Research Results in Pharmacology

Research Results in Pharmacology 11(4): 282–292
UDC 615.015
DOI 10.18413/rrpharmacology.11.863

Research Article

Investigation of behavioral characteristic changes in animals with varying injury severity following brain tissue damage in a mouse model of neonatal hypoxia-ischemia during the juvenile period

Vladimir M. Pokrovsky[1], Ivan V. Chatsky[1], Maria R. Maslinikova[1], Natalia V. Zabolotskikh[2], Arkady V. Nesterov[1], Nikita V. Popov[1], Mikhail V. Korokin[1]

1 Belgorod State National Research University; 85 Pobedy St., Belgorod 308015 Russia,
2 Kuban State Medical University of Public Health Care of Russia; 4 Mitrofana Sedina St., Krasnodar 350063 Russia.

Corresponding author: Vladimir M. Pokrovsky (vmpokrovsky@yandex.ru)

Academic editor: Oleg Gudyrev ♦ Received 12 October 2025 ♦ Accepted 10 December 2025 ♦ Published 29 December 2025

Citation: Pokrovsky VM, Chatsky IV, Maslinikova MR, Zabolotskikh NV, Nesterov AV, Popov. NV, Korokin MV (2025) Investigation of behavioral characteristic changes in animals with varying injury severity following brain tissue damage in a mouse model of neonatal hypoxia-ischemia during the juvenile period. Research Results in Pharmacology 11(4): 282–292. https://doi.org/10.18413/rrpharmacology.11.863

Abstract

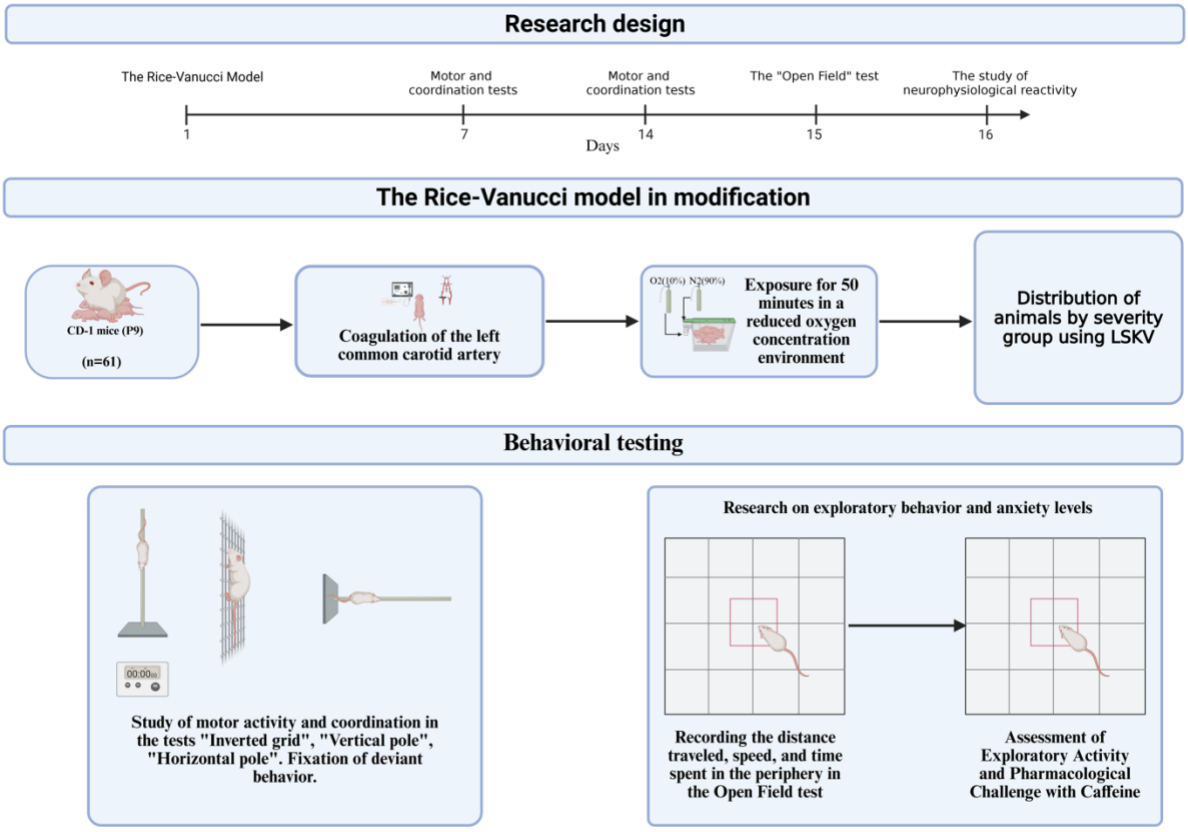
Introduction: Neonatal hypoxia-ischemia (HI) remains one of the most significant causes of perinatal central nervous system injuries and subsequent neurodevelopmental disorders. The development of new therapeutic interventions requires improvements in existing methods for assessing clinical status according to the severity of the pathological process, enabling more precise evaluation of disease correction.

Materials and Methods: Neonatal hypoxia-ischemia was modeled in CD-1 mice at 9 days of age (n=51). Motor and coordination impairments were assessed using the inverted grid, vertical pole, and horizontal pole tests on days 7 and 14. Anxiety-like behavior and exploratory activity were evaluated in the open field test on day 15. Neurophysiological reactivity was examined by administering caffeine (20 mg/kg) one hour prior to the Open field test on day 16.

Results: A statistically significant difference in motor activity was observed between the mild and moderate injury groups compared to the intact group, with reductions of 37% and 57% in the inverted grid test and 27% and 53% in the vertical pole test, respectively. Pharmacological challenge with caffeine stimulated a 1.5-fold increase in speed and distance travelled in the moderate injury group compared to those in the intact group, despite baseline statistically significant differences in the Open field test.

Conclusion: The experimental approach to assessing the clinical status of animals after neonatal hypoxia-ischemia modeling, followed by stratification based on injury severity, revealed statistically significant differences across multiple parameters between the studied groups.

