Journal of Integrative Neuroscience

J. Integr. Neurosci. **2026**; 25(7): 51502
https://doi.org/10.31083/JIN51502

Original Research

The Efficacy of Experimental Therapy for Stroke Induced by Cerebral Air Embolism in Awake Rats Is Affected by Heliox Inhalation Temperature

Arina Kholina[1], Alina Ismailova[1], Victor Palikov[1,2,3,4,5], Natalya Borozdina[1,4,*], Sergey Kozlov[2], Olga Patsap[6], Michael Ignatyuk[6], Dmitry Rzhevsky[1], Gulsara Slashcheva[1], Elvira Shaykhutdinova[1], Igor Dyachenko[1,5], Arkady Murashev[1,4], Nikolay Pavlov[3], Victor Baranov[3]

[1]Department of Biological Testing, Branch of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 142290 Pushchino, Russian Federation
[2]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, 117997 Moscow, Russian Federation
[3]Institute of Biomedical Problems of the Russian Academy of Sciences, 123007 Moscow, Russian Federation
[4]Hospital of the Pushchino Scientific Center of the Russian Academy of Sciences, 142290 Pushchino, Russian Federation
[5]Pushchino Branch of the Federal State Budgetary Educational Institution of Higher Education «Russian Biotechnological University (BIOTECH University)», 142290 Pushchino, Russian Federation
[6]Patrice Lumumba Peoples' Friendship University of Russia, 117198 Moscow, Russian Federation
*Correspondence: borozdina@bibch.ru (Natalya Borozdina)
Academic Editor: Bettina Platt
Submitted: 4 March 2026 Revised: 22 May 2026 Accepted: 29 May 2026 Published: 29 July 2026

Abstract

Background: Previous studies have shown that Heliox, a mixture of 32% oxygen and 68% helium administered at a warm temperature, is effective in treating ischemic brain injury caused by cerebral arterial air embolism. At the same time, therapeutic hypothermia has been shown to lead to a favorable neurological outcome in patients in the acute phase of ischemic stroke; however, there are no published data on the use of Heliox at room temperature after ischemic stroke. All published data indicate only the efficacy of heated Heliox. The most effective temperature regimen for Heliox inhalation to prevent neurological deficits in stroke remains unknown. **Methods:** Three Heliox inhalation temperature regimens were studied at 20–22 °C, 40–50 °C, and 60–70 °C. The mixture was administered immediately after inducing ischemic stroke in awake rats using a model of cerebral arterial air embolism. Respiratory and cardiovascular function, as well as body temperature, coordination, muscle strength, and serum calcium binding protein B (S100B) concentrations were studied 24 hours after embolization. A brain histopathological study was also performed. **Results:** Inhalation of heated Heliox (40–50 °C and 60–70 °C) immediately after cerebral arterial air embolism was shown to be an effective method for maintaining respiratory and cardiovascular function and body temperature, for preventing the development of foci of ischemic brain damage on 2,3,5-triphenyltetrazolium chloride (TTC)-stained sections of the brain, and for partially preserving coordination, muscle strength, locomotor activity, and for a partial reduction in serum S100B concentration during the acute phase of ischemic stroke in rats compared to the untreated group with cerebral arterial air embolism (CAE). Unheated Heliox had a negative effect on experimental animals, and decreased a survival rate to 62.5%. **Conclusions:** Heated Heliox has an equally positive effect at temperatures of 40–50 °C and 60–70 °C, and significantly alleviated the symptoms of ischemic stroke during the acute phase. In contrast, unheated Heliox at room temperature (20–22 °C) had a negative effect on the course of ischemic stroke in rats.

Keywords: mixture of helium and oxygen; embolism; ischemic stroke; S100B protein; Sprague-Dawley rats

1. Introduction

Cerebral arterial air embolism (CAE) is a serious condition that can lead to a stroke. Maintaining oxygenation is an essential part of emergency treatment, directly impacting the reduction of neurological deficits and the success of rehabilitation. Hyperbaric oxygen therapy (HBOT) is the gold standard for CAE treatment, but its limited availability has prompted researchers to seek effective normobaric alternatives [1]. One such alternative is the use of an oxygen-helium mixture known as Heliox. A previous study has shown that, when heated, Heliox has a more pronounced and long-lasting neuroprotective effect in CAE than traditional HBOT [2]. Clinical observations also confirm the effectiveness of heated Heliox in the rehabilitation of post-stroke patients [3]. It is assumed that the neuroprotective effect of Heliox is not so much associated with hemodynamic changes, but rather with the restoration of mitochondrial respiration and decreased mitochondrial membrane permeability under [4]. Due to helium's high heat capacity, Heliox is usually administered in a heated form to prevent hypothermia [5,6]. However, hypothermia it-

self has been considered as a proven neuroprotective factor in stroke [7,8,9,10]. The exact mechanism by which hypothermia exerts its neuroprotective effects remains unknown, but it is believed to influence brain metabolism in multiple ways. These include slowing the breakdown of ATP, reducing glutamate release, and decreasing free radical formation [11,12]. This creates a dilemma. Heliox heating is technically necessary and its effectiveness has already been confirmed [2,3], but the hypothermia caused by the unheated mixture could theoretically also be beneficial.

There is no published data on the use of Heliox at ambient temperature in ischemic conditions. Therefore, the most effective temperature regimen for using Heliox to prevent neurological deficits in stroke remains unknown. The aim of this study is to evaluate the efficacy of Heliox at three different temperatures (20–22 °C, 40–50 °C, and 60–70 °C) in the emergency treatment of experimental stroke induced by CAE in awake rats.

2. Materials and Methods

2.1 Animals

The experiment involved 40 male Sprague-Dawley (SD) rats aged 8–9 weeks, with the mean body weight of 282 grams. Specific pathogen free animals were received from the laboratory of animal breeding center "Pushchino" (Scientific production association "Nursery of laboratory animals" of the M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences). Animal study was supported by Bioresource Collection – Collection of SPF-Laboratory Rodents for Fundamental, Biomedical and Pharmacological Studies #075-15-2025-486.

Before the experiment, the animals were acclimatized for 14 days. During the acclimatization period and the experiment, the animals were kept in the two corridors barrier-type test facility with the automatic change of day and night time (08:00–20:00 - "Day", 20:00–08:00 - "Night") and the renewal of the room air at least 12 times hourly. Temperature (20 °C–24 °C) and humidity (45%–65%) were continuously monitored in each animal housing room automatically using a computerized Eksis Visual Lab system (Practik-NC, Moscow, Russian Federation). Standard Diet for laboratory mice and rats Mucedola 4RF21 autoclavable (Mucedola s. r.l., Via G. Galelei, 4–20019 Settimo Milanese MI, Italy) and filtered tap water (MilliRO, Millipore, Germany) were given *ad libitum*. Animals were housed in cages with red transparent polycarbonate tents (Tecniplast S.P.A., Buguggiate, Italy) and nesting material (Lignocel Nesting Ball, JRS, Rosenberg, Germany).

2.2 Study Design

The groups were randomly assigned based on body weight using a block design. The Sample Size Calculator (ClinCalc LLC, Chicago, IL, USA, https://clincalc.com/Stats/SampleSize.aspx) was used to set the group size at 80% power and 5% alpha values for an unpaired *t*-test.

CAE modeling was performed on awake animals from groups 2–5. Immediately after CAE modeling, the animals in groups 3–5 were placed in an inhalation apparatus and exposed to Heliox inhalation at different temperatures: unheated Heliox (HU) at 20–22 °C, heated Heliox at 40–50 °C (HH50), and heated Heliox at 60–70 °C (HH70). The control group (group 1) was only subjected to catheterization.

