

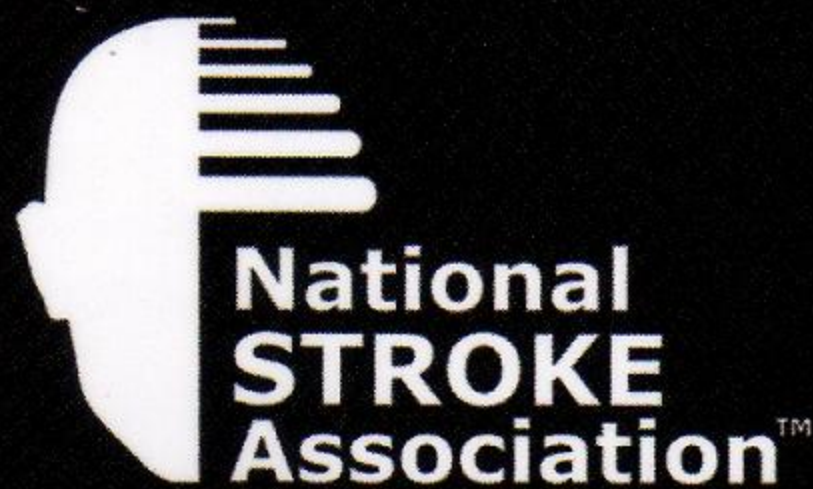
Volume 24

Number 2

February 2015

Journal & of Stroke & Cerebrovascular Diseases

Official Journal of the



Japan
Stroke
Society

This Issue

The Application of Cycling and Cycling Combined with Feedback in the Rehabilitation of Stroke Patients: A Review

Do Octogenarians Still Have a High Risk of Adverse Outcomes after Carotid Endarterectomy in the Era of a Super-aged Society? A Single-center Study in Japan

Paradoxical Brain Embolism Associated with Kimura Disease Mimics Watershed Infarction

ELSEVIER

Combined Exposure to Hypercapnia and Hypoxia Provides Its Maximum Neuroprotective Effect During Focal Ischemic Injury in the Brain

Pavel Tregub, PhD,* Vladimir Kulikov, MD,* Yuri Motin, PhD,†
Andrey Beshpalov, PhD,* and Ilya Osipov, PhD*

Background: In the present research, we compared the neuroprotective efficiency of combined and isolated exposure to hypoxia and hypercapnia preceding focal cerebral ischemic injury in rats. The study was conducted to verify the hypothesis of a possible increase in normobaric hypoxia (NbH; 90 mm Hg) efficiency when combined with permissive hypercapnia (PH; 50 mm Hg). **Methods:** The rats from the test groups were subjected to a 15-fold exposure to NbH (90 mm Hg) and/or PH (50 mm Hg). After the 15th exposure, cerebral ischemic injury was induced by photochemical thrombosis. Seventy-two hours later, neurologic deficit was determined on the Neurological Severity Score scale and by the rotarod test, and the volume of cerebral infarction was measured after focal photochemical thrombosis. **Results:** The neurologic deficit decreased most efficiently in rats that underwent PH and hypercapnic hypoxia (HH) exposure, whereas NbH had no impact on the neurologic status of the animals. On the contrary, motor coordination disturbances were minimal during exposure to hypoxia and HH. All respiratory interventions reduced the cerebral ischemic infarction volume in rats. The smallest infarction volumes were registered in the area of photochemical thrombosis in rats from the hypercapnic-hypoxic impact group, whereas exposure to NbH or PH did not show any cross difference. **Conclusions:** The impact of PH has greater neuroprotective potential compared with NbH. Thus, we can assume that hypercapnia is a predominant factor in providing neuroprotection in combination with hypoxia. **Key Words:** Focal stroke—neuroprotection—hypoxia—hypercapnia—hypercapnic hypoxia.

© 2015 by National Stroke Association

Introduction

Hypoxia is an effective means to increase the tolerance of organs and tissues to acute oxygen deficiency^{1,2} and ischemic damage.³⁻⁵

There are different variants to apply hypoxic exposure, hypoxic pretreatment with reoxygenation intervals showing the highest protective effect.⁵⁻⁷ However, such impacts possess a common disadvantage, that is, a continuous exposure and/or a large number of sessions are necessary; as a rule, it means at least seven sessions of 1- to 15-hour exposure to hypoxic impact.⁷

To date, there has been a significant increasing interest in studies of the therapeutic effect of permissive hypercapnia (PH), and carbon dioxide has been shown to have a protective impact on the brain during ischemic injury.⁸⁻¹⁰

From the *Department of Pathophysiology, Federal Agency for Health and Social Development, Altai State Medical University, Barnaul, Altai Region, Russia; and †Department of Histology, Federal Agency for Health and Social Development, Altai State Medical University, Barnaul, Altai Region, Russia.

Received April 30, 2014; revision received August 22, 2014; accepted September 5, 2014.

Address correspondence to Pavel Tregub, PhD, Department of Pathophysiology, Federal Agency for Health and Social Development, Altai State Medical University, 40 Lenin Prospekt, Barnaul, Altai Region, Russia. E-mail: pfiza_asmu@mail.ru.

1052-3057/\$ - see front matter

© 2015 by National Stroke Association

<http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2014.09.003>

Earlier, we found that the impact of combined exposure to hypoxia and hypercapnia, compared with their isolated effects, increases the acute hypoxia resistance of the organism at most and reduces neurologic disorders during subtotal cerebral ischemia more effectively.^{11,12} However, focal ischemic injury is mostly observed in the brain in clinical conditions,¹³ and no research devoted to the study of neuroprotective efficiency of hypercapnic hypoxia (HH) with this pathology has been conducted previously. Moreover, no study exists in which the neuroprotective efficiency of combined hypercapnia and hypoxia is proved by objective histologic data.