Graphical Abstract



Keywords

neonatal hypoxia-ischemia, motor behavior, caffeine, dopaminergic system, severity stratification, mice

Introduction

Neonatal hypoxia-ischemia (HI) remains one of the most significant causes of perinatal central nervous system injuries and subsequent neuropsychiatric developmental disorders (Zhou et al. 2021; Babbo et al. 2024). In the early postnatal period, hypoxia-ischemia initiates a cascade of pathological processes including energy deficit, excitotoxicity, inflammation and apoptosis, leading to long-term structural and functional brain changes (Odorcyk et al. 2021; Brégère et al. 2022). Despite advances in neuroprotective methods, long-term consequences of perinatal HI such as cognitive and emotional-motor deficits remain difficult to treat and require deeper understanding of their formation and compensation mechanisms (Chen et al. 2025).

Experimental models of neonatal hypoxia-ischemia in rodents, particularly CD-1 mice, remain the most informative tool for studying pathogenesis and evaluating the effectiveness of neuroprotective strategies (Kim et al. 2017). However, most studies focus on acute stages of injury, while the dynamics of functional recovery and behavioral adaptations at later stages remain insufficiently studied (Hamdy et al. 2020). Particularly scarce are data on how emotional-cognitive and motor behavior differs in animals with varying severity of primary injury in adulthood.

Additional interest lies in pharmacological tests using central nervous system stimulants that can reveal hidden imbalances in neurotransmitter balance and plasticity (Weston et al. 2021). Caffeine, an antagonist of A1/A2A adenosine receptors, is a widely used psychostimulant and modulator of dopaminergic activity (Essawy et al. 2017; Ibrahim et al. 2020). Its effects are

largely determined by the state of adenosine-dopamine balance, making caffeine a candidate for functional pharmacological testing to assess compensatory capabilities of neural networks after ischemic brain injury (Ratliff et al. 2019; Zhu et al. 2024). Analysis of behavioral responses to caffeine stimulation can reveal not only motor but also cognitive-emotional deficits that are not apparent in standard behavioral tests.

In this context, a comprehensive study of the behavioral profile in mice after modeling neonatal hypoxia-ischemia during development from early post-stroke stages to adulthood is of particular interest. This approach will allow assessment of the dependence of functional recovery degree on injury severity, features of motor adaptation in the semi-remote period, and changes in behavioral response to caffeine as an indicator of neurochemical plasticity.

Research objective: to evaluate the dynamics of functional and behavioral indicators in mice after modeling neonatal hypoxia-ischemia and determine the nature of behavioral response to caffeine as a potential tool for early assessment of outcomes and compensatory changes in the CNS.

Materials and Methods

Study design

To form experimental groups, CD-1 mice were mated at a male-to-female ratio of 1:3. The presence of a copulatory plug was recorded the following day, with the date of visualization considered the first day of pregnancy. After 20-21 days, 61 offspring were obtained, 10 of which were assigned to the intact group. Post-birth, litters were mixed and cross-fostered to nursing females, with no more than 8 pups per dam, ensuring equal distribution across foster mothers. Neonatal hypoxia-ischemia was induced at postnatal day 9 using a modified Rice-Vannucci model. Animals were stratified into groups based on the severity of brain tissue damage (mild and moderate) using the RFLSI-ZW laser speckle contrast imaging system (RWD Life Science, China) to assess cerebral blood flow. Motor function and coordination tests were performed on days 7 and 14 to evaluate clinical status changes. On day 16, the open field test was conducted to assess general motor activity, anxiety-like behavior, and exploration patterns. To uncover latent emotional-motor alterations and evaluate the functional state of the adenosine-dopamine system, caffeine-induced pharmacological stimulation was applied the following day. The open field test was repeated post-administration to assess neural stimulation.

Animals

The study was approved by the Animal Ethics Committee of Belgorod State National Research University (BelSU), approval No. 01-08i/25 dated 18 August 2025. Animals were housed in the SPF vivarium of BelSU under a controlled 12-hour light/dark cycle at temperatures of +22 to +26°C, with *ad libitum* access to food and water. All procedures followed ethical guidelines for laboratory animal care in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 170). Painful manipulations were performed in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Modeling of neonatal hypoxia-ischemia

Neonatal hypoxia-ischemia was induced at postnatal day 9 using a modified Rice-Vannucci protocol (Sheldon et al. 2018). Briefly, the left common carotid artery was coagulated under inhalation anesthesia with isoflurane (4% induction, 1% maintenance). After surgery, pups were returned to nursing dams for 2 hours, followed by exposure to hypoxic conditions (10% O_2, 90% N_2) for 50 minutes in a chamber accommodating no more than 25 animals per session. Gas composition within the chamber was monitored using a PKG-4 V-K-P gas analyzer (serial no. 62615-15, valid until 12 November 2025, Russia).

Assessment of motor and coordination functions

Motor and coordination functions were evaluated using the Inverted grid, Vertical pole, and Horizontal pole tests, adapted to detect deviant behaviors in juvenile mice (Table 1). The maximum duration for each test was 60 seconds. Each animal was given three attempts with a rest period of at least 30 minutes. Tests were conducted primarily in the morning under consistent “home” lighting to minimize stress. For the inverted grid test, the mesh size was adjusted to 5×5 mm with a wire diameter of 1 mm, and the screen area measured 25×25 cm.

Table 1. Evaluation criteria for time-to-completion and deviant behavior in motor activity tests in mice

Inverted grid test				
Tested parameter	Time to complete the test (seconds)			
Vertical pole test				
Tested parameter	Time to complete the test (seconds)			
Deviant behavior analysis (total score: 12)				
	0 point	1 point	2 points	3 points
Inversion	None	1	2	≥3
Abnormal hindlimb movement	None	–	–	Present
Falls	None	1	2	≥3
Stops during descent	None	1	2	≥3
Horizontal pole test				
Tested parameter	Time to complete the test (seconds)			
Deviant behavior analysis (total score: 12)				
	0 point	1 point	2 points	3 points
Turns	None	1	2	>2
Coordination errors/slipping	None	1	2	>2 or fall
Abnormal hindlimb movement	None	–	–	Present
Total pause time	≤10 sec	10–20 sec	20–30 sec	>30 sec

Assessment of exploratory behavior

Open field test

The open field test was used to evaluate motor activity, exploratory behavior, and anxiety levels. The test was conducted during daylight hours under constant diffuse lighting (40–50 lux) and minimal background noise. Animals were placed in the center of a square arena made of matte plastic (50×50 cm, wall height 40 cm). The surface was cleaned with 70% ethanol before each trial to eliminate odor traces.

