

Decoding the underlying neural activity of neurodegeneration in Alzheimer's Disease

Haolin Li¹, Hong Guo²

¹ Crescent School, Toronto, Canada

² Department of Neurosurgery, Brigham and Women's Hospital, Boston, Massachusetts

SUMMARY

Alzheimer's Disease (AD) is the most prevalent chronic neurodegenerative disease, affecting more than 55 million people worldwide. AD is clinically characterized by a progressive loss of memory and other cognitive functions, which implicate the cholinergic system. Activity-induced neuroprotection based on gamma oscillation–audiovisual stimuli–is shown to be possible. Bulk RNA-seq data from treatment involving sensory stimuli were obtained, and bioinformatics analyses were performed. Activity-induced neuroprotection was found to be strongly associated with the downregulation of synaptic calcium signaling and cholinergic transmission. Candidate genes were screened in consultation with the Allen Brain Atlas Transcriptomics Explorer. We propose that *Chrn4* and *Plcb4* are potential therapeutic targets for AD treatment. *Chrn4* has already been identified as a potential immune-relevant drug target gene using ontology inference, network analysis, and methylation signal, while multiple studies suggest that primary phospholipase C proteins play an integral role in inducing cell death in AD. Hence, both *Chrn4* and *Plcb4* should be considered for future in vitro and in vivo studies as candidate genes.

Keywords: Alzheimer's Disease, cognitive impairment, cholinergic hypothesis, *Chrn4*, *Plcb4*

INTRODUCTION

Alzheimer's Disease (AD) is the most prevalent chronic neurodegenerative disease, affecting more than 55 million people worldwide. It is the sixth leading cause of death in the United States and the fifth leading cause of death among Americans aged 65 and older [1]. AD is progressive and irreversible, clinically characterized by a progressive loss of memory and other cognitive functions—ultimately inducing dementia [2,3]. Although not fully understood, the pathophysiology of AD is largely represented by the neurotoxic events triggered by the beta-amyloid ($A\beta$) cascade and by cytoskeletal abnormalities subsequent to the hyperphosphorylation of microtubule-associated Tau protein in neurons. These processes lead to the formation of neuritic plaques [4] and neurofibrillary tangles [5], respectively, which are recognized as pathological hallmarks of AD [6].

Currently, pharmacological treatments for AD are largely limited to antidementia drugs—for example, memantine [7]. While clinical benefits have been observed, these treatments are limited to temporary, symptomatic support to cognitive functions while the disease progresses. Therapies targeting $A\beta$ -plaques have been the focus of efforts to develop new pharmaceutical compounds with disease-modifying properties, but these approaches have failed to show any clinical promise [8]. On the other hand, neuroinflammation caused by microglial activation has emerged as an active area of research based on risk gene analysis [9,10], but there have yet to be any promising leads. Since AD is only 10% hereditary and 90% sporadic and since its exact cause remains unknown, there is no current method to prevent it.

The progressive cognitive decline of AD has been attributed to the observed cholinergic deficit [11]. Briefly, central cholinergic neurotransmission predominantly provides diffuse innervations, changes neuronal excitability, alters presynaptic release of neurotransmitters, and coordinates the firing of groups of neurons. In AD models, markers within cholinergic basal forebrain (CBF) neurons, which provide the major source of cholinergic innervation to the cerebral cortex and hippocampus, have been found to be downregulated; CBF cell loss and reduced cortical choline acetyltransferase activity were also noted [12,13,14]. Furthermore, CBF cortical projection neurons contain distinctive AD hallmarks and undergo neurochemical phenotypic alterations during disease progression [15]. In fact, delaying this neurodegeneration or minimizing its consequences is the mechanism of action for most currently available drug treatments for cognitive dysfunction in AD. Such cholinesterase inhibitors such as donepezil, rivastigmine, and galantamine reversibly inactivate cholinesterases, thereby inhibiting the hydrolysis of acetylcholine (ACh) and subsequently increasing ACh concentration in synapses. Moreover, since regions of the forebrain also contain non-cholinergic neurons—for instance,

GABAergic interneurons—and since neuropeptides—including inhibitory neuropeptide galanin (GAL) A β —often colocalize with CBF neurons [16], it is therefore likely that understanding neurotransmitter interactions, in particular Ach, may provide novel targets for the development of potential treatments.