The animals underwent clinical examination before catheterization and daily thereafter. Only animals without signs of clinical abnormalities were selected for the experiment. Clinical examinations were performed twice daily during the experiment to detect conditions requiring immediate euthanasia, such as weight loss exceeding 20%, labored breathing, or paralysis after CAE. In the CAE+HU group, three animals died 24 hours after CAE (see Fig. 1B). The results obtained from these three animals are presented at the time points "baseline" and "3 hours after CAE" for the following parameters: body weight, body temperature, neurological deficit score, minute respiratory volume, pulse, systolic pressure, time on the rod in the Rota-Rod test, forelimb grip strength, and vertical and horizontal activity in the open field. These results were also used in the statistical analysis at these time points. The calcium binding protein B (S100B) levels of these three animals are presented only at the baseline time point. At the time point '24 hours after CAE', data from the five to eight deceased animals were used solely to assess neurological deficit score. In the neurological deficit results, these animals received a score of 5, which corresponds to death (Section 2.6). The neurological deficit scores of these three animals 24 hours after CAE were included in the statistical analysis to fully reflect the pathophysiological progression of ischemic stroke. All animals that survived until the end of the experiment were included in the histological analysis. Three deceased animals were excluded from the histological analysis.

The animals were evaluated for physiological and functional parameters 3 and 24 hours after CAE. Twenty-four hours later, the animals were euthanized, necropsied, and examined for brain lesions. Blood samples were collected to measure S100B concentration. The researchers who recorded the animal parameters and performed the histopathological analysis were blinded to group assignment. The study director (VP) randomized the animals for recording of the physiological and functional parameters at each time point. The other researchers who recorded the parameters were blinded to the group assignment. All measurements were performed individually for each animal. The physiological and functional measurements were taken from 10:00 AM to 1:00 PM. The data obtained from the animals after catheterization and before CAE modeling were consistent with baseline results.

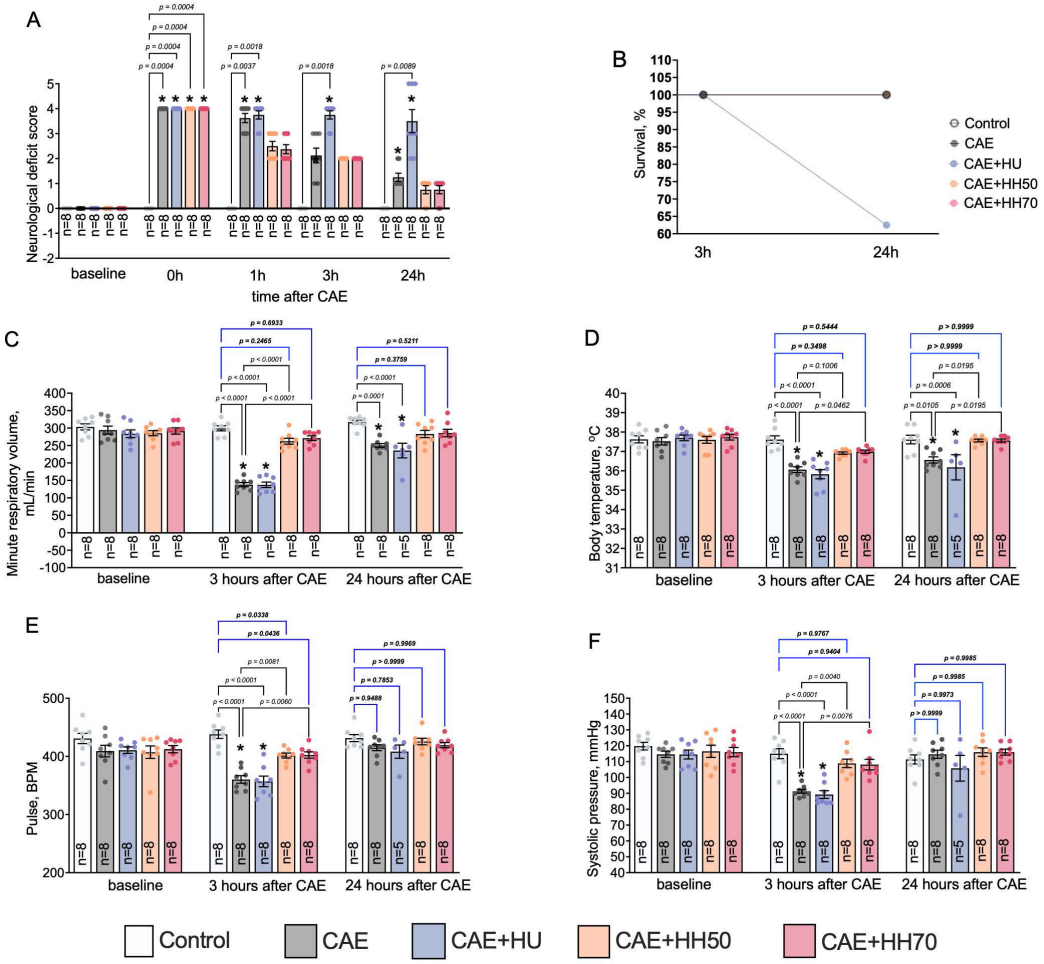

Fig. 1. Physiological parameters before CAE, 3 hours and 24 hours after CAE. (A) The neurological deficit score was significantly increased after CAE modeling compared to the baseline in all groups with CAE (n = 8, Friedman test with post-hoc Dunn's test) and the Control group (n = 8, Kruskal-Wallis test with post-hoc Dunn's test). HH50 and HH70 (n = 8) led to a significant decrease of deficit score, whereas HU did not (n = 8, Kruskal-Wallis test with post-hoc Dunn's test). (B) The deaths of three animals were observed only in the group with HU, 24 hours after CAE, prior to functional parameters registration. (C) The minute respiratory volume was significantly decreased at 3 hours and 24 hours after CAE modeling in the CAE group (n = 8) and CAE+HU group (n = 8 at 3 hours and n = 5 at 24 hours) compared to the baseline (mixed-effects ANOVA with post-hoc Dunnett test) and the Control group (one-way ANOVA with post-hoc Tukey test), while heated HH50 and HH70 reduced respiratory function depression (n = 8, one-way ANOVA with post-hoc Tukey test). HU has not improved respiratory function upon CAE modeling (n = 8, one-way ANOVA with post-hoc Tukey test). (D) Body temperature was significantly decreased at 3 and 24 hours after CAE modeling in the CAE group compared to the baseline (n = 8, mixed-effects ANOVA with post-hoc Dunnett test) and the Control group (n = 8, one-way ANOVA with post-hoc Tukey test), HH50 and HH70 (n = 8) fully restored body temperature 24 hours after CAE, unlike HU (n = 5, one-way ANOVA with post-hoc Tukey test). (E) Pulse and (F) Systolic pressure were significantly decreased at 3 and 24 hours after CAE modeling compared to the baseline (mixed-effects ANOVA with post-hoc Dunnett test) and the Control group (one-way ANOVA with post-hoc Tukey test), HH50 and HH70 had a protective effect on cardiovascular function in all animals 3 hours and 24 hours after CAE modeling (n = 8, one-way ANOVA with post-hoc Tukey test). Significant differences between groups in one-way ANOVA with post-hoc Tukey test are marked above the individual values with indication of *p*-values. Non-significant differences between groups in one-way ANOVA with post-hoc Tukey test are highlighted with a blue connecting line, *p*-values are shown in bold. **p* < 0.05 compared to the baseline of the group in mixed-effects ANOVA with post-hoc Dunnett test. Data are shown as individual values, group means, and SEM. The number of animals in each group is presented on the bars. Abbreviations: CAE, cerebral arterial air embolism; ANOVA, Analysis of Variance; BPM, beats per minute; HU, unheated Heliox; HH50, heated up to 40–50 °C Heliox; HH70, heated up to 60–70 °C Heliox.

Table 1. Study groups.