The animal's behavior was recorded for 5 minutes using an overhead video camera. Analysis was performed using EthoVision software (Noldus Information Technology, Netherlands). The following parameters were analyzed: velocity, total distance traveled, number of entries into the center, and time spent in the peripheral zone.

Pharmacological challenge with caffeine

To investigate neurophysiological reactivity and latent forms of anxiety-like and motor behavior, a functional pharmacological challenge was conducted using caffeine administered intraperitoneally at a dose of 20 mg/kg (Coffeinum-natrii benzoas 20 %; JSC Mosagrogen, Russia), 1 hour prior to the Open field test.

Statistical analysis

Statistical processing and graphical representation of the results were performed using GraphPad Prism Software 8.0 (GraphPad Software Inc, USA). Data were analyzed using one-way ANOVA, Welch's ANOVA, or the Kruskal-Wallis test, followed by post-hoc analysis, as illustrated in the figures. The choice of test depended on the distribution characteristics of the data across groups. Numerical data in the text are presented as mean ± standard deviation (M±SD).

Results

Stratification of animals by severity of brain injury

Three hours after modeling neonatal hypoxia-ischemia, animals were stratified into groups based on injury severity using laser speckle contrast imaging of cerebral blood flow. Based on the difference in mean perfusion values between the affected and contralateral hemispheres, the following groups were formed: slight injury (n=13), moderate injury (n=15), severe injury (n=12), and no visible injury (n=11). An intact control group consisted of 10 animals (Table 2).

Based on perfusion assessment data from the affected and contralateral hemispheres, animals classified with slight and moderate brain injury were selected for further analysis. Animals showing no signs of damage were excluded due to the absence of a lesion focus, which precluded their meaningful inclusion in comparative analysis. In the severe injury group, eight out of twelve animals died by day 7 post-modeling. Given the insufficient number of surviving subjects and the inability to obtain statistically reliable data on behavioral dynamics, this group was excluded from further investigation.

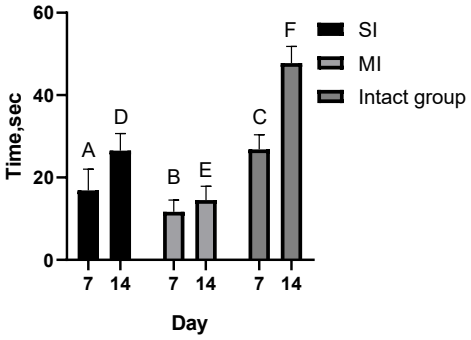
Table 2. Stratification of animals into groups based on the severity of brain tissue damage

Indicator	No visible injury	Slight injury	Moderate injury	Severe injury
Mean Δ perfusion units between the damaged and contralateral hemispheres	31±5	132±41	293±44	511±86
Number of animals per group on day 7	11	13	12	4

Note: Δ – difference between the mean perfusion values of brain tissues.

Assessment of motor activity and coordination on days 7 and 14

Statistically significant differences were observed between the slight and moderate brain injury groups at 7 and 14 days post-modeling of neonatal hypoxia-ischemia (Figs 1, 2, 3).



G

Welch's ANOVA test	
W (DFn, DFd)	53.26 (2.000, 17.17)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

H

Welch's ANOVA test	
W (DFn, DFd)	201.6 (2.000, 17.84)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

I

Games-Howell's multiple comparisons test		
Summary	Adjusted P Value	
*	0.0351	A-B
***	0.0004	A-C
****	<0.0001	B-C

J

Games-Howell's multiple comparisons test		
Summary	Adjusted P Value	
****	<0.0001	D-E
****	<0.0001	D-F
****	<0.0001	E-F

Figure 1. Results of the Inverted grid test on days 7 and 14 in mice, depending on the degree of brain damage after modeling HI. ***Note:*** SD – slight injury; MI – moderate injury; **A, B, C, D, E, F** in graphs – symbols assigned to each group for intergroup comparison; **G, H** – intra- and intergroup variance analysis; **I, J** – *post-hoc* analysis of intergroup comparisons; values in tables imported from Prism 8.0.1 software. Explanations in text.

On day 7 post-modeling, animals with slight (16.9 ± 5.13 s) and moderate (11.7 ± 2.83 s) injuries showed significantly reduced latency to fall in the Inverted grid test compared to that in the intact group (26.8 ± 3.55 s). By day 14, partial recovery of motor function was observed in the slight injury group (26.5 ± 4.17 s), while the moderate injury group (14.5 ± 3.37 s) continued to exhibit substantially impaired performance. These results indicate pronounced deficits in motor coordination and muscle strength, the severity of which correlates directly with the extent of brain injury (Fig. 1).

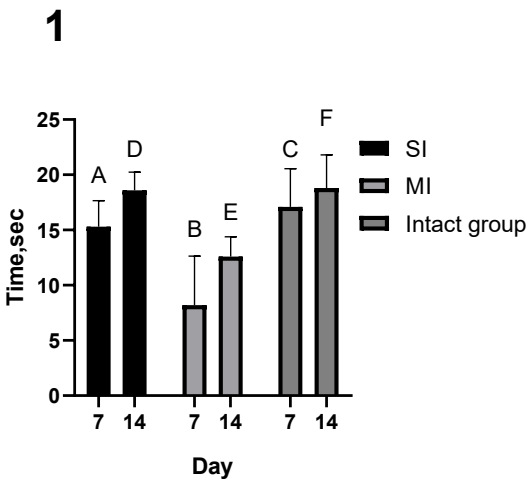
The Vertical pole and Horizontal pole tests revealed significant differences in motor performance and behavioral responses depending on the severity of neurological injury.

