VAGUS NERVE STIMULATION IN RATS WITH ALZHEIMER'S DISEASE-LIKE SYMPTOMS: MORPHOLOGICAL, IMMUNOHISTOCHEMICAL, MOLECULAR AND BEHAVIORAL CHANGES

KAMOGA RONALD
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DECLARATION

I Kamoga Ronald, declare that this thesis titled "Vagus Nerve Stimulation in a rat model of Alzheimer's disease: Morphological, Immunohistochemical, molecular and behavioral changes" is solely my own work, with appropriate references from others. No other university or higher education institution has received it for academic award consideration.

Signature:.....Date 11-09-2025

KAMOGA RONALD

2021/PhD/041/PS

PHD STUDENT

APPROVAL

This is our certification that this thesis labeled "Vagus Nerve Stimulation in a rat model of Alzheimer's disease: Morphological, Immunohistochemical, molecular and behavioral changes," by Kamoga Ronald (2021/PhD/041/PS), was prepared with our oversight and guidance.

Signature **Amadi** Date: ...*25-06-2025*.....

Prof. Amadi Ogonda Ihunwo

School of Anatomical Sciences, Faculty of Health Sciences

University of the Witwatersrand, Johannesburg, South Africa

Signature Date: ...25-06-2025.....

Dr. Godfrey Zari Rukundo

Associate Professor, Department of Psychiatry and Behavioral Neurosciences, McMaster University, Hamilton, Ontario, Canada

Associate Research Professor, Mbarara University of Science and Technology, Uganda

Signature Date: ...25-06-2025.....

Dr. Obungoloch Johnes

Senior Lecturer, Department of Biomedical Engineering, Faculty of Applied sciences and Technology, Mbarara University of Science and Technology

Signature Date: ...25-06-2025.....

Prof. Celestino Obua

Alpha Center for Research Administration, Mbarara,Uganda

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LIST OF ABBREVIATIONS AND ACRONYMS

AD: Alzheimer's disease

ADAM-10: A-disintegrin and metalloprotease 10 levels.

APP- Amyloid precursor protein

Aβ-plaques: Amyloid Beta –plaques

ANOVA : **Analysis of Variance**.

BACE-1- Beta-secretase

CA : Conua Ammonis

DG : Dentate Gyrus

FRC: Faculty of Medicine Research committee

GFAP : Glial Fibrillary Acidic Protein.

H & E : Hematoxylin and Eosin

M : Molecular layer of hippocampus

MWM : Morris Water Maze

SRPX2: Sushi repeat-containing protein X-linked 2

taVNS : Transauricular Vagus Nerve Stimulation

VNS: Vagus Nerve Stimulation

OPERATIONAL DEFINITION OF TERMS

Vagus nerve: This is the tenth cranial nerve, running from the brain stem to the pelvis, abdomen, and thorax. It is responsible for numerous automatic bodily processes and transmits various signals(Breit et al., 2018).

Bioelectronic medicine: The name of a new medical specialty that treats inflammation and other ailments by electrical stimulation (Tracey et al., 2007).

ABSTRACT

Background: Alzheimer's disease (AD) is a gradually worsening neurological illness marked by a decrease in intellectual abilities and hallmark neuropathological changes, including β-amyloid plaque accumulation, tau pathology, gliosis, and neuronal degeneration—particularly within hippocampal circuitry. Amidst global research for non-pharmacological interventions, Vagus Nerve Stimulation (VNS) is showing promise to a way to influence brain activity and has already been used to treat hard-to-manage depression and epilepsy. Nevertheless, its role in modulating AD-associated pathology and cognitive deficits remains poorly understood.

Objective: This study sets out to analyze the morphological, immunohistochemical, molecular, and behavioral impacts of transcutaneous VNS in a Wistar rats with AD caused by chronic acrolein exposure.

Methods: Forty-two Wistar rats (3–6 months old) were divided into acrolein-exposed and non-exposed groups to induce AD-like pathology through gastric gavage of acrolein (2.5 mg/kg/day) for 56 days. Animals were then allocated to control, sham-stimulated, or vagus nerve stimulation (VNS) groups. VNS was applied transcutaneously to the auricular branch of the vagus nerve under anesthesia (25 Hz, 0.8 mA, 50 μs pulses, 10% duty cycle) for 28 days. Cognitive function was assessed using the Morris Water Maze, followed by hippocampal analysis for gene expression (BACE-1, SRPX2), histology (neuronal loss), and immunohistochemistry (GFAP, amyloid). Data were analyzed with one-way ANOVA and Tukey's post-hoc test.

Results: Vagus nerve stimulation (VNS) significantly downregulated **SRPX2 gene expression** ($p < 0.05$) and reduced **astrocytic activation**, while also mitigating **hippocampal neuronal damage** in acrolein-exposed rats. The scoping review highlighted a gap in pre-clinical research on VNS in Alzheimer's models, with available studies—mostly from high-income countries—reporting improvements in cognitive function, neural connectivity, and synaptic resilience.

Conclusion: This study demonstrates that transcutaneous VNS may reduce neuroinflammation and synaptic injury markers in acrolein-induced AD, particularly through modulation of astrocytic activity and SRPX2 expression. While behavioral gains were not significant, the observed neuroprotective effects indicate potential therapeutic value, warranting further research with longer stimulation, broader behavioral testing, and detailed astrocyte subtype analysis.

CHAPTER ONE: INTRODUCTION

1.1.0. Background

The most common form of dementia that affects older persons is Alzheimer's disease (World Health Organization, 2021, Metin, 2015). This disorder progresses over time and affects the nervous system, clinically identified by the existence of atleast two cognitive deficiencies—usually featuring memory impairment along with challenges in reasoning, learning, or decision-making. On a pathogenic level, it is identified by the development of amyloid-containing neuritic plaques and tau

related neurofibrillary tangles in the cortex, hippocampus, and amygdala, among other brain r egions (Sunderland et al., 2003, Verkhratsky et al., 2021, Kelly et al.,, 2019, Amadi et al., 2007, Duyckaerts et al., 2009, Metin et al., 2015, Tarawneh et al., 2012, Wolf et al., 2016, Neugroschl et al., 2011). Approximately 57 million people globally have been impacted by Alzheimer's disease and related dementias (ADRDs), and this figure is projected to climb to about 150 million by 2050 (World Health Organization, 2021, World Health Organisation, 2019). Over 60% of dementia cases occur in countries with low- to middle-income levels, encompassing the entire sub-Saharan Africa region. In 2015, roughly 1.69 million people in this area were affected by dementia, and this figure is predicted to nearly triple, reaching 5.72 million by 2050 (World Health Organization, 2021). Uganda reflects this growing public-health challenge: community-based surveys suggest that roughly one in five older adults—about 20 %—are currently living with ADRDs (Mubangizi et al., 2020). In Alzheimer's disease (AD), the gradual deterioration of cognitive function has been strongly linked to neuroinflammatory processes, notably the activation of microglia and astrocytes in response to the accumulation of amyloid-beta (Aβ) plaques (Barrientos et al., 2002, D'Andrea et al., 2001)). The clinical course of AD varies widely, with the period from diagnosis to death ranging from approximately 3 to over 10 years. Notably, individuals with early-onset AD—often diagnosed before the age of 65—tend to experience a more aggressive and rapidly progressing form of the disease compared to those with late-onset AD.(Sheehan, 2012). These patterns underscore the importance of timely diagnosis and region-specific interventions, particularly in low-resource settings where access to specialized care remains limited.

1.1.1 Animal models of Alzheimer's disease

The rodent models, in particular genetically modified rats, are used as experimental models for AD because they closely resemble human physiological, morphological, and genetic traits(Drummond et al., 2017, Hannan et al., 2016, Bouleau et al., 2015, Newman et al., 2014,

Do Carmo et al. , 2013). However, no animal model accurately reproduces all the neuropathological characteristics of AD, namely, amyloid buildup, neurofibrillary tangles, loss of synaptic connections, increased inflammation and oxidative stress, and neuronal degeneration (Drummond et al.,2017). Essentially, each model enables in-depth analysis of one or two disease components, which would be impractical or ethically inappropriate with human patients(LaFerla et al., 2012). The current study will examine acrolein-induced Alzheimer's like pathology namely, neuron damage, hyper-phosphorylation of the microtubule-associated protein tau which encourages amyloid-β deposition in senile plaques, behavioral disturbances , mild decline in cognition , and hippocampal atrophy(Ying-JuanHuang et al., 2013, Waragai and Tashiro , 2012, Bradley et al., 2010, Nam et al., 2010b, Gómez-Ramos et al., 2003, Kuhla et al., 2007, Singh et al., 2010).

1.1.2. Vagus Nerve Stimulation in Alzheimer's disease

Although several new therapeutic agents have been sanctioned to manage Alzheimer's disease, no treatments have demonstrated effectiveness in halting or reversing the disease's underlying pathology. In addition, many of these treatments are linked to significant side effects (World Health Organization, 2021, Metin et al., 2015, Shafqat et al., 2008). These challenges highlight the pressing need for alternative therapeutic approaches with improved safety and efficacy profiles—a central concern addressed in this study. In recent years, researchers and clinicians have become more interested in using neural stimulation as a possible treatment for AD. Preliminary findings from numerous pilot studies have yielded encouraging outcomes (Chang et al., 2018). Vagus nerve stimulation (VNS) is increasingly recognized as a promising treatment, as it appears to boost the release of important brain chemicals like acetylcholine and norepinephrine. These neurotransmitters are thought to exert beneficial effects by attenuating neuroinflammation—an innate response to neural injury—and enhancing neuroplasticity, thereby supporting neuronal repair and regeneration(Engineer et al., 2019, Pericak-Vance et al., 2000, Pesavento et al., 2002, Van der Thie et al., 2022). In a study conducted in Sweden, seventeen individuals with AD showed improved thinking and memory abilities on standardized tests after undergoing at least one year of VNS. Comparable research has similarly reported that stimulating the vagus nerve with electric current can boost memory in animals and people with AD , as well as following learning experience (Roy et al., 2016, Kosel et al., 2003, Ansar et al., 2007, Vargas-Caballero et al., 2022, Metin et al.,, 2015, Sun et al., 2017, Ghacibeh et al., 2006) . Moreover , VNS is authorized at present to treat illnesses that do not

react well to existing treatments, including epilepsy, depression, cluster headaches, and inflammatory bowel disease (Tarasoff-Conway et al., 2015, Heck et al., 2015, Kumar et al., 2019, Ponce et al., 2016, Ruffoli et al., 2011, Conway et al., 2016, Heck et al., 2002, Lainez et al., 2017, Pisapia et al., 2016) . Furthermore, in the last 25 years, accumulating clinical and preclinical evidence has highlighted the therapeutic promise of VNS in managing a diverse array of medical disorders , including obesity, stroke, and traumatic brain injury, among others (Dere et al., 2007, Rosas-Ballina et al., 2009, Zhang et al., 2009, Bonaz et al., 2018, Cai et al., 2014, Dawson et al., 2000, Guiraud et al., 2016, Kaneshiro et al., 2018). This evidence suggests that VNS could help slow down memory and thinking problems in people with Alzheimer's disease, a focus relevant to the present thesis. Nonetheless, a multicenter investigation conducted by Santos-Lozano (Santos-Lozano et al., 2016) on 42 patients with Alzheimer's disease (AD) receiving chronic deep brain stimulation yielded heterogeneous outcomes. Clinical improvements were observed in patients aged over 65 years, whereas younger patients exhibited a deterioration in clinical status, comparable to the decline seen in non-stimulated patients across all age groups. Although the favorable response in the stimulated cohort reinforces earlier reports, the unexpected worsening in younger patients contradicts previous evidence. Such age-specific discrepancies suggest that neuromodulatory approaches—including DBS and, potentially, VNS —may harbor under-recognized adverse effects that warrant further systematic investigation.

Although VNS is increasingly being utilized in the management of various nervous system and systemic illnesses (Fried et al., 2016) , the underlying mechanisms responsible for its therapeutic and adverse effects remain poorly understood(Cheng et al., 2020).There is limited knowledge regarding how specific brain regions respond to electrical stimulation delivered through the vagus nerve . These gaps in understanding represent critical, yet unresolved, questions that have significant implications for the broader clinical application of VNS. Consequently, this study purposed to evaluate the structural and molecular alterations caused by chronic VNS in the brain, alongside its associated neurobehavioral effects, using a rat model that mimics Alzheimer's disease-like pathology induced by acrolein exposure.

1.2. Problem Statement

Dementia encompasses more than 200 progressive, degenerative, and incurable neurological disorders that impair cognitive functions—such as memory, judgment, understanding, language, and numerical skills—ultimately leading to loss of ability to perform everyday tasks on their own and, in its final stages, leads to death (Organization et

al., 2021 , World Health Organization, 2019) AD contributes between 60 and seventy percent of dementia in older adults. Currently, an estimated 57 million people worldwide are living with AD and other types of dementia. This figure is expected to triple by the year 2050 , with the most significant increases expected within developing economies , driven by enhanced longevity trends and expanding elderly populations(World Health Organization., 2021).Uganda reflects this growing public-health challenge: community-based surveys suggest that roughly one in five older adults—about 20 %—are currently living with ADRDs (Mubangizi et al., 2020).

Although numerous pharmacological agents have been approved for AD, none have successfully modified the disease course, and most are accompanied by adverse effects(TulgarErol and Zafer, 2015). Consequently, there is an urgent need for alternative therapeutic strategies. Brain-stimulation modalities—particularly vagus nerve stimulation (VNS)—have gained substantial clinical and academic attention following promising pilot data suggesting benefits against neurodegeneration , neuroinflammation and cognitive deficits in pre-dementia cognitive changes and rodent models of Alzheimer-like pathology(TulgarErol and Zafer, 2015, Alemany et al., 2021, Wang et al., 2023b, Wang et al., 2022b). Notably, clinical and preclinical investigations of vagus nerve stimulation (VNS) have produced mixed results. While some studies report improvements in memory among both rodent models and human patients, others have found minimal benefits or even adverse effects.(Kaya et al., 2013, Aalbers et al., 2012, Adcock et al., 2020, Adcock et al., 2022). Additionally, even though VNS has received approval for treating conditions such as drug-resistant epilepsy, depression, and cluster headaches, its long-term effects on neural structure and function remain poorly understood. This investigation therefore aimed to provide a comprehensive evaluation of chronic VNS in an AD rat model. Specifically, to characterize histological, immunohistochemical, behavioral, and molecular changes induced by prolonged VNS in AD. The resulting insights could clarify the mechanisms underlying both the beneficial and adverse effects of VNS, informing clinical practice, public health policy, and future research directions.

1.3. General objective

To evaluate the structural, molecular, and behavioral effects of long-term vagus nerve stimulation (VNS) in a rat model of acrolein-induced Alzheimer's disease.

1.3.1. Specific objectives

1) To conduct a comprehensive scoping review of the literature on VNS in Alzheimer's disease and other dementias.
2) To characterize VNS-induced behavioral changes in a rodent model of Alzheimer-like pathology.
3) To examine immunohistochemical, morphological, and molecular alterations in the hippocampus associated with chronic VNS.

1.4. Research questions

1) What is the current scope of research on VNS in the context of Alzheimer's disease and similar forms of dementias, and which stimulation parameters, anatomical targets, and animal models are most frequently utilized in both clinical and pre-clinicalp Investigations?
2) Does VNS affect the neurobehavioral characteristics of a rat model of Alzheimer's-like symptoms after 28 days (4 weeks) of treatment?
3) After 28 days (4 weeks) of treatment, does VNS alter brain morphology, immunohistochemistry, and molecular features in a rat model of Alzheimer's-like symptoms?

1.5. Justication and Significance of the study

Justification of the Study:

This study seeks to synthesize existing evidence on vagus nerve stimulation (VNS) in Alzheimer's disease and related dementias, while also elucidating its cellular and molecular mechanisms. By addressing current gaps in knowledge, the study provides a foundation for understanding how VNS may modulate neurodegenerative processes.

Significance of the Study Findings:

The findings are expected to inform future clinical applications of VNS, guide evidence-based policy development, and shape the design and direction of ongoing and future research in neurodegenerative disease therapeutics.

1.6. Conceptual Framework

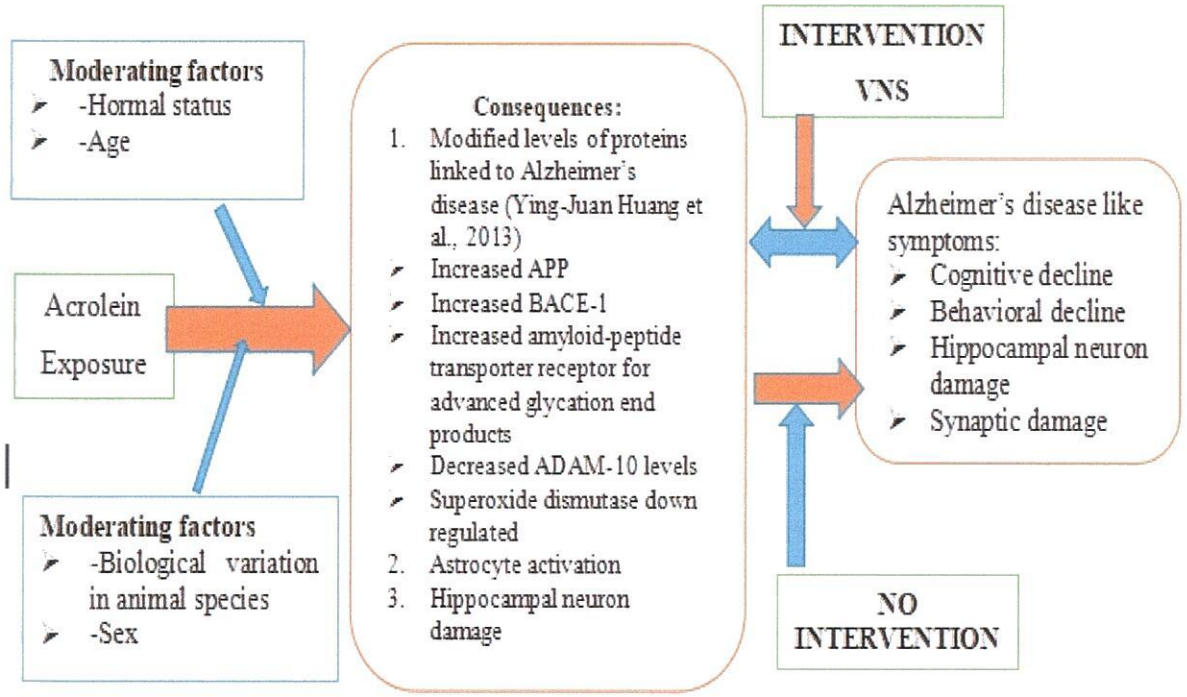

Fig. 1: Conceptual framework: Developed by the researcher based on prior literature review.

APP-Amyloid precursor protein; BACE-1- beta-site APP-cleaving enzyme; ADAM-10 - disintegrin and metalloproteinase 10; VNS- Vagus nerve stimulation. VNS has been shown to enhance cognitive and behavioral outcomes while mitigating acrolein-induced neuro-synaptic damage.

CHAPTER TWO: LITERATURE REVIEW

2.1.0. Alzheimer's disease presentation and prevalence

The leading cause of dementia in older adults is recognized to be Alzheimer's disease (AD)(World Health Organization, 2021, Metin et al., 2015). From a clinical standpoint, AD involves a gradual decline in memory and thinking skills, with diagnostic guidelines typically requiring deficits in at least two cognitive Domains—most often memory, accompanied by challenges in reasoning, learning, or decision-making. The buildup of tau-related neurofibrillary tangles and amyloid-β plaques characterizes AD at the neuropathological level. These pathological alterations are particularly noticeable in brain regions that are important for thinking and controlling emotions, including the cortex, hippocampus, and amygdala (Sunderland et al., 2003, Verkhratsky et al., 2021, Kelly et al., 2019, Amadi et al., 2007, Duyckaerts et al., 2009, Metin et al., 2015, Wolf et al., 2016, Neugroschl et al., 2011, Tarawneh et al., 2012, Sheehan etal., 2012). The prevalence of Alzheimer's disease (AD) and other varieties of dementia (ADRD) is rising worldwide, currently impacting approximately fifty five (55) million individuals globally. It is projected that this number will rise by approximately ten million new cases annually, reaching an estimated total of 152 million worldwide by 2050 (World Health Organization, 2021, World Health Organisation, 2019) . ADRD is the 7th most prevalent cause of death overall and the main cause of disability and dependency among older persons (World Health Organization, 2021, Liu et al., 2009, Musisi et al., 2015) . Economically developing nations account for more than 60% of all dementia cases worldwide. Sub-Saharan Africa is expected to experience a significant rise in dementia cases, with numbers projected to increase from 1.69 million in 2015 to approximately 5.72 million by 2050, indicating around 300% growth (World Health Organization, 2021). In Uganda specifically, the prevalence of ADRD is estimated to be around 20% (Mubangizi et al., 2020) .

Critically, while the global data demonstrate a clear upward trend in ADRD prevalence, there remains a pronounced gap in understanding and addressing dementia in low- and middle-income countries, where healthcare infrastructure, awareness, and access to diagnostic and therapeutic resources are often limited. This underscores the importance of targeted epidemiological studies and contextually appropriate interventions to mitigate the growing individual, societal, and economic burden of dementia in these regions.

2.1.1. Structure and Function of the Vagus nerve

The tenth cranial nerve, the Vagus nerve, connects the brain stem to the pelvis, abdomen, and thorax, and is responsible for numerous automatic bodily processes and transmits various signals (Brei et al., 2018, Hassert , 2014) . The medulla oblongata, which is situated in the basement of the brainstem, is where the vagus nerve originates. It produces ear-supplying auricular branches prior to leaving the cerebral or cranial cavity. Pharyngeal, palatal, and laryngeal areas receive branches from it as it descends into the neck. The larynx, esophagus, lungs, and heart are all innervated by the nerve within the thoracic cavity. The esophagus, stomach, liver, pancreas, and intestines are all innervated via the branches it extends in the abdominal cavity(Breit et al., 2018, Leanage et al., 2020). Roughly eighty percent of the vagus nerve is made up of sensory fibers, and 20% is made up of motor fibers. Afferent signals are sent from the body to the brain by the sensory fibers, while efferent signals are sent from the brain to the body's peripheral organs and tissues by the motor fibers (Breit et al., 2018, Walker et al., 1990). Except for the suprarenal (adrenal) glands, almost every organ from the neck down to the proximal two-thirds of the transverse colon is innervated by the vagus nerve through its motor parasympathetic fibers. In addition to its autonomic functions, it also modulates the activity of select skeletal muscles, notably those of the larynx, pharynx, palate, and tongue (Kenny et al., 2021). Thus, the vagus nerve is essential for controlling an assortment of somatic and autonomic processes.

It contributes to the modulation of heart rate, gastrointestinal peristalsis, and perspiration. Additionally, it facilitates voluntary motor control of oral muscles

Involved in speech. The gag reflex is mediated by its efferent fibers to the throught and posterior oroparynx. Additionary, the vagus nerve plays a role in the physiological mechanisms that underlie emesis and the feeling of fullness after eating (Breit et al., 2018, Eljamel et al., 2011) .

Critically, while the broad anatomical and functional mapping of the VN is well-established, emerging evidence suggests that its **afferent pathways may also influence higher-order brain processes**, including mood regulation, inflammatory modulation, and cognitive functions, highlighting potential therapeutic implications for neurodegenerative disorders such as Alzheimer's disease. This positions the VN not merely as a peripheral conduit but as a **central hub in neurovisceral integration**, capable of mediating complex physiological and behavioral outcomes.

2.1.2. Vagus nerve and bioelectronic medicine.

Bioelectronic medicine is a rapidly developing interdisciplinary field that leverages precise electrical stimulation to influence physiological processes, with a particular focus on managing inflammation and chronic diseases(Tracey et al. 2015). Growing research suggests that vagus nerve stimulation (VNS) can modulate immune function by affecting organs such as the spleen, thereby facilitating the regulation of inflammatory responses through both direct and indirect neural pathways(Koopman et al., 2011). Currently, VNS is an established therapeutic option for several drug-resistant conditions, including refractory epilepsy, major depressive disorder, cluster headaches, and inflammatory bowel diseases (Heck et al., 2015, Ruffoli et al., 2011, Conway et al., 2018, Kumar et al., 2019, Conway et al, 2016, Gaetano et al., 2011) . A significant and expanding volume of clinical and preclinical research conducted over the past 25 years has shown the efficacy and wide-ranging therapeutic promise of VNS in treating a variety of illnesses , including obesity, stroke, and traumatic brain injury among others (Rosas-Ballina et al., 2009, Zhang et al, 2009, Bonaz et al., 2018, Cai et al., 2014, Dawson et al., 2000, Guiraud et al., 2016, Kaneshiro et al., 2018). Acetylcholine, the vagus nerve's primary neurotransmitter, inhibits the secretion of cytokines that trigger inflammation by macrophages, which is how stimulation of the vagus nerve by electricity has been demonstrated to reduce the body's inflammatory response to endotoxins (Koopman et al., 2011 , Tracey et al., 2015). Although the vagus nerve conveys signals from the brain to a variety of intra-abdominal organs, research has predominantly focused on its interaction with the spleen. This focus is due to the spleen's central role in the synthesis of anti-inflammatory mediators, particularly Tumor Necrosis Factor (TNF)(Tracey et al., 2015). Vagal efferent signals synapse at the celiac ganglion, from which postganglionic fibers release norepinephrine within the spleen. Norepinephrine then acts on T lymphocytes, prompting them to release acetylcholine. This acetylcholine binds to α7 nicotinic acetylcholine receptors (α7 nAChR) on splenic macrophages, thereby inhibiting TNF production. This sequence of events constitutes the Cholinergic Anti-Inflammatory Pathway (CAP)(Tracey et al., 2015, Cha et al., 2014 , Pavlov and Tracey, 2012a, Borovikova et al., 2008). The vagus nerve significantly influences neural reflex circuits that regulate innate immune activity and inflammatory processes following infections or tissue injury(Pavlov and Tracey, 2012a, Tracy , 2002, Tracy, 2009). These immune reactions are initiated when immune cells detect molecular patterns linked to pathogens or cellular damage through specialized pattern recognition receptors found on their surfaces or inside cellular compartments. Inflammation, typically a short-term and localized

response, is vital for restoring immune and physiological stability. Constant production of pro-inflammatory cytokines signals long-term or serious inflammation, often caused by disrupted immune system regulatory processes. This abnormal response plays a key role in diseases like sepsis, rheumatoid arthritis, inflammatory bowel disease, and other autoimmune or inflammatory conditions (Pavlov and Tracey, 2012b). In individuals with obesity, chronic low-grade inflammation arises from both immunological and metabolic imbalances, contributing to insulin resistance and a range of metabolic complications (Pavlov and Tracey, 2012a). Moreover, research has shown a link between obesity and decreased vagus nerve activity, prompting interest in vagus nerve stimulation as a possible treatment for managing obesity (Kevin & Tracey, 2016). While the exact mechanism by which gastric electrical stimulation (GES) works remains under investigation ,it is considered to influence the physiological slow-wave activity of the stomach. This modulation may lead to gastric distension, reduced gastric accommodation, and suppression of peristaltic movements, ultimately resulting in delayed stomach emptying and enhanced feeling of fullness (Cha et al . , 2014 , Kevin and Tracey, 2016)

Critically, while VNS demonstrates broad therapeutic potential, much of the research has concentrated on its anti-inflammatory effects via the spleen, leaving other organ-specific pathways and higher-order cognitive and neuroprotective roles relatively underexplored. This gap is particularly relevant in neurodegenerative disorders, such as Alzheimer's disease, where modulation of neuroinflammation, synaptic resilience, and neural plasticity could represent novel therapeutic targets. Thus, bioelectronic interventions like VNS not only exemplify precision modulation of physiological systems but also highlight the need for further mechanistic and translational research across diverse disease contexts.