Thus, the objectives of the present study were to compare the neuroprotective efficiency of the impact of isolated and combined hypoxia and hypercapnia preceding focal cerebral ischemic injury.

Methods

All experimental procedures carried out were approved by the local Animal Protection Committee of the Medical University in Barnaul. The experiments were done on 60 adult male Wistar rats (240-320 g, about 8-9 months old; Cytology and Genetics Institute SD of RAMS, Novosibirsk, Russia). The animals were randomized with the use of the Statistical Package for the Social Sciences (SPSS) 11.5 software (SPSS Inc, Chicago, IL). All rats were preoperatively housed in standard cages under controlled indoor temperature ($\sim 23^{\circ}\text{C}$) and natural daylight conditions. The rats had free access to food and water. The animals were weighed before and after the experiment.

Animal Groups and Experimental Design

The study included four groups of even number of rats. The groups varied in partial pressure of oxygen (PO_2) and partial pressure of carbon dioxide (PCO_2):

1. Normobaric hypoxia (NbH) group (PO_2 90 mm Hg, PCO_2 1 mm Hg; the rest N_2): rats breathed a gas mixture for 20 minutes daily to maintain PO_2 within 90 mm Hg.
2. PH group (PO_2 150 mm Hg, PCO_2 50 mm Hg; the rest N_2): rats breathed a gas mixture for 20 minutes daily, but PO_2 remained within 150 mm Hg and PCO_2 within 50 mm Hg.
3. HH group (PO_2 90 mm Hg, PCO_2 50 mm Hg; the rest N_2): rats breathed a gas mixture for 20 minutes daily, but PO_2 remained within 90 mm Hg and PCO_2 within 50 mm Hg.
4. Reference (R) group (PO_2 150 mm Hg, PCO_2 1 mm Hg; the rest N_2): rats were exposed to all experimental procedures, excluding exposure to hypoxia and/or hypercapnia.

The rats in all groups underwent a 15-session course of respiratory intervention assigned for each group. The day after completion of the intervention course, focal cerebral

ischemic injury was modeled on all the rats. Seventy-two hours after modeling a focal stroke, the rats underwent estimation of neurologic disorders by means of the rotarod test¹⁴ and the scale of neurologic deficit Neurological Severity Score (NSS).¹⁵ Then, the brain of the rats was removed for histologic study and morphometric analysis of the volume of ischemic infarction.

Respiratory Intervention

For respiratory intervention, a flow-type chamber previously described was used.^{11,12} Experimental groups of rats breathed a gas mixture with a composition dependent on the prescription for the group. The R group was placed in a chamber with similar conditions, except that instead of a gas mixture atmospheric air was pumped in by a compressor. The gas composition in the chamber was controlled with a Mikon gas analyzer (Laspek Ltd, Novosibirsk, Russia).

Surgical Manipulation and Photochemical Thrombosis

Surgical Procedure

The day before surgery, the animals were deprived of food but had free access to water. The animals were preoperatively weighed. Each rat received anesthesia by intraperitoneal injection of a mixture of ketamine/xylazine (75 mg ketamine $^{\circ}$ + 10 mg xylazine per 1 kg body weight). A sterile incision was made in the left inguinal region. A sterile catheter was placed into the left femoral vein to infuse a 4% solution of rose bengal (Sigma-Aldrich, Munich, Germany) in .9% NaCl. Rose bengal was injected into rats for 1 minute at a dose of 40 mg/kg body weight. Temperature gauges were positioned in the rectum of each animal. Rectal temperature was maintained at a constant value of $37.5^{\circ} \pm .2^{\circ}\text{C}$ with the help of a servo-controlled heat plate placed on the surgical table and adjusted by a thermostat. The plate kept the table warm during the surgical and postsurgical periods.

Focal Ischemic Injury

Ischemic injury of the right sensorimotor cortex was carried out according to the transcranial photochemical thrombosis technique.¹⁶⁻¹⁹ A scalp incision was made, the periosteum was cleared, and cranial bones were illuminated by a green laser with 532-nm wavelength and 15-mW power for 10 minutes. A region of parietal bone with a diameter of 2 mm was illuminated on the skull. The region was positioned between the bregma and lambdoid suture and 2 mm lateral to the sagittal suture.

Histology and Measurement of Infarct Volume

Histologic Study

After neurologic status assessment, all animals were decapitated. The brains were carefully removed and

prepared for 24 hours in a solution consisting of 10% buffered paraformaldehyde, 96% ethanol, and ice acetic acid at a ratio of 2:7:1 (First Laboratory Company Ltd, St. Petersburg, Russia).

Nissl Staining

The brains were mounted and dehydrated by successive placement into an ethanol solution of increasing concentration, after which they were embedded in paraffin blocks. The blocks were then cut into 10- μ m-thick sections with an infarction intersection gap of 300 μ m. The sections were colored with toluidine blue (First Laboratory Company Ltd) according to the Nissl method and sealed in neutral plastic for observation under a MIKMED 6 light microscope (LOMO Ltd, Moscow, Russia).