Group	Catheterization	CAE modeling	Heliox inhalation	Number of animals at a baseline (after catheterization)
(1) Control	+	-	-	8
(2) CAE	+	24 h after catheterization	-	8
(3) CAE+HU (Unheated Heliox)	+		Immediately after CAE modeling, at a temperature 20–22 °C	8
(4) CAE+HH50 (Heliox Heated up to 40–50 °C)	+		Immediately after CAE modeling, at a temperature 40–50 °C	8
(5) CAE+HH70 (Heliox Heated up to 60–70 °C)	+		Immediately after CAE modeling, at a temperature 60–70 °C	8

2.3 Catheterization

Before CAE modeling, all animals underwent catheterization of the internal carotid artery. The animals were anesthetized with an intramuscular injection of Telazol® (672913, Zoetis Inc., Parsippany, NJ, USA) at a dose of 30 mg/kg and Xyla® (0151, Interchemie Werken "De Adelaar" B.V., Venray, Netherlands) at a dose of 10 mg/kg, and then catheterized. Anesthesia was administered once per study. The animal was considered anesthetized when it showed no corneal reflex and did not respond to tail pinching. Then, a section of the external carotid artery was isolated and secured with two ligatures. Then, an incision was made in the vessel between the ligatures using vascular scissors. The incision formed an opening in the vessel that was spread apart with vascular forceps. Then, a catheter pre-filled with 50 U of heparin (30122, Endopharm. Moscow Endocrine Plant, Moscow, Russian Federation) diluted in 0.9% NaCl was inserted through the opening. After releasing the distal ligature, the catheter was pushed further along the vessel until it entered the common trunk of the carotid artery. Then, the catheter was secured with a shock-absorbing ring. The ligatures were tightened and tied. The catheter was brought out to the withers. The outer catheter sections were 3.5–4.0 cm long. The ventral wound and the tightly fitted dorsal skin around the catheters were sutured. Five groups of eight animals were formed for the experiment after catheterization (Table 1).

2.4 Cerebral Arterial Embolism Modeling

Twenty-four hours after catheterization, CAE was modeled in animals of groups 2–5 using the method described in [13]. In brief, an awake animal was placed in a restrainer. An adapter, which was an additional 100-µL catheter, was connected to the catheter at the rat's withers. The system was then connected to a syringe filled with 0.9% NaCl through the adapter. The syringe was placed in an Aladdin AL-1000 infusion pump (AL-1000, World Precision Instruments, Sarasota, FL, USA). Air was supplied through the catheter at a rate of 10 µL per minute, for a total volume of 100 µL per animal.

2.5 Heliox Treatment

Immediately after CAE modeling, the animals in all groups were placed in the Laboratory Inhalation Complex (LIC) for supplying gas mixtures, which was developed by the Specialized Design Bureau of Experimental Equipment at the Institute of Medical and Biological Problems of the Russian Academy of Sciences. In the groups 3–5, a ready-to-use inhalation mixture of Heliox (32% O_2/68% He) was supplied from a cylinder at a rate of at least 0.5 L/min per individual restrainer house with an animal. The gas was delivered uniformly through a diffuser directly into the individual restrainer houses after passing through a heating circuit. The circuit heated the gas to 40–50 °C for the CAE+HH50 group and 60–70 °C for the CAE+HH70 group. Internal sensors in the restrainer houses monitored the temperature. Animals from the CAE+HH50 and CAE+HH70 groups were placed in a preheated, completely filled Heliox exposure module. The exposure module was not heated for the CAE+HU group. Each inhalation therapy session consisted of three 5-minute inhalation periods with 5-minute breaks in between. The animals from the groups 1 and 2 were placed in individual restrainer house of the LIC for 30 minutes with unrestricted access to air breathing at room temperature.

2.6 Neurological Deficit Evaluation

Neurological deficits were assessed before CAE modeling, immediately after CAE, 1 hour, 3 hours, and 24 hours after CAE. The animal's neurological condition was assessed using a 0–5 point scale: 0 = no neurological deficit, 1 = inability to fully straighten the forelimb, 2 = rotation, 3 = loss of limb support, 4 = no spontaneous walking with depressed level of consciousness and 5 = death, according to the source [14].

2.7 Locomotor Activity

Locomotor activity of animals was assessed before CAE, and 3 and 24 hours after CAE using a computerized Multiple Activity Cage 47420 system with CUB 2005 v.3.0.15 software (Ugo Basile, Gemonio, Italy), recording the vertical and horizontal activity of animals for 3 minutes.

2.8 Respiratory Function

Respiratory function was assessed before CAE and at 3 and 24 hours after CAE using a PowerLab 8/35 system (ADInstruments Pty Ltd., Bella Vista, Australia) which included a spirometer unit FE141 and breathing head. The respiratory rate and tidal volume were recorded for 10 seconds. Minute respiratory volume was calculated by multiplying the respiratory rate by the tidal volume.

2.9 Cardiovascular Function

Cardiovascular parameters were assessed before CAE and 3 and 24 hours after CAE using a PowerLab 8/35 computerized NIBP system (ADInstruments Pty Ltd., Bella Vista, Australia). A pulse sensor with a cuff was placed on the ventral surface of the base of the tail, directly beneath the caudal artery. The systolic blood pressure and pulse rate were recorded.

2.10 Body Temperature

Body temperature was measured before CAE, and 3 and 24 hours after CAE, rectally using a medical digital thermometer (B.Well, Widnau, Switzerland), with a measuring range of 32 °C–42 °C.

2.11 Rota-Rod Test

Coordination was assessed before CAE, 3 hours and 24 hours after CAE using the Rota-rod test on a Rotamex-5 (Columbus Instruments, Columbus, OH, USA). The animals were placed on a horizontal rotating rod with a diameter of 7 cm at a minimum speed of one revolution per minute (RPM). Then, the rod began to rotate at an acceleration of 1 RPM every 10 seconds, up to a maximum speed of 60 RPM.

2.12 Forelimb Grip Strength

Forelimb grip strength was assessed before CAE, 3 hours and 24 hours after CAE, using the Grip Strength Meter (BioMed Easy Technologies Co., Ltd., Shenzhen, China). The animal was allowed to grasp a grate, and the maximum grip strength was recorded over three attempts.

2.13 Necropsy and Brain Examination

The animals that survived until the end of the experiment were euthanized 24 hours after CAE in a CO_2 chamber. During necropsy, brains were removed only from surviving animals and 2-mm-thick coronal sections were prepared. These sections were then placed in a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) (A6246, AppliChem GmbH, Darmstadt, Germany) at 37 °C for 10–12 minutes. The stained sections were examined for brain lesion presence or absence using a photographic record. The brains of prematurely deceased animals in the CAE+HU group were excluded from brain examination and histological analysis.

2.14 Histopathology

Only the brains of animals that survived to the end of the experiment were examined histologically. Brain sections that had been stained with TTC were fixed in formalin, dehydrated, and embedded in paraffin. Serial paraffin sections 5 µm thick were deparaffinized in xylene, rehydrated by sequential incubation with 100%, 95%, 80%, and 60% ethanol. The sections were then stained with hematoxylin and eosin. The stained sections were covered with mounting solution and air-dried. The sections were examined using a KF Bio KF-PRO-005-HI scanner (Helicon Company, Moscow, Russian Federation) at 40× magnification and analyzed using the KFBioScan program v 9.0 (Bioimager Inc., Vaughan, ON, Canada).

2.15 Serum Calcium Binding Protein B Level Measurement

The blood sampling was performed twice: 24 hours after catheterization (baseline) and 24 hours after CAE modeling. For S100B concentration measurement at the baseline, 24 hours after catheterization, blood samples (200 µL) were obtained from a tail vein using a 24 G intravenous catheter (Advamedics, Moscow, Russian Federation) in all animals to obtain serum. Twenty-four hours after CAE, blood samples were collected during necropsy (2 mL) and were used to obtain serum for S100B concentration measurement. The samples were left at room temperature for 40 minutes, after which they were centrifuged at 4 °C for 15 minutes at 1500 g using a laboratory multifunctional centrifuge with cooling (Eppendorf 5804 R, Eppendorf Ltd., Stevenage, UK). The serum was analyzed using an enzyme-linked immunosorbent assay (ELISA) with an ELISA kit for S100B (SEA567Ra, Cloud-Clone Corp., Katy, TX, USA).

2.16 Statistical Analysis

Statistical analysis was performed using Prism v.10 (GraphPad, Boston, MA, USA). The Shapiro-Wilk test was used to determine normality of all distributions. Significant differences were determined at $p < 0.05$. Descriptive statistics (mean and standard error of the mean) are presented for all quantitative data and are shown in the figures.

