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Intergenerational Conditioning via Intermittent Parental Hypoxia Confers Stroke Resilience in Offspring

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Brief summary

Parental intermittent hypoxia induces intergenerational resilience to ischaemic stroke in mice, reducing infarct size in offspring via sex- and lineage-specific molecular adaptations.

Disclourses

None

Number of figures and tables

3 Figures

List of the supplemental materials

Supplemental Tables S1-4
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Abstract

Background:

Intergenerational disease transmission, where parental exposures or experiences influence disease susceptibility in offspring, may represent a crucial layer of stroke risk that extends beyond genetics alone. Environmental conditioning, such as intermittent sub-lethal hypoxia, can induce adaptive protective stress responses in the brain. However, whether such parental conditioning enhances offspring resilience to cerebral ischaemia remains unclear. This study investigates whether intermittent hypoxia in parents acts as an intergenerational conditioning stimulus, conferring resilience to ischaemic stroke in offspring, and explores associated molecular mechanisms.

Methods:

Male and female Balb/C mice (F0; 8-10 weeks old) were exposed to intermittent hypoxia (8% O_2, 2 hours every other day, 16 cycles) prior to mating. To confirm parental neuroprotection, F0 mice underwent transient middle cerebral artery occlusion (tMCAO). Offspring (F1) from hypoxia-exposed F0 were divided into biparental, paternal-only, maternal-only hypoxia, and normoxic groups. Adult F1 (8-10 weeks old) offspring also underwent tMCAO to model ischaemic stroke. Infarct volume and brain swelling were assessed 48 hours post-ischaemia. A subgroup of F0 and F1 brains was analysed by tandem mass tag proteomics to identify molecular pathways linked to neuroprotection.

Results:

Parental intermittent hypoxia significantly reduced infarct size and swelling in F0 mice. These protective effects were inherited by F1 offspring, with biparental exposure producing the greatest reduction in infarct volume, followed by maternal-only and paternal-only groups, and exhibiting sex-specific differences. Proteomic profiling revealed distinct treatment and lineage clusters. Key pathways implicated in offspring neuroprotection included metabolic regulation, immune signalling, cytoskeletal organisation, and cell survival, notably involving PI3K-Akt and EGFR pathways.

Conclusions:

Intermittent hypoxia in parents acts as an intergenerational conditioning stimulus, conferring offspring resilience to stroke. This neuroprotective phenotype is supported by coordinated molecular adaptations in key pathways involved in survival and stress response. These findings highlight the potential for parental environmental conditioning to shape stroke outcomes in offspring, opening new avenues for therapeutic exploration.

Non-standard Abbreviations and Acronyms

Abbreviation	Definition
BALB/c	Inbred mouse strain originally derived from the Bagg albino lineage
DEP	Differentially expressed protein
F0	Parental generation
F1	First filial (offspring) generation
KEGG	Kyoto Encyclopedia of Genes and Genomes
LDF	Laser Doppler flowmetry
MCA	Middle cerebral artery
tMCAO	Transient middle cerebral artery occlusion
RIPC	Remote ischaemic preconditioning

1. Introduction

Ischaemic stroke is a leading cause of adult disability worldwide, and despite advances in acute management, outcomes remain highly variable. While genetic factors undoubtedly contribute to stroke susceptibility, a growing body of evidence suggests that intergenerational disease transmission, in which parental exposures or experiences influence offspring vulnerability, plays an important role in determining stroke risk and severity[1].

One potential mechanism by which parents might influence offspring stroke outcomes is via environmental conditioning[2]. For instance, parental stress or toxin exposure has been linked to altered offspring neurodevelopment and increased susceptibility to neurological disorders via epigenetic modifications[3,4]. Additionally, environmental conditioning such as exercise or caloric restriction in parents can improve offspring cognitive function and resilience to brain injury[5,6].

Intermittent sub-lethal hypoxia is a well-established preconditioning stimulus that can induce neuroprotective adaptations in the brain, largely through activation of stress response pathways, improved metabolic efficiency, and modulation of cell survival mechanisms[2,7].