Infarct Volume

The sections were photographed, and the area of ischemic damage in each section was measured by an investigator blinded to the experimental group. Micrographs were processed with the help of the ImageJ 1.41 software (National Institutes of Health, Bethesda, MD). The infarct volume was assessed according to the following formula: $V = x \times t \times S$, where x is the section rate, t is the serial section thickness, and S is the sum of the ischemic lesion focus areas.²⁰

Statistical Analysis

The size of the general and group samples was calculated based on the results of our previous researches on a similar model according to the quantitative scale method.²¹ Statistical analysis was carried out using the SPSS 11.5 software (SPSS Inc). The hypothesis of normalcy of distribution was confirmed with the help of the Shapiro-Wilk test. Some data did not follow the normal distribution law. Thus, the groups were compared by using a density-free Mann-Whitney test. Differences with the power of the statistical analysis (P) less than .05 were considered statistically significant. The data are presented as median ($\pm$ [25-75] percentiles). Only the data on the animals that had undergone all the experimental procedures in full were subjected to statistical analysis.

Results

The body weight before the experiment, before surgery, and after the experiment did not vary among the animals of all groups (data not given). Some of the animals died during the surgical procedures for modeling focal ischemic injury. Postmortem examination revealed that the principal cause of mortality was cerebral edema. As a result, the rats used in the experiments were grouped as follows: R, $n = 11$; NbH, $n = 12$; PH, $n = 12$, and HH, $n = 15$.

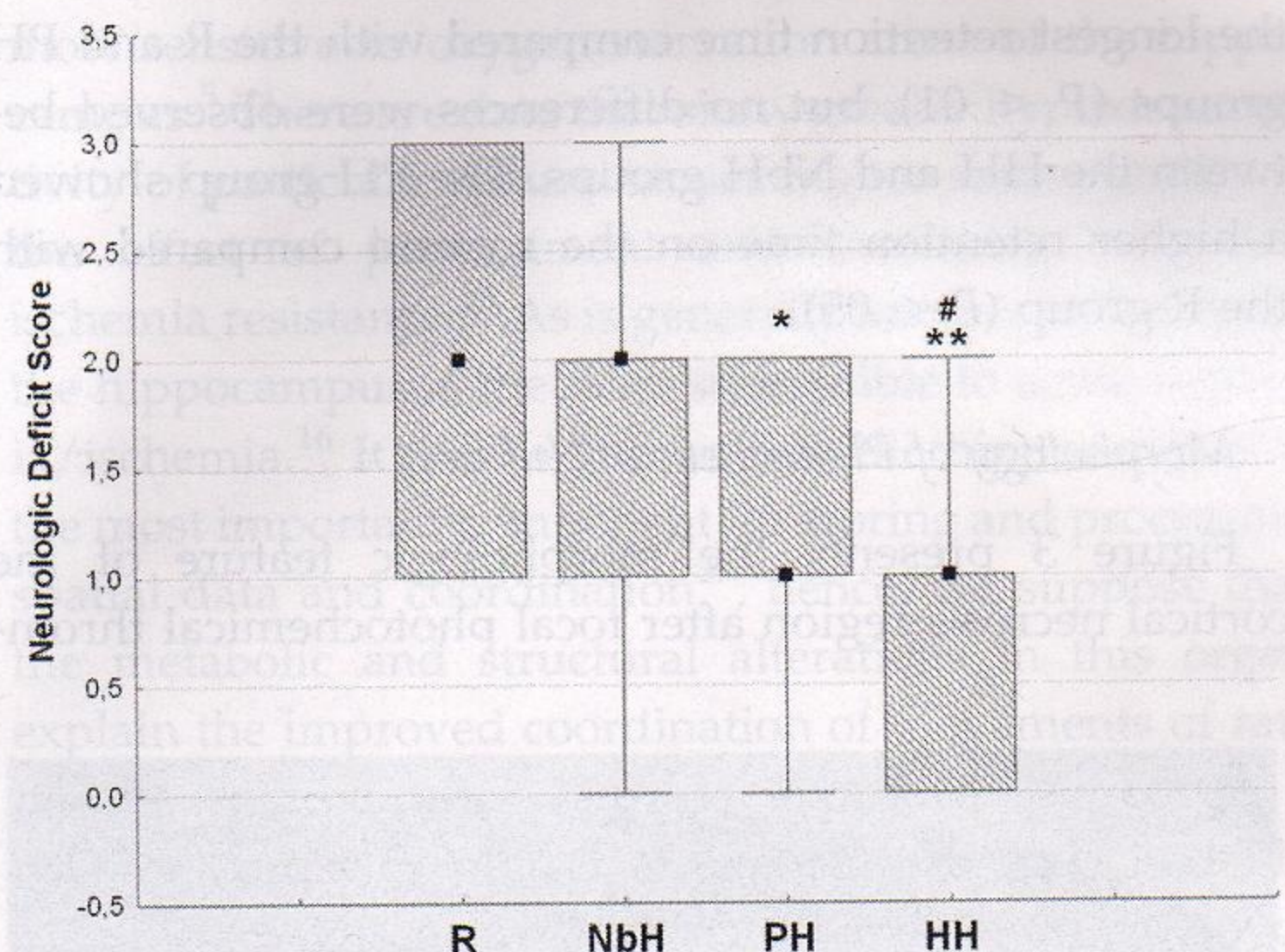


Figure 1. Neurologic deficit on the Neurological Severity Score (NSS) scale. The data are presented as median ($\pm$ [25-75] percentiles). * $P < .05$ difference from the R group, ** $P < .01$ difference from the R group, # $P < .01$ difference from the NbH group. Abbreviations: HH, hypercapnic hypoxia; NbH, normobaric hypoxia; PH, permissive hypercapnia; R, reference.

Neurologic Deficit

Figure 1 presents the neurologic deficit based on the NSS scale. The HH group showed the least neurologic deficit compared with the R and NbH groups ($P < .01$), but no differences were observed between the HH and PH groups. Rats of the NbH group did not show any difference from the R group. The PH group showed a lower neurologic deficit than the R group ($P < .05$) but did not vary from the NbH group.