A parametric one-way ANOVA was used to compare samples across groups within each time point of the study for the following parameters: body weight, body temperature, minute respiratory volume, pulse, systolic pressure, time on the rod in the Rota-rod test, forelimb grip strength, vertical and horizontal activity in the open field, S100B concentration. All groups were compared with each group at each time point. A post-hoc Tukey test was used in this analysis.

Mixed-effects ANOVA was used to compare samples within each group. Data from a single group collected at successive time points during the study were compared with baseline results. A post-hoc Dunnett test was applied in

this analysis. Mixed-effects ANOVA was used for the following parameters: body weight, body temperature, minute respiratory volume, pulse, systolic pressure, time on the rod in the Rota-rod test, forelimb grip strength, vertical and horizontal activity in the open field, S100B concentration.

The neurological deficit score was analyzed using a Kruskal-Wallis test with a post hoc Dunn's test for comparisons between groups at each time point. The Friedman test with post hoc Dunn's test was used for within-group comparisons across time points. The neurological deficit score from a single group, collected at successive time points during the study, was compared with baseline results.

Fisher's exact test was used to assess the presence of ischemic lesions in the brain at necropsy. All groups were compared with the control group and the CAE group. This test compared the number of animals exhibiting foci of ischemic lesions 24 hours after CAE. No post-hoc tests were performed in the Fisher's exact test.

3. Results

3.1 Survival and Neurological Deficit

Neurological deficits in groups 2–5 with CAE modeling was more pronounced immediately after embolization, prior to Heliox inhalation (Fig. 1A). One hour after CAE, animals in the CAE+HH50 and CAE+HH70 groups began to recover mobility, in contrast to the CAE group and the CAE+HU group, where all animals exhibited no locomotor activity. Three hours after CAE, a negative effect was noticeable in the CAE+HU group, where most animals failed to recover locomotor function, in contrast to the CAE+HH50 and CAE+HH70 groups, where all animals were able to move with limb support. Twenty-four hours after CAE, the neurological deficit score decreased in the CAE group. In the CAE+HH50 and CAE+HH70 groups, the neurological deficit score decreased to baseline level 24 hours after CAE. However, in the CAE+HU group, neurological deficits remained severe. Three animals were assigned a deficit score of 5 due to death prior to the 24-hour testing period. During the 24-hour observation period, three out of eight animals (37.5%) died in the CAE+HU group (Fig. 1B). No deaths or conditions requiring immediate euthanasia were observed in the other groups.

3.2 Respiratory Function

Three hours after CAE modeling, a significant decrease in respiratory system parameters was observed in the CAE group and the CAE+HU group compared to baseline level. However, this decrease was not observed in the CAE+HH50 and CAE+HH70 groups (see Fig. 1C). Three hours after CAE modeling, the CAE+HH50 group showed a 90% increase in minute respiratory volume compared to the CAE group. A single inhalation of heated Heliox (60–70 °C) in the CAE+HH70 group also had a positive effect on respiratory parameters three hours after CAE modeling, increasing minute respiratory volume by 97% compared to the CAE group. Twenty-four hours after CAE modeling, minute respiratory volume returned to baseline in all groups except the CAE and CAE+HU groups, where it remained reduced by 15% and 17%, respectively. In the CAE+HH50 and CAE+HH70 groups, minute respiratory volume did not differ significantly from control values at either three or 24 hours after CAE.

3.3 Body Temperature

A statistically significant reduction in body temperature after CAE was observed in animals from the CAE and CAE+HU groups with an average decrease of 4% and 5%, respectively (Fig. 1D). Three hours after CAE, animals in the CAE+HH50 and CAE+HH70 groups had body temperatures that were 0.9 °C higher than those of the CAE group, and 1.0 °C higher at 24 hours after CAE. The CAE+HH50 and CAE+HH70 groups had a higher body temperature than the CAE group at 3 and 24 hours after CAE. Twenty-four hours after CAE, the body temperatures of animals in the CAE and CAE+HU groups decreased by 2.6% and 4%, respectively, compared to the baseline. At 3 and 24 hours after CAE, there was no significant difference in body temperature between the CAE+HH50/CAE+HH70 and control groups.

3.4 Cardiovascular Function

A significant decrease in heart rate (12%) and blood pressure (20%) was observed in the CAE group, 3 hours after CAE (Fig. 1E,F). The HU inhalation did not lead to improvement in the cardiovascular parameters after CAE modeling. Blood pressure and heart rate decreased significantly in the CAE+HU group by 13% and 25%, respectively, compared to the baseline. Three hours after CAE, an increase in heart rate by 11% and in blood pressure by 19% was observed in the CAE+HH50 group compared to the CAE group. HH70 inhalation increased heart rate by 12% and blood pressure by 16%, compared to the CAE group, 3 hours after CAE. The heart rate and blood pressure did not differ significantly from the Control group in the HH50 and HH70 groups 3 hours after CAE. Twenty-four hours after CAE, cardiovascular parameters recovered to the baseline and did not differ among all groups.

3.5 Coordination

Three hours after CAE modeling, coordination in animals was significantly reduced by 94% compared to the Control group and the baseline (Fig. 2A). On average, 3 hours after CAE animals maintained a latency time on the rotating rod of 7 seconds. HU inhalation did not improve coordination. Three hours after CAE modeling, the animals from the CAE+HU maintained a latency time on the rotating rod that was 96% lower than the baseline. In the CAE+HH50 and CAE+HH70 groups, the latency time on the rotating rod was 551.43% and 642.86%, respectively, higher than in the CAE group three hours after CAE modeling.