Alterations to neurochemical states result in changes to neural activity. Many studies have used techniques such as optogenetics and frequency entrainment to investigate the effect of certain sensory stimuli on neural activity and responses. To start, gamma oscillation has been implicated in cognitive processing [17,18,19]. Also, it is prominent in the hippocampus, suggesting its role in memory functions [20,21]. It is therefore not surprising that disorders characterized by memory impairment, such as AD, are associated with aberrant activity of gamma oscillations [22]. In fact, Klein et al. (2016) reported changes in gamma oscillations in the entorhinal cortex at an early stage of AD in animal model [23]. In 2016 and 2019, Tsai lab [45, 46, 47] demonstrated that 40Hz gamma oscillation entrainment through audiovisual stimuli ameliorates AD hallmarks and initiates activity-induced neuroprotection. They hypothesized that their results are due to alternative phenotypic activation of microglia. However, microglial activation must correlate with endogenous processes of neuronal cells. In this study, we analyzed RNA-seq data from Tsai lab [45, 46, 47] and screened for candidate genes using bioinformatics analyses. We aimed to uncover the mechanism underlying gamma oscillation entrainment treatment [45, 46, 47] and identify candidate genes with neuronal functions for further investigation. We hypothesized that cholinergic pathway genes aid in the modulation of neural activity, thereby granting activity-induced neuroprotection. By altering specific subunit interactions, we propose Chrb4 and Plcb4 as potential therapeutic targets for AD treatment. Since audiovisual stimuli could be extended to incorporate music, we, here, present a way to quantify 40Hz content within any sound.

METHODS

Bioinformatics analyses:

Bulk RNA-seq data were obtained from the Gene Expression Omnibus Accession Viewer (GSE77471). Bioinformatics analyses were performed using RStudio. All of the statistical details for each analysis were described in the figure legends. A volcano plot in the form of p-value versus magnitude of change was created using the stat package. Genes with large fold changes that are statistically significant were isolated. GO analysis was subsequently performed with the subset comprising the genes of interest using the gprofiler2 package, and enriched terms were visualized using ggplot2 and GOplot [24]. The genes in the two most significant GO terms were

individually evaluated using the whole cortex and hippocampus single-cell RNA-seq data for mice from the Allen Brain Atlas Transcriptomics Explorer, from which mean neuronal expression levels were weighed against the types of cells with gene expression. 2 candidate genes were then selected.

Soundwave quantification:

R packages `tuneR` [25], `signal` [26], and `oce` [27] were used to cut wave files and build spectrograms. Using Fast Fourier Transform (FFT; an optimized algorithm for implementing Discrete Fourier Transformation), a sound sequence was sampled and divided into its spectral frequency components—single sinusoidal oscillations at distinct frequencies with unique amplitude and phase. The waveform was first plotted, and spectrogram parameters were then defined. After the code was functionalized, spectrograms were generated systematically.

RESULTS

Bioinformatics analyses show enrichment in synaptic nicotinic cholinergic transmission and cell-cell signaling:

To investigate the neuronal mechanism behind Tsai lab's [45, 46, 47] treatment and to uncover potential drug treatment target genes or physical therapy causes, we analyzed their 40Hz gamma oscillation entrainment treatment group bulk RNA-seq data. We began by identifying statistically significant (adj p-value < 0.05) and differentially-expressed (log fold change > 1) genes using a volcano plot (Figure 1A). We found 198 such genes of interest. To interpret biological phenomena underlying 40Hz treatment, we then conducted Gene Ontology (GO) analysis and ranked the GO terms with respect to their negative log adj p-values. This way, we can interpret high throughput molecular data and define enriched gene sets. Here, we see enrichment in synaptic transmission and cell-cell signaling (Figure 1B). To remove redundant and extraneous terms (Figure 1C), we then congregated terms with overlaps greater than 75 percent.

To better visualize significantly enriched terms, a circle plot (Figure 2A) was generated. Each dot, here, represents a specific gene, with blue representing downregulation and red representing upregulation. Most genes were downregulated in Tsai lab's [45, 46, 47] treatment. The two most significant GO terms were synaptic nicotinic cholinergic transmission and cell-cell signaling, with adjusted p-values of 0.00026 and 0.00040 respectively.

Identification of candidate genes from enriched GO terms:

To identify potential therapeutic target genes for AD, we further analyzed genes constituting the two most significant GO terms. Specifically, the 6 genes belonging to synaptic cholinergic transmission and the 32 genes belonging to cell-cell signaling were individually evaluated in consultation with the Allen Brain Atlas. For quantification, we calculated a weighted score for each gene from their mean neuronal expression level and the number of cell subtypes expressing them. Because all 6 cholinergic-related genes were also present in the cell-cell signaling term, we ultimately chose 16 genes out of the total 32 genes for further analysis, and a chord plot (Figure 2B) was generated to depict their relationship with each other. In choosing our final 2 candidate genes, we evaluated these 16 genes by building a protein network of known and predicted interactions using STRING: functional protein association networks (Figure S1).

Chrn4 (cholinergic receptor nicotinic β 4 subunit) was chosen as our first candidate gene because it was the most downregulated after treatment (Figure 2B); Plcb4 (phospholipase C β 4 isoform) was chosen as our second because of its sustained high neuronal expression levels in a wide array of cell types (Figure 2E). Their predicted protein structures (Figure 2C) and their neuronal expression level scatterplots (Figures 2D-E) were generated using AlphaFold and the Allen Brain Atlas, respectively. We note that, in normal mice, Chrn4 is expressed in specific neuron subtypes, while Plcb4 is previously known to be ubiquitously expressed only in neurons.

