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Article

Apelin-13 improves post-ischemic cognition by restoring APLN and microglia rhythmicity in aging

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ABSTRACT

Aging increases susceptibility to cerebral ischemia and weakens endogenous protective pathways. We found that Apelin/APLN expression, especially the bioactive Apelin-13 isoform, is markedly reduced in aged mouse brains, and the normal ischemia-induced upregulation of Apelin is blunted. Glial rhythmicity analysis further showed that Apelin expression exhibits clear oscillations in young astrocytes and microglia but becomes disrupted with aging. Apelin-13 supplementation significantly improved post-ischemic learning and memory in aged mice, increased endogenous Apelin-13 levels, reduced TNF-α and IL-6, and enhanced IL-10 expression.These findings indicate that aging disrupts Apelin-dependent neuroprotective and rhythmic pathways, and that Apelin-13 effectively restores inflammatory balance and cognitive recovery after cerebral ischemia, highlighting its potential as a therapeutic strategy for aging-related stroke vulnerability.

KEYWORDS

aging, Apelin, Apelin-13, cerebral ischemia-reperfusion injury

1 Introduction

Cerebral ischemia–reperfusion injury is a major cause of long–term cognitive impairment and aging significantly exacerbates neurological decline [1, 2]. The aged brain exhibits weakened endogenous protective mechanisms and disrupted glial homeostasis, leading to heightened vulnerability to ischemic insults [3, 4]. Among the molecular systems involved in neuroprotection, APLN-derived Apelin peptides have gained attention for their roles in regulating neuronal survival, vascular stability, and metabolic balance [3, 5].

The APLN gene encodes the precursor preproapelin, which is enzymatically cleaved into several bioactive isoforms, including Apelin-36, Apelin-17, and the highly potent Apelin–13 [4, 6, 7]. These peptides participate in diverse signaling pathways that influence neuronal viability, mitochondrial function, and inflammatory responses [8, 9]. During cerebral ischemia, the expression of APLN-derived Apelin peptides increases, representing an intrinsic compensatory mechanism aimed at limiting neuronal apoptosis, preserving mitochondrial integrity, and reducing inflammatory injury [10].

However, aging markedly disrupts this protective Apelin system [11]. APLN expression declines significantly in the aged cortex and hippocampus, leading to reduced production of Apelin peptides and weakening the endogenous ability to cope with ischemic stress [12]. This age-related decrease contributes to the poor functional recovery and heightened cognitive deficits frequently observed in older individuals after ischemic injury.

Recent findings also indicate that APLN expression in microglia exhibits circadian rhythmicity, which is essential for regulating microglial metabolic activity, inflammatory tone, and phagocytic function [13]. Aging leads to a loss or blunting of this rhythmic pattern, resulting in dysregulated microglial behavior and diminished neuroprotective capacity. The combined effects of APLN downregulation and rhythmic disruption significantly impair the brain's resilience to ischemic damage.

Apelin-13, as the most biologically active isoform of the APLN gene product, has the potential to restore these impaired pathways [14]. Exogenous Apelin-13 has been shown to stabilize mitochondrial function, reduce neuronal injury, and modulate glial activity. Whether Apelin-13 can

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specifically reverse aging-related declines in APLN expression and restore microglial rhythmicity to improve cognitive outcomes after cerebral ischemia remains largely unexplored.

In this study, we investigate whether Apelin-13 can counteract age-related reductions in APLN expression, normalize microglial rhythmic regulation, and consequently enhance cognitive recovery following cerebral ischemic injury.