Motor Coordination Disturbances in Rotarod Test

In all the experimental groups, a decrease in motor coordination disturbances and an increase in retention time on the rotarod were observed compared with the control group (Fig 2). The HH and NbH groups showed

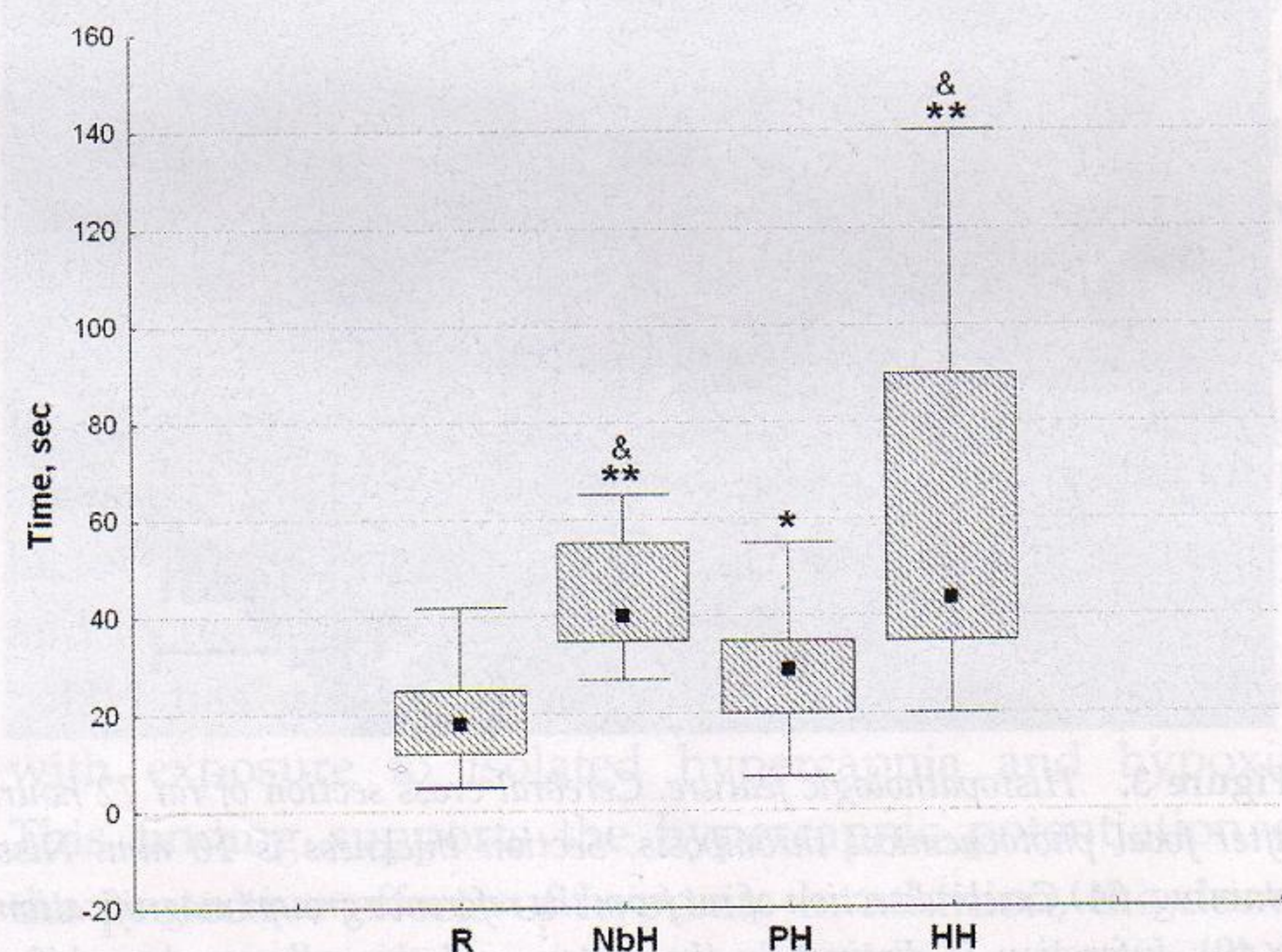


Figure 2. Retention time on bar in the rotarod test. The data are presented as median ($\pm$ [25-75] percentiles). * $P < .05$ difference from the R group, ** $P < .01$ difference from the R group, & $P < .01$ difference from the PH group. Abbreviations: HH, hypercapnic hypoxia; NbH, normobaric hypoxia; PH, permissive hypercapnia; R, reference.

the longest retention time compared with the R and PH groups ($P < .01$), but no differences were observed between the HH and NbH groups. The PH group showed a higher retention time on the rotarod compared with the R group ($P < .05$).

Morphology of Photochemical Infarcts

Figure 3 presents the morphologic feature of the cortical necrosis region after focal photochemical throm-