Soundwave quantification for future treatment design:

Besides identifying potential therapeutic target genes, we could project Tsai lab's [45, 46, 47] treatment with audiovisual stimuli onto music therapy, whereby pieces of music are chosen through quantification of their 40Hz content and utilized as a possible clinical intervention for AD. Therefore, an approach is needed to efficiently analyze and quantify 40Hz content within sound. To achieve this, spectrograms were generated to quantify the amplitude of sound waves over time. We chose to analyze a short 40 Hz test tone to verify the accuracy of our approach (Figure 3A). Then, a random piece of music was chosen to test the effectiveness of the approach in analyzing and distinguishing long, complex sound waves (Figure 3B). As Tsai lab [45, 46, 47] utilized 40Hz gamma oscillation entrainment, we, here, present a possible method to quantify the amount of 40Hz within any piece of music.

DISCUSSION

In this study, we screened for candidate genes using comprehensive bioinformatics analyses. We concluded that activity-induced neuroprotection is strongly associated with the

downregulation of synaptic calcium signaling and cholinergic transmission, specifically the nicotinic acetylcholine receptor (nAChR). We then isolated and identified *Chrnb4* and *Plcb4* as potential therapeutic targets for AD treatment. This study provided a glimpse into the underlying mechanisms of sensory stimuli and activity-induced neuroprotection. Our results may aid in the understanding of AD pathophysiology and contribute to the creation and translation of treatments, thereby helping individuals clinically and potentially improving patient outcomes.

Chrnb4, along with *Chrna3* and *Chrna5*, constitute the important heteromeric $\alpha 3\beta 4$ nAChR gene cluster. The nAChR subunits encoded by this locus form the predominant nicotinic receptor subtypes expressed in key central nervous system (CNS) sites, such as the medial habenula [28]. *Plcb4* codes for the strictly neuronal expressing phospholipase C (PLC) $\beta 4$ isoform, which mobilizes intracellular calcium by depletion of intracellular stores [29].

Because, as established, AD implicates the cholinergic system, it is therefore not surprising that our results indicate an association between AD treatment and restoration of cholinergic function. *Chrnb4* of the $\alpha 3\beta 4$ nAChR cluster has already been identified as a potential immune-relevant drug target gene using ontology inference, network analysis, and methylation signal [30,31]. Also, agonists of another nAChR, the homomeric $\alpha 7$ nAChR, have been shown to rescue A β -induced neurotoxicity and improve cognitive function in AD models [32]. Furthermore, this induced protection is blocked by an $\alpha 7$ receptor antagonist [33].

Regarding *Plcb4*, primary phospholipase C proteins PLC β and PLC γ are activated primarily by neurotransmitters. Among primary PLC isozymes, PLC $\gamma 1$, PLC $\beta 1$, and, more importantly, PLC $\beta 4$ are highly expressed and differentially distributed, suggesting a specific role for each PLC subtype in different regions of the brain. Primary PLCs are key enzymes in maintaining intracellular calcium concentration and sustaining signal, thereby controlling neuronal activity. Dysregulation of primary PLC signaling and disruption of calcium homeostasis have been linked to AD [34,35,36], with recent studies linking intracellular calcium overload to reduction in dendritic spines which, in turn, causes synaptic collapse and mediates neurodegeneration [37,38,39]. Yet, specific pathways and protein interactions remain unclear. For example, PLC- γ was abnormally accumulated in neurofibrillary tangles [40]. Moreover, PLC $\gamma 1$ gene expression was enriched in AD patients [41], and PLC- γ protein level was significantly higher in the cytosolic fraction of AD cortical tissue than in control brains; however, its specific activity is compromised in AD brains [42], suggesting that its inactivation might be related to the pathophysiology of AD. Also, restoration of PLC $\beta 1$ activation ameliorated hippocampal long-term potentiation impaired by A β plaque accumulation [43], but further elevation by A β facilitates calcium overload (Park et al., 2022). Therefore, other genes encoding primary phospholipase C

proteins—with emphasis on Plcb4—should be investigated as potential targets for protecting neurons against excitotoxicity in AD.

ACKNOWLEDGEMENTS

This study would not have been possible without the guidance and support of Dr. Yi Lu. This research received no funding.