2.1.3. Alzheimer's disease and electrical vagal stimulation

Amyloidbeta (Aβ)plaque deposition has been widely linked to the activation of microglia and astrocytes as well as other pro-inflammatory processes,which has been implicated in the progressive loss of memory and thinking abilities seen in Alzheimer's diseases (Barrientos. et al., 2002, D'Andrea et al.,, 2001, Mehlhorn et al., 2000, Yaffe et al., 2003). Although a variety of pharmacological treatments have been approved for AD management, none have proven effective in modifying the disease's progression. Furthermore, these therapies often carry significant side effects, highlighting the urgent necessity for novel therapeutic strategies (World Health Organization, 2021, Metin et al., 2015, Shafqat et al., 2008). Against this backdrop, brain stimulation methods have gained considerable attention as a potential

intervention, attracting both clinical and scholarly interest. Early-stage research has demonstrated promising results in this field (Chang et al., 2018). Specifically, stimulating the vagus nerve has been found to promote the secretion of neuromodulators such as acetylcholine and norepinephrine, which are believed to mediate beneficial effects through the promotion of neuroplasticity and reduction of neuroinflammation.(Engineer et al., 2019, Pesavento et al., 2002).

Previous studies (Merrillet al., 2006, Sjogren et al., 2002) have reported improvements in certain cognitive functions after electrical stimulation targeting the vagus nerve in a sample of 17 individuals in Sweden diagnosed with Alzheimer's , monitored for at least one year. Additional research has consistently demonstrated that VNS enhances memory performance in both animals used in research and people with Alzheimer's, as well as in contexts following learning events. (Roy et al., 2016, Kosel et al., 2003, Ansari et al., 2007) . However, a multicenter research study (Santos-Lozano et al., 2016) involving chronic neural stimulation in 42 AD patients revealed mixed results. Positive clinical outcomes were noted in older participants (65 years and above), while younger patients showed worsening outcomes, and non-stimulated patients had no significant changes regardless of age. The observed clinical improvements in stimulated patients aligned with previous research, but the decline seen in the younger stimulated group contradicted earlier findings. These varying results with VNS in AD may suggest possible side effects of brain stimulation that have not been thoroughly investigated.

Despite the growing application of electrical vagal stimulation in the management of various disorders, several critical and unresolved questions remain regarding its use. Notably, the fundamental mechanisms underlying the therapeutic and unwanted effects of VNS are not yet completely understood (Cheng et al., 2020). Furthermore, there is limited understanding of how targeted parts of the brain respond to vagal electric stimulation (Fried et al., 2016). Accordingly, this study aimed to look at how long-term vagus nerve stimulation affects the brain's structure and molecules, as well as related behavior changes, using rats with features analogous with Alzheimer's disease.

2.1.4: Types, Etiology and Laboratory animals with Alzheimer's-like symptoms

Alzheimer's disease (AD) has two main types: most people (about 99%) have sporadic or late-onset AD, which happens later in life. A smaller group (about 1%) has familial or early-onset AD, which starts before age 65 (LaFerla et al., 2012). Even though the exact cause of Alzheimer's disease (AD) isn't clear, early-onset AD (FAD) has been connected to changes in

three specific genes: presenilin 1 (PSEN1), presenilin 2 (PSEN2), and the amyloid precursor protein (APP) (Cruchaga et al., 2012, LaFerla et al., 2012). Experimental animals with features of AD play a crucial role in helping researchers understand how the disease develops and in testing new treatments before they are used in humans (Drummond et al., 2017) .

Many experimental animals with features of Alzheimer's disease have been used in the desperate search for a medication that could slow or stop its progression , but none of these endeavors have been successful thus far(LaFerla et al., 2012). Animal models of AD vary and include invertebrates like fruit flies (Drosophila melanogaster) and worms (Caenorhabditis elegans), non-mammal vertebrates like chickens, frogs, and zebrafish, as well as mammals like rodents.(Newman et al., 2014, Newman et al., 2017). For research on age-dependent degenerative processes, invertebrates and non-mammalian animals are preferred because they are less expensive, simple to care for, reproduce quickly in large numbers, and have a short life cycle (Newman et al., 2017). However, in contrast to the human brain, they typically have a very simple nervous system, and assessment of their behavioral traits is still under developed (Newman et al. , 2017). On the other hand, primates that are not humans and rodents, because of their greater physiological and anatomical similarity to humans, are preferred as models for AD (Newman et al. , 2017)

It's also worth noting that, because the cause of AD is unknown, all available animal models have relied on using genetic mutations linked to familial AD (fAD), with the rationalization that the events that follow the initial trigger are very parallel (Drummond et al., 2017) . Given that most individuals affected have the idiopathic AD type may explain why it is still difficult to apply preclinical research to humans. Another important consideration is the fact that no animal model accurately reproduces all the neuropathological characteristics of Alzheimer's disease , including amyloid buildup, synaptic loss, and neuronal death among others (Drummond et al., 2017). This could also be another reason for failed translation to clinical application. Essentially, each model enables in-depth analysis of one or two disease components, which would be impractical or ethically appropriate with human patients (LaFerla et al., 2012) .

2.1.5. Acrolein induced Alzheimer's disease-like symptoms

Acrolein is a highly reactive α,β-unsaturated aldehyde that people are often exposed to through various dietary sources, including coffee, cheese, and donuts, as well as from the combustion of organic substances (Srivastava et al., 2011). Research has found that acrolein can cause oxidative stress, which may play a role in the development of brain diseases like Alzheimer's

(Bradley et al., 2010, Nam et al., 2010b). Evidence from previous studies indicates that acrolein can induce hyper phosphorylation of tau, a microtubule-associated protein, and facilitate the buildup of amyloid-beta peptides in brain plaques—both recognized as pathological hallmarks of AD (Gómez-Ramos et al., 2003, Kuhla et al., 2007, Singh et al., 2010). High levels of acrolein have likewise been reported in the brains of people with AD, suggesting a potential role in disease progression (Bradley et al., 2010). Furthermore, long-term exposure of animal models to acrolein has been connected to behavioral changes, memory impairment, hippocampal atrophy, and increased oxidative stress in neural tissue. These effects are accompanied by abnormal expression of proteins involved in amyloidogenic processing, such as higher levels of amyloid precursor protein (APP), β-secretase (BACE-1), and the RAGE receptor, along with lower levels of ADAM10, an enzyme that normally helps prevent amyloid formation (Ying-JuanHuang et al., 2013, Waragai and Tashiro , 2012) Altogether, these findings lend support to the view that acrolein plays a significant role in the pathophysiological processes of AD by enhancing neurotoxicity and promoting neuronal degeneration.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Objective 1: **To conduct a scoping review of literature on vagus nerve stimulation in Alzheimer's-disease and related dementias.**

3.1.1. Scoping review

To systematically undertake an in-depth scoping review of the existing body of literature pertaining to VNS in Alzheimer's disease along with other types of dementia, an in-depth review of published literature was undertaken to determine , summarize, and synthesize the existing body of evidence on the subject (Arksey et al., 2005 , Munn et al., 2018).

3.1.2. Search strategy

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) guidelines(Moher et al., 2009), a systematic search was conducted in PubMed, HINARI, Google Scholar, and the Cochrane Library for studies published between January 1980 and 30 November 2023. The year 1980 was selected as the start point because research on vagus-nerve stimulation (VNS) was minimal before that year (Broncel et al., 2020) and widespread clinical approval of VNS for neurological disorders is comparatively recent (Vargas-Caballero et al., 2022) . Institutional subscription constraints precluded access to additional databases, including Scopus, Embase, and Web of Science; this limitation was recognized a priori. To make sure nothing was missed, a manual search of the reference lists from all the included articles and the grey literature—using web searches, online catalogues, and specialized grey-literature repositories was conducted.

Search terms combined controlled vocabulary (where applicable) and free-text keywords related to vagus-nerve stimulation and dementia, including: "brain stimulation," "dementia," "Alzheimer*," "memory loss," "Deme*," "cognit*," "VNS," "vagal stimulation," and "cranial nerve stimulation." Boolean operators and database-specific filters were applied as appropriate.

3.1.3. Inclusion criteria

Eligibility criteria were restricted to primary research articles that investigated VNS in Alzheimer's disease and other forms of dementia (ADRDs) and included either human or animal subjects in longitudinal or cross-sectional designs. We considered only peer-reviewed papers published in English up to 30 November 2023; studies in other languages were excluded owing to the resources required for translation. We also excluded investigations of VNS for nervous system diseases not related to ADRDs, as well as case reports, conference abstracts or

proceedings, commentaries, and literature review publications . Only records with a readily accessible full text were retained, as abstracts alone could not be critically appraised to the same standard. All eligible study designs were represented, and subgroup analyses were undertaken to address methodological heterogeneity. When a study reported more than one discrete outcome, each outcome was extracted and analyzed separately. For every included article, we recorded the surname of the first author and the year in which the article was published.

3.1.4. Data extraction

Two research assistants , including the PhD candidate, independently conducted the initial screening of articles by reviewing study titles and abstracts to detect research articles that did not meet the predefined inclusion criteria. Following this, EndNote reference management software was utilized to download and organize the full-text articles selected for further evaluation. EndNote was selected for use in this research due to its seamless integration with the chosen word processing software and its proven efficiency in organizing references, managing citations, and generating bibliographies in accordance with academic standards. Subsequently, three independent individuals —comprising two research assistants and the PhD candidate—screened entire research papers and collected relevant information using standardized electronic data extraction forms. In cases where the three individuals disagree regarding study inclusion or exclusion, a content expert (a psychiatrist with subject matter expertise) served as an adjudicator to resolve discrepancies. A matrix table was utilized to systematically organize and present the extracted data. Furthermore, all omitted research articles during the full-article review phase were documented, along with detailed justifications for their exclusion.

3.1.5. Variables to be collected

For every included study, we recorded: (i) study design- the different study designs used in the identified studies for instance experimental studies designs (randomized / non-randomized) and analytical desins (case-control study designs, cohort designs and cross sectional study designs); (ii) sample size; (iii) vagus-nerve-stimulation modality, that is either invasive or non-invasive, or both ; (iv) primary and secondary outcomes—desired or adverse—spanning histomorphological, molecular, and behavioural domains; (v) year of data collection, that is, the year in which the study was carried out ; (vi) geographic setting, that is, the region and country where the study was conducted ; (vii) participant social and demographic

characteristics for studies involving human participants (age, biological sex, religious beliefs , ethnic origin , etc.); and (viii) authorship details and year of publication.

3.1.6. Data analysis

After full-text screening, all eligible studies were independently charted by two research assistants using a pre-piloted extraction form. Key variables captured for each study included: (i) study design and sample size; (ii) type of vagus-nerve-stimulation used (invasive, non-invasive, or both); (iii) desired and adverse results (histomorphological, molecular, behavioural, and cognitive end-points); (iv) year of data collection; (v) geographic setting (country and region); (vi) participant socio-demographics (age, sex, ethnicity, religion, and other reported characteristics); (vii) sampling criteria and recruitment strategy; and (viii) statistical or qualitative analytic methods used. Extracted variables were then grouped inductively into thematic domains reflecting the primary investigative foci across studies. Initial coding was performed in Excel®, followed by iterative discussion to resolve discrepancies. The harmonised dataset was exported to STATA v13.1 (StataCorp, College Station, TX, USA) for quantitative synthesis and descriptive statistics in the next analysis phase.

3.1.7. Quality Assessment

A comprehensive assessment of bias found among the different included studies and a detailed critique of each one weren't done,, since the primary objective of this mapping or preliminary review was to examine the extent and nature of existing research on vagal stimulation (VNS) in dementia and cognitive impairment, irrespective of study quality or risk of bias (Peters et al., 2015, Peter and Marnie , 2017). However, to ensure the review remained focused and consistent, only studies that met the predetermined inclusion criteria were incorporated.(Ocan et al., 2015). Furthermore, three independent reviewers conducted the selection process to enhance reliability and minimize potential selection bias.

3.2.1. Objective 2 and 3

3.2.2. Methods and Materials

3.2.3. Rats

The research involved Wistar rats available from local suppliers.

3.2.3.1. Sample size calculation

The resource equation model (Charan and Kantharia , 2013) , which evaluates the value of E, was used to calculate the required sample size for the study. E represents the degrees of freedom in an Analysis of Variance (ANOVA). A sample size that maintains E within the range of 10

to 20 is generally regarded as sufficient or statistically meaningful. E represents the difference between the total number of animals used and the total number of experimental groups Therefore, X = E + 4 (total number of groups in our study is 4).

But Emin (minimum number of animals) = 10; &

Emax (maximum number of animals) = 20

Hence, Xmin (Minimum number of animals per group) = (10 + 4) / 0.8 (Animal attrition rate) = 14/0.8 = 17.5/ 4 (Total number of groups) = 4.4

Xmax (Maximum number of animals per group) = (20 + 4) /0.8 (Animal attrition rate) = 24/0.8 = 30/4 (Total number of groups) = 7.5

Therefore, our study consisted of four groups (Negative control, Positive control, sham, and Experiment, each consisting of 7 animals. A total of 42 rats were utilized of which 5 died during manipulations across the entire period of experimentation.

Table 1: Distribution of animal groups

<table>
<tr><th>Groups

Study arm</th><th>Negative control (Non-acrolein exposed) group</th><th>Postive control (Control) group</th><th>Sham group</th><th>Test/ Experiment group</th><th>Total</th></tr>
<tr><td>The composition of animal cohorts and corresponding sample sizes employed to explore the impact of vagus nerve stimulation (VNS) on cognitive function.</td><td>7 (1 rat died remaining 6).</td><td>7 (but 1 rat died remaing 6).</td><td rowspan="2">7 (1 rat died remaing 6).</td><td rowspan="2">7 (1 rat died remaing 6).</td><td>24</td></tr>
<tr><td>Animal groups and numbers used to assess effect of VNS on molecular, immunohistochemicaland histomorphological changes associated with acrolein exposure</td><td>7 (1 died remaing 6</td><td>7</td><td>25</td></tr>
</table>

A total of 42 study animals were initially recruited; however, five animals died during the course of the study, resulting in an actual sample size of 37. Animals were assigned to four

groups: Negative Control (n = 14), with no acrolein exposure or stimulation; Positive Control (n = 14), exposed to acrolein only; Sham (n = 7), exposed to acrolein plus electrical stimulation targeting the greater auricular nerve; and Experiment (n = 7), received acrolein exposure along with auricular vagus nerve stimulation. The sham and experimental groups constituted the two intervention arms of the study, both receiving electrical stimulation but targeting different nerve branches

The research was conducted under the supervision of a veterinary surgeon with experience in animal handling, and the least number of animals and the least amount of pain for the animals were ensured throughout the procedures. The study protocol received all relevant ethical approvals. The animals were kept in controlled conditions with a steady temperature and humidity, 12 hours of light and 12 hours of darkness, and unlimited food and water. To ensure acclimatization, every rat was allowed a one-week habituation period prior to acrolein exposure. All electrical stimulation was done for 28 days. Following behavioral observations, the rats underwent brain extraction, and the tissues were carefully processed for histomorphological examinations.

3.2.4. Acrolein

Acrolein is a very reactive chemical that people often come across in meals such as cheese, donuts, and coffee, and also from burning things like wood or plants (Srivastava et al., 2011) . Research shows that acrolein can damage brain cells through oxidation, which is connected to diseases like Alzheimer's (AD) (Bradley et al., 2010, Nam et al., 2010b). Research suggests that acrolein can lead to abnormal changes in the tau protein and promote clumping of amyloid-beta, both key features of AD (Gómez-Ramos et al. , 2003, Kuhla, 2007) (Singh et al., 2010). Higher concentrations of acrolein have been detected in the brains of people with AD (Bradley et al., 2010). In rats, long-term exposure to acrolein causes behavior problems, memory loss, shrinkage of the hippocampus, increased brain oxidative stress, and changes in important brain proteins linked to Alzheimer's disease (Ying-JuanHuang et al., 2013, Waragai and Tashiro , 2012). These findings suggest that acrolein may have a significant role in the pathophysiology of Alzheimer's disease by encouraging neurotoxicity and neuronal degenerat ion.

Moreover, oxidative stress is connected to several diseases that commonly occur with aging, including Alzheimer's disease , and acrolein increases oxidative stress in rat brains by lowering brain SOD activity and increasing MDA activity (Ying-JuanHuang et al., 2013, Belkacemi & Ramassamy , 2012, Black et al., 2008) . Prior research shows that MDA is linked to oxidative

stress in bodily tissues, while SOD activity aids in shielding neurons from oxidative stress-related injury. Stress (Mracskó et al., 2011, Sharma et al., 2011, Hamann & Shi ., 2009, Sultana, 2012, Sultana & Butterfield , 2010).

3.4.5. Acrolein dosing and administration

Everyday newly prepared acrolein was mixed in regular tap water and consumed within two hours after being prepared. Based on previous research, the estimated safe daily intake of unsaturated fatty acids in humans is 5 mg per kilogram per day (Wang et al., 2008) . In this study, animals were exposed to acrolein at a daily dose of 2.5 milligrams per kilogram , which was 50% of the estimated intake. Acrolein was dissolved in 100 mL of tap water and administered once daily by gastric gavage for eight weeks (56 days).

3.2.5. Trans auricular Vagus Nerve Stimulation (atVNS) procedure

Transauricular Vagal Stimulation (taVNS) was carried out under 2% isoflurane anesthesia. Following established methods, a pair of magnetic electrodes with opposite charges (+/–) was applied to each ear, with one electrode placed inside and the other outside the auricular concha area (Wang et al., 2015). Sodium chloride solution was used between the electrode and the skin to boost electrical conductivity (Wang et al., 2023a). The stimulation parameters determined according to prior studies (Smith et al., 2005; Engineer et al., 2011; Porter et al., 2012) included: A one-hour transcutaneous electrical stimulation session delivered at a frequency of 25 Hz, with 0.5 seconds ON and 4.5 minutes OFF intervals , and a current of 0.8 mA applied for 28 days via an electrical stimulator (DDS Signal generator/counter-model: JDS-6600, P/N 1726302922; and a Super phosphor Oscilloscope-SIGLENT SDS1202X-E - Serial number: SDS1ECDD2R1388). The sham stimulation was applied to the auricular margin using comparable stimulation parameters.

3.2.6. Electrical stimulation parameters

The stimulation parameters were 25 Hz frequency, cathodic-leading pulses of 50 microseconds duration and an amplitude of 0.8 milliamps functioning with 10% on-time per cycle—stimulated for 30 seconds with a subsequent 4.5-minute off period —for a total duration of one hour. Clinically, the highest tolerable amplitude is often employed to maximize vagus nerve stimulation (VNS) engagement and therapeutic benefit. However, research on rats has shown an inverted U-shaped correlation between stimulation intensity and cortical plasticity.

Specifically, moderate amplitudes between 0.4 and 0.8 mA produced the most favorable outcomes, while both lower (around 0.4 mA) and higher intensities (above 1.2 mA) proved less effective.(Borland et al., 2016) .

3.2.7. Behavioral analysis (Morris Water Maze)

The animal behavior analyzed in this study was spatial learning and memory, and the assessment was carried out using a Morris Water Maze (MWM) as described by Nunez (Nunez et al., 2008). The MWM is a popular experiment used to evaluate spatial learning and memory. It was made of a white cylindrical tank, with dimensions of 180 cm in diameter and 90 cm in height, containing water to a depth of 80 cm, and kept at 26°C. The tank was divided into four symmetrical sections—north, south, east, and west—by two hypothetical perpendicular lines intersecting at the center.

During the training phase, an elevated platform that was approximately 2.5 cm above the water's surface was positioned in the central part of the pool. To lessen stress, the animals were carefully lowered tail-first into the maze from 1 of 4 predetermined beginning sites (north, south, east, or west). Each animal was given up to 60 seconds to find and climb onto the escape platform, and the time taken (latency) was recorded. If a rat didn't find the platform within 60 seconds, a default latency of 60 seconds was recorded, and the animal was gently guided to the platform. After reaching the platform, animals remained there for 15 seconds to help them associate the platform with escape. Each animal underwent three training trials, each starting from a different position. After completing the training, the animals were dried and the procedure was repeated for all subjects. Once training was completed for all animals, the testing phase commenced.

During testing, the safety platform was submerged about 2.5 cm below the surface of the water, and milk powder was used to make the water opaque. The water temperature was kept consistent with the training conditions. Each animal completed 12 test trials, three from each of the four starting positions, with no consecutive starts from the same location. Each trial lasted up to 60 seconds, during which time the animal was monitored until it located the hidden platform, and the time taken was recorded. Animals that failed to find the platform within the time limit were guided to it, as done during training. After each test, animals were dried and given a 60-second rest period.

To reduce bias, research assistant conducting the MWM did not know which treatment group the animals belonged to. Both the training and testing sessions were conducted on the same day in a sequential manner.

3.2.8. Brain tissue processing

After taVNS, all animals were put under anesthesia and euthanized for brain tissue analysis. Using 3% intraperitoneal sodium pentobarbital, the animals were deeply anesthetized before cardiac perfusion with 0.01 M phosphate-buffered saline (PBS) , followed by brain extraction for hematoxylin and eosin staining. One hippocampal hemisphere from each of the animals was processed for histological analysis to visualize the effects of acrolein exposure on hippocampal neurons. The second hippocampal hemisphere from each animal was prepared for molecular analysis to determine and compared the expression of BACE-1 and SRPX2 genes across the study groups.

3.2.9. Determining cerebral SRPX2 and BACE 1 levels using real time-PCR

Total RNA in the hippocampal tissue for mBACE-1 and SRPX2 gene expression analysis was extracted /isolated using TRIzol® Reagent (Life Technologies), according to the manufacturer's instructions. The resultant RNA was immediately converted to cDNA after elution using RT-PCR kit (solar bio, China) to reduce the effect of RNAses. cDNA was quantified by spectrophotometry using Nano Drop 2000. A total volume of 20ul was used comprising of 10ul of qPCR SYBR green (GDS bio, China), 0.8ul forward, 0.8ul reverse primer, 2ul cDNA and nuclease free water was performed on CFX bio Rad system in a 2 step PCR. The conditions were as follows: 95°C for 3 minutes initial denaturation 40 cycles of denaturation at 95°C for 10sec and annealing at 60°C for 30sec. mBACE-1 and SRPX2 were run along their house keeping genes, each sample being run in triplicates for both targets.

3.2.10. H&E staining

After fixation in 4% paraformaldehyde for 6 hours, the hippocampal hemispheres for H&E staining were cryoprotected in 30% sucrose dissolved in PBS. Hippocampal tissue sections were stained with hematoxylin and eosin, following previously established methods (Milane et al., 2011) . For routine tissue examination, brains were cut into 5-micrometer sections and stained with H&E to check for signs of hippocampal neuron loss, such as pyknotic neurons and atrophy. The histopathologists analyzing changes in the hippocampal tissue were unaware of the treatment groups to reduce the risk of observation bias. Using a digital camera (Leica Bio

systems Imaging , Inc.1360 Park Center Dr. Vista, CA 92081USA), photomicrographs of the hippocampus areas (Conua Ammonis 1-4) were taken.

3.2.11 Immunohistochemistry (GFAP and Beta Amyloid antibodies)

Tissue blocks were cut at 4 microns on charged slides, and they were put at room temperature for one hour. They were then put in an oven for overnight at 52 degrees Celsius. The next day, antigen retrieval, in a bioSB tinto antigen retrieval chamber using the bioSB immuno-DNA retrieval solution with citrate, was performed to expose epitomes which were masked due to formalin fixation (Boston Bioproducts, Inc., 425 fortune BIvd. Milford). Peroxidase blocking agent obtained from Dako Agilent technologies (Dako, Agilent Technologies, Inc. 5301 Stevens creek BIvd. Santa Clara, CA 95051, United States) was used to inhibit immunoreactivity in hippocampus slices. Sections were then washed with tris buffer solution (TBS) PH7.6 from Boston bioproducts, then immersed in GFAP 1034Y (AB68428) or antibeta amyloid 1-42, a primary antibody solution by abcam, and incubated there for an additional hour at room temperature , Sections were incubated with HRP anti-mouse DAKO , a secondary antibody solution , for 30 minutes at room temperature after being washed in TBS. The sections were washed again in 2 changes of TBS and subjected to DAB chromogen by dako for 10 minutes. They were washed in distilled water and counter stained with harris haematoxylin for 30 seconds, they were dehydrated, cleared and mounted with Distyline Platicizer Xylene (D.P.X). Sections were photographed using a Leica Aperio CS2 slide scanner for evidence of hippocampal beta amyloid plaques (Leica Bio systems Imaging, Inc.1360 Park Center Dr. Vista, CA 92081USA).

