

SECTION 4. MEDICAL AND BIOLOGICAL RESEARCHDOI <https://doi.org/10.30525/978-9934-26-413-9-6>**EFFECT OF RHYTHMIC EXTREME CRYOTHERAPY (–120°C)
ON COGNITIVE FUNCTIONS OF SPONTANEOUSLY
HYPERTENSIVE RATS****ВПЛИВ РИТМІЧНОЇ ЕКСТРИМАЛЬНОЇ КРИОТЕРАПІЇ (–120°C)
НА СТАН КОГНІТИВНИХ ФУНКЦІЙ СПОНТАННО
ГІПЕРТЕНЗИВНИХ ЩУРІВ ЛІНІЇ SHR****Babiichuk L. V.**

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According to numerous studies, long-term uncontrolled arterial hypertension (AH) is one of the most significant risk factors of developing the cognitive disorders. Most often, with hypertension, the speedy analysis

of the obtained information and decision-making slows down, and long-term memory decreases [1]. A correlation has been established between the blood pressure level and the risk of central nervous system disorders [2]. Spontaneously hypertensive rats (SHR) are an adequate model of chronic hypertension [3]. Taking into account the effect of hypertension on the development of cognitive dysfunction, these rats are used as a model for studying neurological-behavioral disorders, attention deficit and hyperactivity disorder [4; 5].

Currently, it is known that low and ultra-low temperature effects (-120°C), which are used in medical practice, have a specific, stimulating influence on homeostasis [6]. The active use of cryotherapy (-120°C) in clinical practice to treat a wide range of somatic diseases and psychosomatic disorders allows it to be classified as a promising tool for modern restorative medicine.

Therefore, we believe, that it was reasonable to evaluate the changes in cognitive functions of in SHR against the background of rhythmic extreme cryotherapy (REC) (-120°C).

The studies were performed in sexually mature 12–13-month-old white outbred (normotensive control) and SHR males, weighing 250–300 grams ($n=7$).

REC (-120°C) was performed in a metrologically certified cryochamber for cooling the experimental animals [7]. After the device went into working mode (-120°C), the rats were placed in the cryochamber through the airlock in the main compartment. The total cooling cycle consisted of 9 sessions over 5 days: 3 sessions per day followed by a one-day break [8].

Disturbances of spatial memory, as one of the main way to determine the cognitive functions, were detected in the Morris water maze (MWM) [9]. Laboratory animals were trained to avoid a forced swimming by finding a platform hidden underwater, which was located in one of four quadrants. The water temperature for rats was $26\pm 2^{\circ}\text{C}$.

In the course of the research, we have found (Table 1) that spontaneously hypertensive rats, in comparison with normotensive animals, had significant difficulties in acquiring the skills of finding the platform in the MWM, since the time that passed from the start when the animals of this group were put into the MWM to their stay on the platform was prolonged. It should be noted that the chaotic movements of spontaneously hypertensive rats were more active than those of normotensive rats, that was due, in our opinion, to the presence of hyperactivity syndrome in the former.

Thus, for normotensive rats, from the second attempt the time of finding the platform was reduced by 1.4 times, from the third by 1.7 times, from the fourth and fifth by almost 4 times, and from the sixth and seventh by more than 6 times (Table. 1).

Table 1

Dynamics of changes in cognitive functions of normotensive and spontaneously hypertensive rats depending on time of being at the platform in the MWM

Groups of rats	Attempts						
	1	2	3	4	5	6	7
	Time of being at the platform, seconds						
1 – normotensive control	50±2	36±4	30±2	12±3	13±4	7±2	8±2
2 – SHR as a control	>70 ¹	>60 ¹	58±6 ¹	44±4 ¹	24±2 ¹	18±2 ¹	19±4 ¹

Note: 1 – significant differences from 1 group of rats ($p < 0.05$).

For spontaneously hypertensive rats, the learning process was much more difficult and slower (Table 1). Thus, only from the fourth attempt, the time of finding the platform was reduced by 1.6 times, and the time close to that in normotensive animals was reached only in the sixth and seventh tests (Table 1). Disordered working memory, attention control and decision-making in SHR are apparently related to the changes in their locomotor activity.

