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Transauricular Vagus Nerve Stimulation Attenuates Hippocampal SRPX2 Expression and Reactive Astrocytosis in an Acrolein-Induced Alzheimer's-Like Rat Model

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Abstract

Introduction

Acrolein, a highly reactive aldehyde generated during lipid peroxidation, is elevated in Alzheimer's disease (AD) and has been shown to induce AD-like synaptic and cognitive deficits in experimental models. Transcutaneous auricular vagus nerve stimulation (taVNS) is a noninvasive neuromodulatory intervention with potential cognitive and anti-inflammatory effects. Sushi repeat-containing protein X-linked 2 (SRPX2) is a neuronal complement inhibitor that contributes to synaptic protection by limiting complement-mediated synapse elimination. However, the regulation of SRPX2 in acrolein-induced AD-like pathology and its modulation by taVNS remain unclear. This study investigated the effects of taVNS on hippocampal SRPX2 expression and reactive astrocytosis in an acrolein-induced AD-like rat model.

Methods

Twenty-eight Wistar rats were randomly assigned to an acrolein-exposed cohort (n = 21) or a non-exposed control group (n = 7). Acrolein-exposed rats were further divided into three groups: acrolein-only, sham stimulation of the greater auricular nerve, and vagus nerve stimulation (VNS) of the auricular branch (n = 7 per group). Acrolein was administered by oral gavage at 2.5 mg/kg/day for eight weeks. Sham stimulation and taVNS were subsequently administered for four weeks at 25 Hz, 0.8 mA, and one hour/day. Hippocampal SRPX2 gene expression was quantified using real-time PCR, while reactive astrocytosis was evaluated using glial fibrillary acidic protein (GFAP) immunohistochemistry.

Results

Acrolein exposure significantly increased hippocampal SRPX2 expression and GFAP-defined reactive astrocytosis compared with non-exposed controls. taVNS significantly reduced SRPX2 expression toward control levels (mean ± SD: 0.48 ± 0.17; p = 0.0075). In addition, taVNS reduced the density of GFAP-positive astrocytes and decreased GFAP staining intensity compared with the acrolein-only condition.

Conclusions

taVNS attenuated acrolein-induced AD-like hippocampal pathology, as evidenced by reduced SRPX2 expression and reactive astrocytosis. These findings suggest that taVNS may have therapeutic potential as a noninvasive neuromodulatory intervention targeting complement-related neuron-glia mechanisms in sporadic AD. Further studies are warranted to clarify the mechanistic relationship between SRPX2, complement signaling, astrocytic activation, and the neuroprotective effects of taVNS.

Categories: Neurology, Anatomy, Internal Medicine
Keywords: acrolein-induced neurotoxicity, alzheimer's disease, hippocampus, reactive astrocytes / astrocytosis, srpx2, transauricular vagus nerve stimulation (tavns)

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, amyloid-beta (Aβ) deposition, tau pathology, synaptic loss, and prominent neuroinflammation [1-3]. In addition to classical Aβ and tau lesions, lipid peroxidation-derived aldehydes such as acrolein have emerged as important contributors to sporadic AD pathogenesis [1, 4-6]. Acrolein

levels are elevated in the brains and CSF of patients with AD and exert long-lasting toxic effects on proteins, lipids, and nucleic acids [4, 6-8]. Experimental administration of acrolein in rodents induces synaptic dysfunction, dendritic spine loss, impaired long-term potentiation, gliosis, and memory deficits, thereby reproducing key features of early sporadic AD [4-8].

Glial cells, particularly astrocytes and microglia, are central to the development and progression of AD [2, 3, 9]. Astrocytes become reactive, upregulate glial fibrillary acidic protein (GFAP), and contribute to neuroinflammation, synaptic remodeling, and blood-brain barrier disruption in the brains of patients with AD [2, 3, 9]. Chronic hippocampal astrocytosis correlates with cognitive impairment and the burden of amyloid and tau pathology in both patients and animal models [2, 3, 9]. Complement-mediated synapse elimination is increasingly recognized as a key mechanism in early AD, with aberrant activation of C1q, C3, and microglial complement receptors driving inappropriate synaptic loss [10-12].