3.2.13. Quantitative analysis

To assess glial activation, three high-resolution (200x) images per animal were randomly selected. The intensity of astrogliosis, indicated by astrocytes exhibiting strong Glial Fibrillary Acidic Protein (GFAP) staining in the molecular and cornua ammonis 4 regions in the hippocampus, was quantified using Image-Pro Plus software. The intensity of GFAP-positive astrocytes in each field on each segment were used to express the results. Additionally, the staining intensity of amyloid plaques in the molecular layer and CA4 region were evaluated.3.2.14. Statistical analysis

Data analysis was done using STATA version 17.0 (StataCorp, 2024) statistical analysis software. Gene expression levels of BACE-1 and SRPX2 were expressed as mean ± standard deviation . The expression levels of the acrolein-exposed and non-exposed groups were compared using the Student's t-test. To assess differences in average swimming time and

BACE-1 and SRPX2 expression across groups, a one-way ANOVA was used. A p-value below 0.05 was considered statistically significant. Cognitive test results were shown using boxplots and line graphs, with data presented as mean ± standard deviation. After ANOVA, Tukey's post-hoc test was used to identify differences in SRPX2 expression between groups. A p-value of less than 0.05 was considered significant. Hippocampal neuron changes were also shown using photomicrographs of subregions (Cornua Ammonis 1-4) , captured with a Leica Aperio CS2 slide scanner.

3.2.15. Quality control

The data collection instrument was evaluated through a pilot study. All quantitative assessments and experimental procedures were conducted in triplicate to ensure reliability. For external quality assurance, histological sections were independently reviewed by an experienced histopathologist blinded to the experimental design. Throughout the data collection and documentation of observed changes, only qualified, trained, and certified personnel were involved.

3.2.16. Ethical considerations

The study was approved by relevant ethics committees, including those at Mbarara University and the Uganda National Council for Science and Technology. All animals were cared for under ethical guidelines, with controlled housing and free access to food and water. Approval letters are provided in the appendices.

3.3.1. Dissemination of results

The research findings were compiled into a report submitted to the Faculty of Medicine at Mbarara University of Science and Technology as part of the PhD requirements in Medicine. A copy would be placed in the MUST library. Additionally, the findings were disseminated to the broader scientific community through publication in relevant peer-reviewed journals.

CHAPTER FOUR: RESULTS

This section presents the results arranged according to the specific study objectives.

4:1 Objective 1: To review existing literature on vagus nerve stimulation in Alzheimer's disease and related dementias.

A total of 8,062 articles were found through database searches. After removing duplicates and applying inclusion criteria through title, abstract, and full-text screening, 10 articles were included in the final review. The selection process is shown in the figure below.

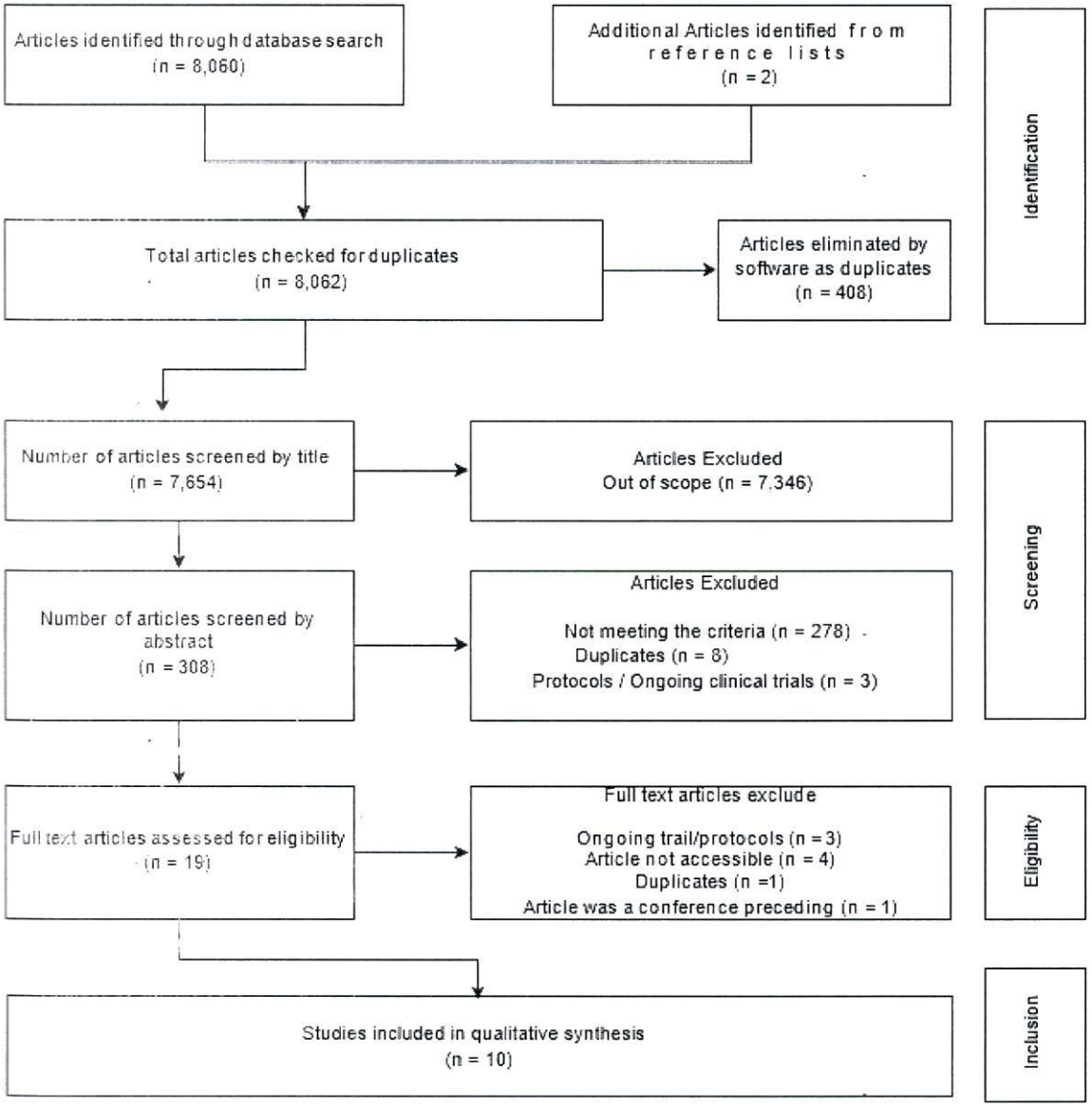

Figure 1: Article selection flow chart

Table 2: Summary of Search Strategy and Outcomes

Digital Repository	Search Plan and Terminology	Selection Criteria Used	Number of Articles Identified
PubMed	(((((("Vagus nerve stimulation") OR ("VNS")) OR ("vagal stimulation")) OR ("cranial nerve stimulation")) OR ("brain stimulation")) AND ((((("dementia") OR ("Alzheimer's disease")) OR ("cognitive impairment")) OR ("memory loss")) OR (deme*)) OR (cogniti*)))	- Publication types: Meta-analysis, Randomized Controlled Trials, Reviews, Systematic Reviews - Publication date: January 1, 1980 – November 30, 2023 - Language: English	819 articles
Cochrane Library	"Vagus nerve stimulation" OR "Vagal stimulation" OR "VNS" OR "Cranial nerve stimulation" OR "Brain stimulation" in Title, Abstract, or Keywords AND "dementia" OR "Alzheimer's" OR "cognitive impairment" OR "memory loss" OR (NEXT deme*) OR (NEXT cognit*)	- Fields searched: Title, Abstract, Keywords	5,744 results (5,722 systematic reviews, 22 trials)
Google Scholar	("Vagus nerve stimulation" OR "VNS" OR "Vagal stimulation" OR "Cranial nerve stimulation") AND ("Alzheimer's disease" OR "Alzheimer disease" OR "Cognitive impairment" OR "Memory loss" OR deme* OR cognit*)	- Language: English - Sorted by relevance - Download limited to top 980 entries	10,900 results (980 articles screened)
JSTOR	((Vagus nerve stimulation) OR (Vagal stimulation) OR (VNS) OR (Cranial nerve stimulation) OR (Brain stimulation)) AND ((Alzheimer's) OR	- Access type: Open access, full-text online - Source type: Scholarly and peer-reviewed	

Digital Repository	Search Plan and Terminology	Selection Criteria Used	Number of Articles Identified
	(Dementia) OR (Cognitive impairment) OR (Memory loss) OR (Deme*) OR (Cognit*))	journal articles - Date range: 1990 – 2023 - Language: English - Fields: Biomedical sciences, neuroscience and zoology	

Table 3: Data extraction matrix

Study & reference	VNS modality & design	Participants	Stimulation protocol*	Principal outcomes
Wang et al., 2023 (China) "Mechanism underlying chronic transcutaneous auricular VNS in MCI"	Transcutaneous auricular VNS (taVNS), parallel-group RCT	60 MCI (55–75 y) + 30 healthy controls; both sexes	Heart (CO15) + kidney (CO10) conchal points; 20 Hz for 10 s then 100 Hz for 50 s each minute; 0.6–1 mA; 30 min/session, 2 ×/day, 5 days/week × 24 weeks	taVNS selectively increased functional connectivity (FC) between left precuneus and parahippocampal gyrus; FC change modestly correlated with MoCA-B and AVLT-N7 gains, indicating cognitive benefit in MCI.
Wang et al., 2022 (China) "Efficacy and safety of taVNS in MCI"	taVNS, double-blind RCT	60 MCI (55–75 y); both sexes	Same montage and duty cycle as Study 1	Relative to sham, taVNS produced significantly greater improvement on MoCA-B ($p = 0.033$) and superior immediate (N5) and delayed (N7) recall. No severe adverse events reported.

Study & reference	VNS modality & design	Participants	Stimulation protocol*	Principal outcomes
Metzger et al., 2012 (Germany) "Vagus somatosensory evoked potentials (VSEPs)"	taVNS, crossover RCT	12 AD, 12 MCI, 27 controls; mean ages 70 ± 6 y	Electrical pulses (0.1 ms, 8 mA, 0.5 Hz) to right or left tragus; EEG sampling 20 kHz	VSEP latencies lengthened progressively from controls to MCI to AD, supporting VSEP latency as a potential diagnostic biomarker.
(Murphy et al., 2023)(USA) "taVNS effects on semantic and hippocampal networks"	taVNS, sham-controlled RCT	50 females with MCI (mean 75 y)	Left tragus (20 Hz, 50 µs pulses) during resting-state fMRI; intensity 80 % of discomfort threshold (≤ 10 mA)	taVNS altered FC within temporal-parietal semantic network and between hippocampus and multiple cortical/subcortical regions, consistent with modulation of networks disrupted early in AD.
Merrill & Bunker, 2004 (USA)	Implanted cervical VNS, 1-year open-label pilot	15 AD	Parameters not reported	At 6 mo median ADAS-cog improved 3 points ($p = 0.011$); CSF-tau fell 7.7 % at 12 mo ($p = 0.003$). One-third showed ≥ 3-point ADAS-cog improvement.
Kaczmarczyk et al., 2017 (Germany) Murine APP/PS1 model	Non-invasive cervical VNS, sham-controlled	6- and 12-month APP/PS1 or WT mice	Two 2-min trains (1 ms bursts, 5 kHz, 25 Hz) at ~1.8 mA	In 12-mo APP/PS1 mice, VNS increased microglial branch number (+3.8 ± 0.9/40 min, $p < 0.001$), indicating a shift toward a neuroprotective phenotype; no effect in 6-mo mice.

Study & reference	VNS modality & design	Participants	Stimulation protocol*	Principal outcomes
Dolphin et al., 2023 (Ireland/UK)	taVNS, double-blind crossover	28 MCI (71 ± 8 y; 17 M/11 F)	Active: 21 ± 3 min at 2.5 ± 0.9 mA; sham: identical montage at 2.0 ± 0.7 mA	Active taVNS improved facial-name associative recall (69 % vs 45 % baseline, p = 0.016) and reduced spatial-navigation time (39 s vs 51 s baseline, p = 0.016). No benefit on SART.
O'Neal et al., 2023 (USA) "taVNS and hippocampal FC"	taVNS, sham-controlled experimental	50 MCI (25 active, 25 sham; 60–89 y)	Parameters not disclosed	taVNS increased left-hippocampal FC to prefrontal and cingulate regions and decreased FC to temporal cortex; right-hippocampal FC to left anterior temporal lobe was reduced, suggesting adaptive network rebalancing.
Merrill et al., 2006 (Sweden)	Implanted VNS, 1-y follow-up	17 AD	Parameters not reported	At 12 mo, 41 % showed no decline or improvement on ADAS-cog and 71 % on MMSE; 12/17 rated as stable or improved on CIBIC+.
Sjogren et al., 2002 (Netherlands)	Implanted VNS, 6-mo pilot	10 AD	Parameters not reported	Responders (≥ 0-point gain): 7/10 on ADAS-cog (median + 2.5) and 7/10 on MMSE (median + 2.5) at 6

Study & reference	VNS modality & design	Participants	Stimulation protocol*	Principal outcomes
				mo, indicating potential cognitive benefit.

*Only salient parameters are shown; see original reports for full protocols.

Abbreviations: *AD = Alzheimer's disease; ADAS-cog = Alzheimer's Disease Assessment Scale–Cognitive Subscale; AVLT-N7 = Auditory Verb Learning Test, delayed recall; CIBIC+ = Clinician's Interview-Based Impression of Change plus Caregiver Input; FC = functional connectivity; MoCA-B = Montreal Cognitive Assessment–Basic; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; RCT = randomised controlled trial; taVNS = transcutaneous auricular VNS; VSEPs = vagus-somatosensory evoked potentials; WT = wild type.*

Study geographical location and study designs

Two of the ten articles reviewed were conducted in China (Wang et al., 2023 et al., Wang, 2022);four in. Europe (Sjogren et al., 2002, Merrill et al., 2006, Kaczmarczyk et al., 2017, Metzger et al., 2012) ,one in the United kingdom(Dolphin et al., 2023); and three in the United States(O'Neal et al., 2023, Merrill and Bunker, 2004, Murphy et al., 2023). Nine studies were clinical, including four randomized controlled trials (RCTs), two experimental studies, and three longitudinal follow-ups. One study was preclinical and used a mouse model of Alzheimer's disease. Among the clinical studies, three focused on patients with Alzheimer's disease. while the other six involved participants with mild cognitive impairment (MCI). Notably, the majority of studies (7 out of 10) employed non-invasive vagus nerve stimulation techniques.

Stimulation Points and Protocols

Non-invasive vagal stimulation was primarily administered via the transauricular route (Wang et al., 2022, Wang et al., 2023) , with the exception of one study that employed trans-cervical stimulation (Kaczmarczyk et al., 2018). Invasive stimulation was done by surgically implanting electrodes in the neck, about 13 mm in front of the neck base and 2 mm to the side of the trachea. Stimulation settings varied by study, with frequencies from 0.5 Hz to 100 Hz and intensities between 0.6 mA and 4.5 mA, adjusted to each participant's tolerance.

Study outcomes

Non-invasive vagus nerve stimulation has been linked to improved functional connectivity in brain regions involved in cognitive processes(Moher et al., 2009, Wang et al., 2023); better immediate and delayed memory performance in individuals suffering from mild cognitive impairment (Wang et al., 2022) and significant microglial structural modification (Kaczmarczyk et al., 2018).

4:2 Objective 2: To assess behavioral effects of vagus nerve stimulation in a rat model of Alzheimer's disease.

Wistar rats were given 2.5 mg/kg/day of acrolein by stomach tube for eight weeks, followed by four weeks of transcutaneous auricular vagus nerve stimulation. Hippocampus changes related to Alzheimer's were examined using H&E staining. Spatial learning and memory were tested with the Morris Water Maze (MWM). The next section summarizes the main results.

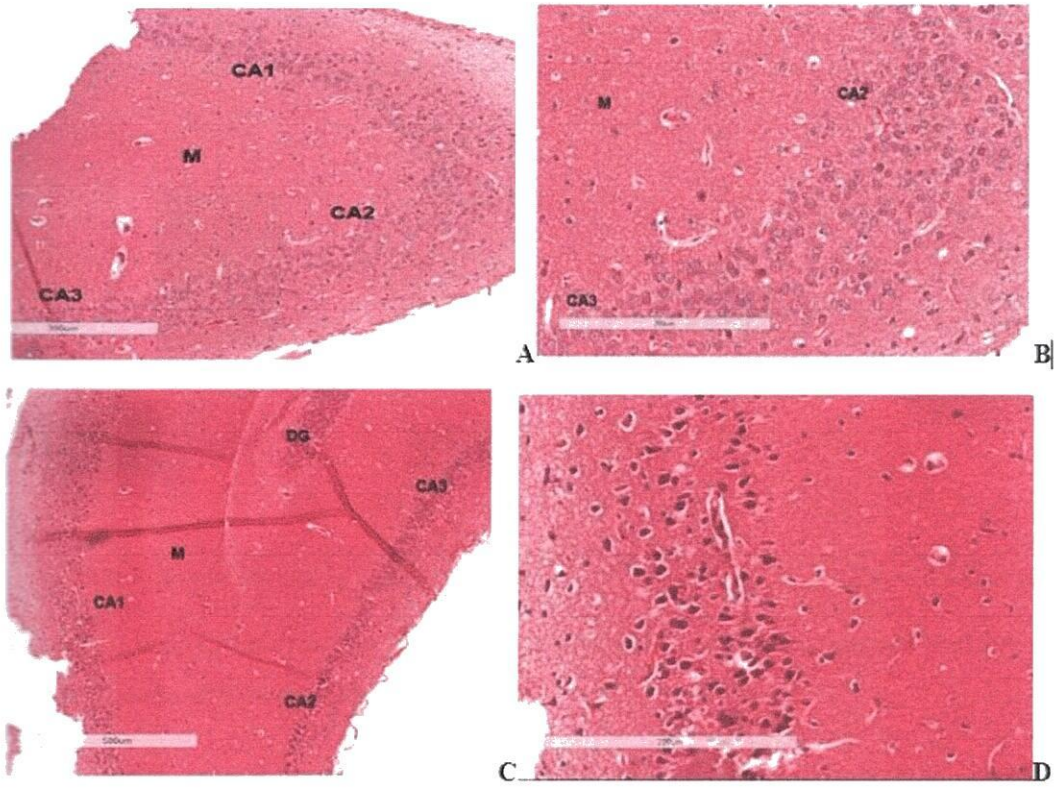

Figure 2: Effect of electrical stimulation on hippocampal expression of BACE 1 gene.

A. *Low-magnification hippocampal image from a non acrolein exposed rat , illustrating preserved neuronal architecture within the CA3, CA2, and CA1 regions. Scattered astrocytes are visible within the molecular layer (M).* ***B.*** *High-magnification view of the hippocampus*

from a control rat, depicting neuronal cell bodies in the CA3 and CA2 regions. The neurons exhibit vesicular chromatin with no evidence of apoptotic changes (Hematoxylin and Eosin stain, ×20). ***C.*** *Low-magnification image of the hippocampus from an acrolein-exposed rat, showing widespread neuronal apoptosis, most pronounced in the CA3 region and extending into CA2 and CA1. (DG: dentate gyrus; M: molecular layer).* ***D.*** *High-magnification image of the hippocampus from an acrolein-treated rat, highlighting pyknotic and apoptotic changes in neurons within the CA1 region. Features include chromatin condensation, cellular shrinkage, and pericellular retraction artifacts.*

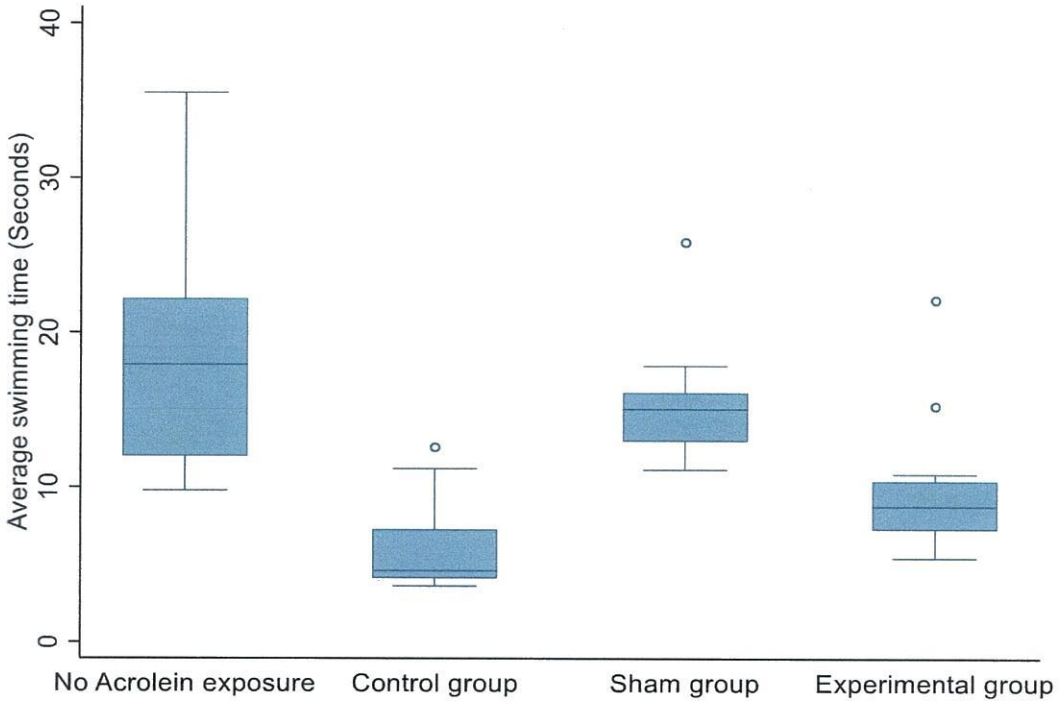

Figure 3: A box plot of average swimming time taken to find the escape platform in a MWM for the different study groups

Legend: *The control group (positive control) exhibited the lowest median swimming time of 4.6 seconds. In contrast, the group not exposed to acrolein (negative control) had the longest average time to find the escape platform, taking 18 seconds. This shows a clear difference between the groups, with the positive control group finding the platform much faster.*

Table 4: Escape latency times (seconds) across Experimental Groups During Morris Water Maze Trials

Trial No.	Negative Control (No Acrolein Exposure, $n = 6$)	Positive Control (Acrolein Only, $n = 6$)	Sham Group (Acrolein + Sham Stimulation, $n = 6$)	Experimental Group (Acrolein + taVNS, $n = 6$)
I	35.50	12.60	25.86	22.14
II	23.25	11.20	16.14	8.00
III	21.14	3.60	17.86	6.14
IV	23.50	4.40	15.43	10.00
V	18.00	4.60	12.14	15.29
VI	11.00	5.60	14.14	9.00
VII	17.83	4.60	15.86	9.00
VIII	13.00	4.20	11.14	8.57
IX	10.33	4.00	13.86	5.43
X	21.17	4.00	14.71	7.00
XI	16.00	7.20	16.14	10.86
XII	9.80	7.40	11.29	7.57
Mean	**18.38**	**6.12**	**15.38**	**9.92**
Median	**18.00**	**4.60**	**15.38**	**9.00**

Legend: *The positive control group demonstrated the shortest average and median swimming times to locate the escape platform, recorded at 6.12 seconds and 4.6 seconds, respectively, whereas the negative control group exhibited the longest times.*

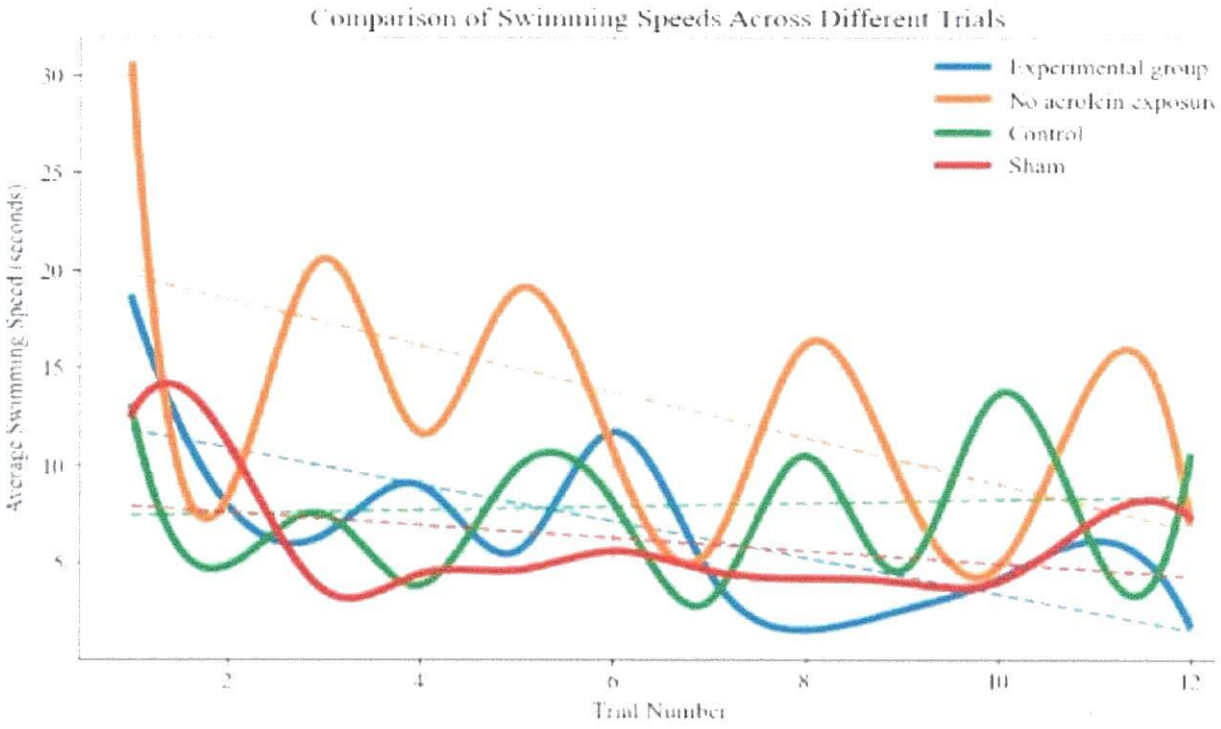

Figure 5: Line graph illustrating the trends in average swimming time across test trials for the different study groups

Legend: *According to the trend lines, the experimental group had a lower latency in detecting the hidden platform than the positive control group, indicating a divergence trajectory. The trend lines, on the other hand, showed a convergent pattern for the experimental and negative control (no acrolein treatment) groups, indicating comparable performance outcomes over time.*

Table 5: Post-Hoc Test Results for Mean Swimming Time Between Study Groups

Group Comparison	Mean Difference	Standard Error	p-value
No Acrolein Exposure vs Control	-12.26	2.03	< 0.001
No Acrolein Exposure vs Sham	-3.00	2.03	0.880
No Acrolein Exposure vs Experimental	-8.46	2.03	0.001
Control vs Sham	9.26	2.03	< 0.001
Control vs Experimental	3.80	2.03	0.405
Sham vs Experimental	-5.46	2.03	0.060

Legend : *A one-way ANOVA revealed a statistically significant difference in mean swimming time among the four study groups ($F = 14.64$, $p < 0.001$). Post hoc comparisons showed that*

the "no acrolein exposure" group had significantly longer swimming times than both the "control" group ($p < 0.001$) and the "experimental" group ($p = 0.001$), indicating improved spatial learning in the latter two groups. A significant difference was also observed between the "control" and "sham" groups ($p < 0.001$), suggesting impaired performance in the sham group. While the "experimental" group outperformed the "sham" group, this difference was not statistically significant ($p = 0.060$). No significant differences were found between the "no acrolein exposure" and "sham" groups ($p = 0.880$), or between the "control" and "experimental" groups ($p = 0.405$).