Table 2

Dynamics of changes in cognitive functions of SHR according to the time of being at the platform in the MWM, on days 7 and 30 after rhythmic extreme cryotherapy

Groups of rats	Attempts						
	1	2	3	4	5	6	7
	Time of being at the platform, seconds						
1 – SHR as a control	>70	>60	58±6	44±4	24±2	18±2	19±4
2 – SHR in a week after REC	58±2 ¹	32±2 ¹	24±2 ¹	12±3 ¹	10±2 ¹	9±2 ¹	8±4 ¹
3 – SHR in a month after REC	38±4 ^{1,2}	31±2 ¹	23±4 ¹	10±3 ¹	9±2 ¹	8±4 ¹	9±3 ¹

Note: 1 – significant differences versus group 1 of rats;
2 – significant differences between groups 2 and 3 of rats ($p < 0.05$).

A week and a month after the REC application (-120°C) (Table 2), qualitative and quantitative changes in search behavioral reactions were observed in spontaneously hypertensive rats. If in the control animals the search response was measured in the first attempt within more than 70 seconds, then after the use of REC (-120°C) (especially in 30 days), the time of searching for the platform in the MWM decreased (by 1.2 and 1.8 times, respectively) and approached the indices of normotensive rats. In the second and third attempts, the time of searching for the platform, both

on the 7th and on the 30th day after the use of REC (–120°C), was reduced by almost 1.9 and 2.4 times, respectively, in the fourth that was by 3.7 times, and in 5th, 6th and 7th about 2 times.

Thus, it was established that compared to normotensive rats, spontaneously hypertensive ones had a cognitive deficit, which was manifested in a reduced rate of learning and memory formation in the MWM. The use of REC (–120°C) had a positive effect on the cognitive functions of spontaneously hypertensive rats. It can be assumed that REC has a neuroprotective effect, increasing the lability of regulatory processes in various brain structures, which contributes to the improvement of cognitive functions and prevents possible deterioration of spatial memory.

Bibliography:

1. Ou Y-N., Tan C-C., Shen X-N., et al. Blood pressure and risks of cognitive impairment and dementia. *Hypertension*. 2020. № 76. P. 217–225.
2. Walker K.A., Power M.C., Gottesman R.F. Defining the relationship between hypertension, cognitive decline, and dementia: a review. *Curr. Hypertens. Rep.* 2017. Vol. 19, 3. P. 24.
3. Айдарова В.С., Наумова О.В., Кудокоцева О.В., и др. Структура мозга крыс линии SHR с генетически детерминированной артериальной гипертензией. *Світ медицини та біології*. 2018. Т. 64, № 2. С. 115–119.
4. Yen P.S.Y., Liu Y.C., Chu C.H., et al. Upregulation of glutamatergic receptors in hippocampus and locomotor hyperactivity in aged spontaneous hypertensive rat. *Cell Mol. Neurobiol.* 2022. Vol. 42. P. 2205–2217.
5. Zhang P., Fang H., Lou C., et al. Enhanced glial reaction and altered neuronal nitric oxide synthase are implicated in attention deficit hyperactivity disorder. *Front. Cell Dev. Biol.* 2022. Vol. 10 901093.
6. Rymaszewska J, Tulczynski A, Zagrobelny Z et al. Influence of whole body cryotherapy on depressive symptoms—preliminary report. *Acta Neuropsychiatrica*. 2003. Vol 15. Vol. 122–28.
7. Кріокамера для екстремального охолодження лабораторних тварин: пат. 40168 Україна, МПК А61В 18/00. № u200812930; заявл. 06.11.2008; опубл. 25.03.2009. Бюл. № 6. 4 с.
8. Бабійчук В.Г. Механізм дії екстремально низких температур на структурно-функціональний стан центральної нервової та серцево-судинної систем у тварин різних вікових груп : дис. ... доктора мед. наук : 14.01.35 «Кріомедицина». Інститут проблем кріобіології і кріомедицини. Харків, 2010. 335 с.
9. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods*. 1984. V. 11, № 3. P. 47–60.