2 Results

2.1 Aging reduces Apelin expression, impairs its ischemia-induced adaptive increase, and disrupts Apelin-13 rhythmicity in glial cells

We first examined whether Apelin expression changes with aging in the mouse brain. Across multiple cohorts, aged mice consistently exhibited a significant reduction in Apelin mRNA levels compared with young controls (Figs. 1A–1C). Western blot analysis further confirmed a robust decline of Apelin protein abundance in the aged brain (Fig. 1D). To determine whether aging exacerbates the response of Apelin to acute ischemic injury, we subjected both young and aged mice to a photothrombotic cortical ischemia model. In young mice, ischemic preconditioning (PC) induced a modest upregulation of Apelin; however, this adaptive increase was markedly blunted in aged animals (Fig. 1E). Among the major splice isoforms of Apelin, Apelin-13 showed the most prominent age-related reduction and the strongest loss of ischemia-induced upregulation (Fig. 1F). In contrast, Apelin-17 and Apelin-36 displayed minimal or no changes across age or ischemic conditions (Figs. 1G and 1H). We next assessed cell-type-specific Apelin expression in astrocytes and

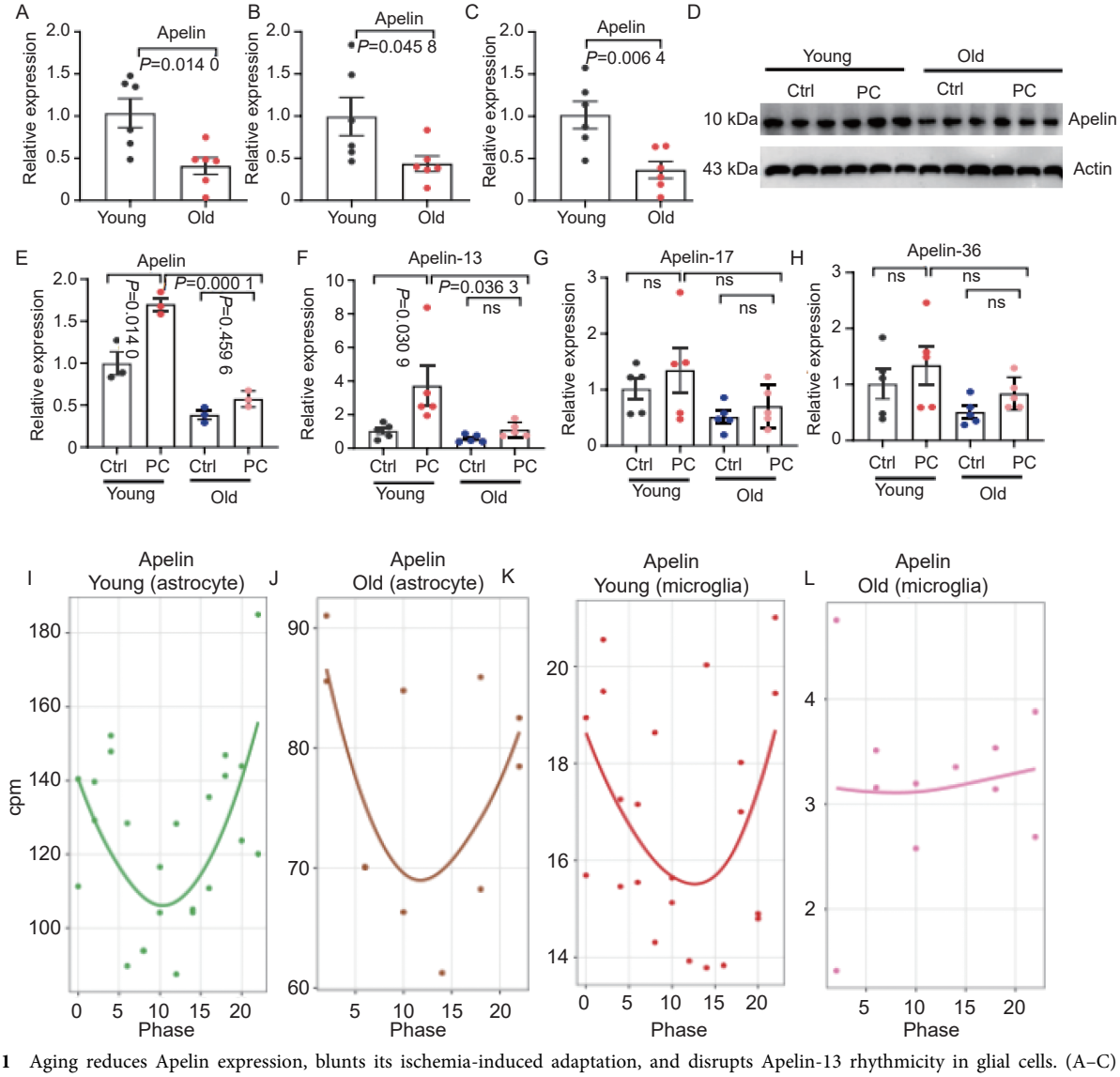

Figure 1 Aging reduces Apelin expression, blunts its ischemia-induced adaptation, and disrupts Apelin-13 rhythmicity in glial cells. (A–C) Apelin mRNA expression is significantly reduced in the brains of aged mice compared with young controls. (D) Representative Western blot showing decreased Apelin protein levels in aged mice; actin served as the loading control. (E) In a photothrombotic ischemia model, ischemic preconditioning (PC) increases Apelin expression in young mice but this adaptive response is markedly attenuated in aged mice. (F–H) Among Apelin splice variants, Apelin-13 shows the strongest age-dependent reduction andloss of PC responsiveness, whereas Apelin-17 and Apelin-36 show minimal age-related changes. Astrocytic Apelin expression exhibits a clear circadian-like rhythmic pattern in young mice (I), which becomes dampened in aged astrocytes (J). Microglial Apelin expression also oscillates in young mice (K), while aged microglia show disrupted and flattened Apelin-13 rhythmicity (L). Data are presented as mean ± SEM; statistical significance was determined as indicated; ns meas "non-significance".