On day 7, the descent time from the Vertical pole was 17.1±3.44 s in intact animals, 12.5±2.01 s in the slight injury group, and 8.2±4.43 s in the moderate injury group. This indicates a reduction in performance by 27% and 53% compared to the intact group, respectively. Concurrently, an increase in deviant behavior scores was observed: 1.0±1.0 points in the intact group, 4.0±0.81 points in the slight injury group, and 6.9±1.37 points in the moderate injury group, suggesting impaired motor coordination and orientation.

By day 14, the descent time was 18.8±3.01 s in intact animals, 20.0±2.21 s in the slight defeat group, and 12.6±1.77 s in the moderate injury group. Animals with slight injury showed a tendency toward normalization of motor activity, unlike the moderate injury group. Deviant

behavior scores at this time point were 0.5±0.5 points in intact animals, 2.4±0.9 points in the slight injury group, and 5.1±1.19 points in the moderate injury group, reflecting persistent behavioral deficits in animals with more severe injuries.

On day 7, the time to complete the Horizontal pole test was 21.11±4.5 s in the intact group, 27.56±2.92 s in the slight injury group, and 38.22±3.52 s in the moderate injury group, reflecting an increase in task completion time by 22% and 81%, respectively. Deviant behavior scores at the same time point were 2.0±0.8 points in intact animals, 4.4±0.9 in the slight injury group, and 7.8±0.9 in the moderate injury group, corresponding to an increase of 24% and 46%, respectively.



G

Kruskal-Wallis test	
P value	0.0001
Exact or approximate P value?	Approximate
P value summary	***
Do the medians vary signif. (P < 0.05)?	Yes
Number of groups	3
Kruskal-Wallis statistic	18.24

H

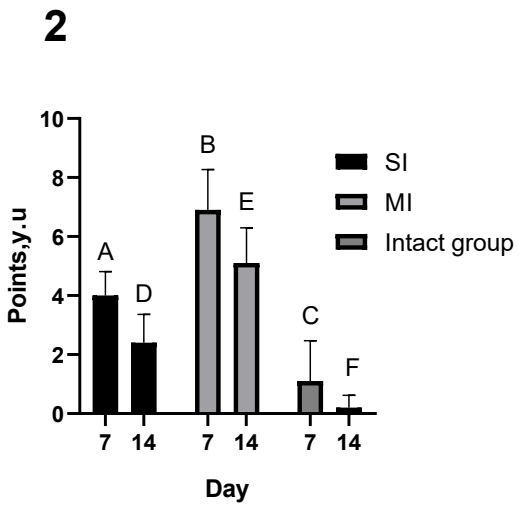
Welch's ANOVA test	
W (DFn, DFd)	37.54 (2.000, 17.30)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

I

Dunn's multiple comparisons test		
Summary	Adjusted P Value	
ns	0.2929	A-B
*	0.0296	A-C
****	<0.0001	B-C

J

Games-Howell's multiple comparisons test		
Summary	Adjusted P Value	
****	<0.0001	D-E
ns	0.5776	D-F
***	0.0002	E-F



G

Kruskal-Wallis test	
P value	<0.0001
Exact or approximate P value?	Approximate
P value summary	****
Do the medians vary signif. (P < 0.05)?	Yes

H

Kruskal-Wallis test	
P value	<0.0001
Exact or approximate P value?	Approximate
P value summary	****
Do the medians vary signif. (P < 0.05)?	Yes

I

Dunn's multiple comparisons test		
Summary	Adjusted P Value	
*	0.0435	A-B
*	0.0314	A-C
****	<0.0001	B-C

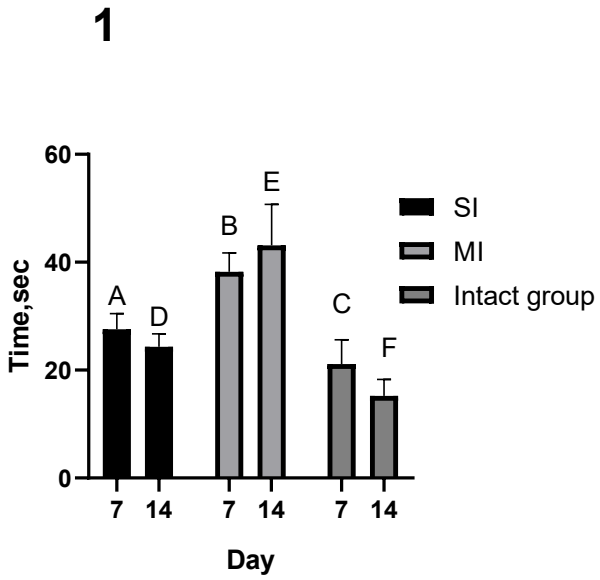
J

Dunn's multiple comparisons test		
Summary	Adjusted P Value	
*	0.0462	D-E
*	0.0299	D-F
****	<0.0001	E-F

Figure 2. Latency to complete the vertical pole test on days 7 and 14 in mice, depending on the degree of brain damage after modeling HI. ***Note:*** 1 – Time to complete the Vertical pole test on days 7-14; 2 – Deviant behavior during the Vertical pole test on days 7-14; SD – slight injury; MI – moderate injury; **A, B, C, D, E, F** in graphs – symbols assigned to each group for intergroup comparison; **G, H** – intra- and intergroup variance comparison; **I, J** – Post-hoc analysis of intergroup comparisons; Values in tables are imported from Prism 8.0.1 software. Explanations in text.

By day 14, a similar trend was observed: the test completion time was 15.22±3.07 s in intact animals, 24.33±2.39 s in the slight injury group, and 43.11±7.59 s in the moderate injury group. This indicates an increase in task completion time by 62% and nearly 2.8-fold compared to

controls, respectively. Deviant behavior scores on day 14 were 0.5±0.5 points in intact animals, 1.9±0.8 in the slight injury group, and 5.6±0.6 in the moderate injury group. Statistically significant differences persisted between all groups, except for the comparison between the intact group and the slight defeat group.