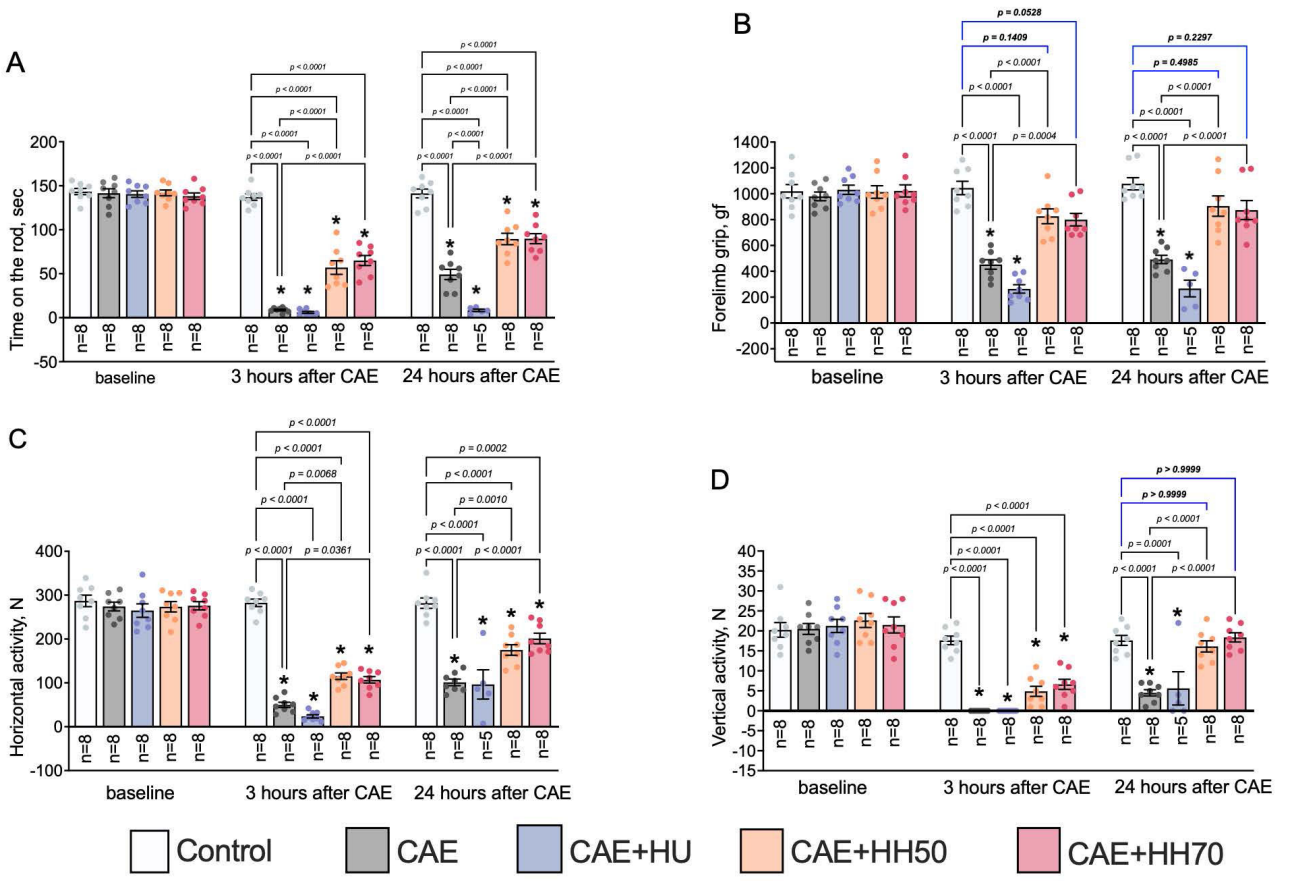

Fig. 2. Functional parameters of coordination, muscle strength and locomotor activity before CAE, 3 hours and 24 hours after CAE. (A) Coordination was significantly impaired in all animals at 3 and 24 hours after CAE modeling in the Rota-Rod test (n = 8, compared to baseline (mixed-effects ANOVA with post-hoc Dunnett test) and the Control group (n = 8, one-way ANOVA with post-hoc Tukey test), HH50 and HH70 (n = 8) inhalation led to gradual recovery of coordination after CAE (one-way ANOVA with post-hoc Tukey test). (B) Forelimb grip strength decreased in the CAE group (n = 8) and CAE+HU group (n = 8 at 3 hours and n = 5 at 24 hours) at 3 and 24 hours after CAE compared to baseline (mixed-effects ANOVA with post-hoc Dunnett test) and the Control group (one-way ANOVA with post-hoc Tukey test), while heated Heliox inhalation in the CAE+HH50 and HH70 groups (n = 8) fully prevented muscle weakness after CAE modeling (one-way ANOVA with post-hoc Tukey test). (C) Horizontal activity and (D) vertical activity were decreased in the open field test at 3 hours in all animals after CAE modeling compared to baseline (n = 8, mixed-effects ANOVA with post-hoc Dunnett test) and the Control group (one-way ANOVA with post-hoc Tukey test). However, at 24 hours after CAE, animals in the CAE+HH50 and CAE+HH70 groups showed a recovery in vertical and horizontal activity (n = 8, one-way ANOVA with post-hoc Tukey test), while animals in the CAE group (n = 8) and the CAE+HU group (n = 5) continued to exhibit reduced locomotor activity (one-way ANOVA with post-hoc Tukey test). Data are shown as individual values, group means, and SEM. Significant differences between groups in one-way ANOVA with post-hoc Tukey test are marked above the individual values with indication of *p*-values. Unsignificant differences between groups in one-way ANOVA with post-hoc Tukey test are highlighted by blue connecting lines, *p*-values are shown in bold. **p* < 0.05 compared to baseline of the group in mixed-effects ANOVA with post-hoc Dunnett test. Data are shown as individual values, group means, and SEM. The number of animals in each group is presented on the bars.

Twenty-four hours after CAE modeling, coordination began to recover in the CAE group, with an average latency time on the rotating rod of 49 seconds; however, values remained 65% lower than baseline. Animals in the CAE+HU group also demonstrated poor performance, maintaining latency time on the rotating rod for an average of 8 seconds, which was 94% lower than baseline values. Heated Heliox in the CAE+HH50 and CAE+HH70 groups enabled the animals to maintain a latency time on the rotating rod for an average of 90 and 92 seconds, respectively, 24 hours after CAE; however, these values were still significantly lower than the baseline by 37% and 35%, respectively.

3.6 Grip Strength

In the CAE group, forelimb grip strength decreased by 57% and 53% in animals at 3 and 24 hours after CAE

modeling, respectively (Fig. 2B). Twenty-four hours after CAE modeling, a trend towards increased grip strength was observed in the CAE group. In the CAE+HU group, grip strength decreased by 74% compared to the baseline at 3 and 24 hours after CAE modeling. HH50 and HH70 inhalation reduced muscle weakness at 3 and 24 hours after CAE. The CAE+HH50 and CAE+HH70 groups did not show significant muscle strength decrease compared to the control group or baseline.

3.7 Locomotor Activity

In animals with CAE modeling, horizontal (Fig. 2C) and vertical (Fig. 2D) activity decreased significantly at 3 and 24 hours after CAE compared to the control group and baseline. In the CAE group, horizontal activity decreased by 82% three hours after CAE modeling compared to baseline. Three hours after CAE modeling, animals in the CAE+HU group exhibited an even greater reduction in horizontal activity compared to the CAE group (53%) and the baseline (91%). Three hours after CAE, horizontal activity was significantly higher in the CAE+HH50 and CAE+HH70 groups by 132% and 115%, respectively, compared to the CAE group. Additionally, vertical activity was observed in all animals in the CAE+HH50 and CAE+HH70 groups three hours after CAE modeling. In contrast, vertical activity was absent in all animals in the CAE and CAE+HU groups. Twenty-four hours after CAE, horizontal activity in the CAE+HH50 and CAE+HH70 groups was at 73% and 99% higher than in the CAE group. Vertical activity in the CAE+HH50 and CAE+HH70 groups was at 258% and 308% higher than in the CAE group, respectively. This contrasted with the CAE+HU group, in which both horizontal and vertical activity remained reduced 24 hours after CAE by 74% and 64%, respectively, compared to baseline. In the HH50 and HH70 groups, vertical activity did not differ significantly from the Control group at 24 hours after CAE. However, horizontal activity had not fully recovered at 24 hours after CAE in the groups with CAE modeling.

3.8 S100B Serum Concentration

Twenty-four hours after CAE modeling, the serum S100B concentration increased significantly by 346.26% in the CAE group and by 385.99% in the CAE+HU group, compared to the Control group (Fig. 3). Serum S100B concentration was also significantly increased by 172.79% and 186.39% in the CAE+HH50 and CAE+HH70 groups, respectively, compared to the Control group. However, the increase in S100B concentration in the CAE+HH50 and CAE+HH70 groups was less pronounced compared to the CAE and CAE+HU groups. Twenty-four hours after CAE, HH50 and HH70 inhalation resulted in a significant decrease in serum S100B concentration by 39% and 36% respectively, compared to the CAE group.

3.9 Histopathological Examination of the Brain

Examination of brain sections stained with TTC revealed clear ischemic brain lesions in all animals from the CAE group (Fig. 4). After HU inhalation, ischemic lesions on TTC-stained sections were observed in three out of five surviving animals (Table 2). No evidence of ischemic stroke lesions on TTC-stained sections was observed in any of the animals from the CAE+HH50 and CAE+HH70 groups (Fig. 4).

Histological examination of the animals' brains after CAE revealed an area of irreversible ischemic damage in the right hemisphere, characterized by neuronal pyknosis and slight neutrophil infiltration. A similar pattern was observed in the CAE+HU group. These pathological changes were absent in the CAE+HH50 and CAE+HH70 groups (Fig. 4).

4. Discussion

Oxygenation plays a significant role in preventing post-stroke complications. Previously, we demonstrated that a single inhalation of heated Heliox (40–50 °C) significantly reduced the development of neurological abnormalities in the physiological parameters of animals with ischemic stroke induced by CAE [2]. Since hypothermia has been shown to be beneficial during stroke rehabilitation, it was interesting to study the effect of room-temperature Heliox (20–22 °C) on rehabilitation, and compare it with one of previously studied Heliox at 40–50 °C and, of warmer Heliox at 60–70 °C.