Both remote ischemic preconditioning (RIPC) and intermittent hypoxia are established methods to induce a prolonged state of ischemic tolerance in preclinical models[8]. Delayed RIPC improves long-term sensorimotor deficits in neonatal rats up to five weeks after hypoxic-ischemic injury[9]. Interestingly, a key study in adult mice demonstrated that intermittent hypoxia conferred protection for up to eight weeks after the end of the conditioning regimen[10]. These results suggest a lasting change in neurovascular physiology, which could potentially serve as the basis for intergenerational transmission of resilience.

However, whether parental exposure to intermittent hypoxia can confer intergenerational resilience to ischaemic injury in offspring has not been fully explored. Here, we investigate whether parental intermittent hypoxia acts as an intergenerational conditioning stimulus to enhance offspring resilience to ischaemic stroke. Using a well-established BALB/c mouse model of transient middle cerebral artery occlusion (tMCAO)[11], we assess infarct volume and brain swelling in offspring from different parental hypoxia exposures.

To further enhance the clinical relevance of our findings, we included both male and female mice to investigate potential sex-specific responses, reflecting the known differences in stroke outcomes observed in human populations.

2. Materials and Methods

Availability of Data

The raw data from this study are available from the corresponding author upon reasonable request.

Animals and Experimental Procedures

All procedures were approved by the Ethics Committee of Istanbul Medipol University (2020/16) and conducted in accordance with the Declaration of Helsinki and relevant institutional guidelines. This study adhered to ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines to ensure transparency, reproducibility, and ethical standards[12]. All experimental procedures occurred in a controlled environment with standard housing conditions, including a 12:12-hour light-dark cycle, temperature control, and environmental enrichment.

BALB/c mice were selected for their extensive use and well-characterised profile in stroke, immune, and epigenetic studies[13-18] (see Supplemental Method 1 for model rationale).

Experimental groups included F0 parental Balb/C mice exposed to intermittent hypoxia or normoxia, and their F1 offspring subdivided by parental exposure (biparental, maternal-only, paternal-only, and normoxic controls).

Details of the experimental design, sample size determination, randomisation procedures, and blinding strategy are provided in Supplemental Method 2.

Intermittent hypoxia was induced using a controlled 8% O_2 exposure paradigm[19] (Figure 1A) adapted from established preconditioning protocols[20-22] (see Supplemental Method 3).

Transient Middle Cerebral Artery Occlusion (tMCAO)

tMCAO was performed using the standard intraluminal filament technique[11] under isoflurane anaesthesia (see Supplemental Method 4).

Laser Doppler flowmetry (LDF) recordings showed a consistent reduction in cerebral blood flow following MCA occlusion across all animals (Supplementary Table S1-3).

Infarct volume (mm^3) and brain swelling (%) were quantified from cresyl violet–stained 2-mm coronal sections at 2 days post-ischaemia using established methods[23,24] (see Supplemental Method 5). Oedema-corrected infarct volumes, expressed as a percentage of the ipsilateral hemisphere, are presented in Supplementary Table S4[25].

Proteomics

Damaged hemisphere samples were collected from the ipsilateral hemisphere at 55 days post-stroke, specifically encompassing both the infarct core and peri-infarct regions. Samples were obtained from F0 (12 weeks old) and F1 (8-10 weeks old) mice. Tissue was dissected, homogenised, and processed for protein extraction[26].

Proteomic profiling was performed by LC-MS/MS, and pathway enrichment analysis was conducted using established bioinformatics tools (see Supplemental Method 6).

Statistical analysis

Data were analysed using t-tests or one-way ANOVA with Dunnett's multiple comparisons test, as appropriate, in GraphPad Prism (v10.4.2) and R. Results are reported as mean ± SEM, with $p < 0.05$ considered statistically significant. All reported results met parametric assumptions.

3. Results

Hypoxic preconditioning in F0 mice reduces ischaemic brain injury

To determine the effect of intermittent hypoxia on stroke outcomes, we compared infarct volumes and brain swelling in F0 mice subjected to tMCAO. As previously demonstrated by us and others, intermittent hypoxia significantly reduced infarct volume (25.01% reduction; $p = 0.0032$) and brain swelling (24.86% reduction; $p = 0.0099$) compared to normoxic controls (Figure 1B).