REFERENCES

- [1] *What Is Alzheimer's Disease?* Centers for Disease Control and Prevention, 26 Oct. 2020, www.cdc.gov/aging/aginginfo/alzheimers.htm.
- [2] Selkoe, Dennis J. "The Molecular Pathology of Alzheimer's Disease." *Neuron*, vol. 6, no. 4, 1991, pp. 487–498., doi:10.1016/0896-6273(91)90052-2.
- [3] Bothwell, Mark, and Edward Giniger. "Alzheimer's Disease." *Cell*, vol. 102, no. 3, 2000, pp. 271–273., doi:10.1016/s0092-8674(00)00032-5.
- [4] Glenner, George, and Caine W. Wong. "Alzheimer's Disease: Initial Report of the Purification and Characterization of a Novel Cerebrovascular Amyloid Protein." *Biochemical and Biophysical Research Communications*, vol. 120, no. 3, 1984, pp. 885–890., doi:10.1016/s0006-291x(84)80190-4.
- [5] Bancher, Christian, et al. "Accumulation of Abnormally Phosphorylated τ Precedes the Formation of Neurofibrillary Tangles in Alzheimer's Disease." *Brain Research*, vol. 477, no. 1-2, 1989, pp. 90–99., doi:10.1016/0006-8993(89)91396-6.
- [6] Serrano-Pozo, Alberto, et al. "Neuropathological Alterations in Alzheimer Disease." *Cold Spring Harbor Perspectives in Medicine*, vol. 1, no. 1, 2011, doi:10.1101/cshperspect.a006189.
- [7] Barthold, Douglas, et al. "Pharmaceutical Treatment for Alzheimer's Disease and Related Dementias: Utilization and Disparities." *Journal of Alzheimer's Disease*, vol. 76, no. 2, 2020, pp. 579–589., doi:10.3233/jad-200133.
- [8] Yiannopoulou, Konstantina G., et al. "Reasons for Failed Trials of Disease-Modifying Treatments for Alzheimer Disease and Their Contribution in Recent Research." *Biomedicines*, vol. 7, no. 4, 2019, p. 97., doi:10.3390/biomedicines7040097.
- [9] Heneka, Michael T., et al. "Neuroinflammation in Alzheimer's Disease." *The Lancet Neurology*, vol. 14, no. 4, 2015, pp. 388–405., doi:10.1016/s1474-4422(15)70016-5.
- [10] Bachiller, Sara, et al. "Microglia in Neurological Diseases: A Road Map to Brain-Disease Dependent-Inflammatory Response." *Frontiers in Cellular Neuroscience*, vol. 12, 2018, doi:10.3389/fncel.2018.00488.
- [11] Hampel, Harald, et al. "Revisiting the Cholinergic Hypothesis in Alzheimer's Disease: Emerging Evidence from Translational and Clinical Research." *The Journal Of Prevention of Alzheimer's Disease*, 2018, pp. 1–14., doi:10.14283/jpad.2018.43.
- [12] Bowen, David M., et al. "Choline Acetyltransferase Activity and Histopathology of Frontal Neocortex from Biopsies of Demented Patients." *Journal of the Neurological Sciences*, vol. 57, no. 2-3, 1982, pp. 191–202., doi:10.1016/0022-510x(82)90026-0.

- [13] Davies, P., and A. J. Maloney. "Selective Loss of Central Cholinergic Neurons in Alzheimer's Disease." *The Lancet*, vol. 308, no. 8000, 1976, p. 1403., doi:10.1016/s0140-6736(76)91936-x.
- [14] Whitehouse, Peter J., et al. "Alzheimer's Disease and Senile Dementia: Loss of Neurons in the Basal Forebrain." *Science*, vol. 215, no. 4537, 1982, pp. 1237–1239., doi:10.1126/science.7058341.
- [15] Scheff, Stephen W., et al. "Synaptic Alterations in CA1 in Mild Alzheimer Disease and Mild Cognitive Impairment." *Neurology*, vol. 68, no. 18, 2007, pp. 1501–1508., doi:10.1212/01.wnl.0000260698.46517.8f.
- [16] Mufson, Elliott J., et al. "Cholinergic System during the Progression of Alzheimer's Disease: Therapeutic Implications." *Expert Review of Neurotherapeutics*, vol. 8, no. 11, 2008, pp. 1703–1718., doi:10.1586/14737175.8.11.1703.
- [17] Cannon, Jonathan, et al. "Neurosystems: Brain Rhythms and Cognitive Processing." *European Journal of Neuroscience*, vol. 39, no. 5, 2013, pp. 705–719., doi:10.1111/ejn.12453.
- [18] Bosman, Conrado A., et al. "Functions of Gamma-Band Synchronization in Cognition: From Single Circuits to Functional Diversity across Cortical and Subcortical Systems." *European Journal of Neuroscience*, vol. 39, no. 11, 2014, pp. 1982–1999., doi:10.1111/ejn.12606.
- [19] Cole, Scott R., and Bradley Voytek. "Brain Oscillations and the Importance of Waveform Shape." *Trends in Cognitive Sciences*, vol. 21, no. 2, 2017, pp. 137–149., doi:10.1016/j.tics.2016.12.008.
- [20] Colgin, Laura Lee, and Edvard I. Moser. "Gamma Oscillations in the Hippocampus." *Physiology*, vol. 25, no. 5, 2010, pp. 319–329., doi:10.1152/physiol.00021.2010.
- [21] Carr, Margaret F., et al. "Transient Slow Gamma Synchrony Underlies Hippocampal Memory Replay." *Neuron*, vol. 75, no. 4, 2012, pp. 700–713., doi:10.1016/j.neuron.2012.06.014.
- [22] Mably, Alexandra J., and Laura Lee Colgin. "Gamma Oscillations in Cognitive Disorders." *Current Opinion in Neurobiology*, vol. 52, 2018, pp. 182–187., doi:10.1016/j.conb.2018.07.009.
- [23] Klein, Alexandra S., et al. "Early Cortical Changes in Gamma Oscillations in Alzheimer's Disease." *Frontiers in Systems Neuroscience*, vol. 10, 2016, doi:10.3389/fnsys.2016.00083.
- [24] Walter, Wencke, et al. "GOplot: An R Package for Visually Combining Expression Data with Functional Analysis: Fig. 1." *Bioinformatics*, vol. 31, no. 17, 2015, pp. 2912–2914., doi:10.1093/bioinformatics/btv300.
- [25] Ligges, Uwe, et al. *TuneR: Analysis of Music and Speech*. Comprehensive R Archive Network (CRAN), 29 Mar. 2022, cran.r-project.org/package=tuneR.