Ischemic stroke was modeled in awake rats by CAE [2,13]. Three hours after CAE, animals without subsequent therapy exhibited symptoms including motor, respiratory, and cardiovascular depression, as well as decreased body temperature, impaired coordination, reduced muscle strength, and decreased locomotor activity. Ischemic stroke lesions were visualized in the middle cerebral artery basin of all animals with CAE after staining with TTC and hematoxylin and eosin. These histopathological changes on TTC-stained sections of the brain and hematoxylin and eosin stained sections were accompanied by an increase in serum S100B concentration, indicating neurodegenerative processes initiated by CAE [15]. Previously, we determined that the average area of ischemic brain lesions stained with TTC in the second frontal section of the brain is 9–11% one day after CAE in rats [2]. However, the present study did not include a quantitative assessment of the volume of ischemic lesions in the brain. We only evaluated the presence or absence of foci of ischemic lesions on TTC-stained sections of the brain, which could be considered a limitation of this study. The pathophysiological picture obtained in the experiment corresponded to the acute phase of ischemic brain injury. This phase was accompanied by loss of consciousness, decreased muscle strength, and an inability to support one's own weight immediately after middle cerebral artery embolization [16]. A clinical study has

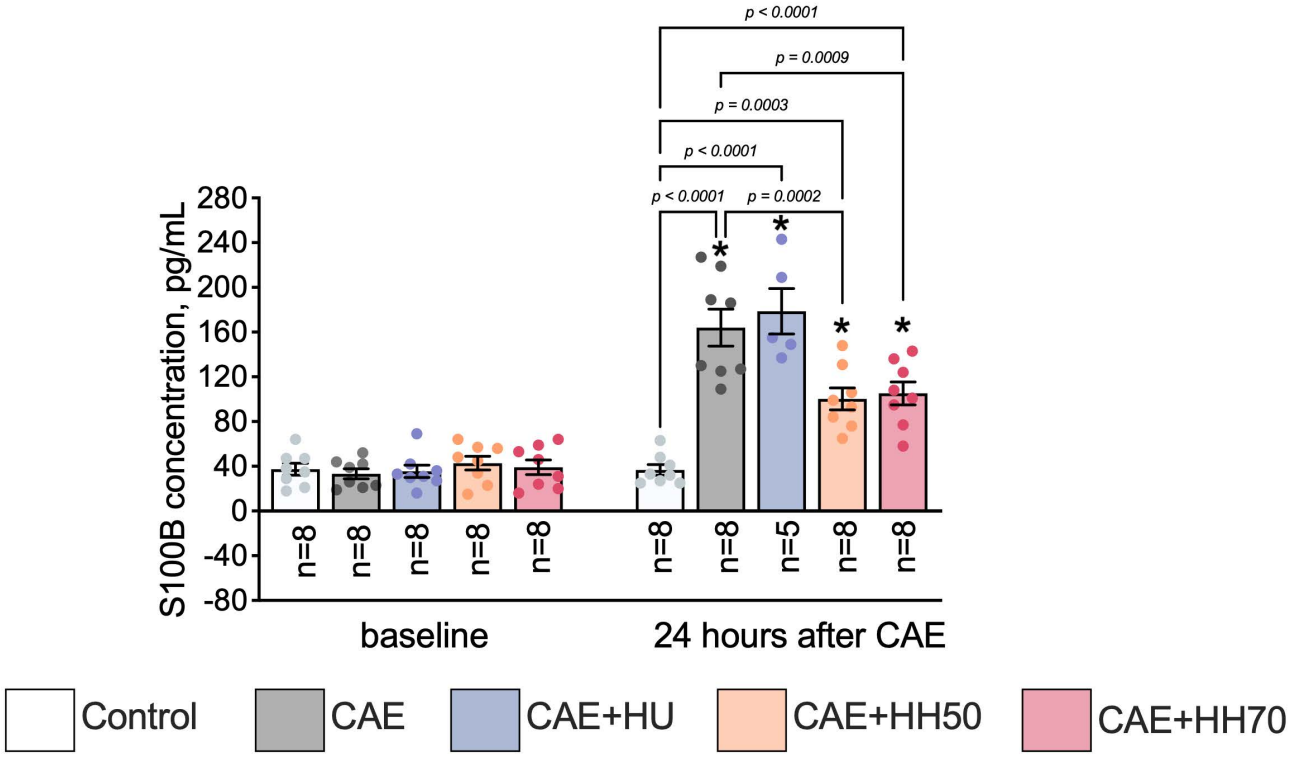

Fig. 3. Serum protein S100B concentrations before and 24 hours after CAE modeling. Twenty-four hours after CAE, serum S100B level was significantly increased after CAE in all groups compared to the Control group (n = 5 in the CAE+HU group, n = 8 in the Control group, CAE group, CAE+HH50 and CAE+HH70 groups, one-way ANOVA with post-hoc Tukey test). Serum S100B level was significantly increased after CAE in all groups compared to the baseline (mixed-effects ANOVA with post-hoc Dunnett test). HH50 and HH70 inhalation (n = 8) decreased S100B level compared to the CAE group (n = 8, one-way ANOVA with post-hoc Tukey test). The S100B concentration in the CAE+HU group did not differ from the CAE group values (one-way ANOVA with post-hoc Tukey test). Significant differences between groups in one-way ANOVA with post-hoc Tukey test are denoted by an asterisk (*) above the individual values with indication of *p*-values. *$p < 0.05$ compared to the baseline of the group in mixed-effects ANOVA with post-hoc Dunnett test. Data are shown as individual values, group means, and SEM. The number of animals in each group is indicated on the bars.

Table 2. Number of animals with ischemic stroke lesions on TTC-stained sections of the brain 24 hours after CAE modeling.

Groups	Control	CAE	CAE+HU	CAE+HH50	CAE+HH70
	Number of cases/Number of animals examined				
Presence of ischemic stroke lesions	0/8	8/8*	3/5	0/8$^{\#}$	0/8$^{\#}$

Significant differences are marked as: * $p < 0.05$ compared to the control group (Fisher's exact test). $^{\#}$ $p < 0.05$ compared to the CAE group (Fisher's exact test). Abbreviations: TTC, 2,3,5-triphenyltetrazolium chloride.

also documented impaired respiratory and cardiovascular function in patients with acute ischemic stroke [17]. The CAE group consisted of animals that received unheated air at room temperature in the LIC following CAE modeling. We previously conducted a study involving a group of animals that breathed heated air after CAE [2]. No significant effect of air heated to 50 °C was observed in preventing ischemic stroke or improving functional and physiological parameters in animals. Therefore, in this study, we limited our analysis to a group of animals that breathed unheated air immediately after CAE modeling.

The use of HH50 and HH70 allowed the animals to prevent a significant decrease in respiratory and cardiovascular parameters and body temperature 3 hours after CAE. Twenty-four hours after CAE, these parameters were similar to those of the control group. Additionally, improved coordination and increased muscle strength were observed with the inhalation of HH50 and HH70 three hours after CAE; however, these results did not align with those of the baseline or control group. Twenty-four hours after CAE, all animals in both CAE+HH50 and CAE+HH70 group showed no brain lesions on TTC-stained sections of

