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Translational Research & Clinical Interventions

SHORT REPORT

CSF proteomics reveals changes in myelin and synaptic biology after Spectris treatment

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Abstract

INTRODUCTION: Brain steady-state gamma oscillations evoked using a non-invasive medical device (Spectris) have shown potential clinical benefits in patients with mild–moderate Alzheimer's disease (AD), including reduced functional and cognitive decline, reduced brain volume and myelin loss, and increased brain functional connectivity. We analyzed changes in cerebrospinal fluid (CSF) proteins after Spectris treatment in mild cognitive impairment (MCI) and their relationship to established biological pathways implicated in AD.

METHODS: Unbiased proteomic analysis of CSF samples from participants with amyloid-positive MCI (n = 10) was conducted from the FLICKER (NCT03543878) clinical trial. Participants used the Cognito Therapeutics medical device (Spectris), confirmed to evoke steady-state gamma oscillations. Participants were instructed to use the device daily for 1 hour each day during the trial. CSF was collected prior to the start of stimulation and after 4 and 8 weeks of treatment. The proteome was analyzed using tandem mass tag mass spectrometry.

RESULTS: Differential expression analysis of proteins at baseline and after 8 weeks of treatment (N = 5) revealed that 110 out of 2951 proteins met the significance threshold (analysis of variance, $P < 0.05$, no false discovery rate). Sixty proteins were upregulated, and 50 proteins were downregulated after treatment. Changes in protein expression were mapped to the consensus human AD protein network, representing co-expressed and functionally linked modules linked to cell type and biochemical pathways. Treatment altered CSF proteins linked to AD-related brain proteome modules, including those involved in myelination (proteolipid protein 1, ecotropic viral integration site 2A), synaptic and neuroimmune functions, and regulation of cellular lipid transportation. Biological pathway analysis revealed that most impacted pathways were associated with lipoproteins, cholesterol, phospholipids processing, and phosphatidylcholine biosynthesis.

DISCUSSION: The CSF proteomic changes observed in this study suggest pleiotropic effects on multiple pathways involved in AD, including myelination, synaptic and neuroimmune function, and lipid transport. These findings are also consistent with observations of white matter and myelin preservation after Spectris treatment of AD.

KEYWORDS
Alzheimer's disease, cerebrospinal fluid proteins, complement system, evoked gamma oscillations, lipid biology, myelin biology

Highlights

- We analyzed changes in cerebrospinal fluid (CSF) proteins in response to sensory-evoked gamma oscillations in individuals with mild cognitive impairment.
- Sensory evoked steady-state gamma oscillations were evoked by Spectris medical device.
- Changes in CSF proteins were observed after 8 weeks of daily 1 hour treatment.
- Affected proteins were related to myelination, synaptic and neuroimmune functions, and regulation of cellular lipid transportation.
- Proteomic changes support clinical outcomes and myelin preservation of Spectris treatment.

1 | INTRODUCTION

Recent experimental and clinical studies have demonstrated the beneficial effects of sensory-evoked 40 Hz steady-state gamma oscillations on Alzheimer's disease (AD) pathologies, including reduced neurodegeneration and brain atrophy, as well as the preservation of synaptic density and function.[1] In a clinical trial (FLICKER; NCT03543878), sensory-evoked gamma oscillations improved functional connectivity and reduced cerebrospinal cytokines and neuroinflammatory markers in mild cognitive impairment (MCI) participants.[2] In AD participants, 40 Hz sensory stimulation reduced brain atrophy and improved brain functional connectivity and performance in cognitive tasks.[3] More recently, the OVERTURE (NCT03556280) clinical trial demonstrated reduced decline in cognitive and functional abilities and reduced brain atrophy in participants with mild to moderate AD, after a 6-month treatment with the Cognito Therapeutics Spectris medical device.[4] Treatment also reduced magnetic resonance imaging (MRI) white matter volume loss and preserved myelin content (T1-weighted/T2-weighted ratio) in several brain regions, most prominently in the entorhinal region.[5] To further explore mechanisms underlying the observed clinical outcomes, we performed an unbiased proteomic analysis of cerebrospinal fluid (CSF) from participants at baseline and after treatment in FLICKER clinical trial participants. We then mapped these changes in the CSF proteome to a consensus brain network to better define the impact of therapy on putative central nervous system–derived protein signatures.