4:3 Objective 3: To assess structural, immunohistochemical, and molecular changes in the hippocampus following chronic vagus nerve stimulation in a rat model of Alzheimer's disease.

In this study, 2.5 mg/kg/day of acrolein was administered by a stomach tube every day for 8 weeks (56 days) to induce hippocampal Alzheimer's disease-like molecular , immunohistochemical and histomorphological features . Acrolein exposure was followed with trans-auricular vagus nerve electrical stimulation (taVNS) every day for 4 weeks (28 days) to analyze the effect of VNS on the acrolein induced hippocampal AD pathological features s. Each stimulation session lasted 1 hour, using 25 Hz frequency and 50-microsecond cathodic-leading pulses at 0.8 mA. The stimulation followed a 10% duty cycle: 30 seconds ON and 4.5 minutes OFF. The results of the histological and immunohistochemical analyses are presented below.

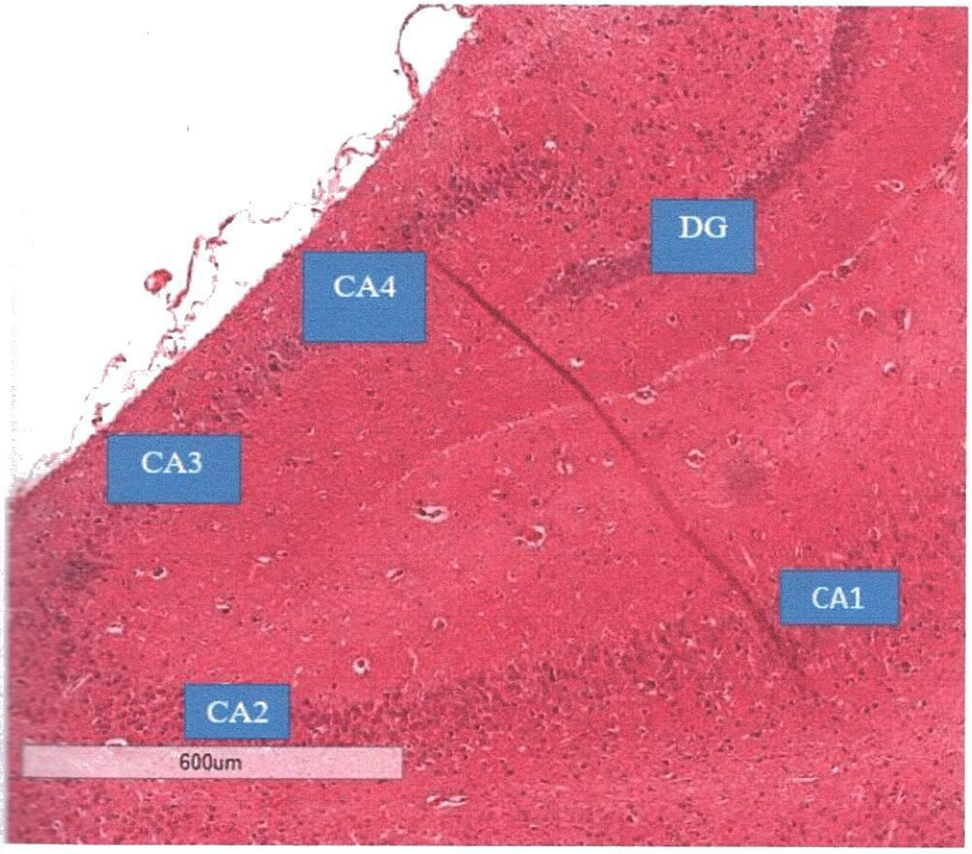

Figure 4: Low power histology section of H& E stained hippocampal images from a non-acrolein exposed rat showing normal CA1-CA4

No evidence of hippocampal neuronal damage.



Figure 5: Low power histology section of H& E stained hippocampal images from a acrolein exposed no vagus stimulation rat

. Shows neuronal damage in all regions of hippocampus.

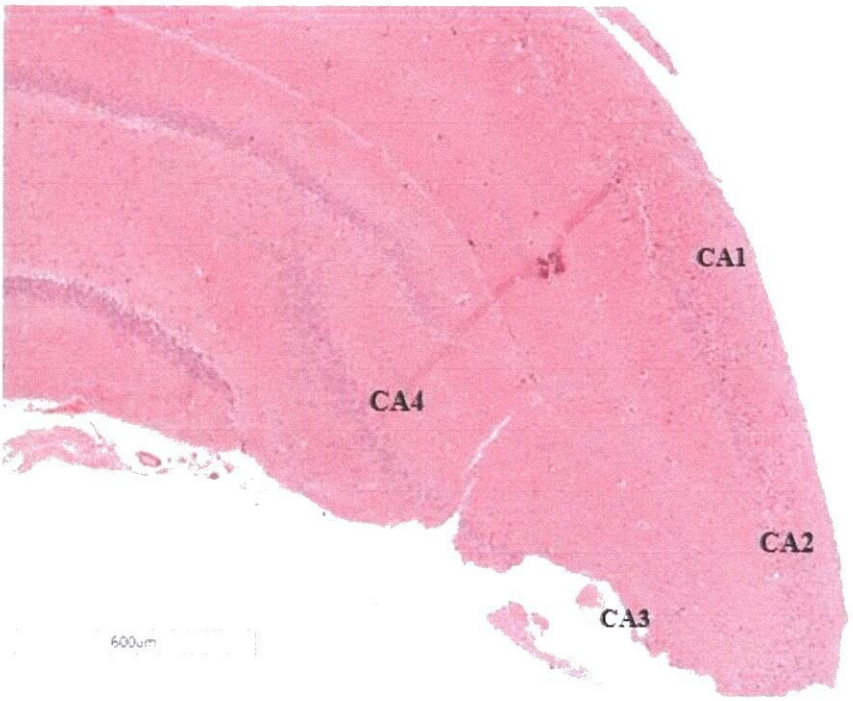

Figure 6: F-AC showing neuronal damage in CA2 and CA1

Note: *A section of the CA3 region was lost in one rat from the acrolein-exposed group that did not receive vagus nerve stimulation.*

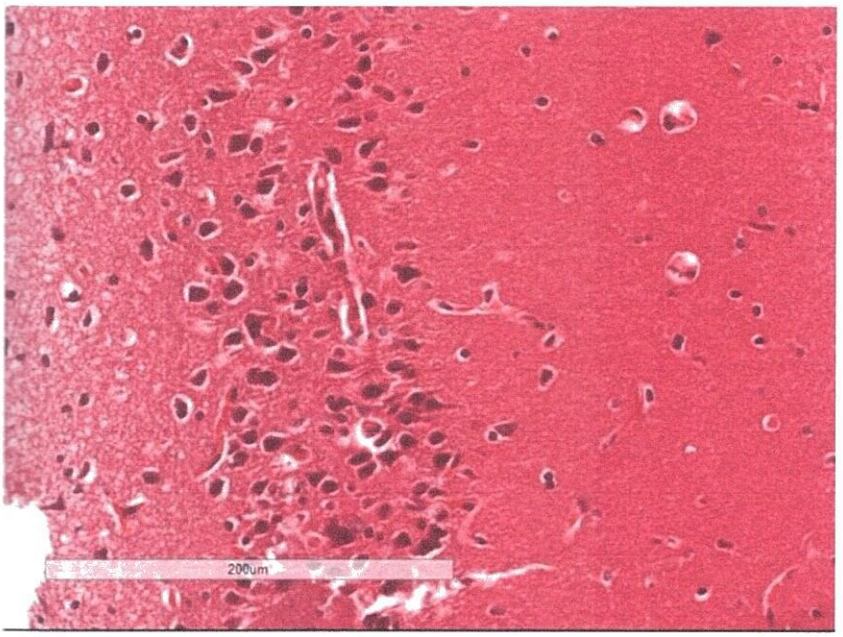

Figure 7: *Acrolein-exposed rat hippocampal high-magnification image exhibiting neuronal degeneration in the CA1 area. The affected neurons*

exhibit condensed chromatin and cellular shrinkage, along with retraction artifacts surrounding the cells.

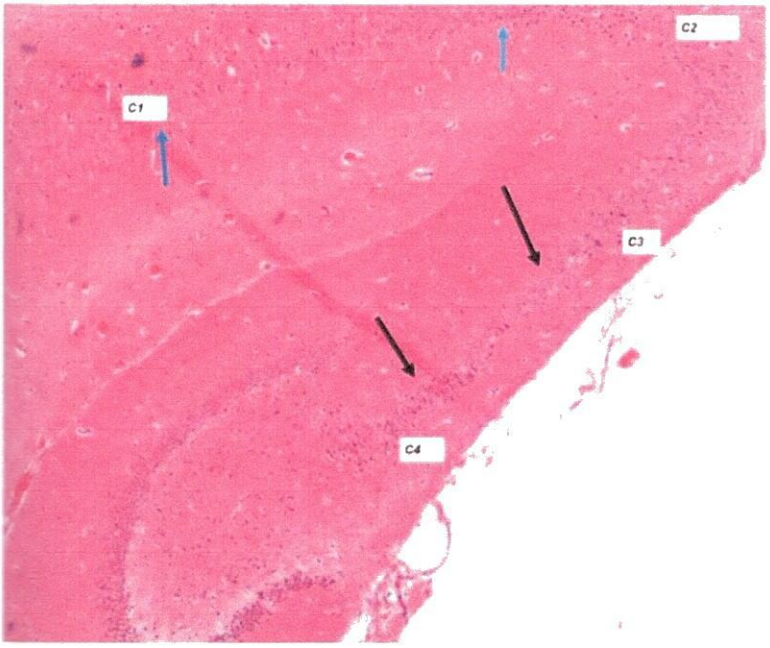

Figure 8: H&E sections of hippocampus of an acrolein exposed rat after vagus nerve stimulation showing minimal disruptions and thinning in regions CA4-CA3 due to neuronal loss (black arrows) and normal regions CA2-CA1 (blue arrows).

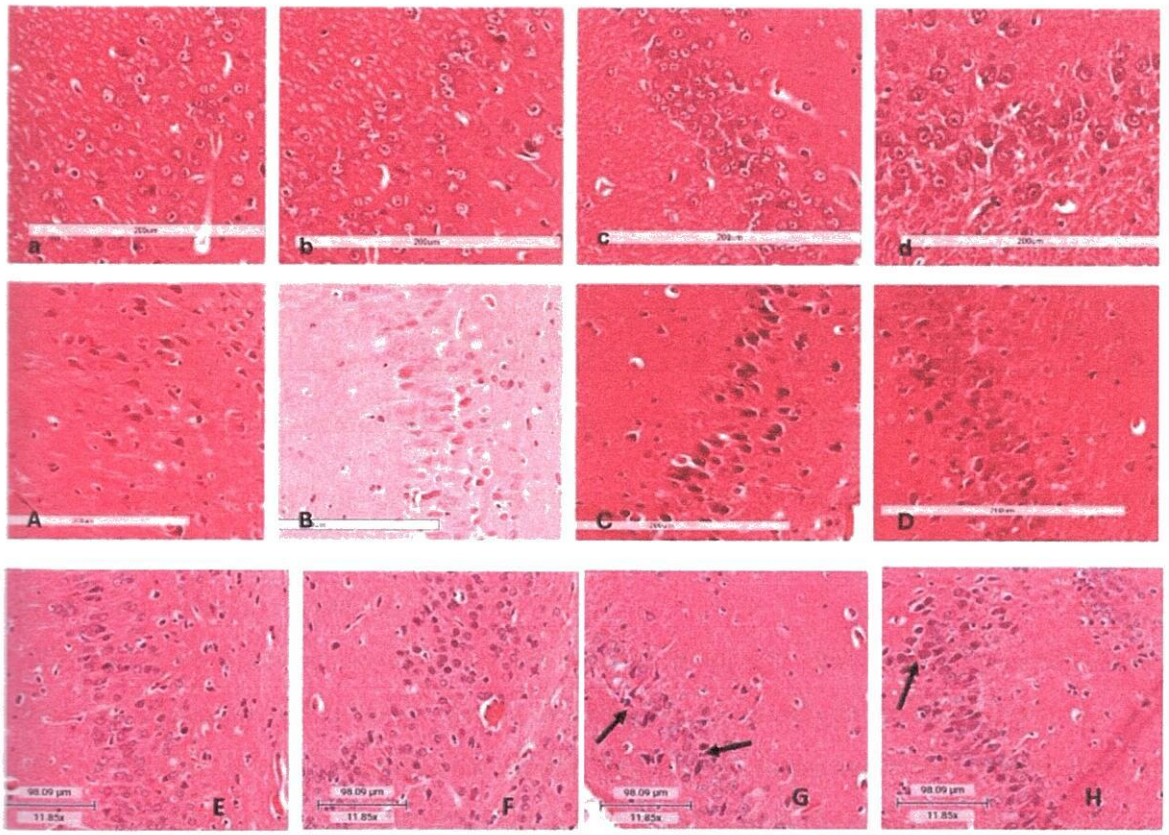

Figure 9: High-power hematoxylin and eosin (H&E) stained images of the hippocampus were used to compare neuronal morphology across different experimental groups.

Images A, B, C, and D (H&E, ×200) depict normal neurons within the CA1, CA2, CA3, and CA4 regions, respectively, in acrolein non-exposed rats, serving as the negative control group. Corresponding images labeled A, B, C, and D illustrate the same hippocampal sub-regions in acrolein-exposed rats prior to nerve stimulation (positive control group), demonstrating evidence of neuronal atrophy and damage. Following acrolein exposure combined with vagus nerve stimulation (VNS), hippocampal sections stained with H&E (images E through H, ×200) reveal differential neuronal loss: extensive neuron loss is evident in the CA3 (G) and CA4 (H) regions (indicated by arrows), whereas the CA1 (E) and CA2 (F) regions exhibit minimal neuronal loss.

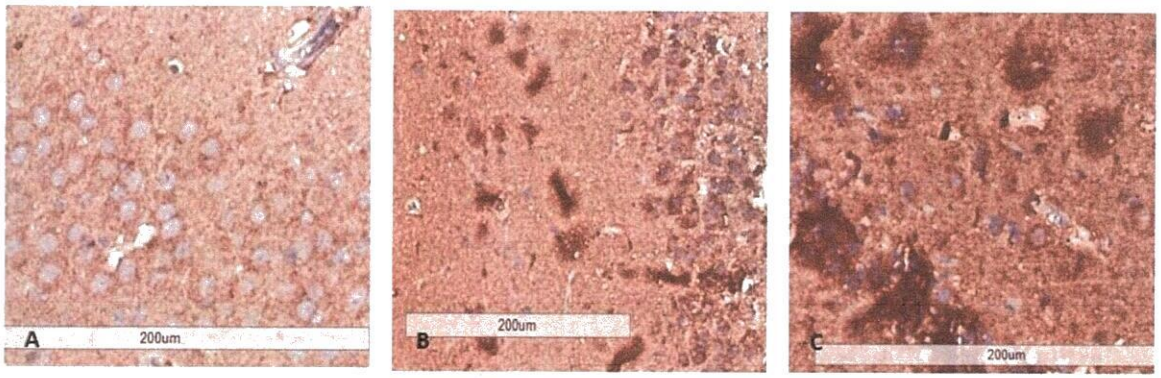

Figure 10:Histological sections of hippocampus stained with B-Amyloid.

Histology sections of the hippocampus stained for β-amyloid reveal distinct differences across experimental groups. A: The control rat shows negative staining (grade 0) for β-amyloid in both the molecular layer and CA4 regions.

B: Following exposure to acrolein, the rat exhibits moderate (grade 2) β-amyloid plaque deposition, characterized by golden-brown staining predominantly in the molecular layer. C: In the rat exposed to acrolein and subsequently subjected to vagus nerve stimulation, there is a strong staining intensity of β-amyloid plaques observed in both the molecular layer and CA4 region.

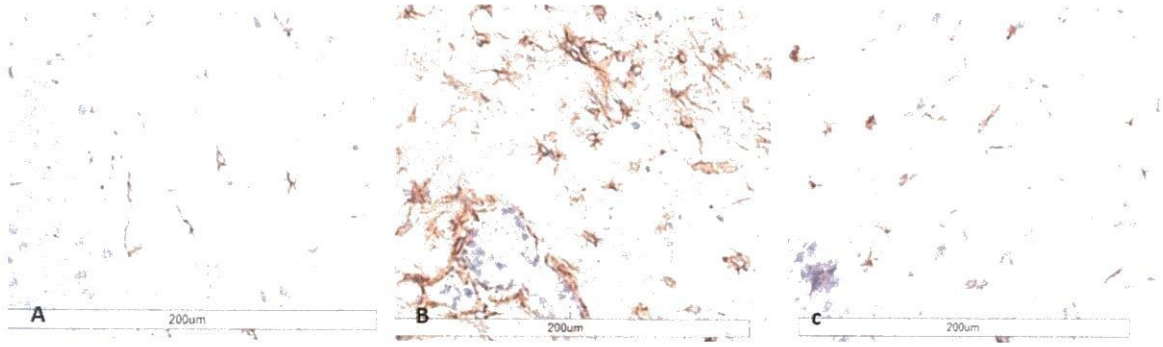

Figure 11: Histological sections of the Hippocampus stained with Glial fibrillary acidic protein (GFAP).

A: Histological section from a control rat showing normally dispersed astrocytes with weak GFAP staining of their processes. **B:** Section from a rat exposed to acrolein, demonstrating intense gliosis characterized by increased density of astrocytes and markedly stronger GFAP staining. **C:** 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.

Table 6:Differences between the mean expression for SRPX2 and BACE1 genes acrolein exposed (positive control) and acrolein non exposed (Negative control) animals

Genes	Total gene expression (n=13)	Groups		*p*-value
		Acrolein exposed (n=7)	Non-acrolein exposed (n=6)	
SRPX2	0.30 ± 0.32	0.50 ± 0.36	0.12 ± 0.07	**0.017**
BACE-1	8.47 ± 7.76	11.02 ± 6.74	5.93 ± 8.37	0.234

The mean hippocampal expression of the SRPX2 gene was significantly higher in the acrolein-exposed rats compared to the non-exposed group (0.50 ± 0.36 vs. 0.12 ± 0.07; p = 0.017). Although the mean expression of the BACE1 gene was also elevated in the acrolein-exposed rats relative to the non-exposed group (11.02 ± 6.74 vs. 5.93 ± 8.37), this difference did not reach statistical significance (p = 0.234).

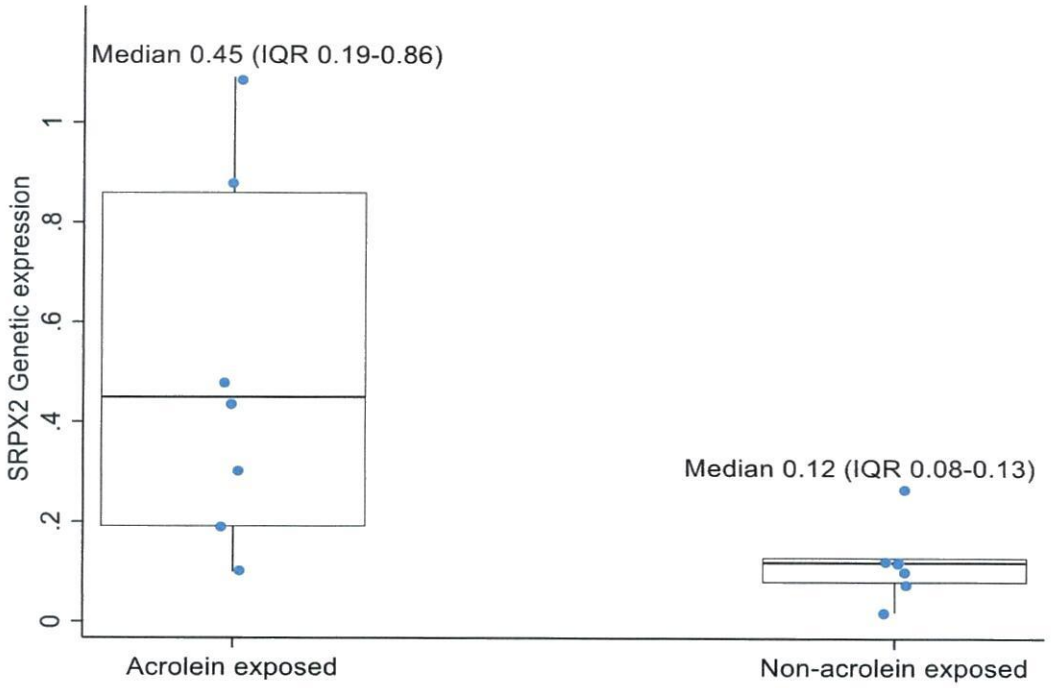

Figure 12: Expression of SRPX2 .

A box plot illustrating SRPX2 gene expression levels in acrolein-exposed and non-exposed rat groups, sacrificed immediately after 8 weeks (56 days) of exposure, revealed a marked difference between the two groups. The median expression level among acrolein-exposed rats was 0.45 (interquartile range [IQR]: 0.19–0.86), compared to 0.12 (IQR: 0.08–0.13) in the non-exposed group.

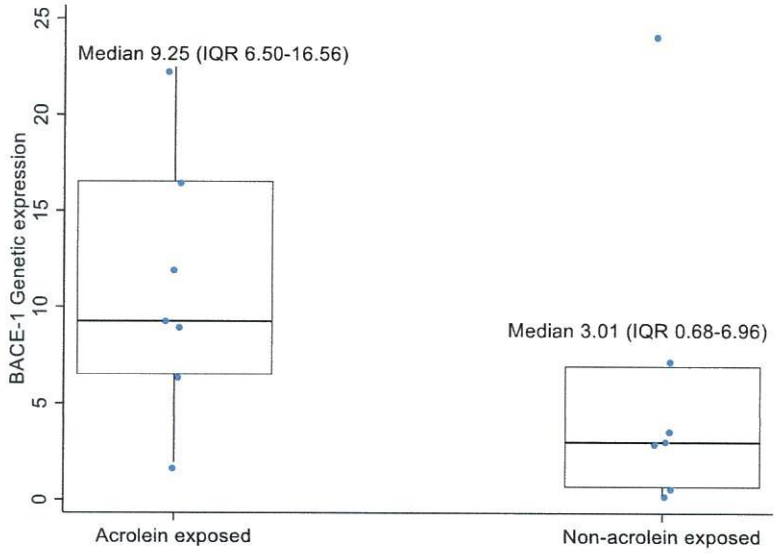

Figure 13: Expression of BACE-1 in hippocampus

A boxplot showing expression of BACE-1 gene in non-acrolein exposed and acrolein exposed rat groups immediately following the 8 weeks of acrolein exposure. Median expression among the acrolein exposed rats was 9.25 (IQR: 6.50, 16.56), whereas that for non-exposed was 3.01 (IQR: 0.68, 6.96).

Table 7: Differences between mean expression of SRPX2 and BACE1 genes among the acrolein exposed rats that experienced different nerve stimulation

Genes	Total gene expression (n=19)	Study groups			*p*-value
		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**
BACE-1	6.72 ± 7.00	7.20 ± 6.81	3.82 ± 2.69	9.05 ± 9.82	0.446

The expression of the SRPX2 gene was significantly reduced in the experimental group (acrolein exposure followed by vagus nerve stimulation) compared to both the control group (acrolein exposure without electrical stimulation) and the sham group (acrolein exposure with greater auricular nerve stimulation). In contrast, BACE1 gene expression did not differ significantly across the groups, indicating that electrical stimulation had no substantial effect on its expression.

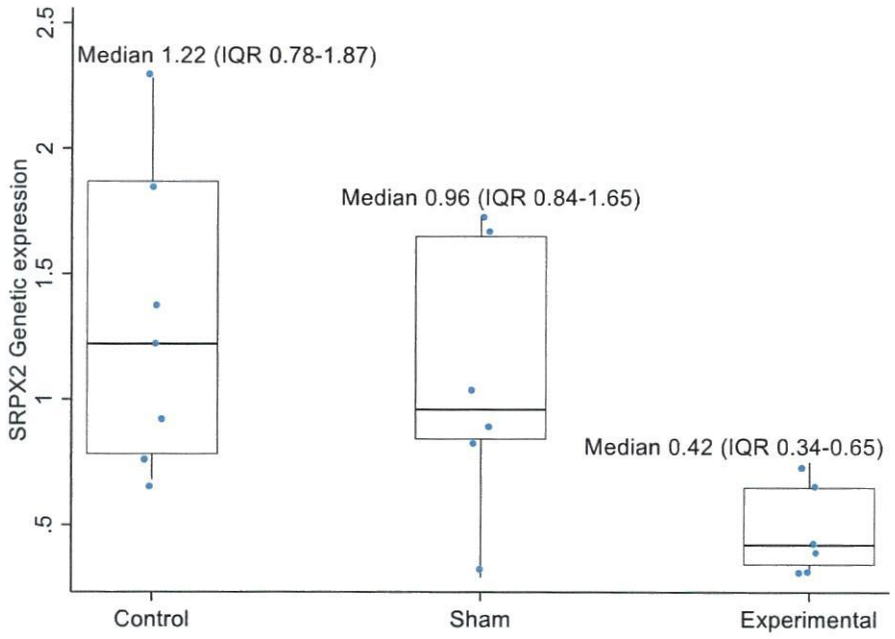

Figure 14: Effect of electrical stimulation on expression of hippocampal SRPX2 gene.