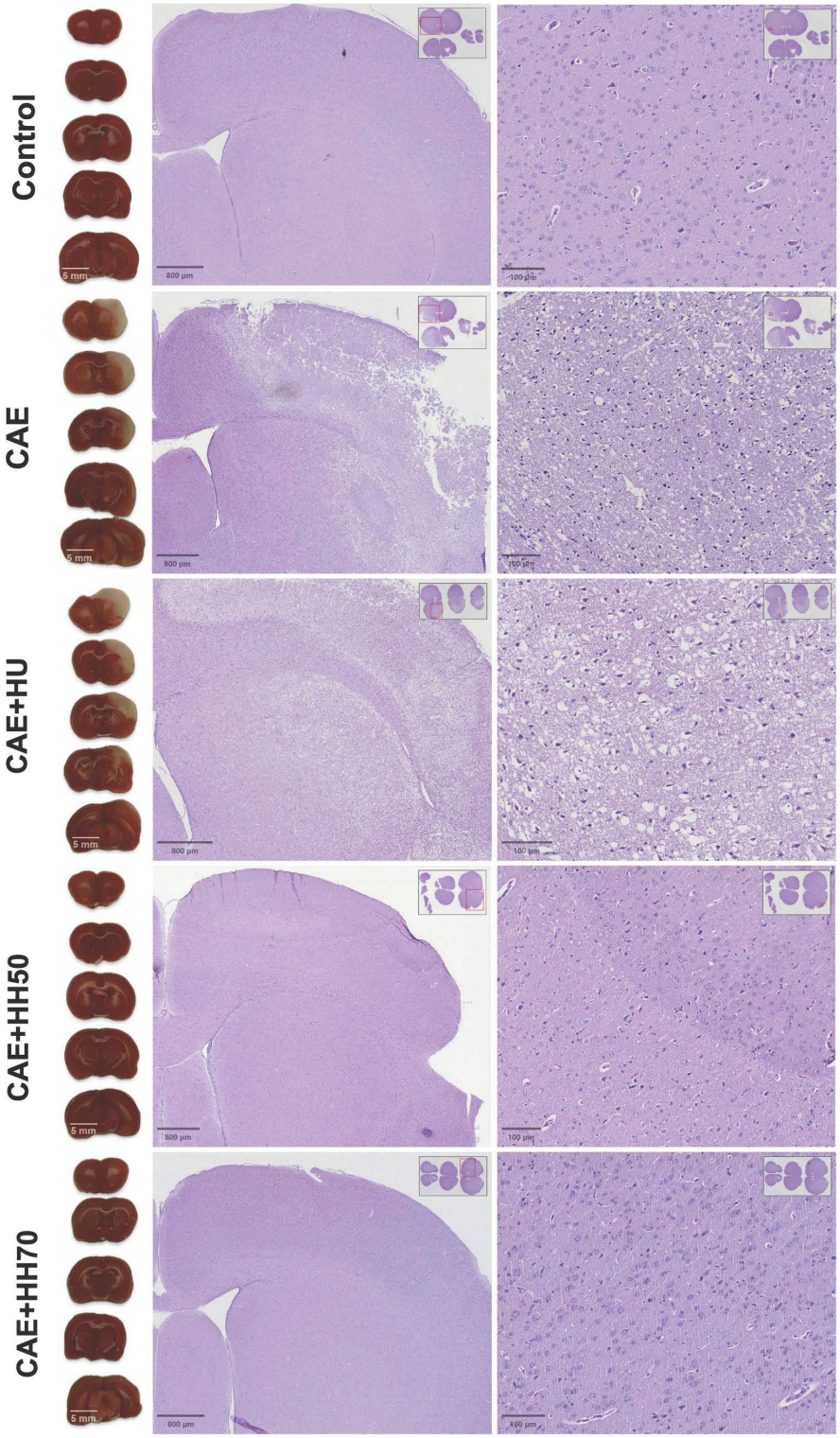

Fig. 4. Histopathological analysis of brain sections 24 hours after CAE. Representative photographs of brain sections stained with TTC are on the left. Scale bar = 5 mm. White areas indicate the infarct zone, and undamaged areas are stained red. Panoramic microphotographs of histological sections (stained with hematoxylin and eosin) show changes in the cortex of the right hemisphere. In the CAE and CAE+HU groups, extensive brain damage was observed on TTC sections, and characteristic signs of ischemic brain damage were observed in the histological analysis. In the groups CAE+HH50 and CAE+HH70, pictures on both TTC and histological sections were comparable to those of the Control group, indicating the absence of morphological signs of infarction. Scale bar = 800 µm and 100 µm.

the brain and hematoxylin and eosin stained sections of the brain. Twenty-four hours after CAE, there is a discrepancy between the lack of foci of ischemic brain lesions on TTC-stained sections of the brain in all animals in the CAE+HH50 and CAE+HH70 groups and the incomplete recovery of their coordination and horizontal activity, as well as the incomplete reduction in neurological deficit scores to baseline levels. This discrepancy may also be related to the staining method used, TTC, which may not be an absolute indicator of the presence or absence of ischemic brain damage.

Twenty-four hours after CAE, serum S100B concentration was significantly lower in the CAE+HH50 and CAE+HH70 groups than in the untreated CAE group. However, S100B levels remained significantly higher in the HH50 and HH70 groups than in the control group 24 hours after CAE. Therefore, it cannot be concluded that ischemic brain damage was completely prevented on TTC-stained sections of the brain. Despite an increase in the neurological injury marker S100B in the CAE+HH50 and CAE+HH70 groups 24 hours after CAE, the animals exhibited recovery of muscle strength, vertical activity in an open field, and respiratory and cardiovascular functions at this time point. Therefore, based on these results, it is suggested that S100B be measured three hours after CAE to better understand the relationship between S100B concentration dynamics and the animals' physiological and functional parameters following CAE.

There were no significant differences in physiological parameters when comparing the two groups (CAE+HH50 and CAE+HH70). Their body temperature, respiratory, and cardiovascular parameters were closer to those of the control group. A previous study has confirmed that inhaling heated Heliox (70 °C) for 20 minutes is safe for human respiratory tract cells [18]. However, the therapeutic effect achieved through such heating does not provide significant benefits in cases of CAE.

Interestingly, inhalation of unheated Heliox at room temperature did not improve physiological, histopathological, or functional parameters, even 24 hours after CAE modelling. Unheated Heliox also failed to restore coordination, muscle strength, or locomotor activity 24 hours after CAE. Meanwhile, functional parameters tended to recover in the CAE group. Furthermore, administering HU immediately after CAE resulted in death of three animals within 24 hours, compared to 100% survival in all other groups. Significant depression of respiratory and cardiovascular function and hypothermia were observed in the deceased animals 24 hours after CAE. Twenty-four hours after CAE, the pulse, blood pressure, and minute respiratory volume of the surviving animals in the CAE+HU group were similar to those of the animals in the CAE group that did not receive therapy (Group 2). Ischemic lesions on TTC-stained sections of the brain were detected in the brains of three out of five surviving animals, and the concentration of the neurodegeneration marker S100B was significantly elevated in their serum. Similarly, ischemic brain lesions on TTC-stained sections of the brain and a significant increase in serum S100B levels were observed in all eight untreated animals from the CAE group, which fully confirmed the danger of unheated Heliox inhalation. Presumably, all the negative effects observed with unheated Heliox are due to its high heat capacity. A clinical study by Pavlov et al. [19] supports this hypothesis, showing that inhaling room-temperature Heliox decreases body temperature, while inhaling Heliox heated to 65 °C increases it. The increase may be caused by the large amount of heat transferred from the Heliox, as well as by the stimulation of receptors in the upper respiratory tract [19]. Thus, based on the results obtained in animals, it is strongly recommended, to use Heliox in a preheated form, at least several degrees above body temperature, to avoid the potential negative effects of it.

A limitation of this study is the short duration of animal observation – 24 hours after CAE. Indeed, it has previously been demonstrated that neurological deficits persist for up to 3 days after CAE and gradually resolve by 7 days after CAE, as published by [13]. The most pronounced impairments in the acute phase of ischemic stroke were observed three hours after CAE. Thus, the present study was limited to the acute phase of ischemic stroke and the effect of the Heliox temperature regime on rat pathophysiology. Only male rats were used in the study, which may affect how these results can be applied to humans.

Nevertheless, the potential for using warmed Heliox during the acute phase of ischemic stroke has been demonstrated, as well as the risk associated with unheated Heliox inhalation after ischemic stroke. Another limitation of this study is the administration of gas mixtures immediately after CAE modeling in rats. While inhalation can be performed immediately after CAE modeling in a laboratory setting, emergency measures following an ischemic stroke are often initiated 30 minutes, an hour, or longer after the event in clinical practice. Future research should investigate the efficacy of heated Heliox at delayed time points following ischemic stroke induction.