Breeding from hypoxia-exposed parents produced four F1 groups: paternal-only, maternal-only, biparental hypoxia, and normoxic controls. Following tMCAO in adult F1 offspring, all hypoxic groups exhibited reduced infarct volume and brain swelling, with sex-specific differences (Figure 1C-D).

In males (Figure 1C), infarct volume was significantly reduced in the paternal (37.5% reduction; p = 0.0030), maternal (45% reduction; p = 0.0005), and biparental (42.5% reduction; p = 0.0007) groups compared to normoxic controls. However, only the maternal (56.52% reduction; $p < 0.0001$) and biparental (34.78% reduction; p = 0.0125) groups showed significantly reduced brain swelling; paternal hypoxia showed a non-significant trend (26.10% reduction; p = 0.0929).

In females (Figure 1D), infarct volume was significantly reduced in the maternal (26.92% reduction; p = 0.0272) and biparental (40.38% reduction; p = 0.0007) groups, with a non-significant trend in the paternal group (19.23% reduction; p = 0.1883). Brain swelling showed a similar pattern, with significant reductions in the maternal (53.33% reduction; p = 0.0008) and biparental (40.0% reduction; p = 0.0185) groups, but no reduction in the paternal group compared to normoxic controls.

Proteomic insights into intergenerational neuroprotection

Building on the observed neuroprotection in both F0 and F1 mice, we performed proteomic analysis to explore underlying molecular mechanisms. For F0 mice, we analysed both male and female brains. F1 analyses focused on females, as the maternal and biparental hypoxia groups consistently exhibited the most robust reductions in infarct volume and brain swelling. This approach aimed to enhance the signal-to-noise ratio and increase the likelihood of identifying molecular signatures associated with intergenerational neuroprotection.

Unsupervised hierarchical clustering of the proteomic data revealed clear segregation by treatment and lineage (Figure 2A). F0 and F1 normoxic female samples clustered together, indicating consistency across generations in the absence of hypoxic preconditioning. In contrast, F1 hypoxic groups (maternal, paternal, and biparental) formed a distinct cluster, suggesting coordinated protein expression changes linked to ancestral hypoxia exposure. Interestingly, F0 hypoxic and normoxic males clustered together, indicating limited proteomic alterations, while F0 hypoxic females diverged from normoxic controls, highlighting a sex-specific response to intermittent hypoxia.

Differential proteomic responses in F0 hypoxic mice

Consistent with the hierarchical clustering, differential expression analysis revealed that hypoxia exposure altered the expression of 31 proteins in F0 males and 477 proteins in F0 females compared to their respective normoxic controls ($p < 0.05$; Figure 2B-E). Seventeen proteins were commonly differentially expressed in both sexes, suggesting a shared molecular response (Figure 2B). Among these, *Gnai3* was upregulated and *Marcksl1* was downregulated in both hypoxic males and females. Notably, *Gnai3* has been implicated in cerebral ischaemic injury and identified as a target of Astragaloside IV in a rat stroke model [27], highlighting its potential relevance to neuroprotection. *Marcksl1* has been identified as a differentially expressed gene in the mouse corpus callosum of ageing mice [28], suggesting a potential role in age-related neurological disorders.

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis of differentially expressed proteins (DEPs) in F0 females revealed significant enrichment in pathways related to protein digestion and absorption, thyroid hormone synthesis, mineral absorption, endocrine signalling, and cytoskeletal regulation (Figure 2F). Although these pathways are not directly linked to ischaemic injury, they may reflect broader systemic or metabolic adaptations to intermittent hypoxia. We focused on F0 females because they showed the most distinct clustering from normoxic controls and the highest number of DEPs, suggesting a more pronounced proteomic response to hypoxic preconditioning.

Differential proteomic responses in F1 hypoxic mice

Differential expression analysis revealed that parental hypoxia exposure altered the expression of 44 proteins in the paternal group, 33 proteins in the maternal group, and 58 proteins in the biparental group compared to F1 normoxic controls (Figure 3A-E). Six proteins were commonly downregulated across all three groups, suggesting a shared molecular response (Figure 2E). These included *Akr1a1*, *Atp12a*, *Csde1*, *Gnaz*, *Impa1*, and *Srgap3*.