A boxplot Showing effect of trans-auricular electrical stimulation of the greater auricular nerve (sham) and vagus nerve (experimental), for 4 weeks following acrolein exposure, on the hippocampal expression of SRPX2 gene : Median for control: 1.22(.78, 1.87); Median for Sham: .96 (.84, 1.65); Median for Experimental: .42 (.34, .65)

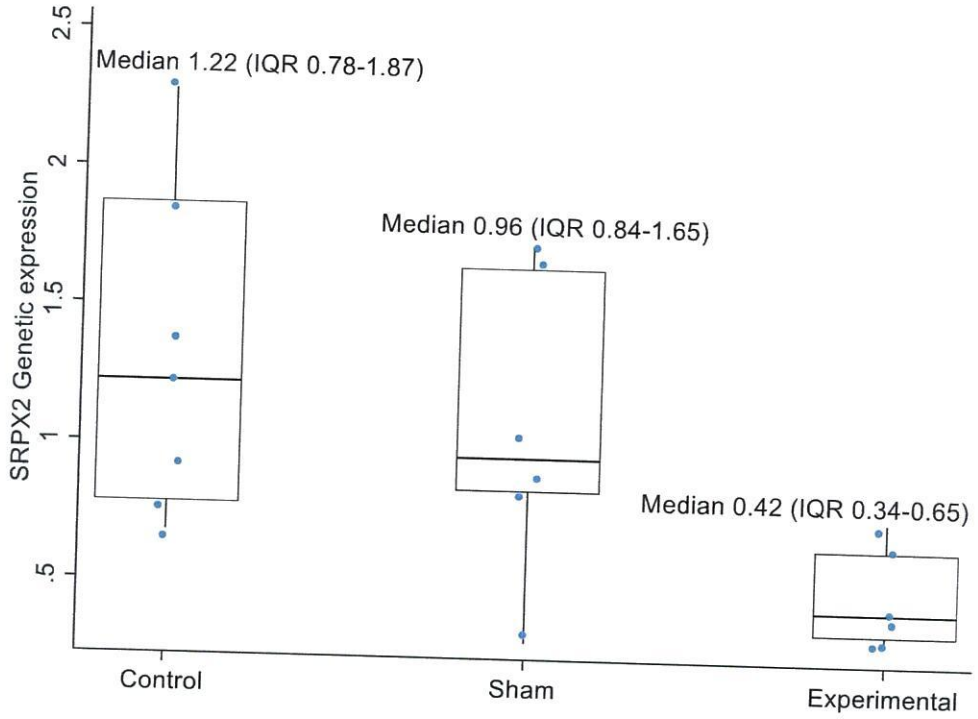

Figure 15: Effect of electrical stimulation on hippocampal expression of SRPX2 gene.

A boxplot illustrating the hippocampal expression levels of the SRPX2 gene following four weeks of post-acrolein exposure electrical stimulation. The intervention included trans-auricular stimulation of the greater auricular nerve (sham group) and the vagus nerve (experimental group). Median BACE1 expression levels were as follows: control group – 4.76 (IQR: 2.25–10.56); sham group – 3.04 (IQR: 2.46–3.39); and experimental group – 4.38 (IQR: 2.58–15.88).

Table 8: Post-hoc analysis of SRPX2 expression across the study groups

Study Group Pairings	Average variation	95% Confidence interval	Standard error	Adjusted *p*-value
Control – Sham	-0.23	-0.80 – 0.33	0.27	1.000
Control Experimental	-0.82	-1.38 – -0.25	0.27	**0.022**
Sham – Experimental	-0.59	-1.18 – -0.0002	0.28	0.150

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

CHAPTER 5: DISCUSSION

This study explored the potential of vagus nerve stimulation (VNS) as a therapeutic intervention for Alzheimer's disease (AD) and related dementias through a multipronged approach: a systematic scoping review of existing literature and an in vivo experimental analysis using an acrolein-induced rat model of AD. The findings offer a nuanced perspective on both the translational promise and biological limitations of VNS, particularly in relation to cognition, neuroinflammatory markers, and neuropathology. In the following sections, the findings are discussed under each study objective, Considering the existing literature, and with reference to methodological considerations and directions for future research.

5.1 Objective 1: **To review existing literature on vagus nerve stimulation in Alzheimer's disease and related dementias.**

The scoping review highlighted the worldwide spread and clinical emphasis of vagus nerve stimulation (VNS) research in dementia. Notably, all the studies identified were carried out in high-income countries such as the United States, the United Kingdom, China, and various European nations, with no research conducted in low- and middle-income countries (LMICs) (Mattap et al., 2022 , Schaller et al. 2015). This geographic skew likely reflects limited access to VNS devices and surgical expertise, low public health prioritization of dementia, and culturally embedded misattributions of symptoms to aging or supernatural beliefs in LMICs(Kamoga et al., 2019). Moreover, even though dementia prevalence is growing rapidly in these regions, the infrastructure for diagnosis and intervention remains underdeveloped(Hojman et al., 2017) . This trend underscores a need to address research equity and technological accessibility globally.

The review also found that transcutaneous VNS (tVNS)—a non-invasive alternative to invasive VNS (iVNS)—was used in 70% of included studies. Unlike iVNS, which involves the surgical implantation of electrodes near the cervical vagus nerve, tVNS delivers stimulation through surface electrodes placed over the auricular or cervical skin, thereby significantly reducing procedural risks(Vargas-Caballero et al., 2022). Indeed, iVNS is associated with complications in 4–30% of cases, including hematomas, infections, vocal cord paralysis, stimulator malfunction, and device failure (Vonck et al., 2014, RévészRydenhag and Ben-Menachem, 2016) . While both forms of stimulation have been shown to influence brain connectivity, cognitive function, and neuroinflammation, some physiological and evoked-response

differences suggest that iVNS might more robustly affect central autonomic circuits due to its proximity to deep vagal fibers(Ventura-Bort et al., 2021, Warren et al., 2019).

Cognitive improvements reported in reviewed studies included enhanced memory consolidation, executive function, and associative memory in patients with mild cognitive impairment(Dolphin et al., 2023, Murphy et al., 2023). Similarly, decreased levels of tau protein and increased microglial reactivity from pro-inflammatory to neuroprotective phenotypes were reported in animal models and human subjects(Merrill et al., 2006, Wang et al., 2022a). Mechanistic investigations attributed these benefits to VNS-mediated release of norepinephrine (NE), brain-derived neurotrophic factor (BDNF), and fibroblast growth factor-1 (FGF-1) in the hippocampus, amygdala, and cortex(Follesa et al., 2007, Rosso et al., 2020). These molecules facilitate long-term potentiation (LTP), reduce glial inflammation, and support synaptic plasticity---key factors in memory and learning(Vonck et al., 2014, Heneka et al., 2024) .

Nevertheless, findings across studies were inconsistent. While some reported marked cognitive and structural benefits of VNS, others failed to detect any significant outcomes or observed worsening symptoms (HelmstaedterHoppe and Elger, 2001, Mertens et al., 2020). This inconsistency could be attributed to differences in stimulation parameters (e.g., duration, frequency, current intensity), disease severity, assessment methods, and species used in preclinical models(ZuoSmith and Jensen, 2007, Olsen et al., 2023). These variables warrant standardized protocols in future clinical trials and experimental investigations.

5.2 Objective 2: To Determine Behavioral Changes Associated with Vagus Nerve Stimulation in a Rat Model of Alzheimer's Disease

This objective focused on assessing spatial learning and memory changes following transauricular VNS in acrolein-exposed Wistar rats using the Morris water maze (MWM). Contrary to expectations, the results showed that VNS at 0.8 mA did not yield statistically significant improvements in spatial memory. Unexpectedly, acrolein-exposed rats performed significantly better than non-exposed controls, spending less time locating the hidden platform.

This paradox highlights a central question in dementia research: is hippocampal neuronal loss directly proportional to behavioral decline? Prior studies suggest that cognitive impairments

may not linearly correlate with neuronal damage, and compensatory mechanisms—especially in early disease stages—may transiently mask behavioral deficits (Rapp & Gallagher, 1996 , Rasmussen et al., 1996) . For instance, exposure to acrolein, a reactive aldehyde, induces oxidative stress, which in turn elevates BDNF expression, particularly in hippocampal glial cells (Huang et al., 2013
, Miranda et al., 2019)
. BDNF interacts with TrkB and p75 neurotrophin receptors to support dendritic growth and synaptic remodeling, potentially accounting for the preserved or even enhanced cognitive performance observed in the acrolein-exposed group(Ming and Song, 2011, Lynch et al., 2007);.

Furthermore, the inverted U-shaped dose-response curve of VNS efficacy must be considered. Zuo et al.,(2007) and Broncel et al. (2020) demonstrated that memory enhancement occurs at moderate stimulation intensities (approximately 0.4 mA), while higher (e.g., 0.8 mA) or lower current amplitudes are less effective or inhibitory. In this study, cognitive testing was performed after VNS administration, whereas prior studies showing benefit often synchronized stimulation with task performance—enhancing hippocampal NE and BDNF availability precisely when most needed(Sanders et al., 2019, Engineer et al., 2015).

Biological variation in animal species, sex, hormonal status, and age may also influence behavioral performance (D'Hooge and De Deyn, 2001, Roof et al., 1993) However, given the controlled age, species, and environment of the study animals, these factors likely had minimal impact. The discrepancy may therefore lie in timing, stimulation parameters, and the severity of neural damage. In APP/PS1 mouse models with less severe neural disruption, Dolphin et al. (Dolphin et al., 2023)reported significant gains in navigation and associative memory following higher-amplitude tVNS, highlighting the need to tailor intervention to disease stage.

These findings reaffirm the importance of defining a neuronal loss threshold at which spatial memory becomes functionally impaired, and suggest that behavioral resilience may persist in the presence of neuropathology due to endogenous repair mechanisms or functional reorganization.

5.3 Objective 3: To Evaluate Morphological, Immunohistochemical, and Molecular Changes Associated with VNS in the Hippocampus

At the molecular level, this study observed a non-significant increase in BACE-1 gene expression and amyloid-beta (Aβ) plaque load following acrolein exposure. These outcomes are supported by earlier studies demonstrating acrolein-induced oxidative stress, amyloidogenesis, and neuronal death (Singh et al., 2010, Huang et al., 2013, Bradley et al., 2010)

; Notably, VNS did not significantly reduce BACE-1 or Aβ levels—an outcome that diverges from studies like Yu et al., (2023) , which reported reduced Aβ42 levels and improved cognition in APP/PS1 mice following 1.8 mA, 40 Hz stimulation. The current study's use of 0.8 mA and 25 Hz likely reflects insufficient current density for engaging amyloid-processing mechanisms(Bok et al., 2022).

Nevertheless, a significant downregulation of SRPX2 gene expression was observed in VNS-treated, acrolein-exposed rats. SRPX2 encodes a peroxiredoxin protein expressed in injured neurons to inhibit complement-mediated synaptic degradation(Cong et al., 2020). Its suppression may imply that fewer neurons were under stress, or alternatively, that VNS directly modulated immune signaling to lower the need for SRPX2-mediated protection. This interpretation is supported by VNS-induced elevations in NE and neurotrophins, which suppress pro-inflammatory cytokine production (e.g., IL-1β, TNF-α) in glial cells and improve neuronal survival(Heneka et al., 2010, Rosas-Ballina et al., 2009).

Additionally, VNS significantly reduced reactive astrogliosis, evidenced by decreased GFAP expression and cell hypertrophy. Astrocyte activation is a well-documented hallmark of chronic neuroinflammation in AD, often preceding overt neuronal loss.

5.5 Limitations and Future Research

This study employed the acrolein-induced Alzheimer's disease (AD) model(Singh et al., 2010, Bradley et al., 2010 , Gómez-Ramos et al., 2003, Zhu et al., 2022) which, while useful in mimicking key pathological elements such as hippocampal neuronal loss and beta-amyloid plaque formation(Miranda et al., 2019 , Sze et al., 1997) ; , does not fully recapitulate the progressive and multifactorial nature of human AD, including tauopathy, age-related decline,

and cortical atrophy. Thus, caution should be exercised when generalizing these results to the broader human disease.

Assessment of amyloid load and astrogliosis was conducted via manual histological staining intensity—a semi-quantitative method subject to inter-observer variability. While evaluation by three independent and experienced histopathologists enhanced reliability, future studies should prioritize quantitative methods, including image analysis software, immunofluorescence quantification, and stereological techniques, to enhance accuracy and reproducibility.

Furthermore, although VNS significantly reduced astrocyte activation in the hippocampus, the study did not distinguish between neurotoxic A1 and neuroprotective A2 reactive astrocyte subtypes. Given the opposing roles of these phenotypes in neurodegeneration and tissue repair, future investigations should incorporate molecular markers (e.g., C3 for A1, S100A10 or BDNF for A2) and transcriptomic approaches to characterize astrocytic heterogeneity and their responses to VNS.

Finally, the stimulation protocol—limited to 25 Hz and 0.8 mA over a four-week period—may not have been sufficient to induce long-term or robust neuroplastic effects. Longer-duration studies that systematically vary stimulation parameters, align VNS with cognitive tasks, and apply VNS at different disease stages may yield more definitive insights into its therapeutic potential.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

This thesis sought to explore the existing research landscape on vagus nerve stimulation in Alzheimer's disease and related dementias, and to experimentally evaluate the behavioral, molecular, and histological effects of VNS in a rat model of acrolein-induced neurodegeneration.

The literature review revealed that virtually all studies on VNS in dementia were conducted in high-income countries and primarily on human subjects. These studies consistently reported VNS-associated improvements in cognitive function, neural connectivity, and neuroimmune modulation, underscoring its promise as an adjunctive therapy for dementive illnesses. However, socio-geographic disparities in research investment and technological accessibility point to a broader need for equitable global engagement in dementia research.

In the experimental phase, transcutaneous auricular VNS did not produce significant improvements in spatial learning and memory in Wistar rats with acrolein-induced hippocampal neuronal loss. Notably , acrolein exposure alone did not impair spatial learning—in fact, it paradoxically improved performance—suggesting that hippocampal neuronal loss may not be linearly related to cognitive decline. These findings challenge long-held assumptions in dementia pathophysiology and highlight the importance of reevaluating hippocampal-centric models of memory loss.

At the molecular and histological levels, VNS at 25 Hz and 0.8 mA failed to significantly alter BACE-1 gene expression or beta-amyloid plaque density, suggesting that VNS-related benefits may operate independently of amyloidogenic pathways or may require different stimulation intensities. Importantly, VNS downregulated SRPX2 gene expression—typically upregulated in neuronal injury—and minimized hippocampal neurodegeneration, indicating potential neuroprotective effects. Furthermore, VNS significantly reduced astrocyte activation, suggesting anti-inflammatory properties. However, the inability to distinguish between astrocyte subtypes limited mechanistic interpretation. In terms of knowledge, this study showed that low-intensity vagus nerve stimulation did not significantly alter amyloid pathology but reduced SRPX2 expression and reactive astrogliosis in acrolein-exposed rats. These findings suggest that VNS may exert its benefits primarily through modulation of neuroinflammatory and glial pathways rather than direct effects on amyloid processing.

Recommendations and translational implications

- **Optimize stimulation parameters.** Future studies should systematically evaluate longer stimulation durations, a range of frequencies and intensities, and protocols that temporally pair VNS with cognitive training tasks to maximize neuromodulatory and behavioural efficacy.
- **Characterize glial phenotypes.** Prioritize detailed profiling of glial responses—especially astrocyte subtypes and microglial activation states—using immunohistochemistry, single-cell transcriptomics, and spatial transcriptomics to clarify how VNS modulates neuroinflammatory pathways.
- **Use comprehensive models and quantitative imaging.** Employ diverse and clinically relevant animal models (e.g., transgenic and toxin-induced AD models) alongside longitudinal, quantitative imaging (SV2A PET, TSPO PET, high-resolution MRI, and small-animal functional imaging) to map VNS-mediated neural remodeling across scales.
- **Promote global collaboration and capacity building.** Support international partnerships, technology transfer, and local training to ensure geographically diverse participation in neuromodulation research and to increase the generalizability of findings across low- and middle-income settings.

Taken together, these findings add to the accumulating evidence that vagus nerve stimulation is a promising neuromodulatory approach for Alzheimer's disease. Although key challenges remain—particularly regarding mechanism elucidation and parameter optimization—this study provides an important foundation for longitudinal, mechanistically informed, and translationally focused research that may ultimately support clinical implementation.

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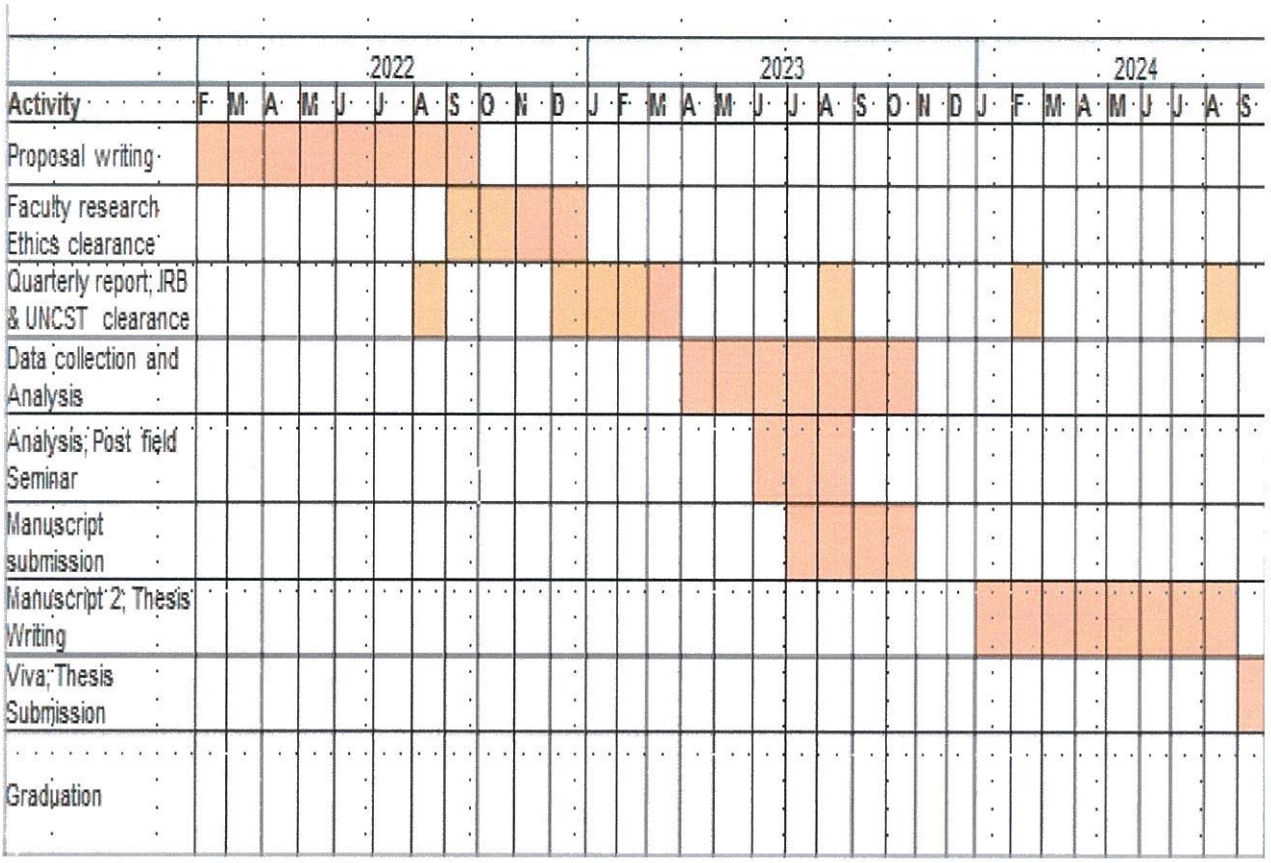
Appendices

Appendix 1: Work plan



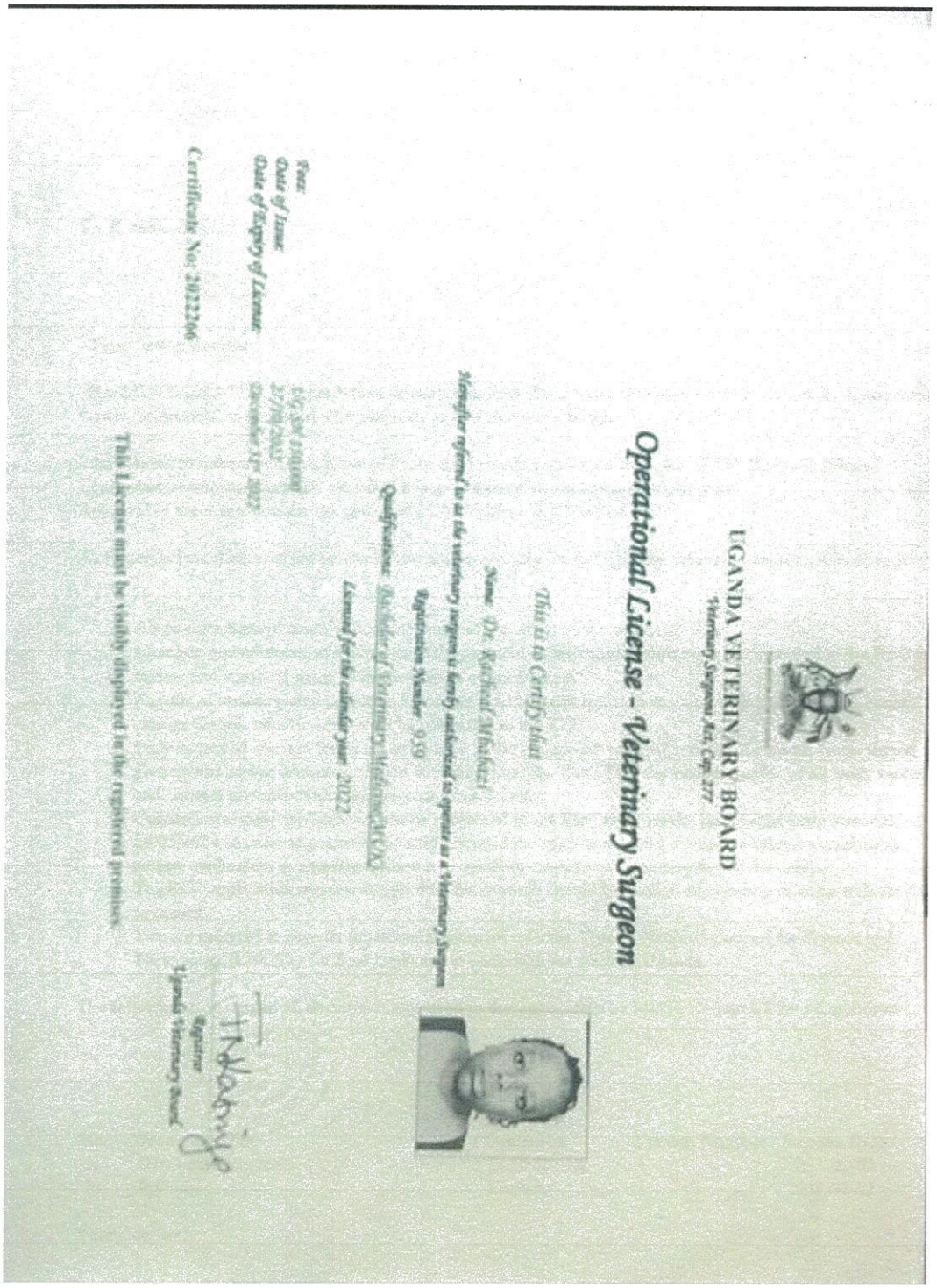
Appendix 2: Data collection form/questionnaire

Variable	Specimen Number (Sham or Control group or Treatment group)		
	Sham (S01-S015)	Control (S01-S015)	Treatment (S01-SO15)
Rat age			
Rat Gender/sex			
Rat Body weight			
Site of VNS			
Stimulation parameters			
Current strength (mA)			
Current duration (ms)			
Frequency (Hz)			
Duty cycle (s)			
Date and Time of stimulation			
Pulse rate			
Blood pressure			
Outcomes of stimulation			
Behavioral outcomes of stimulation			
Gross anatomical changes			
Histological changes observed			

Immunohistochemical changes recorded			
RT –PCR outcome/SRPX2 and BACE1 concentrations			

Appendix 3: Practicing license for supervising veterinary surgeon

(Dr.Racheal Mbabazi-Tel. +256775841382).



UGANDA VETERINARY BOARD

Veterinary Surgeons Act, Cap. 277

Operational License - Veterinary Surgeon

This is to Certify that

Name: Dr. Racheal Mbabazi

Hereafter referred to as the veterinary surgeon is hereby authorised to operate as a Veterinary Surgeon

Registration Number: 959

Qualifications: Bachelor of Veterinary Medicine(BVM)

Licensed for the calendar year: 2022

Fees:

Date of Issue:

Date of Expiry of License:

Certificate No: 2022206

This License must be visibly displayed in the registered premises

Registrar

Uganda Veterinary Board

Appendix 4: Approval letters

4:1 MUST Research Ethics Committee approval letter



MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY

RESEARCH ETHICS COMMITTEE

16/03/2023

To: Kamoga Ronald

Type: Initial Review

Re: MUST-2023-791: "Vagus Nerve Stimulation in A Rat Model of Alzheimer's Disease Like Symptoms: Gross, Immunohistochemical, Histological, and Molecular Changes.", 1, 2023-02-02

I am pleased to inform you that at the 153 convened meeting on 03/03/2023, the MUST Research Ethics Committee, committee meeting, etc voted to approve the above referenced application.
Approval of the research is for the period of 16/03/2023 to 16/03/2024.