- [26] Signal Developers. *Signal: Signal Processing*. R-Forge, 2014, r-forge.r-project.org/projects/signal/.
- [27] Kelley, Dan E. "Oceanographic Analysis with R." 2018, doi:10.1007/978-1-4939-8844-0.
- [28] Improgo, M. R., et al. "From Smoking to Lung Cancer: The CHRNA5/A3/B4 Connection." *Oncogene*, vol. 29, no. 35, 2010, pp. 4874–4884., doi:10.1038/onc.2010.256.
- [29] Glaser, Talita, et al. "Various Facets of Excitotoxicity." *Exploration of Neuroprotective Therapy*, 2022, pp. 36–64., doi:10.37349/ent.2022.00017.
- [30] Han, Zhi-Jie, et al. "Identification of Novel Immune-Relevant Drug Target Genes for Alzheimer's Disease by Combining Ontology Inference with Network Analysis." *CNS Neuroscience & Therapeutics*, vol. 24, no. 12, 2018, pp. 1253–1263., doi:10.1111/cns.13051.
- [31] Li, Zhan-Dong, et al. "Detecting Brain Structure-Specific Methylation Signatures and Rules for Alzheimer's Disease." *Frontiers in Neuroscience*, vol. 16, 2022, doi:10.3389/fnins.2022.895181.
- [32] Ma, Kai-Ge, and Yi-Hua Qian. "Alpha 7 Nicotinic Acetylcholine Receptor and Its Effects on Alzheimer's Disease." *Neuropeptides*, vol. 73, 2019, pp. 96–106., doi:10.1016/j.npep.2018.12.003.
- [33] Shimohama, Shun, and Takeshi Kihara. "Nicotinic Receptor-Mediated Protection against β -Amyloid Neurotoxicity." *Biological Psychiatry*, vol. 49, no. 3, 2001, pp. 233–239., doi:10.1016/s0006-3223(00)01100-8.
- [34] Lechner, Stefan G., et al. "Presynaptic Inhibition via a Phospholipase C- and Phosphatidylinositol Bisphosphate-Dependent Regulation of Neuronal Ca^{2+} Channels." *Molecular Pharmacology*, vol. 68, no. 5, 2005, pp. 1387–1396., doi:10.1124/mol.105.014886.
- [35] Tong, Benjamin Chun-Kit, et al. "Calcium Signaling in Alzheimer's Disease & Therapies." *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1865, no. 11, 2018, pp. 1745–1760., doi:10.1016/j.bbamcr.2018.07.018.
- [36] Alzheimer's Association Calcium Hypothesis Workgroup, and Zaven S. Khachaturian. "Calcium Hypothesis of Alzheimer's Disease and Brain Aging: A Framework for Integrating New Evidence into a Comprehensive Theory of Pathogenesis." *Alzheimer's & Dementia*, vol. 13, no. 2, 2017, p. 178., doi:10.1016/j.jalz.2016.12.006.
- [37] Arbel-Ornath, Michal, et al. "Soluble Oligomeric Amyloid- β Induces Calcium Dyshomeostasis That Precedes Synapse Loss in the Living Mouse Brain." *Molecular Neurodegeneration*, vol. 12, no. 1, 2017, doi:10.1186/s13024-017-0169-9.