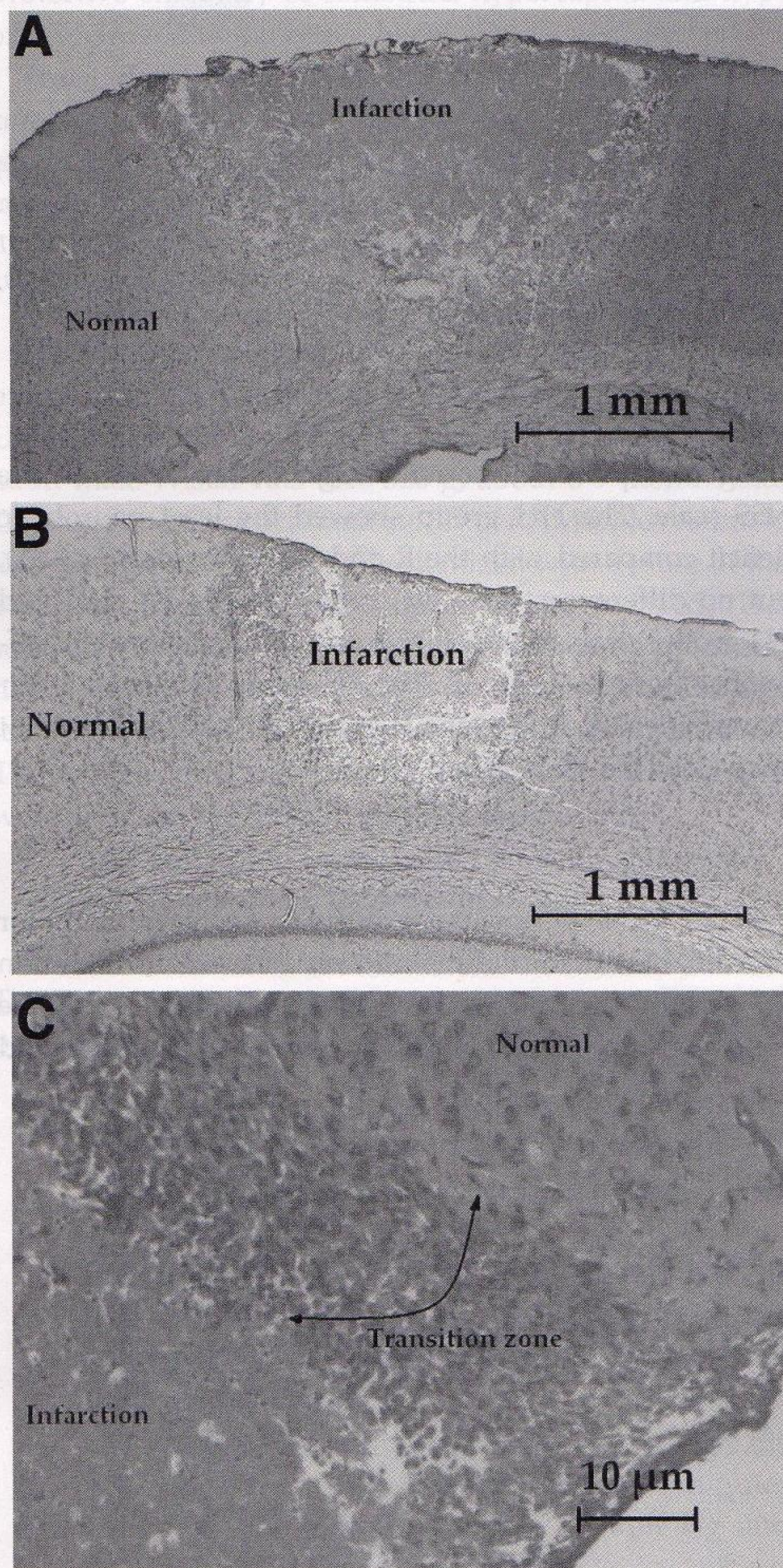


Figure 3. Histopathologic feature. Cerebral cross section of rat 72 hours after focal photochemical thrombosis. Section thickness is 10 mm. Nissl staining. (A) Cerebral section of rat from the reference group (magnification, $\times 40$). Infarction is distinct in the cortex, and the callosum has shifted because of the large infarction area. (B) Cerebral section of rat from the hypercapnic hypoxia group (magnification, $\times 40$). The morphologic alterations are similar, but the infarction area is significantly smaller. (C) Micrograph of transition zone (magnification, $\times 100$). Distinct borders are visible between the necrosis area, transition zone, and normal tissue.

bosis. Cerebral cross sections show distinct borders between the necrosis region, transition zone (Fig 3, C), and normal neural tissue, which allowed assessing the area of infarction on the sections numerically and calculating the infarct volume. In the R group (Fig 3, A), the infarction area could reach the callosum, which shifted position because of the damage expansion. The transition zone (penumbra) was formed by hyperchromic neurons. The NbH, PH, and HH groups showed a similar morphologic feature (Fig 3, B), but the infarction area was smaller and the callosum did not shift.

Infarct Volumetry

Figure 4 presents the results of infarct volumetry. The impact of combined hypoxia and hypercapnia in the HH group showed a maximum neuroprotective effect and reduced infarction by 45.7% compared with the R group ($P < .01$), by 31.5% compared with the NbH group ($P < .01$), and by 27% compared with the PH group ($P < .05$). Exposure to PH and NbH reduced cerebral infarction by 25% ($P < .01$) and 20.5% ($P < .05$), respectively, compared with the R group. However, there was no difference between the NbH and PH groups.

Discussion

In the present research, we compared the neuroprotective efficiency of combined and isolated exposure to hypoxia and hypercapnia preceding focal cerebral ischemic injury in rats. The study was conducted to verify the hypothesis of a possible increase in the NbH protective effect when combined with PH.