Given its robust and consistent phenotypic protection across infarct volume and brain swelling, we focused pathway enrichment analysis on the F1 biparental group. This group also exhibited the highest number of differentially expressed proteins, increasing the power to detect enriched biological pathways.

KEGG pathway enrichment analysis in the F1 biparental group revealed significant involvement of pathways related to HPV infection, focal adhesion, insulin resistance, apoptosis, Hippo signalling, PI3K-Akt signalling, T cell receptor signalling, and EGFR signalling. Collectively, these pathways point towards alterations in cell survival, immune modulation, and intracellular signalling cascades that may underlie the observed intergenerational neuroprotection.

4. Discussion

Taken together, these results demonstrate that intermittent hypoxia reduces infarct volume and brain swelling in F0 mice and that this protective effect is transmitted to F1 offspring. Both maternal and paternal hypoxia exposure contributed to reduced injury, with biparental hypoxia showing the greatest protection.

The observed sex-specific differences suggest distinct mechanisms of inherited resilience. These findings support the idea that intermittent hypoxia induces heritable changes that improve stroke outcomes in offspring, potentially via epigenetic mechanisms and highlighting novel avenues for stroke prevention.

Our proteomic data reveal intra-group consistency that aligns with the observed phenotypes. Normoxic F0 and F1 females clustered together, while hypoxia-exposed F1 groups (maternal, paternal, biparental) formed a distinct cluster, indicating a heritable proteomic shift. F0 females exhibited the most pronounced proteomic changes, consistent with their distinct clustering and higher number of differentially expressed proteins (DEPs), justifying their selection for pathway analysis. In F1 offspring, the biparental group demonstrated the most robust phenotypic protection and the highest number of DEPs, supporting its selection for pathway enrichment analysis. Interestingly, F0 males showed limited proteomic changes despite their protective phenotype, suggesting divergent or subtler molecular mechanisms.

Several of our findings align with existing literature, including the differential expression of *Gnai3* and *Marcksl1*, which are implicated in brain injury and ageing, respectively. Key pathways enriched in the biparental group, such as PI3K-Akt[29] and EGFR signalling[30], are established mediators in ischaemic stroke.

The divergent protein expression patterns between male and female F0 mice suggest sex-dependent molecular adaptations to hypoxic preconditioning[31-34], potentially influenced by hormonal or transcriptional factors[35-37] (see Supplemental Discussion 1 for detailed interpretation).

These results from Balb/C mice may extend to other mammals, including humans, due to conserved stroke-related pathways. Including both sexes improves clinical relevance by reflecting human sex

differences in stroke outcomes. However, further studies are needed to confirm translation to humans and other models.

Findings are limited by a single mouse strain, preclinical stroke and hypoxia models[38,39], female-only proteomic analysis in F1, and absence of causal validation and behavioural outcomes[40-43] (Supplemental Discussion 2).

5. Concluding remarks

This study demonstrates that intermittent hypoxic preconditioning confers a significant reduction in ischaemic brain injury in both treated F0 mice and their untreated F1 offspring. The intergenerational nature of this effect was most pronounced in biparental exposure, with sex-specific differences observed in both the extent of protection and the associated proteomic responses. Proteomic profiling revealed distinct patterns in protein expression patterns linked to ancestral hypoxia, with F0 females and F1 biparental groups showing the most prominent signatures. Pathway enrichment analyses identified processes related to metabolism, immune modulation, and intracellular signalling that may underlie the observed effects.

These findings suggest that parental environmental exposures can prime offspring for improved stroke outcomes through sex- and lineage-dependent proteomic adaptations. This work adds to the emerging evidence supporting the intergenerational impact of preconditioning[44-46] and highlights potential proteins and molecular pathways for future therapeutic exploration in stroke and other neurovascular diseases.