- [38] Mucke, Lennart, and Dennis J. Selkoe. "Neurotoxicity of Amyloid β -Protein: Synaptic and Network Dysfunction." *Cold Spring Harbor Perspectives in Medicine*, vol. 2, no. 7, 2012, doi:10.1101/cshperspect.a006338.
- [39] Patel, Aarti N., and Jack H. Jhamandas. "Neuronal Receptors as Targets for the Action of Amyloid-Beta Protein ($A\beta$) in the Brain." *Expert Reviews in Molecular Medicine*, vol. 14, 2012, doi:10.1017/s1462399411002134.
- [40] Shimohama, Shun, et al. "Aberrant Accumulation of Phospholipase C-Delta in Alzheimer's Brains." *The American Journal of Pathology*, vol. 139, no. 4, 1991, pp. 737–742., doi:10.1016/0197-4580(92)90359-6.
- [41] Kim, Sung-Hyun, et al. "Prediction of Alzheimer's Disease-Specific Phospholipase C Gamma-1 SNV by Deep Learning-Based Approach for High-Throughput Screening." *Proceedings of the National Academy of Sciences*, vol. 118, no. 3, 2021, doi:10.1073/pnas.2011250118.
- [42] Shimohama, Shun. "Apoptosis in Alzheimer's Disease—an Update." *Apoptosis*, vol. 5, no. 1, 2000, pp. 9–16., doi:10.1023/a:1009625323388.
- [43] Lee, Jaedong, and Jeehyun Kwag. "Activation of PLC β 1 Enhances Endocannabinoid Mobilization to Restore Hippocampal Spike-Timing-Dependent Potentiation and Contextual Fear Memory Impaired by Alzheimer's Amyloidosis." *Alzheimer's Research & Therapy*, vol. 13, no. 1, 2021, doi:10.1186/s13195-021-00901-9.
- [44] Park, Jiyu, et al. "Elevation of Phospholipase C- β 1 Expression by Amyloid- β Facilitates Calcium Overload in Neuronal Cells." *Brain Research*, vol. 1788, 2022, p. 147924., doi:10.1016/j.brainres.2022.147924.
- [45] Adaikkan, Chinnakkaruppan, et al. "Gamma Entrainment Binds Higher-Order Brain Regions and Offers Neuroprotection." *Neuron*, vol. 102, no. 5, 2019, pp. 929–943., doi:10.1016/j.neuron.2019.04.011.
- [46] Iaccarino, Hannah F., et al. "Gamma Frequency Entrainment Attenuates Amyloid Load and Modifies Microglia." *Nature*, vol. 540, no. 7632, 2016, pp. 230–235., doi:10.1038/nature20587.
- [47] Martorell, Anthony J., et al. "Multi-Sensory Gamma Stimulation Ameliorates Alzheimer's-Associated Pathology and Improves Cognition." *Cell*, vol. 177, no. 2, 2019, pp. 256–271., doi:10.1016/j.cell.2019.02.014.