SRPX2 is a neuronal complement inhibitor that binds C1q and restricts complement-dependent synapse elimination [10]. In mouse models, loss of SRPX2 enhances microglial phagocytosis and synapse pruning, whereas SRPX2 overexpression preserves synapses in the presence of complement [10, 13]. Dysregulation of SRPX2 and complement pathways has been implicated in neurodevelopmental disorders and is increasingly linked to synaptic vulnerability and neurodegeneration in AD [10-13]. However, the role of SRPX2 in acrolein-induced AD-like pathology and its relationship with astrocytic reactivity in the hippocampus remain poorly defined [4, 10-13].

Vagus nerve stimulation (VNS) and its non-invasive auricular variant modulate brain networks via projections from the nucleus tractus solitarius to the locus coeruleus, dorsal raphe, and other neuromodulatory nuclei, thereby influencing hippocampal plasticity, inflammatory tone, and cognition [5, 6, 14]. Transcutaneous auricular VNS (taVNS) has shown beneficial effects on memory, mood, and autonomic regulation in both animal models and clinical studies [14, 15]. taVNS can improve recognition memory, modulate amygdala and prefrontal activity, and reduce markers of neuroinflammation and gliosis in preclinical models [14, 15]. Recent experimental work indicates that taVNS restores spatial learning and memory and modulates hippocampal plasticity-related proteins in rodent models of cognitive impairment [14, 15].

Given the central role of acrolein in sporadic AD-like pathology and the emerging importance of complement-mediated synapse elimination and glial activation, this study investigated whether transauricular VNS can modulate hippocampal SRPX2 expression and astrocytic reactivity in an acrolein-induced AD-like model in Wistar rats as a preventive and/or reversal intervention in relation to the eight-week acrolein exposure. Based on this rationale, it was hypothesized that taVNS would downregulate SRPX2 expression, reduce GFAP-defined astrocytosis, and thereby mitigate and/or reverse hippocampal AD-like pathology associated with acrolein exposure.

Materials And Methods

Study design and setting

This was a randomized, controlled, preclinical experimental study with a factorial exposure-intervention design. Animals were first allocated to acrolein-exposed or non-exposed groups; acrolein-exposed rats were then randomized to receive VNS, sham stimulation, or no stimulation, allowing separation of exposure effects from stimulation-specific effects [4, 6-8].

Animals and experimental design

Adult male and female Wistar rats aged 12-16 weeks (males: 280-450 g; females: 200-320 g) were used. Sample size was estimated using the resource-equation method to ensure adequate power while minimizing animal use [16]. Rats were housed under standard conditions (12-h light/dark cycle, 22 ± 2 °C, ad libitum access to food and water). All procedures were approved by the Mbarara University of Science and Technology Research Ethics Committee (MUST-2023-791) and the Uganda National Council for Science and Technology (HS3781ES) and conformed to international guidelines for the care and use of laboratory animals [17].

Rats were randomly assigned to acrolein-treated (n = 21) or non-treated control groups (n = 7). Within the acrolein-treated cohort, animals were further randomized into three subgroups (n = 7 each): acrolein control (acrolein exposure without electrical stimulation), sham (acrolein exposure plus transauricular stimulation over the greater auricular nerve territory/pinna), and experimental (acrolein exposure plus transauricular stimulation over the concha, corresponding to the auricular branch of the vagus nerve). Outcome assessment was conducted by investigators blinded to group allocation [4, 6-8].

Acrolein-induced Alzheimer's-like pathology

Acrolein (CPI International, Santa Rosa, CA, USA; catalog Z-020229-05 513389) was administered according to established protocols for inducing AD-like hippocampal pathology [4, 6-8]. Rats in the acrolein groups

received acrolein at 2.5 mg/kg/day for 8 weeks by gastric gavage, a regimen previously shown to impair memory, reduce synaptic markers, and increase glial activation [4-8]. Non-treated control rats received equivalent volumes of vehicle for the same duration.

Transauricular VNS

Transauricular VNS was delivered under 2% isoflurane anesthesia [14, 15]. Bipolar clip magnetic electrodes (Dongguan Heymed Technology Co., Ltd., Guangdong, China) were placed bilaterally on the concha (auricular branch of the vagus nerve) in the experimental group and on the pinna (greater auricular nerve territory) in the sham group, with the cathode positioned externally and the anode internally. Stimulation parameters were adapted from previous animal taVNS studies: a frequency of 25 Hz, cathodic-leading pulses of 50 µs duration, an amplitude of 0.8 mA, and a 10% duty cycle (30 s on followed by 4.5 min off) for a total of 1 h/day over 4 consecutive weeks [14, 15]. Stimulation was generated using a DDS signal generator/counter (JDS-6600, P/N 1726302922; JUNCTEK, Hangzhou Junce Instrument & Meter Co., Ltd., Hangzhou, China) and monitored with a digital phosphor oscilloscope (SDS1202X-E, SIGLENT Technologies, Shenzhen, China).