microglia. Single-cell rhythmicity analysis revealed that Apelin expression in astrocytes retained a clear daily oscillation pattern in young animals (Fig. 1I), but this rhythmicity was diminished with aging (Fig. 1J). In microglia, Apelin expression also oscillated in young mice (Fig. 1K), whereas aged microglia showed a disrupted and flattened Apelin13 rhythm (Fig. 1L), indicating that aging impairs both the amplitude and phase coherence of Apelin rhythmicity in glial populations. Together, these data demonstrate that Apelin expression declines with aging, that the adaptive Apelin response to ischemic injury is selectively impaired in aged mice, and that Apelin—particularly the Apelin-13 isoform—undergoes age-dependent downregulation and rhythm disruption in astrocytes and microglia.

2.2 Apelin-13 supplementation improves cognitive recovery and modulates inflammatory responses in aged mice after cerebral ischemia

To determine whether supplementation of Apelin-13 can restore functional outcomes after acute cerebral ischemia, we administered Apelin-13 to young and aged mice subjected to photothrombotic injury. In the Morris water maze test, aged ischemic mice exhibited markedly impaired learning and memory compared with young animals. Remarkably, Apelin-13 treatment significantly improved performance in aged mice, resulting in a progressive reduction in escape latency across training days, and largely restoring cognitive function toward the level of young controls (Fig. 2A). At the molecular level, Apelin-13 administration effectively increased endogenous Apelin-13 expression in aged ischemic brains (Fig. 2D). We next examined the inflammatory response, given the established role of Apelin signaling in modulating neuroinflammation. Aged ischemic mice showed elevated expression of pro-inflammatory cytokines TNF-α and IL-6, while treatment with Apelin-13 significantly reduced both markers (Figs. 2E and 2F). Conversely, the anti-inflammatory cytokine IL-10 was markedly increased upon Apelin-13 treatment (Fig. 2G), indicating a shift toward a more anti-inflammatory milieu in the aged ischemic brain.

These findings demonstrate that exogenous Apelin-13 supplementation restores cognitive performance, enhances endogenous Apelin-13 levels, and rebalances inflammatory responses in aged mice undergoing acute ischemic injury, suggesting that loss of Apelin-13 may be a key contributor to impaired recovery during aging.

3 Discussion

Our findings demonstrate that aging profoundly weakens the endogenous Apelin/APLN protective system and increases brain vulnerability to ischemic injury. Aged mice showed marked reductions in Apelin expression and a loss of the ischemia-induced adaptive upregulation normally present in young animals. Among the Apelin isoforms, Apelin-13 exhibited the strongest age-dependent decline, indicating that reduced availability of this highly active peptide may contribute to impaired recovery after stroke.