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight** weeks prior to the expiration date of **16/03/2024** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by MUST Research Ethics Committee:

No.	Document Title	Language	Version Number	Version Date
1	Data collection tools	English	1	2023-02-02
2	Protocol	English	1	2023-02-02

Yours Sincerely

Dr. Paul Alele
For: MUST Research Ethics Committee

4:2 MUST VC administrative clearance letter

MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
Plot 8-18, Kabale Road, P.O. Box 1410, Mbarara, Uganda.
Telephone: +256-4856-60208, Fax: 256-4854-20782 TIN: 1000397456
Office of the Vice Chancellor

Your Ref:

Our Ref: MUST13/7

February 5, 2024

The Executive secretary
Uganda National Council for Science and Technology

Dear Sir,

RE: PERMISSION TO CONDUCT RESEARCH AT MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY (MUST)

Warm greetings to you.

The purpose of this letter is to kindly inform you that Dr.Kamoga Ronald (MBChB; MSc) is a registered PhD student at Mbarara University of Science and Technology **(Reg. No.: 2021/PHD/041/PS)**.

He intends to carry out his research project at the animal research facility of MUST. He has my permission to access the MUST animal research facility to carry out his PhD research activities.

Any assistance rendered to him by UNCST is highly appreciated.

Yours Sincerely,

Professor Celestino Obua
VICE CHANCELLOR

4: 3 Approval letter from UNCST

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS3781ES **26 February 2024**

Kamoga Ronald
Mbarara University of Science and Technology
Mbarara

Re: Research Approval: **VAGUS NERVE STIMULATION IN A RAT MODEL OF ALZHEIMER'S DISEASE-LIKE SYMPTOMS: MORPHOLOGICAL, IMMUNOHISTOCHEMICAL, MOLECULAR AND BEHAVIORAL CHANGES**

I am pleased to inform you that on **26/02/2024**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **26/02/2024** to **26/02/2025**.

Your research registration number with the UNCST is **HS3781ES**. Please, cite this number in all your future correspondences with UNCST in respect of the above research project. As the Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. Keeping all co-investigators informed of the status of the research.
2. Submitting all changes, amendments, and addenda to the research protocol or the consent form (where applicable) to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local REC for review with copies to the National Drug Authority and a notification to the UNCST.
4. Unanticipated problems involving risks to research participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST notification after review by the REC.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

Please note that this approval includes all study related tools submitted as part of the application as shown below:

No.	Document Title	Language	Version Number	Version Date
1	Data collection tool	English	1	08 February 2023
2	Project Proposal	English	2	
3	Approval Letter	English		
4	Administrative Clearance	English		

Yours sincerely,

Hellen Opolot
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

4:4 Faculty Research Committee approval letter.

MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
FACULTY OF MEDICINE RESEARCH COMMITTEE

Date 27th January, 2023

Kamoga Ronald
2021/PhD/041/PS
Department of Anatomy
MUST

Dear Kamoga
Submitted Proposal: "***Vagus Nerve Stimulation in A Rat Model of Alzheimer's Disease Like Symptoms: Gross, Immunohistochemical, Histological and Molecular Changes.***"

Reference is made to the above protocol you re-submitted to the Faculty Research Committee (FRC) for review.

The Committee reviewed and approved your proposal.

You are now advised to proceed to produce enough copies of the Protocol Form and Research Proposal for eventual submission to the Institutional Research Ethics Committee. The Secretary to that Committee will advise you on the number of copies you are to submit.

Please remember to attach a letter from the Head of Department or your supervisor when submitting.

Sincerely

Dr. Conrad Muzoora
Chairperson, Faculty Research Committee

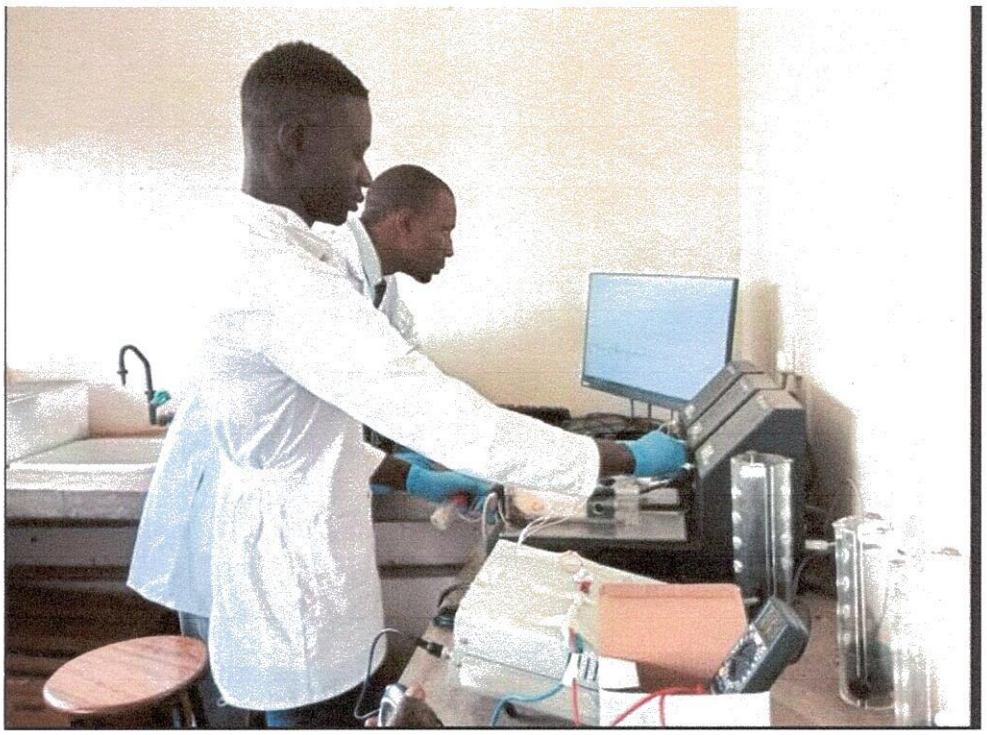
Copy: Prof.Amadi Ogonda Ihunwo, Dr Godfrey Rukundo and Dr.Johnes Obongoloch
Supervisors

Appendix 5: Images related to data collection

Appendix 5: 1 Image about VNS







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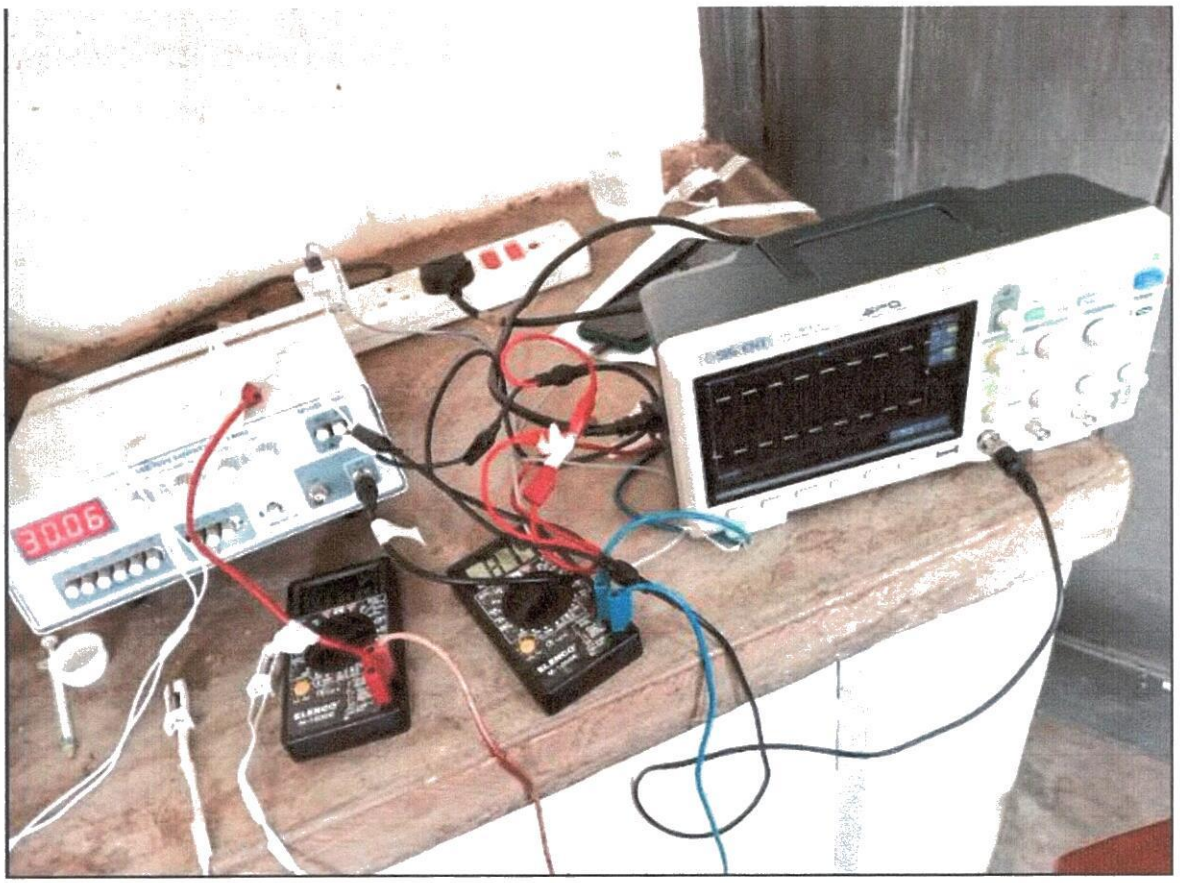
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Appendix 5.2: Primer sequences for Gene analysis

The primer sequences were: mBACE forward primer 5′CCCACAGACGCTCAACATCC3′ reverse primer 5′CGAGTGGTCGATACCTCCAATG3′; and SRPX2 forward primer 5′GGCTCTGCCATTTTCTCACGA3′ reverse primer 5′GCTGCTTGACTCCCGTTGTG3′. The formula used for the expression determination was $2^{-\Delta\Delta CT}$.

Appendix 6: Study publications

SPRINGER NATURE

Open Access Original Article

Impact of Transcutaneous Auricular Vagus Nerve Stimulation on Spatial Learning and Memory in Acrolein-Induced Alzheimer's Disease-Like Hippocampal Neuronal Damage in Wistar Rats

Review began 01/10/2025
Review ended 01/28/2025
Published 01/31/2025

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DOI: 10.7759/cureus.78285

Ronald Kamoga [1], Godfrey Z. Rukundo [2], Samuel Kalungi [3], Johnes Obungoloch [4], Celestino Obua [5], Amadi Ihunwo [6]

1. Department of Anatomy, Mbarara University of Science and Technology, Mbarara, UGA 2. Department of Psychiatry, Faculty of Medicine, Mbarara University of Science and Technology, Mbarara, UGA 3. Department of Pathology, School of Health Sciences, Makerere University, Kampala, UGA 4. Department of Biomedical Engineering, Mbarara University of Science and Technology, Mbarara, UGA 5. Department of Pharmacology and Therapeutics, Mbarara University of Science and Technology, Mbarara, UGA 6. School of Anatomical Sciences, University of the Witwatersrand, Johannesburg, ZAF

Corresponding author: Ronald Kamoga, rkamoga@must.ac.ug

Abstract

Background: Data about the utility of vagus nerve stimulation (VNS) as a potential therapy for neurodegenerative disorders are still inconclusive. We used a rat model of acrolein-induced hippocampal neurodegeneration to investigate the effect of VNS on spatial learning and memory.

Methods: A total of 24 Wistar rats were randomly allocated to one of the four groups: no acrolein exposure (n = 6), control (n = 6), sham (n = 6), and experimental (n = 6). The control, sham, and experimental groups were exposed to acrolein 2.5 mg/kg/day by gastric gavage for eight weeks. After acrolein exposure, the experimental and sham groups received transcutaneous auricular VNS and greater auricular nerve stimulation, respectively, under 2% isoflurane anesthesia for four weeks. Then, all animal groups were assessed for spatial learning and memory in a Morris water maze before being euthanized for hippocampus histological examination.

Results: The mean time to find the hidden platform varied significantly between the no acrolein exposure group and each of the acrolein-exposed groups. The results of one-way ANOVA indicated a significant difference in the average swimming time between the four study groups ($F = 14.64$, $p < 0.001$). Results from the post-hoc analysis indicated that the mean difference was statistically significant between the "no acrolein exposure" and "control" groups ($p < 0.001$), the "no acrolein exposure" and "experimental" groups ($p = 0.001$), and between the "control" and "sham" groups ($p < 0.001$). There was no statistically significant difference in swimming time to find the hidden escape platform between the sham and experimental groups ($p = 0.060$).

Conclusion: Transcutaneous auricular VNS has no significant effect on spatial learning or memory in Wistar rats with acrolein-induced hippocampus neuronal damage, indicating the need to review the long-standing notion that hippocampal neuronal loss causes spatial navigation deficits.

Categories: Neurology, Psychiatry, Pharmacology
Keywords: acrolein, alzheimer's disease, brain stimulation, dementia, morris water maze, neurodegeneration, rats, spatial learning, spatial memory, vagus nerve stimulation

Introduction

Vagus nerve stimulation (VNS), an approved therapy for a variety of neurological disorders, including refractory epilepsy and treatment-resistant depression, among others, is gaining attention as a potential treatment for neurological dementive diseases such as Alzheimer's disease (AD) and associated dementias [1]. There are two forms of VNS stimulation, i.e., invasive and non-invasive (transcutaneous), with the former involving surgical implantation of stimulator electrodes around the nerve and the latter, which is the most popular due to its minimal side effects, involving stimulation via an intact skin, either at the auricular concha region of the auricular branch of the vagus nerve (ABVN) or in the cervical region of vagus verve distribution [2]. Beyond the concha region, the auriculotemporal nerve innervates the ear region superiorly and the greater auricular nerve inferolaterally [3], but research into the effects of electrical stimulation on these nerves on the body is sparse.

Despite the enormous number of innovative medicines approved to treat AD, none have been effective in changing the course of the disease, and they are also associated with side effects, prompting the search for alternative treatments [4]. This has sparked a great deal of interest in brain stimulation techniques as

potential treatments for AD in the clinical and academic domains, and some pilot experiments have shown promising results [5]. AD, the most common type of dementia accounting for 65-75% of dementia cases [2], is a progressive neurodegenerative disease characterized by the accumulation of abnormal beta-amyloid plaques, neurofibrillary tangles, brain atrophy, hippocampal neuronal loss, and impairment of at least two cognitive functions, including memory loss [2]. Currently, there are about 50 million people living with dementia globally, which is expected to increase by approximately 300% by 2050 [6].

Numerous studies on humans and animals have been conducted. Some focused on populations that are not cognitively impaired [7], while others involved dementive conditions like AD [8-10]. These studies reported VNS to be associated with decreased neuroinflammation, decreased cerebral spinal fluid tau proteins, neuroprotective microglial structural changes, and increased neuroplasticity in terms of increased functional connectivity, as well as improvement across a range of cognitive domains. Many cognitive domains have been studied, but study findings on the effects of VNS on cognition are still equivocal, with many studies reporting improvement while others reporting no changes or a decline in cognitive performance [8,10]. Moreover, not all cognitive domains have been fully studied, leaving our understanding of how VNS affects cognition limited. Attention, arousal, semantic memory, recall memory, mood, and decision-making are some of the cognitive domains that have been extensively studied in terms of how they are modulated by VNS [8,10]. However, there is limited information about the effects of VNS on spatial learning and memory. Therefore, this study aimed to evaluate the effect of VNS on spatial learning and memory in a rat model of acrolein-induced AD-like pathology [11]. Acrolein is a strong electrophilic alpha, beta-unsaturated aldehyde that causes AD-like oxidative damage pathology in the brain, including hyperphosphorylation of the microtubule-associated protein tau, beta-amyloid aggregation, hippocampal atrophy, behavioral dysfunction, and cognitive decline, including impaired place navigation, among others [12,13]. The findings from this study may add to our understanding of the potential use of VNS to manage dementive conditions such as AD.

Materials And Methods

Study setting

The study was conducted at the Animal Research Laboratory of the Faculty of Medicine of Mbarara University of Science and Technology, located along the Kabale-Mbarara road, approximately 260 km southwest of Kampala, the capital city of Uganda. It was an experimental animal study conducted between October 2023 and March 2024. The study was carried out in accordance with the European Council Directive 86/609/EEC of November 24, 1986 [14] and the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals [15]. The Research Ethics Committees of Mbarara University of Science and Technology (Ref. No.: MUST-2023-791) and the Uganda National Council for Science and Technology (Ref. No.: HS3781ES) approved the study.

Animals

A sample size of 24 outbred Han Wistar rats aged between three and six months without any evidence of ill health was included in this sub-study. The sample size was determined by the resource equation model [16]. Except for the time they spent training and testing in the Morris water maze (MWM), the animals were housed in a regulated setting with regulated humidity, temperature, and a 12-hour cycle of light and dark. Every two days, the cages and litter were cleaned and replenished. At 12 weeks of age, the rats were randomly assigned to one of the four groups: non-acrolein exposed group (n = 6); control group (n = 6) - exposed to acrolein but no electrical stimulation; sham group (n = 6) - acrolein exposed with electrical stimulation of the greater auricular nerve; experimental group (n = 6) - exposed to acrolein with transauricular vagus nerve stimulation. The acrolein-exposed groups were exposed to acrolein for 56 days, after which the sham and experimental groups were subjected to electrical stimulation for 28 days. The spatial learning and memory were assessed using the MWM memory test administered to each animal group after the end of the electrical stimulation of the sham and experimental groups.

Administration of acrolein

Acrolein solution (Catalog No.: Z-020229-05 513389, CPI International, Santa Rosa, CA) was freshly dissolved in daily-prepared tap water and administered within two hours. For eight weeks, each of the rats received 2.5 mg/kg/day of acrolein by gastric lavage, which was half of the estimated daily acrolein intake in people [17], and had previously been administered to induce AD-like pathologies in mice (Table 1) [18].

Groups	No. of rats	Acrolein exposure	Electrical stimulation
Non-acrolein exposed	6	0 mg/kg/day	Nil
Control	6	2.5 mg/kg/day	Nil
Sham	6	2.5 mg/kg/day	Greater auricular nerve
Experimental	6	2.5 mg/kg/day	Periauricular vagus nerve

TABLE 1: Details of acrolein exposure and electrical stimulation to all groups of rats with their doses.

Electrical stimulation was conducted at the concha region of the periauricular vagus nerve in the experiment group and at the peripheral region of the greater auricular nerve in the sham group.

Cognitive behavioral analysis (Morris water maze test)

The MWM, which measures spatial learning and memory, was used based on a previously outlined methodology [12]. The maze was made up of a white circular pool (180 cm in diameter and 90 cm high) filled with water at 26°C to a depth of 80 cm. The center of the tank served as the intersection of two imaginary perpendicular lines, dividing the water maze into four equal quadrants marked north, south, east, and west. An escape platform was placed in the center of the tank. For the trial or pre-experiment training, the escape platform was exposed about an inch above the water's surface. The water maze had four starting positions: north, south, east, and west. The animal was gently lowered to one of the starting positions tail in first. We avoided stressing animals by not damping them head first. Animals were allowed to swim around for a maximum of 60 seconds. Once the animal reached and climbed the escape platform, the timer was stopped and the time taken to reach the escape platform was recorded. If it failed to reach the platform in one minute, the time was recorded for the trial as one minute. The animal was then gently guided to the escape platform with the assessor's hand. The animal would be allowed to rest for 15 seconds on the platform so that it learned to stay on the platform for rescue from the water. The same procedure was repeated for two more rounds starting at a different location for each. The animal was let to understand that it had to stay on the platform to be rescued from the pool. Once the animal completed all three trials, it was dried up with a towel. The three trials' training process was repeated for all the animals. Once all the animals were trained, then the water maze test was started. For the water maze test, the escape platform was covered at least 1 inch below the surface of the water. We used milk powder to make the water opaque. The water temperature was maintained as that of the pre-experiment/trial training. Each animal was made to make 12 tests, three from each starting direction, each test lasting a maximum of 60 seconds (one minute), without starting from one point in raw. The animal under test was tracked until it reached the escape platform and the time it took was recorded. If the animal did not reach the platform in 60 seconds, it was guided to the platform as in the training. The animal was then dried up and let to rest for 60 seconds. The names of the treatment groups were not disclosed to the assessor administering the MWM to avoid bias. Both the trial experiments and the experiments were conducted on the same day, sequentially.

Electrical stimulation of the auricular vagus nerve and the greater auricular nerve

Electrical stimulation of the vagus nerve was performed on animals under 2% isoflurane inhalation anesthesia by applying two oppositely charged magnetic electrodes (+/-) to the inside and outside of the auricular concha (auricular vagus nerve distribution) and the helix and scapha auricular areas (great auricular nerve distribution), as previously described [19]. In the study, the great auricular nerve (GAN), a superficial branch of the cervical plexus that innervates the skin covering the parotid gland, ear lobe, and posterior auricular region, was stimulated as a sham [20]. To boost electrical conductivity, salty water was placed between an electrode and the skin. Based on prior studies [21], the stimulation parameters included one hour of transcutaneous electrical stimulation using a Super Phosphor Oscilloscope - SIGLENT SDS1202X-E (Serial number: SDS1ECDD2R1388; SIGLENT Technologies, Shenzhen, China) and a Beetech 1603-3MHz Function Generator (Shreyans Enterprises, Bengaluru, India) at a frequency of 25 Hz and 50 microseconds cathodic-leading pulses with an amplitude of 0.8 mA, administered at a 10% duty cycle of 30 seconds ON and 4.5 minutes OFF. This was done once a day for four weeks.

Brain tissue preparation and H&E staining for histological analysis

Following vagal stimulation, all animal groups were deeply sedated with 3% intraperitoneal sodium pentobarbital before receiving intracardial perfusion with 0.01 M phosphate-buffered saline (PBS) for H&E staining. The hippocampus was then extracted and prepared for histological investigation (H&E staining). The hippocampus was fixed in 4% paraformaldehyde for six hours before being cryopreserved in PBS with

30% sucrose. H&E staining of hippocampal slices was performed as previously described [22]. To conduct a normal histomorphological investigation, a 5-micrometer-thick serial piece of hippocampus tissue was prepared, and H&E staining was applied to evaluate hippocampal neuronal loss or atrophy. To reduce observation bias, the histopathologists who evaluated the alterations in the hippocampus histology were not aware of the treatment groups.

Statistical analysis

The statistical analysis was carried out using Stata version 17.0 (StataCorp LLC, College Station, TX). The findings from the cognitive performance test were presented as the mean and standard deviation on boxplots and line graphs. To detect the statistical significance in average swimming time between groups, one-way analysis of variance (ANOVA) was used considering p-values less than 0.05 as statistically significant for observed differences. Tukey's post-hoc analysis was run to compare all possible pairs of group means to identify specific significant differences. The results on hippocampal neuronal changes were presented as photomicrographs of hippocampus areas (CA1, CA2, CA3, and CA4) captured with a digital camera and an Olympus IX71 microscope (Olympus, Tokyo, Japan).

Results

Wistar rats were given acrolein 2.5 mg/kg/day via stomach gavage for eight weeks, followed by four weeks of transcutaneous auricular VNS. H&E staining was used to obtain evidence of hippocampal neuronal injury by assessing histological evidence of hippocampal neuron damage in the different animal groups. The results showed features of neuron damage in the hippocampus of rats that were exposed to acrolein, a known neuron-damaging chemical, and no evidence of hippocampal cell damage in rats that were not exposed to acrolein (Figure *1*).

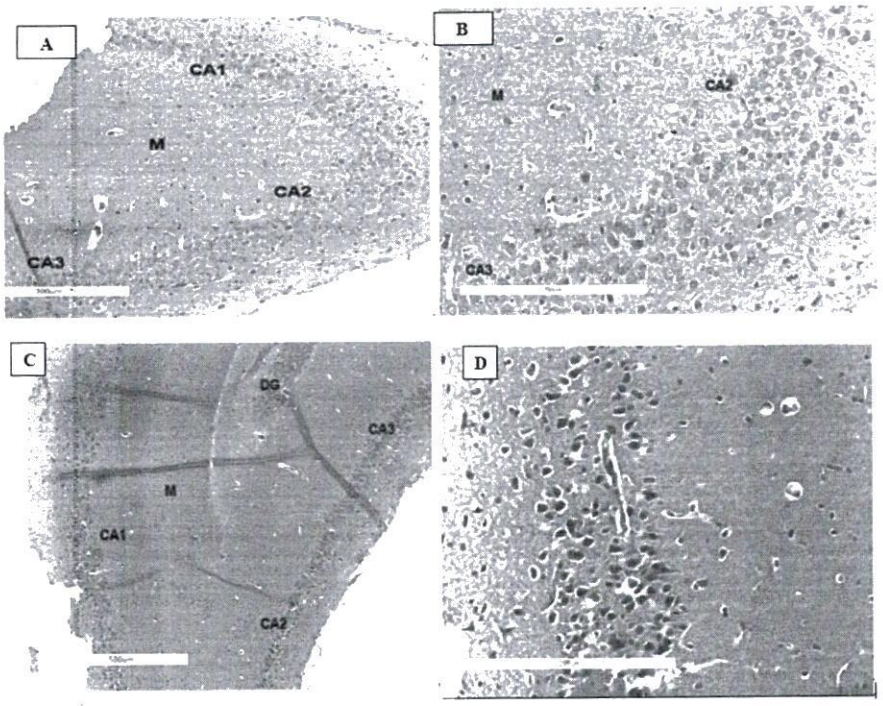

FIGURE 1: H&E staining of the hippocampus cornu ammonis regions CA1, CA2, CA3, and CA4, molecular layer (M), and the dentate gyrus (DG), providing histologic evidence of acrolein-induced hippocampal neuronal damage.

(A) Low-power section (300 µm) of the hippocampus of a rat that was not exposed to acrolein showed normal neurons in regions CA3, CA2, and CA1, with some scatter astrocytes in M. (B) High-power image (200 µm) of the hippocampus of a normal rat showing neuronal cell bodies in CA3 and CA2, with vesicular chromatin pattern. Both slides A and B from non-acrolein exposed animals had no evidence of apoptosis on H&E staining (x20). (C) Low-power images (500 µm) of the hippocampus of a rat exposed to acrolein showed extensive apoptosis in regions CA3 extending through CA2, CA1, DG, and M. (D) High-power image (200 µm) of the hippocampus of a rat exposed to acrolein showing apoptosis of neurons in region CA1. The neurons had condensed chromatin and were shrunken with a retraction artifact around the neurons. Slides C and D demonstrated that acrolein exposure was associated with hippocampal neurocellular damage.