Figures and Figure Captions

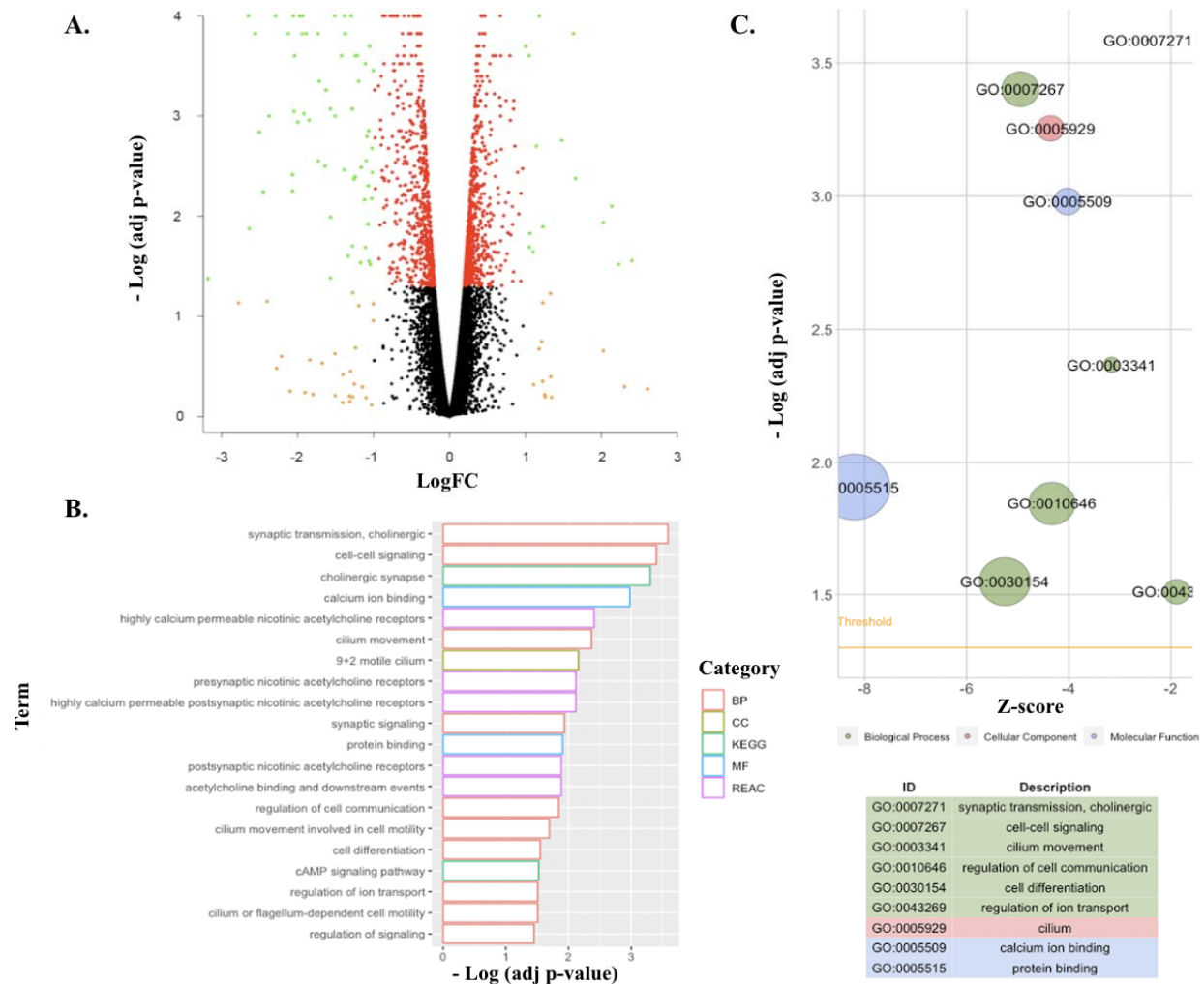


Figure 1. Analyses show enrichment in synaptic nicotinic cholinergic transmission and cell-cell signaling. A. Volcano plot determining the 198 genes of interest. Red: adj p < 0.05. Orange: |LogFC| > 1. Green: adj p < 0.05 and |LogFC| > 1. B. Bar plot ranking the GO terms by statistical significance (adj p < 0.05). C. Extraneous and redundant terms removed via term reduction and z-score added to predict regulation.