We also show that Apelin expression in glial cells displays rhythmic regulation, which becomes disrupted with aging. The loss of Apelin rhythmicity in microglia likely reflects broader defects in glial homeostasis and may diminish the capacity of aged glial cells to respond effectively to ischemic stress. Importantly, exogenous Apelin-13 supplementation restored multiple aspects of this impaired system. Apelin-13 improved learning and memory recovery in aged ischemic

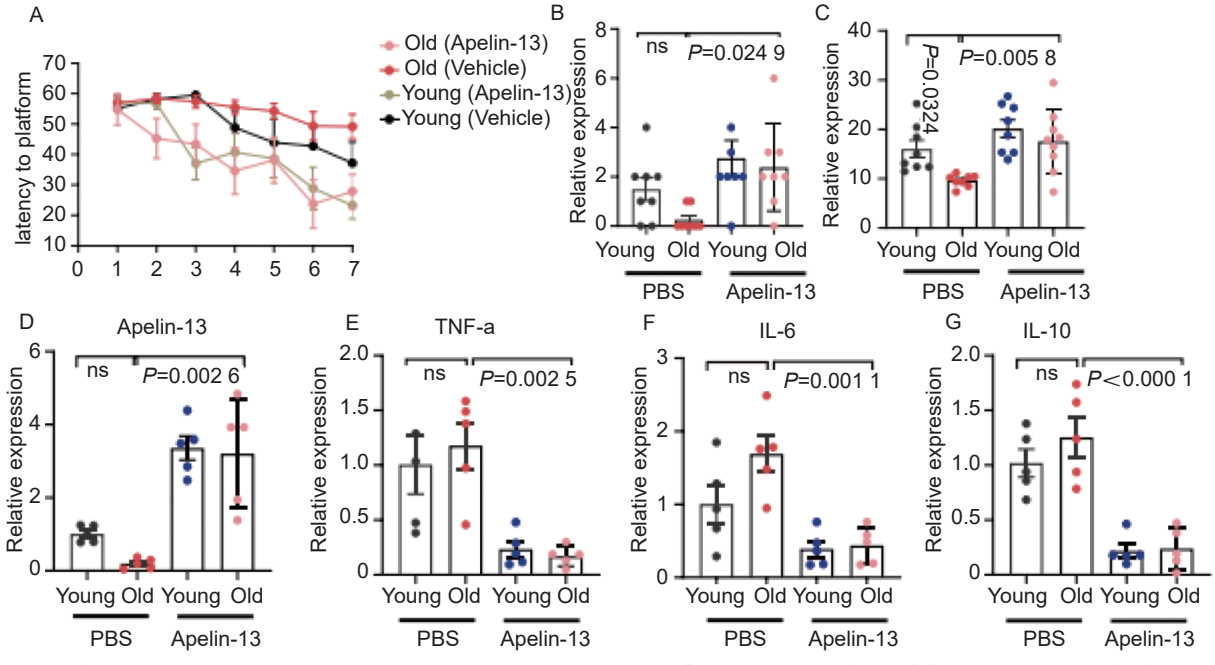

Figure 2 Apelin-13 supplementation improves cognitive recovery and mitigates inflammation in aged mice following acute cerebral ischemia. (A–C) Morris water maze test showing impaired learning and memory in aged ischemic mice, and significant improvement after Apelin-13 treatment. Young mice show normal learning curves with or without vehicle. (D) Apelin-13 treatment increases endogenous Apelin-13 levels in aged ischemic mice. Pro-inflammatory cytokines TNF-α (E) and IL-6 (F) are elevated in aged ischemic mice and significantly reduced by Apelin-13. (G) Anti-inflammatory cytokine IL-10 is increased following Apelin-13 supplementation in aged ischemic mice. Data are presented as mean ± SEM; statistical significance is indicated in each panel; ns meas "non-significance".

mice, enhanced endogenous Apelin-13 expression, reduced pro-inflammatory cytokines, and increased IL-10. These results suggest that Apelin-13 acts through both anti-inflammatory and neuroprotective mechanisms to promote functional recovery in aging brains.

