sudakov, 1983; Sudakov and Umruhen, 2010). It was shown that previous

stress influenced biochemical parameters of the body during stroke development. The stress load before the experimental stroke differently changed the conformational properties of some blood proteins (e.g. albumin) in rats, differing in the parameters of behavior and response to stress (Kalinina *et al.*, 2012).

Previous special experiments showed that active rats exhibiting in the "open field" a short latent period of the first movement and exit to the center, and also high locomotor activity on the periphery and, especially, in the center, are animals predictively resistant to stress loads. At the same time, rats that demonstrate a long latent period of the first movement and exit to the center, low activity, both in the center and on the periphery and having high vegetative balance indicators, are animals predictively predisposed to stress loads.

In this regard, rats that demonstrate

different locomotor activity in the "open field" test can be divided into two groups: resistant (highly active - VA) and predisposed (low active - NA) to emotional stress influences (Koplik, 2002; Koplik *et al.*, 2005).

Neurochemical and morphological changes in the brain of rats with different behavioral parameters occurring at various stages of the post-stroke period have not been fully studied. The dynamics of neurological symptoms in behaviorally passive (NA) and active (VA) animals with intracerebral hemorrhage (hemorrhagic stroke) are not completely studied (Koplik and Klassina, 2018; Koplik and Pertsov, 2019; Bogolepov *et al.*, 2001; Koplik *et al.*, 2001).

It has been shown that animal resistance to emotional stress closely correlates with genetically determined and individually acquired properties of neurotransmitter processes in various brain structures (Sudakov and Umryukhin, 2010). Thus, in VA and NA due to individual behavior parameters, preliminary emotional stress load changes dynamics of norepinephrine, dopamine, and to a lesser extent, serotonin content in the sensorimotor cortex after modeling hemorrhage in the caudate nucleus of the brain (Ivannikova *et al.*, 2012).

However, the neural mechanisms providing neuromediator regulation of responses to stressful effects are still little studied (Bogolepov *et al.*, 2004). Considering the above, the purpose of this study was to analyze neurochemical sensitivity of neurons in the sensorimotor cortex of rats having different activity according to behavior parameters, i.e., resistance to emotional stress influences after hemorrhagic stroke.

2. MATERIALS AND METHODS

The experiments were carried out in 24 male rats of the Wistar strain (12 — VA and 12 — NA) with body weight 190–210 g, on the background of experimental hemorrhagic stroke (EHS) before and after stress influence. EHS was modeled by injecting 60 µl of own blood into the caudate nucleus of the left hemisphere (Deinsberger *et al.*, 1996). All control rats underwent a false operation for hemorrhagic stroke 1 day before the experiments.

Two days before the experiments, in which impulse activity was recorded and microionophoretic addition of neuroactive substances into the perineuronal space done, scalping and trepanation of the animal skull were performed under air-ether anesthesia. A

micromanipulator was placed on the bones of the skull to introduce 3 channel pencil-type glass microelectrodes into the brain with a tip diameter of 2–4 µm, with the help of which extracellular registration of neurons impulse activity and microionophoretic addition of physiologically active substances to them was done. The recording channel of pencil-type glass three-channel microelectrodes was filled with 3M NaCl solution. For MIP neurotransmitters, 10⁻⁶ M solutions of Ach and NA in distilled water were used.

On the third (72 hours) and seventh (168 hours) days of influence (Koplik and Klassina, 2018) after EHS development the impulse activity of neurons of the sensorimotor (SM) cortex and microionophoretic (MIP) neurotransmitters behavior (acetylcholine (Ach) and norepinephrine (NA) were recorded: strength current 15–25 nA, with intervals between summing up 2–3 min.) to nerve cells of the SM cortex. The experiments were performed in anesthetized animals - urethane 1g / kg in 3–4 ml of 0.9% NaCl solution, i / p.

The obtained experimental data were statistically treated using nonparametric statistical methods and "Statistica 6" programs.

The character of neuronal activity pattern was analyzed by time-frequency characteristics: the duration of the interpulse intervals was measured. After analog-to-digital conversion, neuronograms were recorded on electronic media. Subsequently, a computer analysis of neurons activity of was carried out according to specially developed programs with the construction of graphs of interval histograms with a piecewise-uneven time scale (Sudakov, 1983; Timoshin, 1992).

A total of 57 neurons were recorded in VA and 48 in NA rats. The number of registered neurons in groups of experimental animals and the experimental scheme are shown in Table 1. Number of SM neurons in the cortex of rats with different states in groups

3. RESULTS AND DISCUSSION

The performed studies have shown that after EHS SM cortex neurons of the rat are characterized by a single- arrhythmic type of spontaneous impulse activity. In the patterns of the discharge activity of the overwhelming majority (96%) of neurons of both VA and NA rats, the pulse intervals of 600–800 ms duration dominate (Figure 1). Moreover, patterns in the region of 600–700 ms are more characteristic for VA rats, and for NA - 700–800 ms.

Our previous experiments showed that the most difficult period in hemorrhagic stroke in NA rats was day 3. During this period, there was a sharp increase in lipid peroxidation indicators and an increase in the antioxidant index, whereas by day 7, opposite *мытеы* are observed in VA rats compared to NA rats, indicating a higher neurological status in VA rats (Bogolepov *et al.*, 2004).