G

Welch's ANOVA test	
W (DFn, DFd)	43.14 (2.000, 15.55)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

H

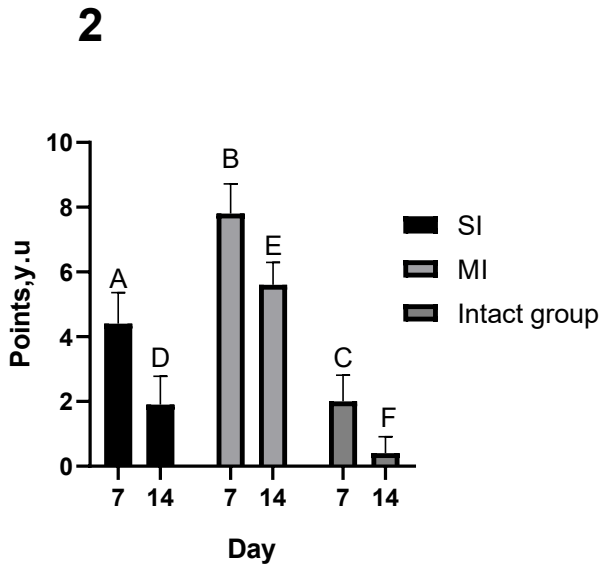
Welch's ANOVA test	
W (DFn, DFd)	58.24 (2.000, 14.48)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

I

Games-Howell's multiple comparisons test		
Summary	Adjusted P Value	
****	<0.0001	A-B
**	0.0079	A-C
****	<0.0001	B-C

J

Games-Howell's multiple comparisons test		
Summary	Adjusted P Value	
***	0.0001	D-E
****	<0.0001	D-F
****	<0.0001	E-F



G

Welch's ANOVA test	
W (DFn, DFd)	43.14 (2.000, 15.55)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

H

Kruskal-Wallis test	
P value	<0.0001
Exact or approximate P value?	Approximate
P value summary	****
Do the medians vary signif. (P < 0.05)?	Yes

I

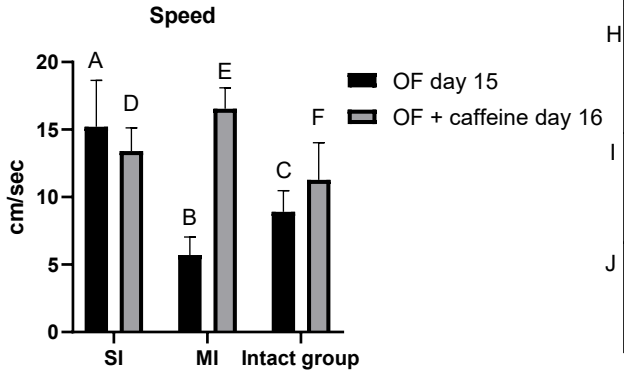
Games-Howell's multiple comparisons test		
Summary	Adjusted P Value	
****	<0.0001	A-B
**	0.0079	A-C
****	<0.0001	B-C

J

Dunn's multiple comparisons test		
Summary	Adjusted P Value	
*	0.0157	D-E
ns	0.0896	D-F
****	<0.0001	E-F

Figure 3. Latency to complete the Horizontal pole test on days 7 and 14 in mice, depending on the degree of brain damage after modeling HI. ***Note:*** 1 – Time to complete the Horizontal pole test on days 7-14; 2 – Deviant behavior during the Horizontal pole test on days 7-14; SI – slight injury; MI – moderate injury; **A, B, C, D, E, F** in graphs – symbols assigned to each group for intergroup comparison; **G, H** – intra- and intergroup variance comparison; **I, J** – Post-hoc analysis of intergroup comparisons; Values in tables are imported from Prism 8.0.1 software. Explanations in text.

1



G

ANOVA summary	
F	43.70
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.7640

H

ANOVA summary	
F	14.71
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.5612

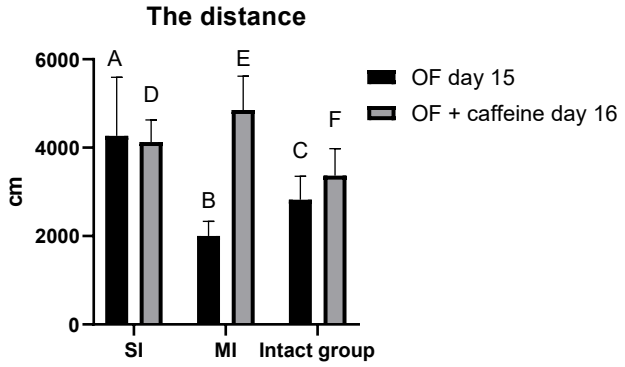
I

Tukey's multiple comparisons test		
Summary	Adjusted P Value	
****	<0.0001	A-B
****	<0.0001	A-C
*	0.0120	B-C

J

Tukey's multiple comparisons test		
Summary	Adjusted P Value	
**	0.0062	D-E
ns	0.2054	D-F
****	<0.0001	E-F

2



G

ANOVA summary	
F	20.47
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.6807

H

ANOVA summary	
F	12.29
P value	0.0002
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R square	0.5060

I

Tukey's multiple comparisons test		
Summary	Adjusted P Value	
****	<0.0001	A-B
**	0.0021	A-C
ns	0.0651	B-C

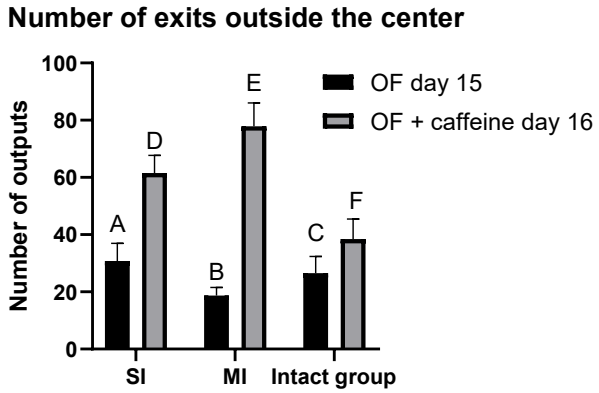
J

Tukey's multiple comparisons test		
Summary	Adjusted P Value	
ns	0.0577	D-E
*	0.0468	D-F
***	0.0001	E-F

Figure 4. Velocity and total distance traveled in the Open field test in mice, depending on the degree of brain damage after modeling HI. ***Note:*** 1 – Velocity in the open field test on days 15 and 16 with caffeine stimulation; 2 – Distance traveled in the Open field test on days 15 and 16 with caffeine stimulation; SI – slight injury; MI – moderate injury; **A, B, C, D, E, F** in graphs – symbols assigned for intergroup comparisons; **G,H** – intra- and intergroup variance analysis; **I, J** – Post-hoc analysis of intergroup comparisons; Values imported from Prism 8.0.1 software. Explanations in text.