Figures

Figure 1. Parental intermittent hypoxia reduces infarct volume and brain swelling following ischaemic stroke in F0 and F1 mice. A) Schematic of the in vivo mouse model. F0 mice were exposed to intermittent hypoxia prior to tMCAO. A subset was bred to generate F1 offspring, which also underwent tMCAO to assess intergenerational effects. Image created with BioRender. **B**) **Top** = Representative coronal brain sections stained with cresyl violet showing infarcted regions in normoxic and hypoxia-exposed F0 mice (scale bar = 2.5 mm). **Bottom** = Quantification of infarct volume and brain swelling in F0 mice (N=20-21; equal male and female distribution). Data are shown as mean ± SEM. Statistical analysis: unpaired *t*-test. **C) Top =** Representative cresyl violet-stained brain sections from F1 male offspring of normoxic and hypoxia-exposed breeders (scale bar = 2.5 mm). **Bottom =** Quantification of infarct volume and brain swelling in F1 male offspring (N=11-12). Analyses are one-way ANOVA with Dunnett's multiple comparisons test. **D) Top =** Representative cresyl violet-stained brain sections from F1 female offspring (scale bar = 2.5 mm). **Bottom =** Quantification of infarct volume and brain swelling in F1 female offspring (N=14-15).

Figure 2. Proteomic analysis of hypoxia-induced changes in F0 mice brain cortex. **A)** Heatmap displaying scaled average protein expression across sample groups (N=6-8). Each row represents a protein, and each column represents a sample group. Expression values were averaged per group and scaled by protein (row-wise z-score). **B)** Venn diagram illustrating the number of differentially expressed (DE) proteins common to F0 male and female hypoxic mice compared to normoxic controls. **C-D)** Volcano plot showing DE proteins in hypoxic vs normoxic F0 male **(C)** and female **(D)** mice. Differential expression was defined by p-value < 0.05. **E)** Scatter plot showing the 17 DE proteins shared between hypoxic F0 male and female mice. **F)** KEGG pathway enrichment analysis showing the top 10 significantly enriched pathways for proteins differentially expressed in hypoxic vs normoxic F0 female mice.

Figure 3. Proteomic analysis of hypoxia-induced changes in F1 mice brain cortex A-C) Volcano plot showing differentially expressed (DE) proteins in paternal-hypoxic vs normoxic F1 female mice **(A)**, maternal-hypoxic vs normoxic F1 female mice **(B)**, and biparental-hypoxic vs normoxic F1 female mice **(C)**. Differential expression was defined by p-value < 0.05. **D)** Venn diagram displaying commonly DE proteins shared among paternal-, maternal-, and biparental-hypoxic F1 mice compared to normoxic controls. **E)** Bar plot illustrating the six DE proteins that are consistently altered across paternal-, maternal-, and biparental-hypoxic F1 groups relative to normoxic controls. **F**) KEGG pathway enrichment analysis showing the top 10 significantly enriched pathways for proteins differentially expressed in biparental-hypoxic vs normoxic F1 female mice.