At present, when selecting a model of experimental cerebral ischemia, preference is given to focal ischemic injury methods because they present the clinical features

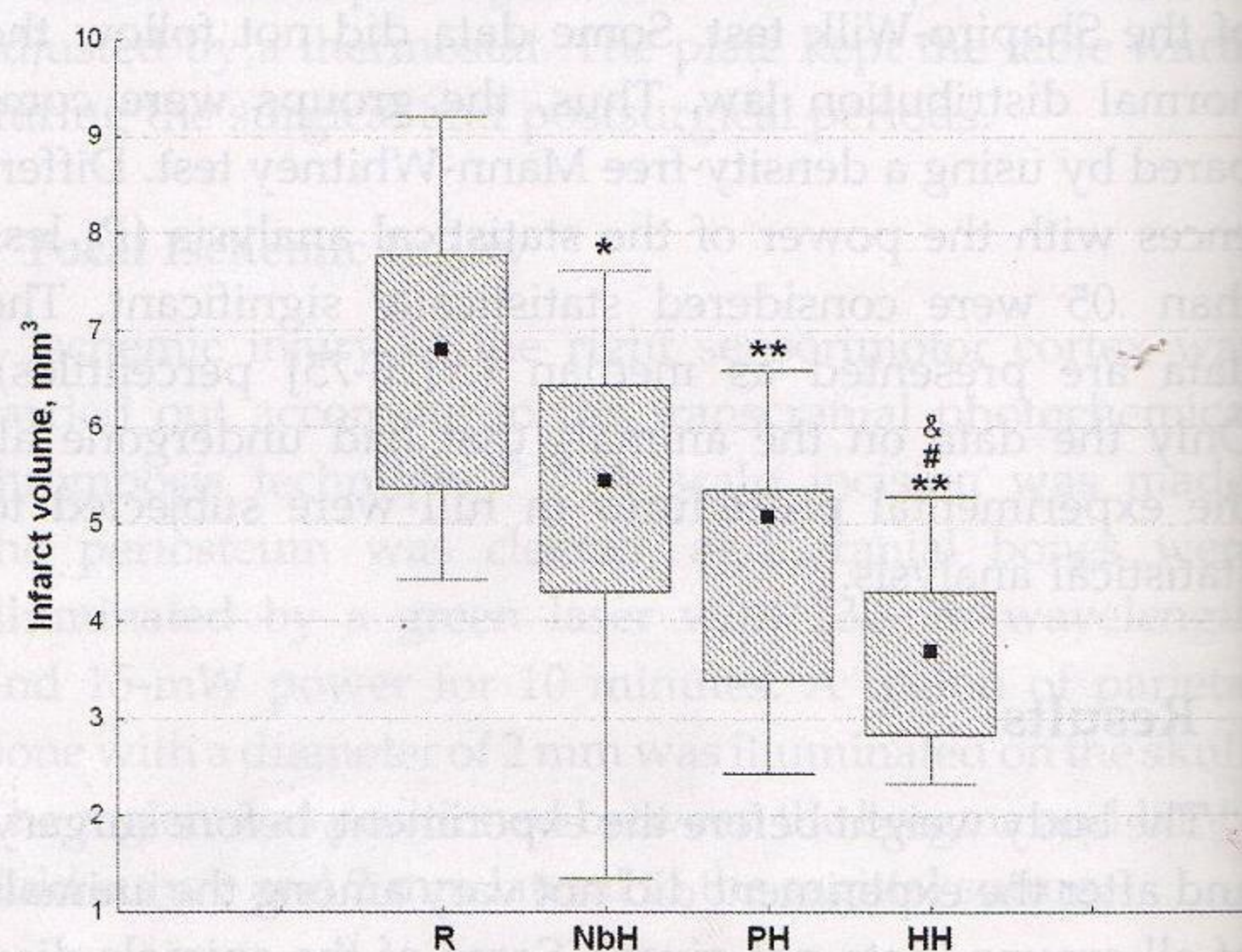


Figure 4. Cerebral infarction volume in rats. The data are presented as median ($\pm$ [25-75] percentiles). * $P < .05$ difference from the R group, ** $P < .01$ difference from the R group, # $P < .01$ difference from the NbH group, & $P < .05$ difference from the PH group. Abbreviations: HH, hypercapnic hypoxia; NbH, normobaric hypoxia; PH, permissive hypercapnia; R, reference.

of ischemic stroke most adequately.^{18,22} The principal requirement for such models includes producing a constant location and ischemic focus volume replicable in a series of experiments, which is essential for numerical assessment of the degree of cerebral damage and the protective effect of neuroprotector use. The noninvasive method of photochemical thrombosis of cortical vessels meets this requirement because it allows selecting the necessary localization.¹⁹ To assess the volume of ischemic damage with the use of the given model, numerical assessment of infarction volume^{23,24} and estimation of a normal and necrotizing tissue volume ratio^{18,25} are used equally.

All the animals in the present research received typical photochemical infarcts affecting all layers of the sensorimotor cortex. In the infarction zone, fibrous alterations and microvessels with thrombi were distinguished in all sections from the region of ischemic injury, which is typical of the photochemical thrombosis method.¹⁶⁻¹⁹ The morphologic feature was similar across all experimental groups, whereas differences were observed in the size of the cerebral infarction zone.

The neurologic manifestations of the focal cerebral infarct differed in the experimental groups exposed to combined and isolated hypercapnia and hypoxia. The neurologic deficit decreased most efficiently in rats that underwent PH and HH exposure, whereas NbH had no impact on the neurologic status of the animals. Different mortality rates in the experimental groups of animals generally show different intensity of the protective effect of combined and isolated exposure to hypoxia and hypercapnia. In the HH group, mortality rate was the lowest, whereas in the R group, the number of animals that died of cerebral edema was the highest.