Together, our data identify Apelin-13 as a promising therapeutic candidate for mitigating age-related vulnerability to cerebral ischemia and improving cognitive outcomes in older individuals.

4 Methods

4.1 Animals

Young (3–4 months) and aged (20–22 months) C57BL/6J mice were obtained from the institutional animal facility.

4.2 Photothrombotic ischemia model

Focal cortical ischemia was induced using a standard photothrombotic procedure. Mice were anesthetized with isoflurane and injected intraperitoneally with rose bengal (30 mg/kg). The skull was illuminated with a cold light source for 15 min to induce localized thrombosis. Control mice underwent identical procedures without illumination. Ischemic preconditioning (PC) was performed by applying a subthreshold photothrombotic stimulus 24 h before the main ischemic event.

4.3 Apelin-13 administration

Synthetic Apelin-13 was dissolved in sterile saline and administered intraperitoneally at 10 μg/kg once daily beginning 2 h after ischemia and continuing for 5 consecutive days. Vehicle groups received equal volumes of saline.

4.4 Morris water maze

Cognitive function was assessed 7–12 days after ischemia using the Morris water maze. Mice underwent four trials per day for six days to locate a hidden platform. Escape latency was recorded and averaged per day. All behavioral testing was performed by experimenters blinded to treatment conditions.

4.5 qPCR analysis

Brain tissue from the peri-ischemic cortex was collected 24–48 h after ischemia. RNA was isolated using TRIzol, and cDNA was synthesized using a standard reverse transcription kit. qPCR was performed using SYBR Green master mix. Relative expression of APLN, Apelin-13, Apelin-17, Apelin-36, TNF-α, IL-6, and IL-10 was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as the internal control.

4.6 Western Blotting

Protein lysates were prepared from cortical tissue, separated by SDS–PAGE, and transferred to PVDF membranes. Membranes were incubated with primary antibodies against Apelin and β-actin followed by HRP-conjugated secondary antibodies. Signals were visualized using chemiluminescence and quantified with ImageJ.

4.7 Single-cell rhythmicity analysis

Public single-cell RNA-seq datasets and in-house sorted astrocytes/microglia were analyzed to assess circadian-like rhythmic expression of Apelin. Expression values were plotted against circadian phase, and rhythmicity was evaluated using harmonic regression and cosine-fit models. Young and aged groups were compared for oscillation amplitude and phase stability.

4.8 Statistical analysis

Data are presented as mean ± SEM. Two-group comparisons used unpaired Student's *t*-tests. Multi-group analyses used one-way or two-way ANOVA with Tukey's post hoc tests when appropriate. $P < 0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism.

Ethics approval

All procedures followed institutional guidelines for animal care and were approved by the local ethics committee.

Consent to participate

Not applicable.

Data availability

All the data presented in the study are included in the article.

Author contributions

Min-Hui Tan designed the study, performed the experiments, analyzed the data, and wrote the manuscript. Ou-Hai Liang and Xin-Lin performed the experiments and collected data. Yanhao Huang supervised the study and revised the manuscript.

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Conflict of interests

The authors declare no potential conflict of interests.

References

[1] Schaller, B., Graf, R. Cerebral ischemia and reperfusion: The pathophysiologic concept as a basis for clinical therapy. *Journal of Cerebral Blood Flow & Metabolism*, **2004**, 24(4): 351–371. https://doi.org/10.1097/00004647-200404000-00001

[2] Zhang, Q., Jia, M., Wang, Y. F., Wang, Q., Wu, J. P. Cell death mechanisms in cerebral ischemia–reperfusion injury. *Neurochemical Research*, **2022**, 47(12): 3525–3542. https://doi.org/10.1007/s11064-022-03697-8