Reference list

1. Casciaro C, Hamada H, Bloise E, Matthews SG. The paternal contribution to shaping the health of future generations. *Trends Endocrinol Metab*. 2025;36:459-471. doi: 10.1016/j.tem.2024.10.007
2. Thushara Vijayakumar N, Sangwan A, Sharma B, Majid A, Rajanikant GK. Cerebral Ischemic Preconditioning: the Road So Far…. *Mol Neurobiol*. 2016;53:2579-2593. doi: 10.1007/s12035-015-9278-z
3. Bale TL. Epigenetic and transgenerational reprogramming of brain development. *Nat Rev Neurosci*. 2015;16:332-344. doi: 10.1038/nrn3818
4. Franklin TB, Russig H, Weiss IC, Gräff J, Linder N, Michalon A, Vizi S, Mansuy IM. Epigenetic transmission of the impact of early stress across generations. *Biol Psychiatry*. 2010;68:408-415. doi: 10.1016/j.biopsych.2010.05.036
5. Mandolesi L, Polverino A, Montuori S, Foti F, Ferraioli G, Sorrentino P, Sorrentino G. Effects of Physical Exercise on Cognitive Functioning and Wellbeing: Biological and Psychological Benefits. *Front Psychol*. 2018;9:509. doi: 10.3389/fpsyg.2018.00509
6. Robinson AM, Bucci DJ. Maternal Exercise and Cognitive Functions of the Offspring. *Cogn Sci (Hauppauge)*. 2012;7:187-205.
7. Bae ON, Rajanikant K, Min J, Smith J, Baek SH, Serfozo K, Hejabian S, Lee KY, Kassab M, Majid A. Lymphocyte cell kinase activation mediates neuroprotection during ischemic preconditioning. *J Neurosci*. 2012;32:7278-7286. doi: 10.1523/jneurosci.6273-11.2012
8. Lang JA, Kim J. Remote ischaemic preconditioning - translating cardiovascular benefits to humans. *J Physiol*. 2022;600:3053-3067. doi: 10.1113/jp282568
9. Drunalini Perera PN, Hu Q, Tang J, Li L, Barnhart M, Doycheva DM, Zhang JH, Tang J. Delayed remote ischemic postconditioning improves long term sensory motor deficits in a neonatal hypoxic ischemic rat model. *PLoS One*. 2014;9:e90258. doi: 10.1371/journal.pone.0090258
10. Stowe AM, Altay T, Freie AB, Gidday JM. Repetitive hypoxia extends endogenous neurovascular protection for stroke. *Ann Neurol*. 2011;69:975-985. doi: 10.1002/ana.22367
11. Jain S, Kim ES, Kim D, Burrows D, De Felice M, Kim M, Baek SH, Ali A, Redgrave J, Doeppner TR, et al. Comparative Cerebroprotective Potential of d- and l-Carnosine Following Ischemic Stroke in Mice. *Int J Mol Sci*. 2020;21. doi: 10.3390/ijms21093053
12. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *J Pharmacol Pharmacother*. 2010;1:94-99. doi: 10.4103/0976-500x.72351
13. Barone FC, Knudsen DJ, Nelson AH, Feuerstein GZ, Willette RN. Mouse strain differences in susceptibility to cerebral ischemia are related to cerebral vascular anatomy. *J Cereb Blood Flow Metab*. 1993;13:683-692. doi: 10.1038/jcbfm.1993.87
14. Doyle KP, Fathali N, Siddiqui MR, Buckwalter MS. Distal hypoxic stroke: a new mouse model of stroke with high throughput, low variability and a quantifiable functional deficit. *J Neurosci Methods*. 2012;207:31-40. doi: 10.1016/j.jneumeth.2012.03.003
15. Ellenbroek B, Youn J. Rodent models in neuroscience research: is it a rat race? *Dis Model Mech*. 2016;9:1079-1087. doi: 10.1242/dmm.026120
16. Hartmann W, Blankenhaus B, Brunn ML, Meiners J, Breloer M. Elucidating different pattern of immunoregulation in BALB/c and C57BL/6 mice and their F1 progeny. *Sci Rep*. 2021;11:1536. doi: 10.1038/s41598-020-79477-7
17. Rocha-Ferreira E, Phillips E, Francesch-Domenech E, Thei L, Peebles DM, Raivich G, Hristova M. The role of different strain backgrounds in bacterial endotoxin-mediated sensitization to neonatal hypoxic-ischemic brain damage. *Neuroscience*. 2015;311:292-307. doi: 10.1016/j.neuroscience.2015.10.035
18. Yang G, Kitagawa K, Matsushita K, Mabuchi T, Yagita Y, Yanagihara T, Matsumoto M. C57BL/6 strain is most susceptible to cerebral ischemia following bilateral common carotid