Tissue collection and processing

At the end of the stimulation period, rats were deeply anesthetized with 3% intraperitoneal pentobarbital and perfused transcardially with 0.01 M phosphate-buffered saline (PBS) [18]. Brains were removed, post-fixed in 4% paraformaldehyde, and cryoprotected. One hemisphere was processed for histology and immunohistochemistry, while hippocampi from the contralateral hemisphere were dissected and stored for molecular analysis of SRPX2 gene expression [18].

Immunohistochemistry for GFAP

Coronal hippocampal sections (4 µm) were cut and placed onto charged slides, air-dried at room temperature for 1 h, and incubated overnight at 52 °C [19]. The following day, antigen retrieval was performed using a BioSB Tinto chamber (catalog no. BSB7008; BioSB, Santa Barbara, CA, USA) and citrate-based immuno-DNA retrieval solution (Boston Bioproducts, Milford, MA, USA) to unmask formalin-fixed epitopes. Endogenous peroxidase activity was blocked with a peroxidase-blocking reagent (Dako, Agilent Technologies, Santa Clara, CA, USA). Sections were rinsed in Tris-buffered saline (TBS, pH 7.6; Boston Bioproducts), incubated for 1 h at room temperature with a primary antibody against GFAP (GFAP 1034Y, ab68428, Abcam, Cambridge, UK), and then washed in TBS.

Slides were incubated for 30 min with an HRP-conjugated anti-mouse secondary antibody (catalog no. P0447; Dako Denmark A/S, Glostrup, Denmark), rinsed in TBS, and developed with DAB chromogen (Dako) for 10 min. After washing in distilled water, sections were counterstained with Harris hematoxylin for 30 s, dehydrated, cleared, and mounted with DPX mounting medium. Stained sections were scanned using a Leica Aperio CS2 slide scanner (Leica Biosystems Imaging, Vista, CA, USA) to evaluate astrocyte activation in the dentate gyrus and CA4 regions of the hippocampus [19].

SRPX2 gene expression analysis

Total RNA was extracted from hippocampal tissue using TRIzol® Reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was immediately reverse-transcribed to cDNA using a commercial RT-PCR kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) to minimize RNA degradation. cDNA concentration and purity were assessed by spectrophotometry (NanoDrop 2000, Thermo Scientific, Wilmington, DE, USA). The experimental procedures and reporting of results followed the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines [20].

Quantitative real-time PCR was performed in a 20 µL reaction containing 10 µL of qPCR SYBR Green master mix (Guangzhou Dongsheng Biotech Co., Ltd., Guangzhou, China), 0.8 µL of forward primer, 0.8 µL of reverse primer, 2 µL of cDNA template, and nuclease-free water, using a Bio-Rad CFX system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) in a two-step protocol. The cycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. SRPX2 amplification was run in parallel with a housekeeping gene, and each sample was analyzed in triplicate for both targets. The primer sequences for SRPX2 were as follows: forward, 5′-GGCTCTGCCATTTTCTCACGA-3′, and reverse, 5′-GCTGCTTGACTCCCGTTGTG-3′. Relative gene expression was calculated using the 2^−ΔΔCt method [10].

Quantification of glial activation

Glial activation was quantified from three randomly selected high-resolution (200×) fields per animal in the molecular layer and CA4 region of the hippocampus. Astrocytes exhibiting strong GFAP immunoreactivity were considered reactive [19]. The intensity of GFAP-positive staining and the area occupied by GFAP-positive astrocytes were measured using Image-Pro Plus software (Media Cybernetics, Rockville, MD, USA), and mean values per animal were used for subsequent analyses.

Statistical analysis

Statistical analyses were performed using Stata version 17.0 (StataCorp, College Station, TX, USA). SRPX2 expression levels were summarized as mean ± SD. One-way ANOVA was used to compare SRPX2 gene expression across the three acrolein-exposed subgroups (control, sham, and experimental). When the ANOVA indicated significant differences, Tukey's post hoc test was applied to identify pairwise group differences. A two-sided p-value of <0.05 was considered statistically significant [21].