Velocity measurements showed an increase in the slight injury group (15.21±3.44 cm/s) and a decrease in the moderate injury group (5.70±1.33 cm/s) compared to those in intact animals (8.91±1.56 cm/s). Significant differences were also observed in distance traveled: slight injury group – 4267±1107 cm, moderate injury group – 2004±329 cm, and versus intact animals – 2823±527 cm. Hyperactivity was observed in the slight injury group, while the moderate injury group showed markedly reduced locomotion, with statistically significant intergroup differences. Center zone entries numbered 26±5 in intact animals, 30±6 in the slight injury group, and 18±2 in the moderate injury group. The significant reduction in this parameter in the moderate injury group compared to both control and slight injury groups indicates increased anxiety and reduced exploratory behavior. Time spent in the peripheral zone averaged 272.4±9.2 s in intact animals, 245.5±19.1 s in the slight injury group, and 217.1±9.5 s in the moderate injury group. Reduced peripheral zone time suggests altered behavioral strategies and disrupted balance between exploration and defensive behavior, particularly pronounced in moderate injury.

1



G

ANOVA summary	
F	123.2
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9277

H

ANOVA summary	
F	70.57
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.8547

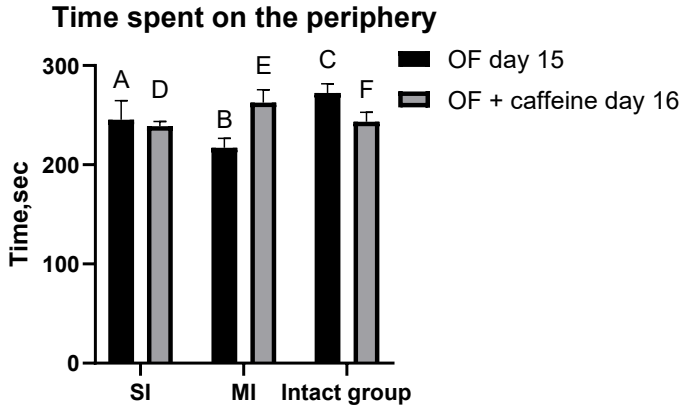
I

Tukey's multiple comparisons test		
Summary	Adjusted P Value	
***	0.0001	A-B
ns	0.2170	A-C
**	0.0098	B-C

J

Tukey's multiple comparisons test		
Summary	Adjusted P Value	
***	0.0001	D-E
****	<0.0001	D-F
****	<0.0001	E-F

2



G

ANOVA summary	
F	22.02
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.6964

H

ANOVA summary	
F	20.93
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.6356

I

Tukey's multiple comparisons test		
Summary	Adjusted P Value	
***	0.0005	A-B
***	0.0008	A-C
****	<0.0001	B-C

J

Tukey's multiple comparisons test		
Summary	Adjusted P Value	
****	<0.0001	D-E
ns	0.0725	D-F
**	0.0012	E-F

Figure 5. Time spent in peripheral zone and number of center entries in mice, depending on the degree of brain damage after modeling HI. ***Note:*** 1 – Number of center entries in the Open field test on days 15 and 16 with caffeine stimulation; 2 – Time spent in peripheral zone in the Open field test on days 15 and 16 with caffeine stimulation; SI – slight injury; MI – moderate injury; **A, B, C, D, E, F** in graphs – symbols for intergroup comparisons; **G, H** – intra- and intergroup variance; **I, J** – Post-hoc analysis; Values from Prism 8.0.1. Explanations in text.

The caffeine challenge test revealed significant differences in spatial-orientation activity depending on neurological injury severity (Figs 4, 5). Velocity increased in the slight injury (13.41±1.72 cm/s) and more markedly in the moderate injury group (16.54±1.55 cm/s) compared to that in intact animals (11.8±2.08 cm/s). Distance traveled increased from 3370±608 cm in intact animals to 4126±503 cm in the slight injury group and 4852±763 cm in the moderate injury group. The moderate injury group showed statistically significant increases in distance traveled, reflecting enhanced motor reactivity under caffeine. Center entries significantly increased in both injury groups: intact – 39±6, slight defeat – 61.6±6, moderate injury – 77±8, indicating reduced anxiety and enhanced exploration following caffeine stimulation, particularly pronounced in more severely affected animals. Time in peripheral zone was 243.5±9.3 s (intact), 244.3±9.2 s (slight injury), and 262.7±12.9 s (moderate injury). Despite similarities in controls in slight injury, moderate injury animals showed significant increases, possibly reflecting impaired spatial orientation and heightened motor vigilance.

The Open field and caffeine challenge tests indicate substantial motor and exploratory deficits in moderate injury compared to intact and slight injury groups. Slight injury preserves mobility but partially reduces exploratory behavior. Caffeine predominantly enhanced motor responses in moderate injury, suggesting altered neurophysiological sensitivity to stimulants.

Discussion

The obtained results demonstrate that the severity of neonatal hypoxic-ischemic brain injury significantly affects motor and exploratory activity in animals. As early as 7 and 14 days post-modeling, mice with moderate injury showed substantial reductions in velocity, distance traveled, and number of center entries in the Open field test compared to intact and slightly injured animals. Such hypoactivity and anxiety-like behavior have previously been associated with functional suppression of the dopaminergic system, which regulates motor activity, motivation, and responses to stress stimuli (Qian et al. 2013).