occlusion among seven mouse strains: selective neuronal death in the murine transient forebrain ischemia. *Brain Res*. 1997;752:209-218. doi: 10.1016/s0006-8993(96)01453-9
19. Boroujerdi A, Milner R. Defining the critical hypoxic threshold that promotes vascular remodeling in the brain. *Exp Neurol*. 2015;263:132-140. doi: 10.1016/j.expneurol.2014.10.007
20. Burtscher J, Citherlet T, Camacho-Cardenosa A, Camacho-Cardenosa M, Raberin A, Krumm B, Hohenauer E, Egg M, Lichtblau M, Müller J, et al. Mechanisms underlying the health benefits of intermittent hypoxia conditioning. *J Physiol*. 2024;602:5757-5783. doi: 10.1113/jp285230
21. Damgaard V, Mariegaard J, Lindhardsen JM, Ehrenreich H, Miskowiak KW. Neuroprotective Effects of Moderate Hypoxia: A Systematic Review. *Brain Sci*. 2023;13. doi: 10.3390/brainsci13121648
22. Navarrete-Opazo A, Mitchell GS. Therapeutic potential of intermittent hypoxia: a matter of dose. *Am J Physiol Regul Integr Comp Physiol*. 2014;307:R1181-1197. doi: 10.1152/ajpregu.00208.2014
23. Caglayan AB, Beker MC, Sertel Evren E, Caglayan B, Kilic Ü, Ates N, Caglayan A, Dasdelen MF, Doeppner TR, Saarma M, et al. The Unconventional Growth Factors Cerebral Dopamine Neurotrophic Factor and Mesencephalic Astrocyte-Derived Neurotrophic Factor Promote Post-ischemic Neurological Recovery, Perilesional Brain Remodeling, and Lesion-Remote Axonal Plasticity. *Transl Stroke Res*. 2023;14:263-277. doi: 10.1007/s12975-022-01035-2
24. Kilic E, Kilic U, Matter CM, Lüscher TF, Bassetti CL, Hermann DM. Aggravation of focal cerebral ischemia by tissue plasminogen activator is reversed by 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor but does not depend on endothelial NO synthase. *Stroke*. 2005;36:332-336. doi: 10.1161/01.STR.0000152273.24063.f7
25. Reglodi D, Tamás A, Somogyvári-Vigh A, Szántó Z, Kertes E, Lénárd L, Arimura A, Lengvári I. Effects of pretreatment with PACAP on the infarct size and functional outcome in rat permanent focal cerebral ischemia. *Peptides*. 2002;23:2227-2234. doi: 10.1016/s0196-9781(02)00262-0
26. Yelkenci HE, Degirmenci Z, Koc HI, Bayirli S, Baltaci SB, Altunay S, Oztekin N, Kocak M, Kilic E, Beker MC. Vinpocetine Ameliorates Neuronal Injury After Cold-Induced Traumatic Brain Injury in Mice. *Mol Neurobiol*. 2025;62:3956-3972. doi: 10.1007/s12035-024-04515-8
27. Zhu XH, Yu X, Kong XW, Zhang Y, Jiang SL, Chai JH, Liang J, Kuang HX, Xia YG. Insight of action mechanism of Astragaloside IV for relieving of cerebral ischemic injury in a rat model of middle cerebral artery occlusion reperfusion via proteomics and network pharmacology. *J Nat Med*. 2025;79:591-607. doi: 10.1007/s11418-025-01892-9
28. Hoshino T, Takase H, Hamanaka G, Kimura S, Fukuda N, Mandeville ET, Lok J, Lo EH, Arai K. Transcriptomic changes in oligodendrocyte lineage cells during the juvenile to adult transition in the mouse corpus callosum. *Sci Rep*. 2024;14:22334. doi: 10.1038/s41598-024-72311-4
29. Liu T, Li X, Zhou X, Chen W, Wen A, Liu M, Ding Y. PI3K/AKT signaling and neuroprotection in ischemic stroke: molecular mechanisms and therapeutic perspectives. *Neural Regen Res*. 2025;20:2758-2775. doi: 10.4103/nrr.Nrr-d-24-00568
30. Peng DH, Liu YY, Chen W, Hu HN, Luo Y. Epidermal growth factor alleviates cerebral ischemia-induced brain injury by regulating expression of neutrophil gelatinase-associated lipocalin. *Biochem Biophys Res Commun*. 2020;524:963-969. doi: 10.1016/j.bbrc.2020.02.025
31. Chandra PK, Cikic S, Baddoo MC, Rutkai I, Guidry JJ, Flemington EK, Katakam PV, Busija DW. Transcriptome analysis reveals sexual disparities in gene expression in rat brain microvessels. *J Cereb Blood Flow Metab*. 2021;41:2311-2328. doi: 10.1177/0271678x21999553
32. Distler U, Schumann S, Kesseler HG, Pielot R, Smalla KH, Sielaff M, Schmeisser MJ, Tenzer S. Proteomic Analysis of Brain Region and Sex-Specific Synaptic Protein Expression in the Adult Mouse Brain. *Cells*. 2020;9. doi: 10.3390/cells9020313