5. Conclusions

Heated Heliox (40–50 °C or 60–70 °C) inhalation immediately after CAE prevented the formation of ischemic brain lesions on TTC-stained sections of the brain, significantly alleviated the suppression of respiratory and cardiovascular functions, maintained body temperature 3 hours after CAE. Heated Heliox (40–50 °C or 60–70 °C) inhalation immediately after CAE promoted the recovery of muscle strength, and reduced serum S100B concentration 24 hours after CAE in rats. Three hours after CAE during the acute phase of ischemic stroke, heated Heliox (40–50 °C or 60–70 °C) partially restored coordination, locomotor activity, and neurological deficits. In contrast, inhaling unheated

Heliox at room temperature (20–22 °C) immediately after CAE negatively affected physiological and functional parameters and failed to eliminate ischemic brain lesions on TTC-stained brain sections.

Abbreviations

BPM, beats per minute; CAE, cerebral arterial air embolism; ELISA, enzyme-linked immunosorbent assay; HH50, heated up to 40–50 °C Heliox; HH70, heated up to 60–70 °C Heliox; HBOT, hyperbaric oxygen therapy; HU, unheated Heliox; LIC, Laboratory Inhalation Complex; RPM, revolution per minute; SPF, Specific-pathogen-free; SD, Sprague-Dawley; S100B, calcium binding protein B; TTC, 2,3,5-triphenyltetrazolium chloride.

Availability of Data and Materials

All data reported in this paper will be shared by the corresponding author upon request.

Author Contributions

VP and NB wrote the original draft. VB, AM, ID, ES, GS, DR contributed to the conceptualization and discussion of the analyzed data. AI, MI, DR, GS, NP and ES performed the cerebral arterial embolism modeling, functional tests, physiological parameters measurement and ELISA. OP and MI performed histopathological analysis. NP and VB performed test objects administration. NB, OP, MI and SK performed statistical analysis. AK, SK, AM and ID contributed to the investigation, draft correction, and conceptualization. VB, ES, NP contributed to the review and editing conception. AK, AI and VP contributed to the design of the study. ID contributed to the resources. SK contributed to the funding. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the standard operating procedures of the laboratory and was approved by the bioethical commission of the Institutional Animal Care and Use Committee of The Branch of the M.M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences (Protocol No 922/22 from 27.12.2022). All experiments were carried in accordance with the following regulations: 'Guide for the Care and Use of Laboratory Animals' (NIH 80-23, revised 1978); The Guide for Care and Use of Laboratory Animals, National Academy Press, Washington, D.C., 2011; European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Council of Europe (ETS 123); Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes; the ARRIVE 2.0 guidelines (**Supplementary Material**).

Acknowledgment

The authors thank the Closed Joint Stock Company "Specialized Design Bureau of Experimental Equipment at the Institute of Medical and Biological Problems of the Russian Academy of Sciences" and its General Director Alexsey Timofeevich Logunov for providing the gas mixture.

Funding

This work was supported by the Russian Science Foundation (grant number 25-15-00037).

Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/JIN51502.

References

[1] Ganti L. Management of acute ischemic stroke in the emergency department: optimizing the brain. International Journal of Emergency Medicine. 2025; 18: 7. https://doi.org/10.1186/s12245-024-00780-5

[2] Palikov VA, Pavlov NB, Amirov RR, Ismailova AM, Borozdina NA, Palikova YA, et al. Effect of a helium and oxygen mixture on physiological parameters of rats with cerebral arterial air embolism. Frontiers in Physiology. 2024; 15: 1388331. https://doi.org/10.3389/fphys.2024.1388331

[3] Kim T, Chuchalin A, Martynov M, Gusev E, Shogenova L, Panin A. Efficacy and safety of thermic helium-oxygen (t-He/O2) mixture in reducing hypoxemia in acute ischemic stroke patients. European Respiratory Journal. 2019; 54: PA2284. https://doi.org/10.1183/13993003.congress-2019.PA2284

[4] Ke H. Progress in the clinical application of heliox. Medical Gas Research. 2025; 15: 546–547. https://doi.org/10.4103/mgr.MEDGASRES-D-25-00026

[5] Tugusheva MP, Dyachenko AI, Shulagin YA. Research on the physiological effect of heated Heliox on human external respiration parameters. Human Physiology. 2010; 36: 889–892. https://doi.org/10.1134/S036211971007025X

[6] Majchrzycka A. Thermodynamic properties of helium – oxygen mixtures. Bulgarian Chemical Communications. 2018; 50: 99–103.

[7] Huang J, Wang P, Wen H. The safety and efficacy of hypothermia combining mechanical thrombectomy or thrombolysis in the treatment of ischemic stroke: A systematic meta-analysis. Clinics. 2023; 78: 100218. https://doi.org/10.1016/j.clinsp.2023.100218

[8] Huang ZX, Hu M, Zhang P, He X, Ma X, Hong Q, et al. Intra-arterial selective hypothermia for acute ischemic stroke neuroprotection: A multicenter pilot trial in China. PLoS Medicine. 2025; 22: e1004668. https://doi.org/10.1371/journal.pmed.1004668

[9] Jeong JH, Hong JH, Sohn SI, Park H, Chang JY, Yum KS, et al. Mild Hypothermia After Endovascular Treatment for Acute Ischemic Stroke: A Pilot Randomized Controlled Trial. Stroke.

2025; 56: 3100–3107. https://doi.org/10.1161/STROKEAHA.124.049762

[10] You JS, Kim JY, Yenari MA. Therapeutic hypothermia for stroke: Unique challenges at the bedside. Frontiers in Neurology. 2022; 13: 951586. https://doi.org/10.3389/fneur.2022.951586

[11] Shintani Y, Terao Y, Ohta H. Molecular mechanisms underlying hypothermia-induced neuroprotection. Stroke Research and Treatment. 2010; 2011: 809874. https://doi.org/10.4061/2011/809874

[12] Hemmen TM, Lyden PD. Hypothermia after acute ischemic stroke. Journal of Neurotrauma. 2009; 26: 387–391. https://doi.org/10.1089/neu.2008.0574

[13] Palikov VA, Ismailova AM, Kazakov VA, Kholina AV, Borozdina NA, Mikhaylov ES, et al. Awake Rat Model of Ischemic Stroke with Neuropsychiatric Complications During a Seven-Day Observation. Journal of Visualized Experiments. 2025; e69194. https://doi.org/10.3791/69194

[14] Schäbitz WR, Li F, Irie K, Sandage BW, Jr, Locke KW, Fisher M. Synergistic effects of a combination of low-dose basic fibroblast growth factor and citicoline after temporary experimental focal ischemia. Stroke. 1999; 30: 427–432. https://doi.org/10.1161/01.str.30.2.427

[15] Gnyliukh N, Wei J, Neuhaus W, Boukherroub R, Szunerits S. The role of S100B protein as a diagnostic biomarker for brain injury. Sensing and Bio-Sensing Research. 2025; 50: 100888. https://doi.org/10.1016/j.sbsr.2025.100888

[16] Müller HHO, Czwalinna K, Wang R, Lücke C, Lam AP, Philipsen A, et al. Occurence of Post-Traumatic Stress Symptoms, Anxiety and Depression in the Acute Phase of Transient Ischemic Attack and Stroke. The Psychiatric Quarterly. 2021; 92: 905–915. https://doi.org/10.1007/s11126-020-09873-9

[17] Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke. 2019; 50: e344–e418. https://doi.org/10.1161/STR.0000000000000211

[18] Varfolomeev SD, Panin AA, Ryabokon AM, Kozyr AS, Kononikhin AS, Shogenova LV, et al. Thermal Heliox proteome. High-temperature Heliox does not cause destruction of human respiratory system cells. Terapevticheskii Arkhiv. 2020; 92: 69–72. https://doi.org/10.26442/00403660.2020.06.000769 (In Russian)

[19] Pavlov BN, D'yachenko AI, Shulagin YA, Pavlov NB, Buravkova LB, Popova YA, et al. Studies of Physiological Effects of Breathing Heated Oxygen–Helium Mixtures. Human Physiology. 2003; 29: 576–580. https://doi.org/10.1023/A:1025863900451 (In Russian)