Comparison between slight and moderate injury groups revealed that the severity of motor impairments increases proportionally with injury extent. This aligns with recent studies demonstrating dose-dependent reductions in tyrosine hydroxylase (TH) and dopamine transporter (DAT) expression in the midbrain alongside hypoactivity in models of neuroinflammation and moderate hypoxic damage (Kweon et al. 2024). Slight injury was characterized by preserved partial motor activity and exploratory behavior, suggesting compensatory mechanisms despite moderate dopaminergic suppression. In contrast, animals with moderate injury exhibited insufficient compensation, manifesting as markedly reduced locomotor performance. Recent studies attribute such changes to impaired striatonigral pathway function and reduced neuronal activity in the substantia nigra (Wang et al. 2024).

The effects of caffeine are of particular interest. In our study, stimulation with this A1/A2A adenosine receptor antagonist primarily enhanced motor activity in moderately injured animals. According to recent data, adenosine receptor blockade potentiates dopaminergic transmission by increasing D2 receptor sensitivity and dopamine release (Lopes et al. 2019). The observed caffeine effect likely reflects compensatory hypersensitivity of postsynaptic dopamine receptors under conditions of reduced presynaptic neurotransmitter production (Salvi et al. 2020).

Reduced time spent in the peripheral zone and decreased center entries in moderately injured animals may indicate impaired cognitive and anxiety-related behavioral components. The combined behavioral and caffeine challenge results confirm that neonatal hypoxia-ischemia causes persistent alterations in dopaminergic regulation. These changes include reduced activity of dopamine metabolic and synthetic enzymes alongside compensatory upregulation of D2 receptor expression (Kozina et al. 2017).

Thus, the identified patterns indicate that motor impairment severity depends on the extent of cerebral damage and involvement of dopaminergic pathways. The caffeine effect reflects residual neural plasticity potential amid partially preserved dopaminergic neurons, suggesting therapeutic prospects for adenosine antagonists in rehabilitation strategies post hypoxic-ischemic events.

To expand and correlate these findings with molecular processes, future studies should investigate neuroplasticity markers (GFAP, IL-1β, BDNF) and molecular validation of behavioral changes through TH and DAT gene expression analysis in midbrain and striatal regions. This would clarify dopaminergic system involvement in post-hypoxic impairments.

From a practical point of view, the identified differences in behavioral characteristics in animals with different degrees of severity of HIE will allow for a more detailed assessment of the effectiveness of neuroprotective therapy with various pharmacological compounds in preclinical studies. This is particularly important, as the same drugs may demonstrate therapeutic potential in mild damage, but may not have a significant effect in moderate pathology.

Conclusion

The experimental approach assessing behavioral changes in animals after neonatal hypoxia-ischemia modeling, followed by severity-based stratification, revealed statistically significant differences across multiple parameters. Pharmacological testing with caffeine (20 mg/kg) uncovered specific neurophysiological response patterns. Slight brain injury was characterized by reversible changes, while moderate injury led to persistent dopaminergic suppression and significant motor deficits. Notably, caffeine induced substantial motor enhancement primarily in moderately injured animals, suggesting adaptive reorganization in dopaminergic regulation and heightened sensitivity to psychostimulation despite substantial brain tissue damage.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

Funding

This work was supported by the Ministry of Science and Higher Education of the Russian Federation, agreement No. FZWG-2021-0016.

Ethical statement

The study was approved by the Animal Ethics Committee of Belgorod State National Research University (BelSU), approval No. 01-08i/25 dated 18 August 2025.

Data availability

All of the data that support the findings of this study are available in the main text.