At the same time, the activity patterns of SM cortex neurons had no significant differences on the third and seventh days after EHS, both in VA and in NA animals.

However, the acute stress influence exerted before EHS development changes the character of the impulse activity of the cortical SM cortex neurons in VA and NA rats on the third and seventh days after EHS (Figures 2, 3). Therefore, the analysis of changes in the sensitivity of neurons to neurotransmitters - Ach and NA on the background of EHS was later performed.

Ach MIP was shown to induce activation of the neuron activity in VA and NA rats, and these changes are of the same nature on the third and seventh days after EHS. On the third day, as well as on the seventh day, after stroke, Ach MIP leads to domination of neuron activity patterns in VA rats at intervals of 70-90 ms (Figure 2). At the same time, a discrete dominance of the interpulse intervals within the range of 100-300 ms is characteristic for NA rats. Cancellation of MIP Ach is accompanied by a gradual restoration of the initial activity of neurons within 180 seconds.

Acute stressful influence results in the activation of the discharge activity of neurons in both groups of animals, which is reflected in the dominance in the activity patterns of intervals within the limits of 10-30 ms.

Stressful influence before EHS development does not change the character of the responses of neurons to MIP NA in VA animals. In NA rats, acute stressful influence, before EHS development, is accompanied by a loss of sensitivity of SM cortex neurons to MIP NA on the third and seventh days after stroke (Figure 4).

After EHS, NA unidirectionally changes the impulse activity of SM cortex neurons in VA and NA rats on the third and seventh day. The character of changes in the patterns of neuronal activity has no specific features in animals of both groups, which indicates the absence of differences in the internal noradrenergic mechanisms during EHS development in VA and NA animals (Figures 3, 4).

4. CONCLUSION

Individual characteristics of rats' behavior in the "open field", specifying their resistance to emotional stress, significantly affect the severity of neurological symptoms in case of intracerebral hemorrhage. Predictably stress-resistant rats are characterized by a faster recovery of neurological status, motor, and coordinatory disorders by the seventh day after unilateral hemorrhagic stroke in the caudate nucleus compared with stress-predisposed animals. (Koplik, 2015).

It is the structures of the sensorimotor cortex that participate in the restoration of motor activity after a stroke and are associated with restoring the balance of asymmetric interhemispheric inhibition and corticomotor excitability (McDonnell and Stinear, 2017).

The spontaneous impulse activity of SM cortex neurons in VA and NA rats on the background of EHS is of the same type with weakly expressed specificity.

Acute stressful influence leads to activation of neurons discharge activity in both groups of animals, which is reflected in the dominance in the activity patterns of intervals within 10-30 ms. Moreover, acute stress influence, before EHS development, changes the activity pattern of SM cortex neurons in VA and NA rats on the third and seventh days after EHS.

SM cortex neurons sensitivity to Ach is unidirectional in VA and NA rats on the third and seventh days after EHS on the background of stress. In VA rats, intervals of 70-90 ms duration dominate, and in NA rats – 100-300 ms.

MIP NA unidirectionally changes the spontaneous impulse activity of SM cortex neurons in VA and NA rats on the third and seventh days after EHS. The character of changes in the activity patterns of nerve cells has no specific features in VA and NA animals. This may indicate the identity of noradrenergic mechanisms during EHS development in VA and NA animals. However, prior to EHS development, in NA rats, acute stress influence is accompanied by a loss of sensitivity of SM cortex neurons to MIP NA on the third and seventh day after stroke.

Thus, due to neural activity, the recovery process after post-stress stroke has specific differences in VA and NA animals. EHS leads to changes in neurons sensitivity to NA in VA and NA rats. Acute stress influence before stroke development does not change the character of the neuron responses to MIP mediator in VA animals. On the contrary, in NA rats there was a loss of neurons sensitivity to NA during EHS

development on the background of acute stress. Therefore, acute stress influence before EHS development can change the activity of the noradrenergic system of neurons of the SM cortex in rats - prognostically predisposed to stress effects. The data obtained once again emphasize the plasticity of the central nervous structures in development of a response to stressful effects.

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TABLES

Table 1. The scheme of the experiment

State of the experimental animals		VA rats after 72 hours	NA rats after 72 hours	VA rats after 168 hours	NA rats after 168 hours	
EHS		15	12	18	12	
Stress influence - EHS		12	12	12	12	

The scheme of the experiments (stages in seconds)						
Background 180	Ach 30	After Ach 180	Break 300	Background 180	NA 30	after NA 180

Total time of one neuron activity registration – 1 080 sec.

FIGURES

Figure 1. Histogram of interpulse interval distribution in the patterns of SM cortex neurons activity in the rat after EHS.

Figure 2. Histograms of interpulse intervals distribution in the activity patterns of SM cortex neurons in VA rats on the 7th day (168 hours) after EHS with Ach MIP.

Figure 3. Histograms of interpulse intervals distribution in the activity patterns of the SM cortex neurons in VA rats on the 7th day (168 hours) after EHS with MIP NA.

Figure 4. Histograms of interpulse intervals distribution in the activity patterns of the SM cortex neurons in NA rats on the 7th day (168 hours) after EHS with MIP NA.