Ethical approvals

The experimental protocol was formally approved by the Research Ethics Committee of Mbarara University of Science and Technology (ref. no.: MUST-2023-791); and the Uganda National Council for Science and Technology (ref. no.: HS3781ES), the Uganda national research ethics review board. The experimental procedures were in conformity with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and the European Communities Council Directive of November 24, 1986 (86/609/EEC) [17, 22].

Results

taVNS downregulates hippocampal SRPX2 expression in acrolein-treated rats

Real-time PCR gene expression analysis revealed that acrolein significantly increased hippocampal SRPX2 gene expression levels compared with controls ($p < 0.05$). taVNS in acrolein-treated rats significantly reduced SRPX2 gene expression toward control values ($p < 0.05$). These findings are shown in Table *1*.

Gene	Total gene expression (n = 13)	Groups		p-value	Test statistic
		Acrolein-exposed (n = 7)	Non-acrolein-exposed (n = 6)		
SRPX2	0.30 ± 0.32	0.50 ± 0.36	0.12 ± 0.07	0.017*	F-statistic

TABLE 1: Mean SRPX2 gene expression in acrolein-exposed (positive control) and acrolein-non-exposed (negative control) rats.

Values are presented as mean ± SD; *p < 0.05, as determined by one-way ANOVA.

Hippocampal SRPX2 expression was significantly higher in acrolein-exposed rats than in non-exposed controls (0.50 ± 0.36 vs. 0.12 ± 0.07; $p = 0.017$).

Figure *1* shows a box plot illustrating SRPX2 gene expression levels in acrolein-exposed and non-exposed rat groups sacrificed immediately after eight weeks (56 days) of exposure.

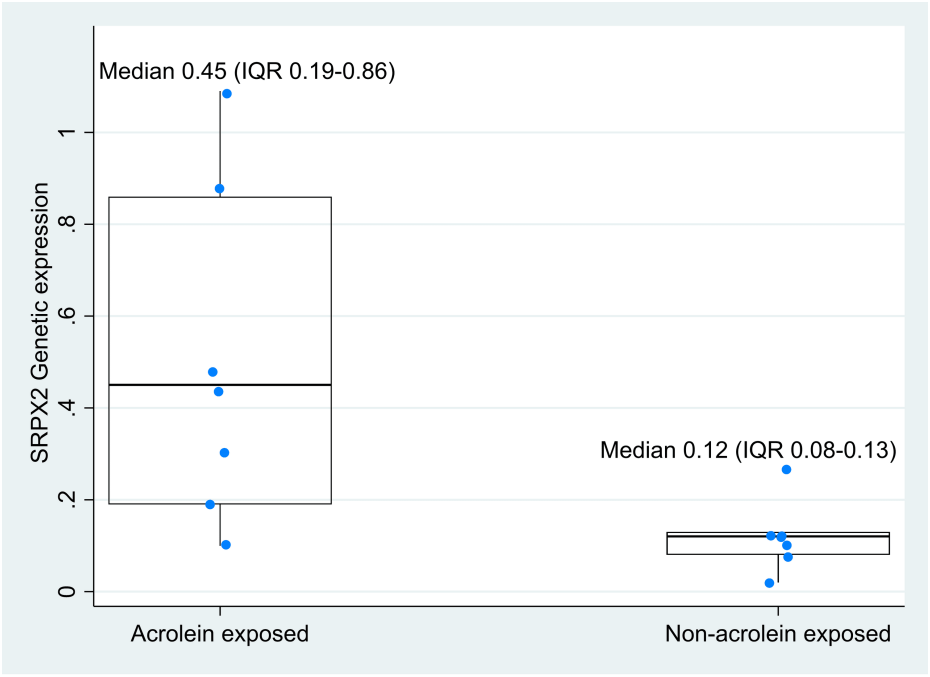


FIGURE 1: SRPX2 gene expression in acrolein-exposed and non-exposed rats.

SRPX2 expression was higher in acrolein-exposed rats (median: 0.45; IQR: 0.19-0.86) than in non-exposed controls (median: 0.12; IQR: 0.08-0.13).

Differences in mean SRPX2 expression among acrolein-exposed rats that underwent different nerve stimulation procedures were also assessed, as shown in Table 2.