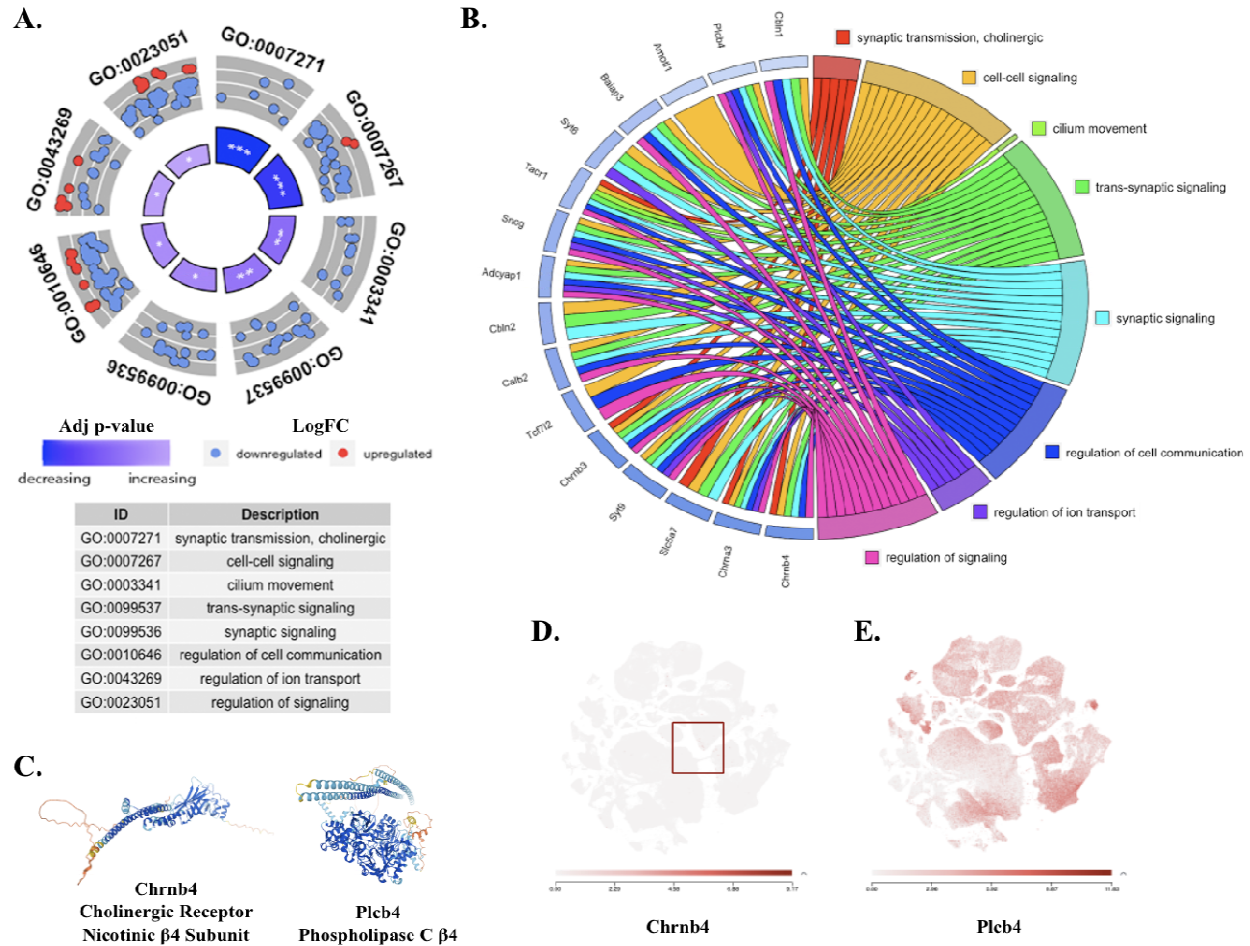


Figure 2. Identification of candidate genes from enriched GO terms. A. Visualization of individual differentially expressed genes in chosen GO terms. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. B. Selected candidate genes mapped. Blue outlines represent the degree of downregulation. Final candidate genes: Chrnb4 and Plcb4. C. Predicted protein structures of candidate genes. D-E. Neuronal expression levels of candidate genes.

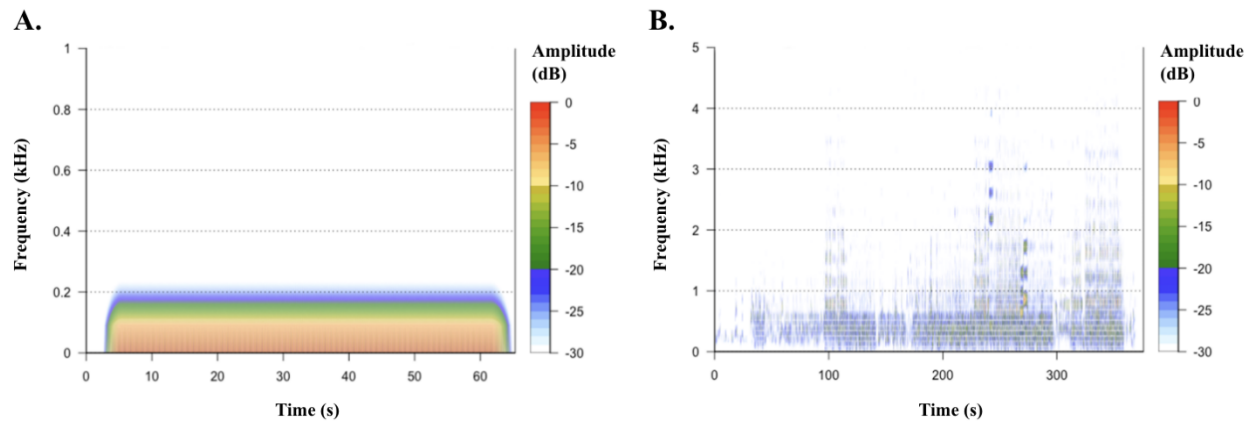
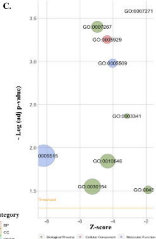
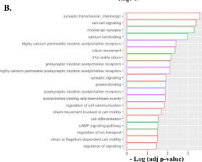
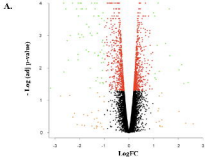
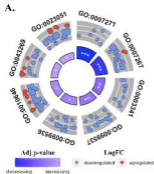


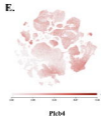
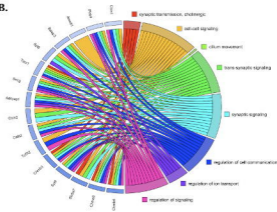
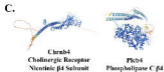
Figure 3. Soundwave quantification for future treatment design. A. 40 Hz test tone. B. Piece of music.

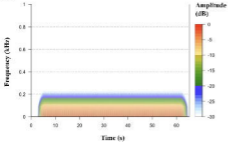


ID	Description
GO:0003271	synaptic transmission, cholinergic
GO:0003267	cell-cell signaling
GO:0003041	cellular movement
GO:0003048	regulation of cell communication
GO:0003044	cell differentiation
GO:0003049	regulation of ion transport
GO:0003046	cellular ion binding
GO:0003045	protein binding



ID	Description
GO:0007271	synaptic transmission, cholinergic
GO:0007267	cell-cell signaling
GO:0003041	cellular movement
GO:0099537	trans-synaptic signaling
GO:0099536	synaptic signaling
GO:0010644	regulation of cell communication
GO:0043269	regulation of ion transport
GO:0023051	regulation of signaling



A.**B.**