It has been reported in the literature that hypoxia reduces neurologic damage during ischemic injury in the brain. However, the studies in which it was earlier demonstrated^{3,4} were conducted with the use of higher degrees of hypoxia (57-71 mm Hg) and longer exposure (1-6 hours) than in the present experiment. Besides, we were totally aware that the reduction in neurologic damage under less intensive conditions of exposure in the NbH group could have failed. However, the choice of lower hypoxia degree was conditional on such parameters, having shown high efficiency when combined with PH to prevent experimental ischemia in rats.¹² Neurologic status assessment data show that positive effects of PH prevail over hypoxia effects in their combination. On the contrary, motor coordination disturbances were minimal during exposure to NbH and HH, whereas in the group with PH, the disturbances were less reduced than during other respiratory interventions. This finding can be related to the lesser impact of carbon dioxide deficiency compared with oxygen deficiency on the coordination function of the brain because the hypoxic impact more quickly activates protection of the regions

most sensitive to oxygen-starvation areas of the hippocampus.⁵ There are data that show greater hypoxic sensitivity of particular tissues causes higher susceptibility to the effect of pretreatment and increasing hypoxia/ischemia resistance.²⁶ As is generally known, in the brain, the hippocampus is the most susceptible to acute hypoxia/ischemia.¹⁶ It is also known that the hippocampus is the most important component for storing and processing spatial data and coordination²⁷; hence, we suppose that the metabolic and structural alterations in this organ explain the improved coordination of movements of rats on the rotarod after hypoxic exposure. Moreover, a different protective effect of exposure to NbH and PH with improving neurologic status and coordination of movements shows that the highest protective effect of those factors combined requires further studies and presents a complicated natural phenomenon.

All respiratory interventions reduced the infarction volume in cerebral ischemic damage focus areas in rats. The smallest infarction volumes were registered in the area of photochemical thrombosis in rats from the hypercapnic-hypoxic impact group, whereas exposure to NbH or PH did not result in any reciprocal alterations. Thus, a conclusion can be made about the potentiation of the neuroprotective effects of the combined exposure.

The use of HH as a neuroprotector is not described in the scientific literature except in our study, which showed a reduction in neurologic disturbances during cerebral ischemia under the influence of hypercapnic-hypoxic exposure.¹² Carbon dioxide has been proven to have a neuroprotective impact on the cerebrum during hypoxia/ischemia injury⁹ and ischemia/reperfusion injury.^{8,10} Existing data show a positive impact of hypercapnia on mitochondrial K_{ATP} channels,²⁸ the antioxidative system,²⁹ angiogenesis,³⁰ and pulmonary circulation.³¹ Other data indicate inhibition of apoptosis under the influence of PH.^{8,10} These support our hypothesis that hypercapnic potentiation of hypoxia increases neuroprotective efficiency during focal cerebral ischemic injury. The principal mechanisms of these hypoxic effects possibly include an increase in messenger RNA expression in the early gene family,³² activation of mitochondrial K_{ATP} channels under the influence of protein kinase C,³³ activation of succinate oxidase oxidation,³⁴ and genome reprogramming by redox-sensitive protein hypoxia-inducible factor-1 α ,³⁵ modulating of cerebrovascular reactivity, and increase of perfusion in the brain.³⁶

HH has greater neuroprotective potential compared with exposure to isolated hypercapnia and hypoxia. This finding supports the hypercapnic potentiation of the protective effects of hypoxia. In addition, the dominant factor is hypercapnia, given that its protective effect exceeded that of NbH, as shown in the assessment of neurologic disturbances. The absence of difference between HH and NbH on the rotarod, as demonstrated in our research, does not contradict this statement. To our

opinion, the results of the rotarod are less significant for interpretation than the data of neurologic status assessment and measurement of infarct volume in the brain. Rotarod test shows only motor-coordinating disturbances, whereas neurologic status assessment includes the determination of neural sensitivity, reflexes, motional, and behavioral activity, and reduction in the size of necrosis area visually proves the neuroprotective effect.

Acknowledgment: We wish to thank Dr. Igor Barskov, Nikolay Rucheykin, and Ekaterina Belova for great help in the work on this study.