Gene	Total gene expression (n = 19)	Study groups			p-value	Test statistic (ANOVA)
		Control (n = 7)	Sham (n = 6)	Experimental (n = 6)		
SRPX2	0.97 ± 0.58	1.30 ± 0.59	1.07 ± 0.54	0.48 ± 0.18	0.022*	F-value

TABLE 2: Differences in mean SRPX2 gene expression among acrolein-exposed rats that underwent different nerve stimulation procedures.

Values are presented as mean ± SD; *p < 0.05, as determined by one-way ANOVA.

The expression of the SRPX2 gene was significantly reduced in the experimental group (acrolein exposure followed by VNS) compared with both the control group (acrolein exposure without electrical stimulation) and the sham group (acrolein exposure with greater auricular nerve stimulation).

The effect of transauricular electrical stimulation of the greater auricular nerve (sham) and vagus nerve (experimental) for four weeks following acrolein exposure on hippocampal SRPX2 gene expression was also examined, and the findings are illustrated in Figure 2.

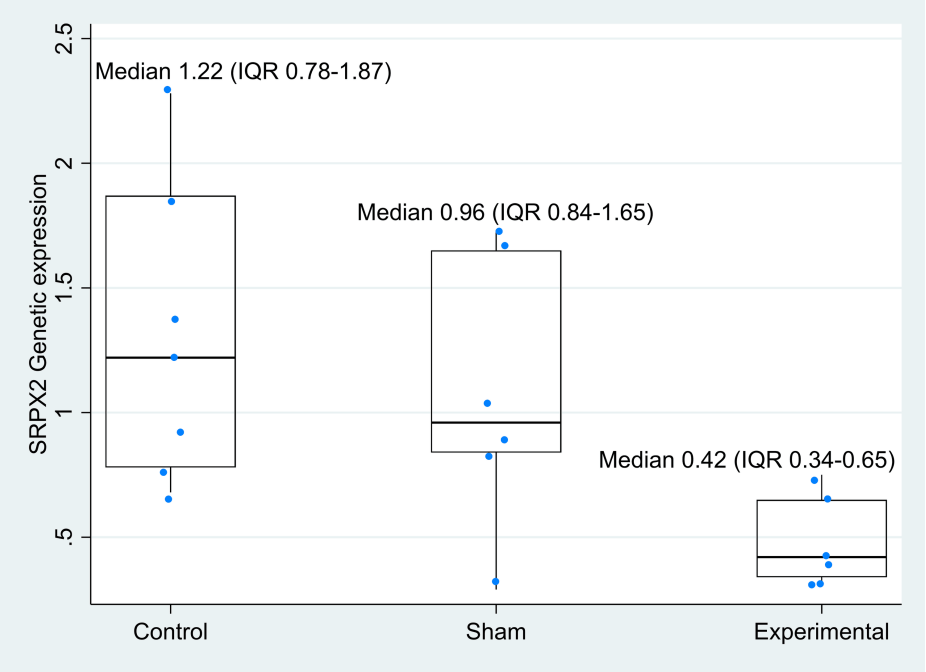


FIGURE 2: Effect of electrical stimulation on hippocampal SRPX2 gene expression.

From the box plot above, the median for the control group was 1.22 (0.78, 1.87), while that for the sham group was 0.96 (0.84, 1.65), and the median for the experimental group was 0.42 (0.34, 0.65).

Post hoc analysis of SRPX2 expression across the study groups was also performed to assess differences in mean values between the groups, as shown in Table 3.

Study group comparisons	Mean difference	95% CI	Standard error	Adjusted p-value	Test statistic (Tukey's post hoc test)
Control-Sham	-0.23	-0.80 to 0.33	0.27	1	q-statistic
Control-Experimental	-0.82	-1.38 to -0.25	0.27	0.022*	
Sham-Experimental	-0.59	-1.18 to 0.0002	0.28	0.15	

TABLE 3: Post hoc analysis of SRPX2 expression across the study groups.

Values are presented as mean ± SD; *p < 0.05, as determined by Tukey's post hoc test.

The analysis revealed a significant difference in mean values between the control and experimental groups, indicated by a p-value of 0.022.

taVNS reduces hippocampal reactive astrocytes

Acrolein markedly increased GFAP-positive astrocyte density and GFAP-occupied area in the dentate gyrus, indicating pronounced astrocytosis. taVNS significantly decreased GFAP immunoreactivity and reactive astrocyte counts in the hippocampal subfields of acrolein-treated rats ($p < 0.05$). These findings are shown in Figure 3.