33. Guneykaya D, Ivanov A, Hernandez DP, Haage V, Wojtas B, Meyer N, Maricos M, Jordan P, Buonfiglioli A, Gielniewski B, et al. Transcriptional and Translational Differences of Microglia from Male and Female Brains. *Cell Rep*. 2018;24:2773-2783.e2776. doi: 10.1016/j.celrep.2018.08.001
34. Shi L, Zhang Z, Su B. Sex Biased Gene Expression Profiling of Human Brains at Major Developmental Stages. *Sci Rep*. 2016;6:21181. doi: 10.1038/srep21181
35. Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, et al. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell*. 2010;143:1084-1096. doi: 10.1016/j.cell.2010.12.008
36. Ragsdale A, Ortega-Recalde O, Dutoit L, Besson AA, Chia JHZ, King T, Nakagawa S, Hickey A, Gemmell NJ, Hore T, et al. Paternal hypoxia exposure primes offspring for increased hypoxia resistance. *BMC Biol*. 2022;20:185. doi: 10.1186/s12915-022-01389-x
37. Rodgers AB, Morgan CP, Leu NA, Bale TL. Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress. *Proc Natl Acad Sci U S A*. 2015;112:13699-13704. doi: 10.1073/pnas.1508347112
38. Liu F, Schafer DP, McCullough LD. TTC, fluoro-Jade B and NeuN staining confirm evolving phases of infarction induced by middle cerebral artery occlusion. *J Neurosci Methods*. 2009;179:1-8. doi: 10.1016/j.jneumeth.2008.12.028
39. Türeyen K, Vemuganti R, Sailor KA, Dempsey RJ. Infarct volume quantification in mouse focal cerebral ischemia: a comparison of triphenyltetrazolium chloride and cresyl violet staining techniques. *J Neurosci Methods*. 2004;139:203-207. doi: 10.1016/j.jneumeth.2004.04.029
40. Ates N, Caglayan A, Balcikanli Z, Sertel E, Beker MC, Dilsiz P, Caglayan AB, Celik S, Dasdelen MF, Caglayan B, et al. Phosphorylation of PI3K/Akt at Thr308, but not phosphorylation of MAPK kinase, mediates lithium-induced neuroprotection against cerebral ischemia in mice. *Exp Neurol*. 2022;351:113996. doi: 10.1016/j.expneurol.2022.113996
41. Beker MC, Caglayan AB, Altunay S, Ozbay E, Ates N, Kelestemur T, Caglayan B, Kilic U, Doeppner TR, Hermann DM, et al. Phosphodiesterase 10A Is a Critical Target for Neuroprotection in a Mouse Model of Ischemic Stroke. *Mol Neurobiol*. 2022;59:574-589. doi: 10.1007/s12035-021-02621-5
42. Liang X, Lin L, Woodling NS, Wang Q, Anacker C, Pan T, Merchant M, Andreasson K. Signaling via the prostaglandin E_2 receptor EP4 exerts neuronal and vascular protection in a mouse model of cerebral ischemia. *J Clin Invest*. 2011;121:4362-4371. doi: 10.1172/jci46279
43. Rousselet E, Kriz J, Seidah NG. Mouse model of intraluminal MCAO: cerebral infarct evaluation by cresyl violet staining. *J Vis Exp*. 2012. doi: 10.3791/4038
44. Bowers ME, Yehuda R. Intergenerational Transmission of Stress in Humans. *Neuropsychopharmacology*. 2016;41:232-244. doi: 10.1038/npp.2015.247
45. Entringer S. Impact of stress and stress physiology during pregnancy on child metabolic function and obesity risk. *Curr Opin Clin Nutr Metab Care*. 2013;16:320-327. doi: 10.1097/MCO.0b013e32835e8d80
46. Wu L, Lu Y, Jiao Y, Liu B, Li S, Li Y, Xing F, Chen D, Liu X, Zhao J, et al. Paternal Psychological Stress Reprograms Hepatic Gluconeogenesis in Offspring. *Cell Metab*. 2016;23:735-743. doi: 10.1016/j.cmet.2016.01.014