[3] Dagamajalu, S., Rex, D. A. B., Philem, P. D., Rainey, J. K., Keshava Prasad, T. S. A network map of apelin-mediated signaling. *Journal of Cell Communication and Signaling*, **2022**, 16(1): 137–143. https://doi.org/10.1007/s12079-021-00614-6

[4] Wang, Y. H., Liu, W. C., Geng, P. P., Du, W. H., Guo, C., Wang, Q., Zheng, G.-Q., Jin, X. C. Role of crosstalk between glial cells and immune cells in blood-brain barrier damage and protection after acute ischemic stroke. *Aging and Disease*, **2023**, 15(6): 2507–2525. https://doi.org/10.14336/AD.2023.1010

[5] Chen, J. W., Li, Z. Y., Zhao, Q., Chen, L. X. Roles of apelin/APJ

system in cancer: Biomarker, predictor, and emerging therapeutic target. *Journal of Cellular Physiology*, **2022**, 237(10): 3734–3751. https://doi.org/10.1002/jcp.30845

[6] Demoures, B., Soulet, F., Descarpentrie, J., Galeano-Otero, I., Sanchez Collado, J., Casado, M., Smani, T., González, A., Alves, I., Lalloué, F. et al. Repression of apelin Furin cleavage sites provides antimetastatic strategy in colorectal cancer. *EMBO Molecular Medicine*, **2025**, 17(3): 504–534. https://doi.org/10.1038/s44321-025-00196-5

[7] Karimi, M., Shirsalimi, N., Sedighi, E. Apelin-13 as a novel diagnostic laboratory biomarker in thromboembolic disorders: A review of literature with prospective insights. *International Journal of Emergency Medicine*, **2024**, 17: 190. https://doi.org/10.1186/s12245-024-00774-3

[8] Fibbi, B., Marroncini, G., Naldi, L., Peri, A. The Yin and Yang effect of the apelinergic system in oxidative stress. *International Journal of Molecular Sciences*, **2023**, 24(5): 4745. https://doi.org/10.3390/ijms24054745

[9] Kaminska, B., Kurowicka, B., Kiezun, M., Dobrzyn, K., Kisielewska, K., Gudelska, M., Kopij, G., Szymanska, K., Zarzecka, B., Koker, O. et al. The role of adipokines in the control of pituitary functions. *Animals*, **2024**, 14(2): 353. https://doi.org/10.3390/ani14020353

[10] Li, A., Zhao, Q., Chen, L. X., Li, Z. Y. Apelin/APJ system: An emerging therapeutic target for neurological diseases. *Molecular Biology Reports*, **2023**, 50(2): 1639–1653. https://doi.org/10.1007/s11033-022-08075-9

[11] Aminyavari, S., Zahmatkesh, M., Farahmandfar, M., Khodagholi, F., Dargahi, L., Zarrindast, M. R. Protective role of Apelin-13 on amyloid β25–35-induced memory deficit; Involvement of autophagy and apoptosis process. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, **2019**, 89: 322–334. https://doi.org/10.1016/j.pnpbp.2018.10.005

[12] Tian, Y. J., Chen, R. J., Jiang, Y. L., Bai, B., Yang, T. J., Liu, H. Q. The protective effects and mechanisms of apelin/APJ system on ischemic stroke: A promising therapeutic target. *Frontiers in Neurology*, **2020**, 11: 75. https://doi.org/10.3389/fneur.2020.00075

[13] Guzmán-Ruiz, M. A., Guerrero-Vargas, N. N., Lagunes-Cruz, A., González-González, S., García-Aviles, J. E., Hurtado-Alvarado, G., Mendez-Hernández, R., Chavarría-Krauser, A., Morin, J. P., Arriaga-Avila, V. et al. Circadian modulation of microglial physiological processes and immune responses. *Glia*, **2023**, 71(2): 155–167. https://doi.org/10.1002/glia.24261

[14] Wang, X. Y., Liu, X. F., Song, Z. F., Shen, X., Lu, S. Y., Ling, Y., Kuang, H. B. Emerging roles of APLN and APELA in the physiology and pathology of the female reproductive system. *PeerJ*, **2020**, 8: e10245. https://doi.org/10.7717/peerj.10245