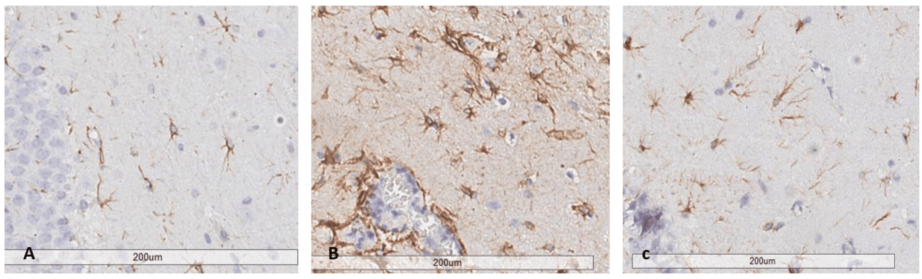


FIGURE 3: Histological sections of the hippocampus stained for glial fibrillary acidic protein (GFAP).

Histological sections of the dentate gyrus. (A) A section from a control rat showing normally dispersed astrocytes with weak GFAP staining of their processes. (B) A section from a rat exposed to acrolein, demonstrating intense gliosis characterized by an increased density of astrocytes and markedly stronger GFAP staining. (C) A section from a rat exposed to acrolein followed by vagus nerve stimulation, showing only mild gliosis, with GFAP staining intensity comparable to that of the control group.

Discussion

This study evaluated the therapeutic potential of VNS in an acrolein-induced rat model of AD, focusing on neuroinflammatory markers and complement-related synaptic regulation. The findings indicate that transauricular VNS attenuates acrolein-induced hippocampal pathology in Wistar rats, as evidenced by downregulation of SRPX2 expression and reduced reactive astrocytosis. These results integrate three converging lines of evidence: the contribution of acrolein to sporadic AD, the role of SRPX2 in complement-mediated synapse elimination, and the neuromodulatory and anti-inflammatory actions of taVNS [4-8, 10, 11, 14, 15].

Acrolein is a highly reactive endogenous aldehyde that is elevated in the AD brain and has been shown to induce synaptic dysfunction, dendritic spine loss, and memory impairment, partly via pathways such as RhoA/ROCK2 [4-8]. The present immunohistochemical and molecular data are consistent with this literature, demonstrating that chronic acrolein exposure increases hippocampal SRPX2 expression and GFAP-defined astrocytosis, supporting a robust glial response that may exacerbate synaptic and neuronal injury [4-7, 9]. The observed pattern aligns with the concept of a “cellular phase” of AD, in which glial activation and complement dysregulation play central roles in early disease [2, 3, 9-12].

A key observation is the significant downregulation of SRPX2 gene expression in acrolein-exposed rats receiving taVNS. SRPX2 is a neuronal complement inhibitor that binds C1q and protects synapses from complement-mediated elimination [10, 13]. In developmental and disease models, loss of SRPX2 enhances microglia-mediated synaptic pruning, whereas SRPX2 overexpression confers synaptic protection [10, 13]. In the context of acrolein-induced injury, elevated SRPX2 likely reflects a compensatory synapse-protective response to heightened complement activity [4, 6-8, 10]. The reduction in SRPX2 expression after taVNS may therefore indicate either that fewer neurons are under complement-mediated stress or that neuromodulation directly dampens upstream inflammatory and complement signals, reducing the need for SRPX2 upregulation [10, 13, 14].

This interpretation is compatible with the known effects of VNS on central neuromodulatory systems. VNS and taVNS activate vagal afferents projecting to the nucleus tractus solitarius, which in turn influence the locus coeruleus and other neuromodulatory nuclei, enhancing noradrenergic and cholinergic tone in the hippocampus and cortex [14, 15, 23]. These pathways contribute to cholinergic anti-inflammatory mechanisms and can suppress pro-inflammatory cytokine release (e.g., IL-1β, TNF-α) from glial cells while promoting neurotrophin signaling and neuronal survival [12, 14, 15]. The current findings of normalized SRPX2 expression and reduced GFAP-defined astrocytosis after taVNS in acrolein-treated rats are consistent with prior work showing that auricular VNS attenuates neuroinflammation and improves memory in other models of brain injury and cognitive dysfunction [14, 15, 23].