References

- Babbo CC, Mellet J, van Rensburg J, Pillay S, Horn AR, Nakwa FL, Velaphi SC, Kali GTJ, Coetzee M, Masemola MYK, Ballot DE, Pepper MS (2024) Neonatal encephalopathy due to suspected hypoxic ischemic encephalopathy: pathophysiology, current, and emerging treatments. World Journal of Pediatrics 20(11): 1105–1114. https://doi.org/10.1007/s12519-024-00836-9 [PubMed] [PMC]
- Brégère C, Schwendele B, Radanovic B, Guzman R (2022) Microglia and stem-cell mediated neuroprotection after neonatal hypoxia-ischemia. Stem Cell Reviews and Reports 18(2): 474–522. https://doi.org/10.1007/s12015-021-10213-y [PubMed] [PMC]
- Chen A, Teng C, Wei J, Wu X, Zhang H, Chen P, Cai D, Qian H, Zhu H, Zheng X, Chen X (2025) Gut microbial dysbiosis exacerbates long-term cognitive impairments by promoting intestinal dysfunction and neuroinflammation following neonatal hypoxia-ischemia. Gut Microbes 17(1): 2471015. https://doi.org/10.1080/19490976.2025.2471015 [PubMed] [PMC]
- Essawy SS, Tawfik MK, Korayem HE (2017) Effects of adenosine receptor antagonists in MPTP mouse model of Parkinson's disease: mitochondrial DNA integrity. Archives of Medical Science 13(3): 659-669. https://doi.org/10.5114/aoms.2017.67284 [PubMed] [PMC]
- Hamdy N, Eide S, Sun HS, Feng ZP (2020) Animal models for neonatal brain injury induced by hypoxic ischemic conditions in rodents. Experimental Neurology 334: 113457. https://doi.org/10.1016/j.expneurol.2020.113457 [PubMed]
- Ibrahim MK, Kamal M, Tikamdas R, Nouh RA, Tian J, Sayed M (2020) Effects of chronic caffeine administration on behavioral and molecular adaptations to sensory contact model induced stress in adolescent male mice. Behavior Genetics 50(5): 374–383. https://doi.org/10.1007/s10519-020-10003-1 [PubMed]
- Kim M, Yu JH, Seo JH, Shin YK, Wi S, Baek A, Song SY, Cho SR (2017) Neurobehavioral assessments in a mouse model of neonatal hypoxic-ischemic brain injury. Journal of Visualized Experiments (129): 55838. https://doi.org/10.1080/19490976.2025.2471015 [PubMed] [PMC]
- Kozina EA, Kim AR, Kurina AY, Ugrumov MV (2017) Cooperative synthesis of dopamine by non-dopaminergic neurons as a compensatory mechanism in the striatum of mice with MPTP-induced Parkinsonism. Neurobiology of Disease 98: 108–121. https://doi.org/10.1016/j.nbd.2016.12.005 [PubMed]
- Kweon S.H, Ryu HG, Park H, Lee S, Kim N, Kwon SH, Ma SX, Kim S, Ko HS (2024) Linking Gba1 E326K mutation to microglia activation and mild age-dependent dopaminergic neurodegeneration. BioRxiv 21:2023.09.14.557673. https://doi.org/10.1101/2023.09.14.557673 [PubMed] [PMC]
- Lopes JP, Pliássova A, Cunha RA (2019) The physiological effects of caffeine on synaptic transmission and plasticity in the mouse hippocampus selectively depend on adenosine A1 and A2A receptors. Biochemical Pharmacology 166: 313–321. https://doi.org/10.1016/j.bcp.2019.06.008 [PubMed]
- Odorcyk FK, Ribeiro RT, Roginski AC, Duran-Carabali LE, Couto-Pereira NS, Dalmaz C, Wajner M, Netto CA (2021) Differential age-dependent mitochondrial dysfunction, oxidative stress, and apoptosis induced by neonatal hypoxia-ischemia in the immature rat brain. Molecular Neurobiology 58(5): 2297–2308. https://doi.org/10.1007/s12035-020-02261-1 [PubMed]
- Qian Y, Chen M, Forssberg H, Diaz Heijtz R (2013) Genetic variation in dopamine-related gene expression influences motor skill learning in mice. Genes, Brain and Behavior 12(6): 604–614. https://doi.org/10.1111/gbb.12062 [PubMed]
- Ratliff WA, Saykally JN, Mervis RF, Lin X, Cao C, Citron BA (2019) Behavior, protein, and dendritic changes after model traumatic brain injury and treatment with nanocoffee particles. BMC Neuroscience 20(1): 44. https://doi.org/10.1186/s12868-019-0525-5 [PubMed] [PMC]
- Sheldon RA, Windsor C, Ferriero DM (2018) Strain-related differences in mouse neonatal hypoxia-ischemia. Developmental Neuroscience 40(5–6): 490–496. https://doi.org/10.1159/000495880 [PubMed] [PMC]
- Wang Q, Wang M, Choi I, Sarrafha L, Liang M. Ho L, Farrell K, Beaumont KG, Sebra R, De Sanctis C, Crary JF, Ahfeldt T, Blanchard J, Neavin D, Powell J, Davis DA, Sun X, Zhang B, Yue Z (2024) Molecular profiling of human substantia nigra identifies diverse neuron types associated with vulnerability in Parkinson's disease. Science Advances 10(2): eadi8287. https://doi.org/10.1126/sciadv.adi8287 [PubMed] [PMC]
- Weston NM, Rolfe AT, Freelin AH, Reeves TM, Sun D (2021) Traumatic brain injury modifies synaptic plasticity in newly-generated granule cells of the adult hippocampus. Experimental Neurology 336: 113527. https://doi.org/10.1016/j.expneurol.2020.113527 [PubMed] [PMC]

- Zhou KQ, McDouall A, Drury PP, Lear CA, Cho KHT, Bennet L, Gunn AJ, Davidson JO (2021) Treating seizures after hypoxic-ischemic encephalopathy-current controversies and future directions. International Journal of Molecular Sciences 22(13): 7121. https://doi.org/10.3390/ijms22137121
- Zhu H, Bai S, Ma W, Qian H, Du P (2024) A combined effect of fish-originated collagen peptides and caffeine on the cognitive function of sleep-deprived mice. Food & Function 15(2): 917–929. https://doi.org/10.1039/d3fo03841f [PubMed]

Author Contributions

Vladimir M. Pokrovsky, Junior Researcher, Research Institute of Pharmacology of Living Systems, Belgorod State National research University, Belgorod, Russia; e-mail: vmpokrovsky@yandex.ru; **ORCID ID:** https://orcid.org/0000-0003-3138-2075. The author participated in the conceptualization and development of the research direction, defining key goals and objectives, conducting experimental work, analyzing materials, and writing the article.

Ivan V. Chatsky, Laboratory research assistant, Research Institute of Pharmacology of Living Systems, Belgorod State National research University, Belgorod, Russia; e-mail: 1212327@bsuedu.ru; **ORCID ID**: https://orcid.org/0009-0001-0072-4426. The author prepared experimental cohorts and conducted behavioral research.

Maria R. Maslinikova, Laboratory research assistant, Research Institute of Pharmacology of Living Systems, Belgorod State National research University, Belgorod, Russia; e-mail: maria.maslinikova@gmail.com; **ORCID ID**: https://orcid.org/0009-0003-4042-7631 The author prepared experimental cohorts and conducted behavioral research.

Natalia V. Zabolotskikh, Doctor Habil. of Medical Sciences, Professor of the Department of Clinical Pharmacology and Functional Diagnostics, Associate Professor of the Department of Neurology and Neurosurgery, Kuban State Medical University of Public Health Care of Russia, Krasnodar, Russia; e-mail: optimaznv@mail.ru; **ORCID ID:** https://orcid.org/0000-0002-2409-4684. The author participated in the development of the research direction and material analysis.

Arkady V. Nesterov, PhD in Medical Sciences, Associate Professor, Belgorod State National research University, Belgorod, Russia; e-mail: nesterov_a@yandex.ru; **ORCID ID:** https://orcid.org/0000-0003-3822-4213. The author participated in pathology modeling.

Nikita V. Popov, undergraduate student, Institute of Medicine, Belgorod State National Research University, Belgorod, Russia; e-mail: 1450552@bsuedu.ru. The author prepared experimental cohorts and conducted behavioral research.

Mikhail V. Korokin, Doctor Habil. of Medical Sciences, Professor of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: mkorokin@mail.ru; **ORCID ID:** https://orcid.org/0000-0001-5402-0697. The author participated in the development of the research direction and material analysis.