References

1. Agadzhanyan NA, Bragin LKh, Davydov GA, et al. Dynamics of external respiration and gas exchange during combined exposure to hypoxia and hypercapnia. *Hum Physiol* 1984;10:282-288.
2. Meerson FZ, Ustinova EE, Orlova EH. Prevention and elimination of heart arrhythmias by adaptation to intermittent high altitude hypoxia. *Clin Cardiol* 1987;10:783-789.
3. Chen WJ, Chen HW, Yu SL, et al. Gene expression profiles in hypoxic preconditioning using cDNA microarray analysis: altered expression of an angiogenic factor, carcinoembryonic antigen-related cell adhesion molecule 1. *Shock* 2005;24:124-131.
4. Neckar J, Papousek E, Novakova O, et al. Cardioprotective effects of chronic hypoxia and ischemic preconditioning are not additive. *Basic Res Cardiol* 2002;97:161-167.
5. Yang CC, Lin LC, Wu MS, et al. Repetitive hypoxic preconditioning attenuates renal ischemia/reperfusion induced oxidative injury via upregulating HIF-1 alpha-dependent bcl-2 signaling. *Transplantation* 2009;88:1251-1260.
6. Glazychev OS. Optimization of clinical application of interval hypoxic training. *Med Tekh* 2013;3:21-24.
7. Lukyanova LD, Germanova EL, Kopaladze RA. Development of resistance of an organism under various conditions of hypoxic preconditioning: role of the hypoxic period and reoxygenation. *Bull Exp Biol Med* 2009;147:400-404.
8. Tao T, Liu Y, Zhang J, et al. Therapeutic hypercapnia improves functional recovery and attenuates injury via antiapoptotic mechanisms in a rat focal cerebral ischemia/reperfusion model. *Brain Res* 2013;1533:52-62.
9. Vannucci RC, Towfighi J, Heitjan DF, et al. Carbon dioxide protects the perinatal brain from hypoxic-ischemic damage: an experimental study in the immature rat. *Pediatrics* 1995;95:868-874.
10. Zhou Q, Cao B, Niu L, et al. Effects of permissive hypercapnia on transient global cerebral ischemia-reperfusion injury in rats. *Anesthesiology* 2010;112:288-297.
11. Tregub P, Kulikov V, Besspalov A. Tolerance to acute hypoxia maximally increases in case of joint effect of normobaric hypoxia and permissive hypercapnia in rats. *Pathophysiology* 2013;20:165-170.
12. Tregub PP, Kulikov VP, Besspalov AG, et al. Neuroprotective effects of individual or combined exposure to hypoxia and hypercapnia in the experiment. *Bull Exp Biol Med* 2013;155:327-329.
13. Glazier SS, O'Rourke DM, Graham DI, et al. Induction of ischemic tolerance following brief focal ischemia in rat brain. *J Cereb Blood Flow Metab* 1994;14:545-553.
14. Balduini W, De Angelis V, Mazzoni E, et al. Long-lasting behavioral alterations following a hypoxic/ischemic brain injury in neonatal rats. *Brain Res* 2000;859:318-325.
15. Chen J, Li Y, Wang L, et al. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 2001;32:1005-1011.
16. Barth AM, Mody I. Changes in hippocampal neuronal activity during and after unilateral selective hippocampal ischemia in vivo. *J Neurosci* 2011;31:851-860.
17. Chen F, Suzuki Y, Nagai N, et al. Rat cerebral ischemia induced with photochemical occlusion of proximal middle cerebral artery: a stroke model for MR imaging research. *MAGMA* 2004;17:103-108.
18. Pevsner PH, Eichenbaum JW, Miller DC, et al. A photothrombotic model of small early ischemic infarcts in the rat brain with histologic and MRI correlation. *J Pharmacol Toxicol Methods* 2001;45:227-233.
19. Watson BD, Dietrich WD, Busto R, et al. Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann Neurol* 1985;17:497-504.
20. De Ryck M, Van Reempts J, Borgers M, et al. Photochemical stroke model: flunarizine prevents sensorimotor deficits after neocortical infarcts in rats. *Stroke* 1989;20:1383-1390.
21. Dell RB, Holleran S, Ramakrishnan R. Sample size determination. *ILAR J* 2002;43:207-213.
22. Jackman K, Kunz A, Iadecola C. Modeling focal cerebral ischemia in vivo. *Methods Mol Biol* 2011;793:195-209.
23. Keiner S, Wurm F, Kunze A, et al. Rehabilitative therapies differentially alter proliferation and survival of glial cell populations in the perilesional zone of cortical infarcts. *Glia* 2008;56:516-527.
24. Liebigt S, Schlegel N, Oberland J, et al. Effects of rehabilitative training and anti-inflammatory treatment on functional recovery and cellular reorganization following stroke. *Exp Neurol* 2012;233:776-782.
25. Kleinschnitz C, Braeuninger S, Pham M, et al. Blocking of platelets or intrinsic coagulation pathway-driven thrombosis does not prevent cerebral infarctions induced by photothrombosis. *Stroke* 2008;39:1262-1268.
26. Lukyanova LD, Kirova YI. Effect of hypoxic preconditioning on free radical processes in tissues of rats with different resistance to hypoxia. *Bull Exp Biol Med* 2011;151:292-296.
27. O'Keefe J, Dostrovsky J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res* 1971;34:171-175.
28. Lindauer U, Vogt J, Schuh-Hofer S, et al. Cerebrovascular vasodilation to extraluminal acidosis occurs via combined activation of ATP-sensitive and Ca²⁺-activated potassium channels. *J Cereb Blood Flow Metab* 2003;23:1227-1238.
29. Zakyntinos S, Katsaounou P, Karatza MH, et al. Antioxidants increase the ventilatory response to hyperoxic hypercapnia. *Am J Respir Crit Care Med* 2007;175:62-68.
30. Siafakas NM, Jordan M, Wagner H, et al. Diaphragmatic angiogenic growth factor mRNA responses to increased ventilation caused by hypoxia and hypercapnia. *Eur Respir J* 2001;17:681-687.
31. Chuang IC, Dong HP, Yang RC, et al. Effect of carbon dioxide on pulmonary vascular tone at various pulmonary arterial pressure levels induced by endothelin-1. *Lung* 2010;188:199-207.

32. Das DR, Maulink N. Cardiac-genomic response following preconditioning stimulus. *Cardiovasc Res* 2006; 70:254-263.
33. Murphy E. Primary and secondary signaling pathways in early preconditioning that converge on the mitochondria to produce cardioprotection. *Circ Res* 2004; 94:7-16.
34. De Silva MM, Sartori A, Belisle E, et al. Ischemic preconditioning inhibits mitochondrial respiration, increase H_2O_2 release, and enhances K^+ transport. *Am J Physiol Heart Circ Physiol* 2003;285:1154-1162.
35. Lukyanova LD. Current issues of adaptation to hypoxia. Signal mechanisms and their role in system regulation. *Patol Fiziol Eksp Ter* 2011;1:3-19.
36. Kulikov VP, Besspalov AG, Yakushev NN. The state of cerebral hemodynamics in conditions of prolonged adaptation to hypercapnic hypoxia. *Neurosci Behav Physiol* 2009;39:269-273.

H₂O₂ release, and enhances K⁺ transport. *Am J Physiol Heart Circ Physiol* 2003;285:1154-1162.

35. Lukyanova LD. Current issues of adaptation to hypoxia. Signal mechanisms and their role in system regulation. *Patol Fiziol Eksp Ter* 2011;1:3-19.

36. Kulikov VI, Bespalov AG, Yakushev NN. The state of cerebral hemodynamics in conditions of prolonged adaptation to hypercapnic hypoxia. *Neurosci Behav Physiol* 2009;39:269-273.