CA: cornu ammonis regions (CA1, CA2, CA3) of the hippocampus; DG: dentate gyrus; M: hippocampal molecular layer; µm: micrometer.

The results of one-way ANOVA indicated a significant difference in the average swimming time between the four study groups ($F = 14.64$, $p < 0.001$). Results from post-hoc analysis have indicated that there were significant differences between some study groups (Table 2).

Study groups combinations		Mean difference	Standard error	P-value
No acrolein exposure	Control group	-12.26	2.03	<0.001
	Sham group	-3.00	2.03	0.880
	Experimental group	-8.46	2.03	0.001
Control group	Sham group	9.26	2.03	<0.001
	Experimental group	3.80	2.03	0.405
Sham group	Experimental group	-5.46	2.03	0.060

TABLE 2: Post-hoc test results showing differences in average swimming time between the study groups.

The mean difference was statistically significant between the "no acrolein exposure" and "control" groups ($p < 0.001$), between the "no acrolein exposure" and "experimental" groups ($p = 0.001$), and between the "control" and "sham" groups ($p < 0.001$). There was no statistically significant difference in swimming time to find the hidden escape platform between the sham and experimental groups ($p = 0.060$).

Below is a box and whisker plot (Figure 2) of the data for the average swimming time to find the escape platform by the different study groups.

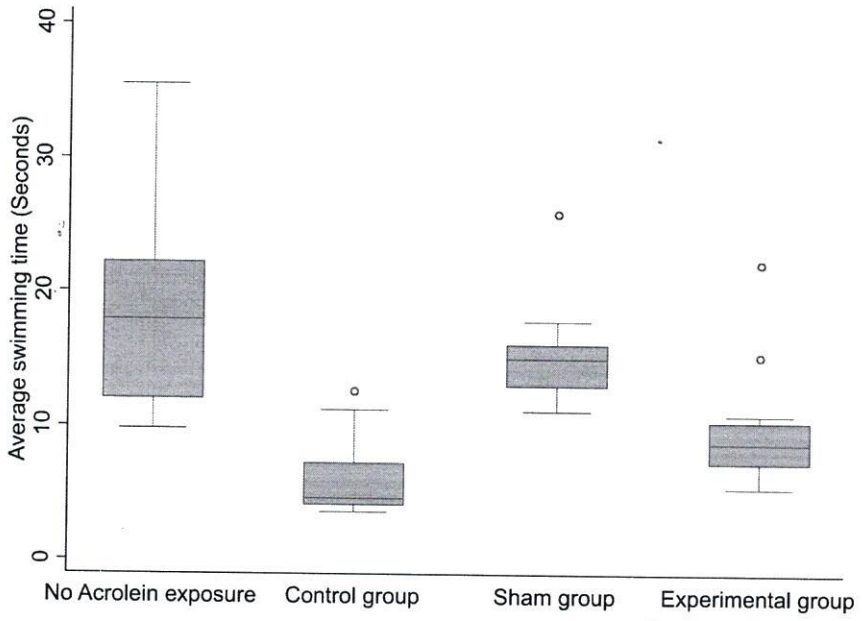

FIGURE 2: A box plot of average swimming time taken to find the escape platform in a Morris water maze (MWM) for the different study groups.

The lowest median swimming time for the control group was 4.6 seconds. The median swimming time to find the escape platform was significantly higher in the no acrolein exposure group (18 seconds) than for each of the other study groups. Data in both sham and experimental groups were skewed to the right but the median swimming time to find the escape platform was shorter in the experiment group (about nine seconds) compared to the sham group (about 15 seconds).

The data were presented on a line graph (Figure 3), which indicated a divergent tendency between the control and experimental groups with the latter spending less time to locate the hidden platform, and a convergent tendency between the no acrolein exposure and experimental groups (Table 3).

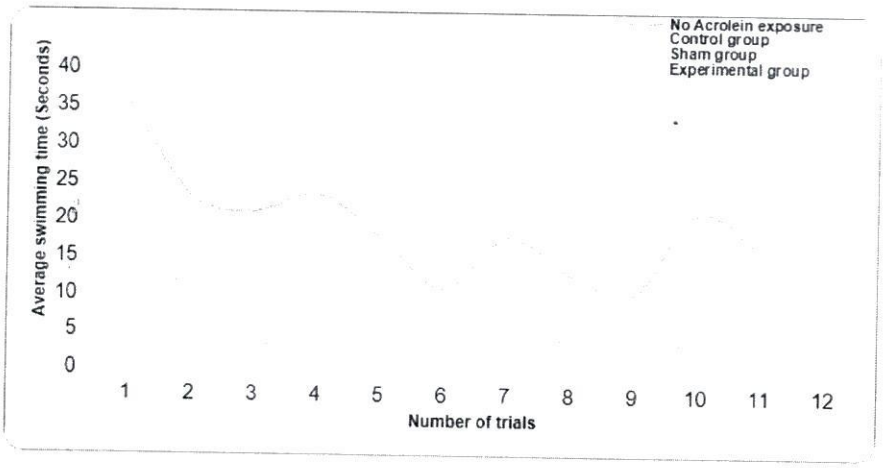

FIGURE 3: Line graph showing trends of average swimming time for the different study groups across the test trials.

The trend lines showed a divergent tendency between the control and experimental groups with the latter spending less time to locate the hidden platform, and a convergent tendency between the no acrolein exposure and experimental groups.

Trial number	No Acrolein exposure	Control group	Sham group	Experimental group
1	35.50	12.60	25.86	22.14
2	23.25	11.20	16.14	8.00
3	21.14	3.60	17.86	6.14
4	23.50	4.40	15.43	10.00
5	18.00	4.60	12.14	15.29
6	11.00	5.60	14.14	9.00
7	17.83	4.60	15.86	9.00
8	13.00	4.20	11.14	8.57
9	10.33	4.00	13.86	5.43
10	21.17	4.00	14.71	7.00
11	16.00	7.20	16.14	10.86
12	9.80	7.40	11.29	7.57
Overall average	18.38	6.12	15.38	9.92
Median time	18.00	4.60	15.38	9.00

TABLE 3: The swimming time to find the escape platform for rats in the four study groups across the 12 trials.

The control group had the shortest median (4.6 seconds) swimming time to find the escape platform while the no acrolein exposure group had the highest median (18 seconds) swimming time to locate the escape platform.

Discussion

This study aimed to investigate the effect of transauricular VNS on spatial learning and memory (place navigation) in Wistar rats with acrolein-induced hippocampal neuronal damage. VNS did not have a statistically significant effect on spatial learning and memory in Wistar rats with acrolein-induced

hippocampal neuronal damage. Incidentally, acrolein-exposed rats spent less time locating the hidden escape platform in the MWM test than the non-exposed rats, indicating better spatial learning and memory, and the difference was statistically significant.

Our study revealed that acrolein exposure caused neuronal injury, and these results collaborated with many previous reports that linked chronic acrolein exposure to neuronal damage and hippocampal atrophy [11,13]. This link between acrolein and neuronal injury may explain why acrolein is noticeably increased in the brains of AD patients [13] and underlines the necessity for policy guidelines to minimize acrolein exposure through the consumption of certain foods like cheese, donuts, and coffee as well as combustion of organic materials that have significant acrolein content.

Furthermore, our results indicated that VNS had no statistically significant effect on spatial learning and memory, and these outcomes were consistent with many previous research studies [23,24]. However, there are previous studies that reported enhancement of specific cognitive functions with VNS in people with mild cognitive impairment and AD; for instance, Dolphin et al. [25] reported enhanced associative memory and spatial navigation, Murphy et al. [26] reported enhanced semantic memory, and Wang et al. [27] reported improvement in immediate and delayed recall memory. The inconsistencies could be caused by variable stimulation protocols (duration, intensity, and frequency), timing in relation to cognitive assessment, dementia status of participants, and administration sites, among other factors [28]. For instance, with regard to stimulation protocols and timing in relation to cognitive assessment, cognitive assessment in our study was performed sequentially after stimulation at a mean amplitude of 0.8 mA, whereas in Dolphin et al.'s [25] study, cognitive assessment was paired with active stimulation at a mean amplitude of 2.0 mA. Previous investigations showed that the effect of VNS on cognitive function follows an inverted U-shaped curve, whereby only current intensities between 0.2 mA and 0.8 mA produce an effect on cognition, with maximal effects observed at 0.4 mA and inhibitory effects at about 3 mA [29-31]. It has been reported that VNS increases recognition performance and increases expression of brain-derived neurotrophic factor (BDNF) mRNA in the hippocampus when it is timed around the specific learning or performance task [32]. This could explain why the two studies had inconsistent results. With regard to study participants, the extent of neuronal damage could affect outcomes of VNS on cognition. VNS affects excitability in memory-associated pathways, such as the locus coeruleus (LC), leading to the release of neuromodulatory molecules like norepinephrine (NE), BDNF, and basic fibroblast growth factor (FGF-1) in the hippocampus and other brain areas related to learning and memory [33]. These neuromodulators enhance memory through a number of mechanisms, including reduction of inflammatory signaling by acting on astrocytes and neuroglia; induction of long-term potentiation (LTP), a form of synaptic plasticity; stimulation of mitogenesis in glial cells; and secretion of the nerve growth factor (NGF) whose expression is linked to motor nerve regeneration in the hippocampus [29-31,34-36]. If neuronal damage is substantial, as it is most likely with acrolein exposure because its effects are not restricted to the hippocampus, the brain areas activated by VNS to improve cognition may be destroyed. This may have caused the difference in outcomes between the current study with more severe and diverse acrolein-induced neuronal damage and studies by Dolphin et al. [25] and Murphy et al. [26], whose participants had mild cognitive impairment.

Additionally, in our study, acrolein-exposed rats performed better on spatial learning and memory MWM tests because they spent significantly less time locating the hidden escape platform compared to the non-exposed rats. This outcome was consistent with prior research studies, indicating that neuronal damage is not directly correlated with cognitive impairment [37,38]. There is a need for research to determine the critical hippocampal neuronal loss threshold at which deficits in spatial learning and memory occur. Neuronal damage long before the onset of cognitive deficits may be utilized as a potential biomarker to screen and monitor individuals at risk of neurodegenerative diseases like AD. This observation may indicate the effects of compensatory mechanisms triggered by acrolein-induced neuronal injury to restore neuronal structure and function in the hippocampus, and other brain areas that control spatial learning and memory [11,13,39]. Acrolein exposure is linked to increased oxidative stress in the brain [11], and oxidative stress triggers the expression of numerous neuroprotective biomolecules, for instance, BDNF by glial cells throughout the brain, but most abundantly in hippocampal neurons [40]. BDNF is a key molecule that plays a vital role in regulating synaptic plasticity and fostering learning and memory activities through several trophic activities on hippocampal neurons via its interactions with the tropomyosin-related kinase B (TrkB) and p75 cellular receptors [26,41]. The other possible cause of the disparity could be differences in gender, age, body weight, animal species, stress, disease, and nutritional status of the study animals in the acrolein-exposed and the no acrolein exposed groups [40]. Males, in general, tend to perform better than females, which may be attributed to differences in muscle strength or endurance, as well as the influence of sex hormones [42]. Roof [43] proved the effect of sex hormones on MWM performance by showing that testosterone administration during the first week after birth was linked with the equivalent performance of male and female rats in MWM acquisition training. Similarly, Daniel et al. [44] demonstrated that ovariectomized rats performed better in the MWM test than gonadally intact female rats. Bucci et al. [45] reported that the effect of gender differences on cognitive performance in MWM is insignificant in rats below six months old. Additionally, a number of earlier studies demonstrated that distinct rodent species and strains within a given species differed in their performance on the MWM test owing to differences in visual abilities, fundamental cellular and molecular processes involved in learning and memory, and differences in how they responded to stress and anxiety [42,46-48]. In this study, all the experimental

animals were of the same species, younger than six months, and were kept under similar conditions with regard to nutrition and environmental parameters throughout the study period. As such, the differences reported in cognitive performance among the study groups may be attributable to acrolein exposure and VNS.

Study limitations and strengths

There were some limitations to this study that should be considered when interpreting these results. The relatively short duration of VNS, spanning only four weeks, may have been insufficient to observe significant changes in spatial learning and memory. Furthermore, we used an acrolein-induced AD-like animal model [13,49,50], implying only a limited portion of the neuropathological features of AD, such as neuronal death and synapse loss, could be replicated by our model. Consequently, our findings may not accurately represent the effects of VNS in AD patients and may only be extrapolated to comparable models. Nonetheless, loss of synapses and/or neurons is sometimes seen as AD pathology and is a better indicator of cognitive impairment than the existence of plaques or tangles [40,51]. Furthermore, the experimental design placed VNS after learning in sequence rather than pairing learning and memory, which is reportedly associated with stimulation-specific neuroplasticity [7].

Conclusions

Transcutaneous auricular VNS did not have a statistically significant effect on spatial learning or memory in Wistar rats with acrolein-induced hippocampus neuronal damage, and acrolein-induced hippocampal neuronal loss was not directly related to spatial learning and memory impairments. Overall, the data added to our understanding of the effects of VNS on spatial learning and memory and highlighted the need to review the long-standing notion that hippocampal neuronal loss causes spatial navigation deficits in the elderly. Future longer-term studies matching VNS with learning and memory are needed to evaluate the effects of VNS on a broader range of cognitive domains in addition to spatial learning and memory and to generate more conclusive findings about the effects of VNS on cognitive performance in neurodegenerative disorders.

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.

Concept and design: Ronald Kamoga, Johnes Obungoloch, Celestino Obua, Amadi Ihunwo

Acquisition, analysis, or interpretation of data: Ronald Kamoga, Godfrey Z. Rukundo, Samuel Kalungi, Amadi Ihunwo

Drafting of the manuscript: Ronald Kamoga, Godfrey Z. Rukundo, Celestino Obua

Critical review of the manuscript for important intellectual content: Ronald Kamoga, Samuel Kalungi, Johnes Obungoloch, Amadi Ihunwo

Supervision: Godfrey Z. Rukundo, Amadi Ihunwo

Disclosures

Human subjects: All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** The Research Ethics Committee of Mbarara University of Science and Technology and the Uganda National Council for Science and Technology Issued protocol number MUST-2023-791 and HS3781ES. **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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gus nerve stimulation in dementia: A scoping review of clinical and -clinical studies

ald Kamoga[1,*], Godfrey Zari Rukundo[2], Samuel Kalungi[3], Wilson Adriko[4], Gladys idde[5], Celestino Obua[6] Johnes Obongoloch[7] and Amadi Ogonda Ihunwo[8]

partment of Anatomy, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, anda

partment of Psychiatry, Mbarara University of Science and Technology, P.O.Box 1410, Mbarara, anda

ıkerere University, School of health sciences, Department of Pathology. Kampala, Uganda

rary department, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara anda

ulty of Nursing and Health Sciences, Bishop Stuart University, Mbarara, Uganda

partment of Pharmacology, Mbarara University of Science and Technology, P.O.Box 1410, arara, Uganda

partment of Biomedical engineering, Mbarara University of Science and Technology, P.O.Box 0, Mbarara, Uganda

versity of the Witwatersrand, School of Anatomical Sciences, Faculty of Health Sciences, annesburg, South Africa

respondence: Email. rkamoga@must.ac.ug; Tel: +256772853821.

ıct: Background: Dementia is a prevalent, progressive, neurodegenerative condition with ctorial causes. Due to the lack of effective pharmaceutical treatments for dementia, there are g clinical and research interests in using vagus nerve stimulation (VNS) as a potential non- ıcological therapy for dementia. However, the extent of the research volume and nature into the of VNS on dementia is not well understood. This study aimed to examine the extent and nature ırch activities in relation to the use of VNS in dementia and disseminate research findings for

tential utility in dementia care. **Methods:** We performed a scoping review of literature searches ıMed, HINARI, Google Scholar, and the Cochrane databases from 1980 to November 30th, 2023, ing the reference lists of the identified studies. The following search terms were utilized: brain ation, dementia, Alzheimer's disease, vagal stimulation, memory loss, Deme*, cognit*, VNS, ranial nerve stimulation. The included studies met the following conditions: primary research s pertaining to both humans and animals for both longitudinal and cross-sectional study designs ıblished in English from January 1st, 1980, to November 30th, 2023; investigated VNS in either ıtia or cognitive impairment; and were not case studies, conference proceedings/abstracts, entaries, or ordinary review papers. **Findings and conclusions:** We identified 8062 articles, and screening for eligibility (sequentially by titles, abstracts and full text reading, and duplicate al), 10 studies were included in the review. All the studies included in this literature review were cted over the last three decades in high-income geographical regions (i.e., Europe, the United , the United Kingdom, and China), with the majority of them (7/10) being performed in humans. ıain reported outcomes of VNS in the dementia cases were enhanced cognitive functions, an sed functional connectivity of various brain regions involved in learning and memory, microglial ıral modifications from neurodestructive to neuroprotective configurations, a reduction of al spinal fluid tau-proteins, and significant evoked brain tissue potentials that could be utilized gnose neurodegenerative disorders. The study outcomes highlight the potential for VNS to be s a non-pharmacological therapy for cognitive impairment in dementia-related diseases such as imer's disease.

vords: cognitive impairment; Dementia; Alzheimer's disease; vagus nerve stimulation; brain ation

ntroduction

Dementia is a group of neurodegenerative disorders characterized by features of cognitive ment such as memory loss, impaired judgment, and reduced ability to carry out activities of living, among others [1,2]. There are several causes of dementia; however, Alzheimer disease which is a condition characterized by Amyloid plaques and neurofibrillary tangles in the brain, most common cause of dementia in older people, accounting for approximately 60–70% of [3,4]. Due to the lack of effective treatments against Alzheimer's disease and a number of related ıtias (ADRDs), there is growing clinical and research interests in using vagus nerve stimulation) as a potential non-pharmacological therapy for ADRDs [5,6]. VNS is a Food and Drug ıistration (FDA) approved therapy to manage drug resistant epilepsy, major depression, cluster ches, and inflammatory bowel disease [7–10]. There are a number of clinical and preclinical s that have demonstrated VNS to be associated with reduced neuroinflammation, an increased lasticity, and an improved cognitive performance, though it is mainly in non-cognitively ed populations [11–14]. The scope and extent of research on the effect of VNS in cognitively ed populations is scanty. Moreover, a number of studies about the effects of VNS in dementia

e reported inconsistent outcomes, including either the absence of an effect or the worsening of nitive functions [3,15–20]. The contradictory findings about the effects of VNS in dementia could consequence of the differences in the methods of investigation, the stimulation protocols, and the archer's knowledge and skills, among others. Thus, due to a growing interest to use VNS to iage various diseases including dementia [21], and the variability of the study results, there is an ent need to summarize the available literature about VNS in dementia so as to decide the direction uture research and to guide policies and practices with regard to the use of VNS as a potential apy for dementia. Therefore, this scoping review was conducted to systematically map the research has been performed, to identify the nature of the existing literature, to summarize and disseminate ings for the potential utility in dementia care policies, and to practice and research guidelines. The ew process was guided by the following question: what is known from the existing literature about use of VNS in dementia or cognitive impairment?

Methods and materials

Protocol and registration

The protocol for this scoping review was drafted using the Preferred Reporting Items for tematic Reviews and Meta analyses—extension for scoping reviews (PRISMA-ScR) [22,23], and revised by the team. However, the protocol is not publicly available, though it can be accessed on uest from the corresponding author.

Eligibility criteria

Only primary articles pertaining to both humans and animals were included for both longitudinal cross-sectional study designs. All English-language articles about VNS in dementia that had been lished from 1st January, 1980 to November, 30th 2023, were included. The start date of 1980 was sen because research activities regarding VNS as a potential therapy for various conditions were y scanty before 1980 [24], and because the approval and use of VNS as a therapy for various rological conditions is relatively recent [25]. All articles in languages other than English were luded because of the cost and time constraints in translating the material. All published articles on S in conditions other than AD and case studies, conference proceedings/abstracts, commentaries, review papers were excluded. We only used studies with readily available full articles. Abstract-y articles were also included. All study designs were represented in order within the review article btain as in-depth and broad results as possible.

Information sources and search strategy

In order to identify potentially relevant literature, the following electronic databases were rched from 1980 to 30th November 2023: PubMed, HINARI, Google Scholar, and Cochrane. abases. The following keywords were used to search the various databases: Brain stimulation, ementia, Alzheimer's disease, vagal stimulation, memory loss, Deme*, cognit*, VNS, and Cranial

ve stimulation. The search strategies and the definition of key concepts were developed from the earch question with the guidance of a qualified librarian (WA), who helped us to identify the evant keywords and who advised us on what databases were most likely to produce the type of dies we sought. Additionally, the librarian devised the initial search strategy, which was later refined light of the early results. However, some databases such as Scopus, EMBSAE, and Web of science re inaccessible due to the lack of subscriptions. The final search results were exported into EndNote, l duplicates were removed by the research team. The Endnote reference manager software was used e to its compatibility with the word processing package we were using, and it was relatively quick l easy to use to generate lists of references to include in the final literature review report. The ctronic database search was supplemented by manually searching the reference lists/bibliographies the identified studies through the database searches, and by scanning relevant reviews and grey rature to ensure they had been included in the scoping exercise. The search strategy and databases t were used are summarized in Table 1.

. Data charting process

A standardized data extraction form was jointly developed by three reviewers to ensure that all evant data based on the study objectives were captured. Then, each of the three reviewers lependently screened and charted all the eligible articles by title. After the title screening, the three iewers compared their results. Any disagreements were resolved through discussions and nsultations with a content expert (GZR), who is a senior consultant psychiatrist. Then, the reviewers lependently reviewed the eligible studies by reading through the abstracts and charting. They osequently compared their results, and any disagreements were resolved as previously described. xt, they each independently read the full articles of the eligible studies and charted the data. Finally, y compared the results and resolved the differences as previously described. The data extraction m was continuously updated in an iterative process. A matrix table was used to display the data, l all articles excluded based on the full text review were recorded and the reasons for exclusion were cumented.

5. Variables to be collected

The following variables were collected and recorded for the analysis: the study design, the sample es, the type of intervention (invasive or non-invasive or both), the study outcomes istomorpholgical, molecular, and behavioral outcomes), the year when the data was collected, the ographic location/region/country where the study was performed, socio-demographics for the nical studies (e.g., age, gender, religion, ethnicity, etc.), the authors' names, the year of publication, e stimulation site, and the stimulation parameters (Table 2).

5. Quality assessment

A risk of bias across the studies and a critical appraisal of the individual sources of evidence were t performed because the purpose of this scoping review was to provide a general overview of the

ent and nature of the existing evidence pertaining to use of VNS in either dementia or cognitive airment, regardless of the methodological quality or risk of bias [27,28].

Data analysis

Following the data extraction, a thematic analysis was performed whereby the studies were anized according to the investigated study variables. The key elements of each study were noted, uding the study design, the sample sizes, the type of intervention (invasive or non-invasive or both), ly outcomes (wanted and unwanted outcomes), the year when the data was collected, the graphic location/region/country where the study was conducted, and the socio-demographics (e.g., , gender, religion, ethnicity, etc.) used in the participant selection.

Results

We identified 8062 articles through the database search. After removing 408 duplicates, 7654 cles remained, of which 7346 articles were eliminated because they did not fit the inclusion criteria ed on the title screening. Of the remaining 308 articles, 289 articles were excluded after screening abstracts due to the following reasons: not meeting the inclusion criteria (n = 278); duplicated (n = and protocols/ongoing clinical trials (n = 3). Of the remaining 19 articles, 9 were excluded after the text screening due to the following reasons: ongoing trials/protocols (n = 3); duplicated (n = 1); a ure to access either the full article or relevant information from abstract (n = 4); and one article was onference proceeding (n = 1). The figure below illustrates the iterative process of the article ction. Having read the articles in full, 8 articles were selected for inclusion in the review.

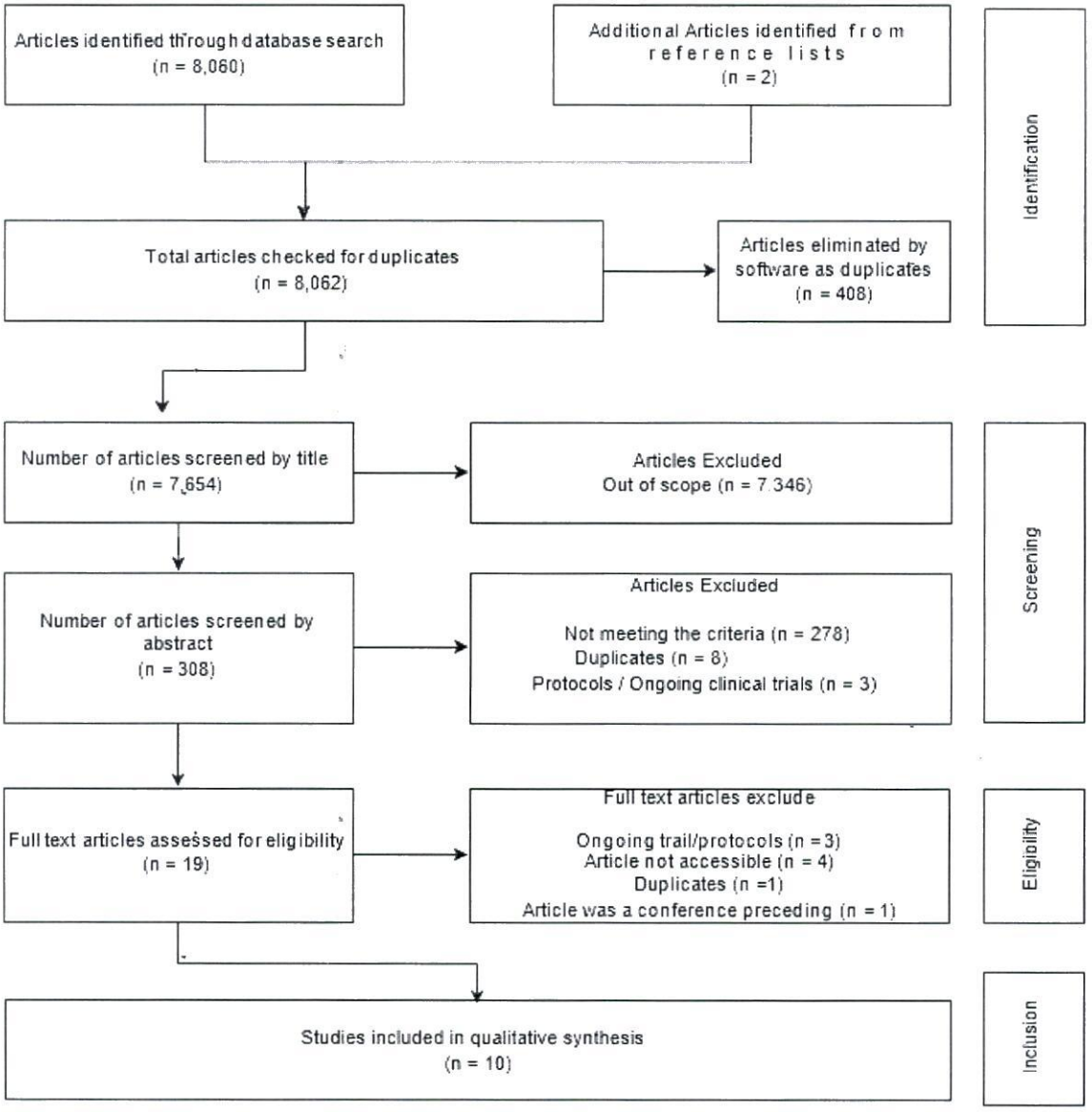

Figure 1. Article selection flow chart.