Additionally, this study shows that taVNS significantly reduces reactive astrogliosis, reflected by decreased GFAP expression and reduced astrocyte hypertrophy in hippocampal subfields. Astrocyte activation is a well-recognized hallmark of chronic neuroinflammation in AD and often precedes overt neuronal loss [2, 3, 9]. By attenuating astrocytic activation, taVNS may prevent or delay the transition from a reactive, potentially neurotoxic environment to irreversible neurodegeneration [9]. The combined reduction in SRPX2 upregulation and astrocytosis suggests that taVNS modulates a complement-glia axis increasingly viewed as central to early synapse loss in AD [10-13].

The study's strength lies in its novel mechanistic intersection, combining lipid peroxidation-derived

aldehyde toxicity (acrolein) with a complement-inhibiting marker (SRPX2) and noninvasive vagus nerve neuromodulation (taVNS), which is original and biologically compelling. Second, the active sham control, involving greater auricular nerve (pinna) stimulation, provides a rigorous control for the nonspecific somatic and tactile effects of electrical stimulation and repeated daily anesthesia. Lastly, the investigators conducting histological and molecular assessments were blinded to treatment allocation.

Several limitations were also identified. First, only a single acrolein dose and one stimulation protocol (25 Hz, 0.8 mA, 4 weeks) were tested; different intensities, frequencies, or treatment windows might yield distinct neuroprotective profiles [4-8]. Second, the study did not directly quantify complement components (e.g., C1q, C3) or microglial phagocytic activity, limiting mechanistic attribution of SRPX2 changes to specific complement pathways [10-13]. Future work should include direct measures of complement activation and microglia-mediated synapse engulfment to better define the causal chain from taVNS to SRPX2 modulation and synaptic preservation [10-13]. Third, the assessment of astrogliosis relied on semi-quantitative evaluation of GFAP staining intensity, which is susceptible to interobserver variability despite the use of experienced histopathologists. More robust quantification using standardized image-analysis pipelines, immunofluorescence with co-labeling, and stereological sampling would strengthen the evidence. Fourth, the study did not distinguish between neurotoxic A1 and neuroprotective A2 astrocyte phenotypes, which have divergent roles in neurodegeneration and repair [9, 15, 19]. Future experiments should incorporate phenotype-specific markers (e.g., C3 for A1; S100A10 or BDNF for A2) and transcriptomic profiling to characterize astrocyte heterogeneity and its modulation by taVNS [9]. Sex-disaggregated data were also not provided, and the small sample size may limit the clinical implications of the findings, highlighting the need for studies with larger sample sizes.

In summary, this work demonstrates that transauricular VNS mitigates acrolein-induced AD-like hippocampal pathology in Wistar rats by downregulating SRPX2 expression and reducing reactive astrocytosis, supporting a model in which taVNS targets complement-related neuron-glia mechanisms in early sporadic AD [4-8, 10-15]. The results suggest that low-intensity taVNS confers neuroprotective effects primarily through modulation of neuroinflammatory and glial pathways in this model [1-4, 7, 12, 14, 15, 23]. These observations provide a rationale for further preclinical and early clinical studies testing taVNS as a noninvasive adjunctive strategy to preserve synapses and limit neuroinflammation in the prodromal stages of AD [12, 14, 15, 23].

Conclusions

Transauricular VNS attenuated acrolein-induced AD-like hippocampal pathology by reducing SRPX2 expression and reactive astrocytosis. These findings suggest that taVNS modulates complement-related neuron-glia interactions and neuroinflammatory pathways and may have therapeutic potential as a noninvasive strategy for limiting early AD-related synaptic and glial dysfunction. Further studies are needed to establish the causal relationships among SRPX2, complement activation, microglial synaptic pruning, and the neuroprotective effects of taVNS.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Drafting of the manuscript: Ronald Kamoga

Critical review of the manuscript for important intellectual content: Ronald Kamoga, Godfrey Z. Rukundo, Samuel Kalungi, Johnes Oungoloch, Amon Tayebwa, Gladys Nakidde, Celestino Obua, Amadi O. Ihunwo

Supervision: Godfrey Z. Rukundo, Johnes Oungoloch, Celestino Obua, Amadi O. Ihunwo

Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Mbarara University of Science and Technology Research Ethics Committee and Uganda National Council for Science and Technology (UNCST) issued approval MUST-2023-791 and HS3781ES. **Animal subjects:** The experimental procedures were in conformity with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) and the European

Communities Council Directive of November 24, 1986 (86/609/EEC). issued protocol number NIH Publications No. 80–23. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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