Table 1. Summary of the data bases, search strings and the number of articles obtained from each data base.

atabase	Search strategy	Number of articles obtained	Comments
ıbMed	(((((Vagus nerve stimulation) OR (VNS)) OR (vagal stimulation)) OR (cranial nerve stimulation)) OR (brain stimulation) AND ((((((dementia) OR (Alzheimer's disease)) OR (cognitive impairment)) OR (Memory loss)) OR (deme*)) OR (cogniti*))))	819 articles	Filters included: meta-analysis; randomized controlled trials; Review; systematic review; 1980/1/1:2023/11/30; English
ɔchrane	"vagus nerve stimulation" OR "Vagal stimulation" OR "VNS" OR "Cranial nerve stimulation" OR "Brain stimulation" in Title Abstract Keyword OR "dementia" OR "Alzheimer's" OR "cognitive impairment" OR "memory loss" OR (NEXTdeme*) OR (NEXT cognit*)	5744	5722 were Cochrane systematic reviews while 22 were clinical trials
ɔogle holar	("vagus nerve stimulation" OR "VNS" OR "vagal stimulation"OR"Cranial nerve stimulation") AND ("Alzheimer's disease" OR "Alzheimer disease" OR "Cognitive impairment" OR "memory loss OR deme* OR cognit*")	980	The search returned 10,900 English results. However, there was a limitation of how many results can be downloaded. The first 980 articles sorted according to relevance were obtained.
inari	((vagus nerve stimulation) OR (vagal stimulation) OR (vns) OR (cranial nerve stimulation) OR (brain stimulation)) AND ((alzheimer's) OR (dementia) OR (cognitive impairment) OR (memory loss) OR (deme*) OR (cognit*))	517	Additional filters included; Open access, Full text online, Scholarly and peer reviewed, Journal articles, Publications, 1990 to 2023, English Fields searched included; medicine, biology, pharmacology, therapeutics, zoology, occupational therapy, rehabilitation, neurosciences, neurology, nerve stimulation studies.

Table 2. Electronic data extraction form showing the extracted variable from the selected studies.

Tudy tile	Author(s), year/ Country	Type of VNS intervention /Study deign	Type of study/Gender / age range	Sample size	Stimulation parameters: sites; invasive/non-invasive stimulation; intensity; frequency and duration/duty cycles and timing in relation to learning stimulation	Outcomes/Conclusions
1. The mechanism underlying chronic transcutaneous auricular vagus nerve stimulation in patients with mild cognitive impairment through the enhancement of the functional connectivity between the left precuneus and parahippocampus gyrus	Wang et al., 2023/ China [29]	Noninvasive /Randomized Controlled Trial (RCT)	Clinical study/Males& females/55-75 years	60 with MCI and 30 healthy control participants	**Sites** ➢ Experimental taVNS group- two auricular acupoints were stimulated, including heart (CO15) and kidney (CO10), in the distribution of vagus nerve. ➢ Sham taVNS group- another two auricular acupoints were stimulated, including elbow (SF3) and shoulder (SF4, 5), out of the distribution of vagus nerve. **Stimulation** ➢ taVNS group, a pulse train of 20 Hz for 10 s and 100 Hz for 50s in each minute, intensity from 0.6 mA to 1.0 mA, determined by individual tolerance of the patients, 30 min in each session, two sessions every day, once in the morning, the other in the afternoon or evening, five consecutive days per week with an interval of 2 days for rest, treatment period for 24 weeks	➢ Increased functional connectivity (FC) between the left medial prefrontal lobe and right lingual gyrus at the baseline in patients with MCI. ➢ Declined FC between the left/right hippocampus and middle/upper frontal gyrus, and between the left/right precuneus and parahippocampal gyrus. ➢ Only the FC between the left precuneus and parahippocampus was enhanced, marginally positive correlated with the overall score of MoCA-B and AVLT-N7, after 24 weeks of taVNS ➢ There was no other reverse regulation of the FC between each pair of brain regions within the DMN. ➢ taVNS can improve cognition in patients with MCI through enhancing the FC between the left precuneus and parahippocampus.
2. The efficacy and safety of transcutaneous auricular vagus nerve stimulation in patients with mild cognitive impairment: A double blinded	Wang et al., 2022/ China [30]	Noninvasive /Randomized Controlled Trial (RCT)	Clinical study/Males& females/55-75 years	60	**Site** ➢ In taVNS group, a pair of auricular acupoints were stimulated, including heart (concha, CO15) and kidney (CO10), in the distribution of vagus nerve. ➢ In sham VNS group, another pair of auricular acupoints were stimulated, including elbow (scaphoid fossa, SF3) and shoulder (SF4,5), out of the	➢ Significant difference in the overall scores of MoCA-B between taVNS group and sham taVNS group ($p = 0.033 < 0.05$). ➢ In taVNS group, compared with those before intervention, the overall scores of MOCA-B increased significantly after intervention ($p < 0.001$); and in sham taVNS group, compared with those before intervention, there was no significant difference in the overall scores of MoCA-B after intervention ($p = 0.338$).

	randomized clinical trial					distribution of vagus nerve. **Stimulation** ➢ A pulse train of 20 Hz for 10s and 100Hz for 50s in each minute, intensity from 0.6 Ma to 1.0 mA, determined by individual tolerance of the patients, 30 min in each session, two sessions every day; once in the morning, the other in the afternoon or evening, five consecutive days per week with an interval of 2 days for rest, treatment period for 24 weeks.	➢ Compared with sham taVNS, there was also significant difference in the difference value of pre- and post-intervention in taVNS group ($p < 0.001$). ➢ For immediate recall, there was significant increase in N5 post-intervention compared to pre-intervention ($p < 0.001$) within the taVNS group; and no significant difference in N5 post-intervention compared to pre-intervention ($p = 0.059$) within the sham taVNS group. ➢ For delayed recall, Pre-intervention: No significant difference between taVNS and sham taVNS groups ($p = 0.470$). Post intervention: No significant difference between taVNS and sham taVNS groups ($p = 0.056$). Significant increase in N7 post-intervention compared to pre-intervention ($p < 0.001$) within taVNS group. No significant difference in N7 post-intervention compared to pre-intervention ($p = 0.051$) within sham taVNS group ➢ Significant difference in the change from pre- to post-intervention between taVNS and sham taVNS groups ($p = 0.005$).
3.	Vagus Somatosensory Evoked Potentials (VSEPs) – A Possibility for Diagnostic Improvement in Patients with Mild Cognitive Impairment?	Metzger et al.,2012/ German[31]	Noninvasive / Randomized Controlled Trial (RCT)	Clinical study/Males& females/ Mean age 73.5 ± 8.4 years for AD group, mean age 69.5 ± 5.0 years for MCI group, mean age 70.1 ± 5.7 years for healthy controls	51 participants (12 patients with AD, 12 patients with MCI, 27 healthy subjects)	**Site** • Right and left tragus **Stimulation** ➢ Electrical square impulses of 0.1 ms duration, 8 mA intensity, and a frequency of 0.5 Hz were applied at the left and right tragus in separate trials. The electrical brain activity was recorded with a sampling rate of 20 KHz, a band-pass of 0.1–1 KHz, and an epoch length of 10 ms separately for right and left stimulation.	➢ Identifiable evoked potentials noted during stimulation at the right tragus with increasing latencies from healthy controls to mild cognitive impairment, indicating the potential diagnostic value of VSEPs in Alzheimer's disease.
4.	The effects of transcutaneous vagus nerve stimulation on functional	Murphy and Aidan, 2023/USA [32]	Noninvasive / Randomized Controlled Trial (RCT)	Clinical study/Females / Average age 75	50	**Sites** ➢ Experimental group- Left tragus-left auricular branch of the vagus nerve	➢ During unilateral left taVNS, compared with ear lobe stimulation, patients with MCI showed alterations in functional connectivity between regions of the brain critical for semantic and salience functions including regions of the

	connectivity within semantic and hippocampal networks in mild cognitive impairment					➢ Control group- electrodes were placed on opposite sides (mesial and lateral faces) of the earlobe. ➢ The return electrode for tVNS was placed anterior to the tragus to minimize off-target stimulation, and the sham return electrode was placed on the mesial face of the ear lobe. **Stimulation** ➢ Positive pulses were delivered at a 20 Hz, 50 μs pulse. ➢ Stimulation was delivered continuously during one fMRI resting state condition. ➢ Stimulus intensity for sham and tVNS was progressively increased from 0 to the threshold of discomfort, then reduced to 80% of threshold, as per prior investigations. Due to device limitations, stimulation intensity was capped at 10 mA, which most subjects reached without discomfort.	temporal and parietal lobes. Furthermore, connectivity from hippocampi to several cortical and subcortical clusters of ROIs also demonstrated change with tVNS compared with ear lobe stimulation. ➢ In conclusion, tVNS modified the activity of brain networks in which disruption correlates with worsening in Alzheimer's disease.
5.	P3-032 Effects of vagus nerve stimulation on cognition, CSF-Tau and cerebral blood flow in patients with Alzheimer's disease: results of a 1 year pilot study	Merrill & Bunker 2004 [33]/USA	iVNS/Single arm follow-up study	n=15	Clinical study	Stimulation parameters inaccessible	➢ Improvement or no decline from baseline on the ADAS-cog score in 33% and on the MMSE in 64% of participants. ➢ Significant improvement from baseline was sustained at 6 months for the ADAS (median improvement of 3 points, $p = 0.011$, $n = 16$) and MMSE (median improvement of 2 points, $p = 0.012$, $n = 16$). ➢ After 12 months of VNS, the median change in CSF-tau was a reduction of 7.7% ($p = 0.003$, $n = 15$) ➢ Conclusion: a positive effect of VNS on cognition in Alzheimer's disease after one year of treatment

6.	Microglia modulation through external vagus nerve stimulation in a murine model of Alzheimer's disease	Kaczmarczyk et al., 2017/German (Europe) [34].	Noninvasive / Experimental study	Male/female/ 6 month old mice to 12 month old mice	Pre-clinical study	**Site** ➢ Stimulation was performed 13mm anterior to the neck's base and 2mm from the trachea over the vagus nerve. **Stimulation** ➢ The mouse was stimulated twice for two minutes with a three-minute break in between. ➢ The signal consisted of 1 ms duration bursts of 5 kHz sine waves, repeated at 25 Hz. ➢ The signal amplitude was increased until there was strong muscle stimulation, corresponding to approximately 1.8 mA. After the stimulation or sham-treatment, the same three regions were imaged five times. ➢ Sham treatment was performed in the same manner as the nVNS only without turning on the stimulator.	➢ Significant changes in microglial morphology were dependent on stimulation parameters were noted ($p = 0.001$). ➢ The effects of nVNS were slightly but significantly different between young and old animals ($p = 0.024$) ➢ 12-Month-Old APP/PS1 Mice: nVNS caused a significant increase in the number of branches compared to sham-treated controls, with an increase of 3.82 ± 0.90 branches every 40 minutes ($p < 0.001$) ➢ 12-Month-Old Wild Type (WT) Mice: There was a non-significant trend showing an increase of 2.07 ± 0.88 branches every 40 minutes ($p = 0.071$). ➢ 6-Month-Old Mice (Both Groups): No significant differences in microglial morphology between nVNS and sham-treated mice. ➢ tVNS significantly associated with morphological changes related to a neuroprotective phenotype in microglia
7.	Improvements in associative memory and spatial navigation with acute transcutaneous Vagus Nerve Stimulation in Mild Cognitive Impairment: preliminary data.	Dolphin et al., 2023[36]./ Ireland, United Kingdom	Non-invasive/Experimental study	n=28; Mean age 71.5 (range 55-85) years; 17M/11F;	Clinical study/	The mean stimulation time pre-cognitive assessments was 21.2 minutes, with mean amplitude setting during active stimulation of 2.5 mA (1.8–4.5) and sham of 2.0 mA (0.9–3.1).	➢ During FNAT, active tVNS had no effect on facial recognition or reaction times, however recall accuracy was significantly improved (69.2% ±3.13) compared to baseline (44.7% ±3.51 $p = 0.016$) and sham (50.1% ±3.28 $p = 0.021$) and during active tVNS spatial navigation (38.94 sec [±1.68]) was quicker than baseline (51.49 sec (±3.2) $p = 0.0164$) and sham (51.9 sec (±3.15) $p = 0$ 0.0038) ➢ noted no significant improvements in SART or other cognitive tests performance during tVNS
8.	Transcutaneous Vagus Nerve Stimulation Effects on Functional Connectivity of	O'Neal et al., 2023[37]/ USA	Non-invansive/ Experimental study	n=50(sham n=25, experimental n=25); F28/M22; age	Clinical study	➢ Stimulation parameters inaccessible	➢ Contrasting tVNS and sham stimulation, whole-brain seed-to-voxel analysis demonstrated significant changes in connectivity from the left hippocampus to several cortical and subcortical regions bilaterally.

	the Hippocampus in Mild Cognitive Impairment.			years.			and the prefrontal regions and cingulate gyri, and decreased connectivity to anterior and medial temporal lobes. ➢ A seed-to-voxel analysis from the right hippocampus indicated significant decrease in connectivity to a single cluster of regions in the left anterior temporal lobe in response to tVNS. ➢ tVNS modified connectivity from the hippocampus to multiple brain regions involved in learning and understanding, which disruption correlates with deterioration in AD
9.	Vagus nerve stimulation in patients with Alzheimer's disease: additional follow-up results of a pilot study through 1 year	Merrill et al., 2006 [39]/ Europe (Sweden)	iVNS / Longitudinal follow-up study	Clinical study	n= 17	Stimulation parameters not accessible	➢ Improvement or no decline from baseline of 41.2% and 70.6% on the ADAS-cog and MMSE, respectively after 1 year. ➢ Twelve of 17 patients were rated as having no change or some improvement from baseline on the Clinician Interview-Based Impression of Change (CIBIC+)
10.	Cognition-enhancing effect of vagus nerve stimulation in patients with Alzheimer's disease: a pilot study	Sjogren et al., 2002[40]/ Europe (Netherlands)	iVNS/ Longitudinal follow-up study	Clinical study	n =10	Stimulation parameters inaccessible	➢ After 3 months of treatment, 7 of 10 patients were responders according to the ADAS-cog (median improvement of 3.0 points), and 9 of 10 patients were responders according to the MMSE (median improvement of 1.5 points). ➢ After 6 months of treatment, 7 patients were responders on the ADAS-cog (median improvement of 2.5 points), and 7 patients were responders on the MMSE (median improvement of 2.5 points).

Legend: AVLT-H- auditory verbal learning test-HuaShan version; MOCA-B - Montreal cognitive assessmentbasic; MCI- mild cognitive impairment; CO-Concha ; taVNS- transcutaneous auricular vagus nerve stimulation; VNS- vagus nerve stimulation; SF- scaphoid fossa; DMN- default-mode network; Nvns – Noninvasive vagus nerve stimulation; VSEPs - Vagus Somatosensory Evoked Potentials; FNAT- Face-Name Association Task; SART- Sustained Attention Response Test; MADRS- Montgomery-Asberg Depression Rating Scale; MMSE-Mini Mental State Examination; ADAS-cog -Alzheimer Disease Assessment Scale, cognitive section.

3.1. *Study geographical location and study designs*

Of the 10 studies included, two studies [29,30] were conducted in China, four studies [34] were conducted in Europe [39–42], one study was conducted in the United Kingdom [36], and three studies were conducted in the United States [33,37,43]. Regarding the study designs, there were 9 clinical studies, four Randomized Controlled Clinical Trials (RCTs), two experimental studies, and three longitudinal follow-up studies. There was one pre-clinical experimental study that involved a murine model of Alzheimer's disease. Of the clinical studies, 3 involved people with Alzheimer's disease, while 6 involved people with a mild cognitive impairment. Most studies (7/10) utilized the non-invasive VNS (nVNS) technique.

3.2. *Stimulation sites and parameters*

Non-invasive vagus stimulation was trans-auricular [29,30], except in one study, where it was within the trans-cervical region [34]. For invasive stimulations, implantation was performed in the cervical region at 13 mm anterior to the neck's base and 2 mm from the trachea. The stimulation parameters varied from study to study, with current frequencies between 0.5 Hz to 100 Hz and intensities from 0.6 mA to 4.5 mA, depending on the participants' tolerance.

3.3. *Study outcomes*

In general, nVNS was associated with an increased functional connectivity in the brain regions that are critical for cognitive functions [26,29], an improved immediate and delayed memory/cognition among people with mild-cognitive impairments (MCI) [30] and significant microglial structural changes [34].

4. Discussion

This scoping review aimed to examine the extent and nature of research activities in relation to the use of VNS in dementia and to summarize and disseminate the research findings. All the studies included in this literature review were conducted over the last three decades in high-income geographical regions (i.e., Europe, the United States, the United Kingdom, and China), with the majority of them (7/10) being performed in humans. The main reported outcomes of VNS in the dementia cases were enhanced cognitive functions, an increased functional connectivity of various brain regions involved in learning and memory, microglial structural modifications from neurodestructive to neuroprotective configurations, a reduction of cerebral spinal fluid tau-proteins, and significant evoked brain tissue potentials that could be utilized to diagnose neurodegenerative disorders.

All studies identified for inclusion in this review were conducted in high-income countries. This may be due to the absence of necessary competencies such as surgical skills to perform invasive VNS procedures, or financial limitations that may prevent access to VNS devices. Additionally, despite the higher prevalence of dementia in lower-income countries, the estimated costs of dementia are substantially higher in higher-income countries [44], thus making dementia research a higher health-care priority in those countries. Lower income nations appear to have lower medical, non-medical, and

indirect costs associated with dementia compared to higher income countries, which is most likely due to disparities in services and cultural views of ageing and dementia [45–47]. For instance, lower income countries have lower dementia diagnostic coverages, possibly due to a healthcare workers' lack of knowledge and skills in dementia assessments and diagnoses, as well as the influence of cultural beliefs that attribute signs of dementia to either aging or witchcraft [48]. These factors can lead to missed diagnoses, which consequently minimize the disease burden and the estimated costs of dementia, as well as the need for prioritizing dementia research in low-income countries [44]. Moreover, in lower-income economies, the sick elderly are typically cared for by family members within their homes, and it is this indirect home-based care that is projected to be the major driver of dementia costs in higher-income countries [44]. The aforementioned factors may explain why dementia research has received less attention in lower-income countries. Additionally, we noted that all the identified studies were conducted among human subjects except for one study that evaluated the effects of VNS on the structure of microglia in a rat model of Alzheimer's disease [34]. The financial burden associated with animal studies could be the major reason for the limited number of animal studies identified, especially in relation to the preparation of transgenic animal models of cognitive impairment [49]. Furthermore, because VNS is already an established treatment for a number of human diseases, obtaining ethical clearance to conduct research is much easier [50]. Moreover, because animal models have shorter life spans, they may not faithfully mimic all of the time-dependent pathophysiological changes of a human disease that develop over extended periods of time such as Alzheimer's disease [51].

We further noted that 70% (7/10) of the studies included in this review utilized non-invasive (trans-cutaneous) electrical vagus stimulation (tVNS). The greater preference for tVNS was most probably due to its better safety profile since it does not involve surgery; hence, surgery related complications can drive up the expenses [25,52,53]. There are two approaches of VNS: 1) Invasive VNS (iVNS), in which a pulse generator is implanted beneath the skin in the upper chest under the clavicle, and the cuff electrodes are connected to the left cervical vagal nerve[25,40,54–57]; and 2) the tVNS, which involves transcutaneously stimulating the vagus nerve through the auricular branches of either the vagus nerve (atVNS) or the cervical vagus nerves (ctVNS) [58–60]. Research has shown that between 4 to 30% of patients who undergo iVNS experience unfavorable side effects that may necessitate repeat surgeries for correction [25,61]. These side effects may include hematomas, infections, vocal cord paralysis, hoarseness, parasthesias (tingling sensations in the neck region), a shortness of breath, spontaneous turnoff, lead breakage, and stimulator malfunctions, among other things [60–62]. Both iVNS and tVNS have been reported to be equally effective [2,63–67], although other studies have found differences in which specific psychophysiological responses were absent in tVNS compared to iVNS [68,69]. This indicates that tVNS may have weaker benefits when compared to iVNS, most likely because tVNS does not directly stimulate the vagus nerve, which is located deep within the carotid sheath of the neck.

Regarding outcomes of electrical VNS in dementia, the following VNS effects were reported: an enhanced cognitive function, a functional connectivity between different brain areas, a reduced cerebral spinal fluid tau-proteins, neuroprotective microglial structural changes, and neuroprotective morphological changes [33,36,39,40,74,75]. Similar results have been reported in related studies conducted in various disease conditions other than dementia [25,37,43,76–78], in healthy volunteers [38,79,80], and in animal models [35,38,81,82]. The mechanism by which VNS affects cognitive functions is not fully understood; however, according to prior research, VNS is associated

with increased levels of Norepinephrine (NE) and neutrophins, specifically, the fibroblast growth factor (FGF-1) and the brain-derived neurotrophic factor (BDNF), within the cerebral cortex, the amygdala, and the hippocampus [83–85]. BDNF and FGF-1 reportedly promote enhanced cell signaling through interactions with p75 cellular receptors and tyrosine kinase receptors, as well as a reversal of synaptic loss among other functions, thus resulting in long-term potentiation (LTP), which is a key mechanism of learning and memory [62,86,87]. These effects may account for the enhanced cognition and functional connectivity reported in this study. Moreover, NE lowers the inflammatory gene expression in glial cells, thereby lowering the glial expression of pro-inflammatory molecules such as cytokines, tumor necrosis factor, and cell adhesion molecules [88]. Furthermore, decreased levels of NE are associated with microglial structural changes collectively called dystrophic microglia, including decreased branches, cellular shrinkage, and an increased stroma volume that makes the cell more oval-shaped [89]. These changes reduce the microglial motility and phagocytic functions, and hence reduce the ability of the microglia to protect neurons against foreign agents [88,90]. When dystrophic microglia fail to destroy foreign bodies, they remain in a state of chronic activation and produce excessive proinflammatory molecules, which begin to destroy synaptic connections, and hence weaken the neuronal functioning and cause neuronal death [25,89], which is a characteristic feature of neurodegenerative diseases. Therefore, it inhibits the release of proinflammatory molecules by dystrophic microglia because VNS increases the release of NE into the brain [83–85]; thus, it may be protective against neuronal loss. Additionally, VNS has been associated with an increased microglial length and microglial branching [41], as well as a reduction in the amounts of cerebral spinal fluid-tau proteins [33]. The transformation of the neurodestructive dystrophic microglial structures to a neuroprotective structural configuration, as well as a reduction in the CSF concentration of tau-proteins, which are proteins implicated in the pathogenesis of many neurodegenerative diseases, may suggest that VNS could potentially be used in the treatment of neurodegenerative conditions. However, the aforementioned effects of VNS related to structural changes and the consequent improvement of cognitive functions have not always been reproducible. For instance, there are numerous studies where VNS did not have any effect on cognition in general or in specific areas of cognitive abilities [62,91–94]. The inconsistent outcomes could be due to variations in the stimulation protocols. For instance, it has been demonstrated that the electrical VNS for memory enhancement exhibits an inverted U-shaped curve, wherein a stimulation at a moderate intensity of 0.4 mA was linked to memory enhancement, while no or very little positive effect was seen at lower intensities such as 0.2 or higher intensities such as 0.8 mA [60,95].

4.1. Strengths and limitations

There were some limitations to this review study. First, we only reviewed research published in English. The English language barrier may have resulted in the exclusion of several potentially relevant studies. However, a greater diversity in our publication sample could have provided a broader perspective on the use of VNS in dementia. Second, as a scoping review, we did not assess the methodological quality of the included research reports; therefore, we did not ascertain whether specific studies produced robust or generalizable results. This should be considered when interpreting the findings. However, the effects of VNS on cognitive function observed in multiple investigations were generally consistent, thus lending a degree of credibility to the findings. Furthermore, we worked hard to adequately document the review process so that future investigators might replicate the study,

thus increasing both the reliability of the findings and the methodological rigor [96,97]. This scoping review identified and summarized studies on the use of VNS in cognitive impairments based on the volume, nature, and characteristics of the original research that can be used in policies, practice, and future investigations.

5. Conclusion

The majority of published research on the impact of VNS in dementia have been undertaken on human participants in high-income nations. The overall effect of VNS in dementia is an enhanced cognitive performance, which suggests that it may be an effective non-pharmacological therapy for cognitive impairment in dementia-related diseases such as Alzheimer's disease.

Use of AI tools declaration

Not applicable.

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Authors contribution

Contributions to the scoping review's conceptualization and design came from RK, GZR, GN, AOI, and JO. WA and RK performed the systematic search across all databases. The data extraction and article screening were carried out by GN, RK, GZR and SK. Each author made a contribution to the data's analysis and interpretation. The scoping review was originally written by RK, and all authors edited the document to bring it to its current state and approved the submitted version.

Conflict of interest

All authors declared no conflict of interest.

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