2 | METHODS

Details of the FLICKER trial design and participants' baseline characteristics have been published previously[2] and summarized in the Methods S1 in supporting information. In brief, participants were randomly assigned to undergo sensory stimulation using Cognito Therapeutics' Spectris device (description in Methods S1) for 1 hour per day for either 8 weeks or 4 weeks. Here, we compared changes in CSF proteomics between baseline and 8 weeks of treatment, the only time point showing significant changes in CSF cytokines and immune factors[2]. Furthermore, these protein abundance changes were compared to those in participants from the delayed-start group, who did not receive stimulation during the first 4 weeks and were referred to as the "no-stimulation" group or condition. Proteomics were measured using unbiased tandem mass tag mass spectrometry methods published previously[6] (Methods S1 and Figure S1 in supporting information). We used previously established co-expression protein modules in the brain as a reference[7] to assess the impact of Spectris treatment on pathophysiological pathways related to AD and GO Elite (version 1.2.5).

3 | RESULTS

3.1 | Changes in CSF protein in response to treatment

Protein abundance data were tabulated, and a total of 2785 CSF proteins were selected for further analysis after excluding all proteins that were not reliably detected in at least 50% of the CSF samples. The data were normalized[8] and log transformed. Using a $P < 0.05$ threshold for significance, without applying false discovery rate (FDR) correction, 110 proteins were found to be nominally significantly differentially expressed between the no-stimulation and 8-week treatment conditions. Sixty proteins were upregulated, and 50 proteins were downregulated after treatment (Figure 1A). Many of the most significantly changed proteins were related to oligodendrocyte function and myelination, such as proteolipid protein 1 (PLP1), ecotropic viral integration site 2A (EVI2A), and synapse/neuronal function (e.g., teneurin transmembrane protein 3 [TENM3]). The highest number of changed proteins were related to the complement/acute phase pathway (including complement factor properdin [CFP], carbonic anhydrase 3 [CA3], apolipoprotein 1 [APOL1], afamin [AFM], and apolipoprotein A-II [APOA2]; Figure 1B, Figure S2A in supporting information). Some of the proteins that were most affected after 8 weeks of treatment also showed a similar trend after 4 weeks of treatment (Figure S3 in supporting information).

3.2 | Treatment effects on brain protein modules associated with AD

Changes in CSF protein levels were mapped to previously established modules representing co-expressed and functionally linked networks of proteins in human brain tissue, which have been shown to be associated with AD pathology.[7] Among the 110 proteins differentially expressed by the treatment, 42 were mapped to previously identified AD modules.[7] Sensory-evoked gamma oscillations primarily altered those proteins associated with M26-complement/acute phase module (10 proteins); M3 oligodendrocyte/myelination module (4 proteins); and modules related to synaptic or neuronal functions, such as M1-synapse/neuron (4 proteins), M5 postsynaptic density (3 proteins), M4 synapse/neuron (2 proteins), and M36-neurotransmitter regulation (2 proteins; Figure 2A and Figure S2A). The M3 oligodendrocyte/myelination module showed significant changes in the abundance of four proteins (PLP1, EVI2A, lectin galactoside-binding soluble 3 binding protein, tenascin C). Changes were observed in other proteins regulating synaptic function, synaptic plasticity, and neuronal network development, including leucine rich glioma inactivated 1 (LGI1), TENM3, ephrin type-A receptor 7 (EPHA7), and Down syndrome cell adhesion molecule (DSCAM). We also found changes in leukotriene A4 hydrolase (LTA4H) and carbonic anhydrase-related protein 8 (CA8), proteins in the mitogen-activated protein kinase (MAPK) module strongly linked to cognition,[8] and SNCG, a protein linked to neurodegeneration (Figure 1B, Figure S2A).

RESEARCH IN CONTEXT

1. **Systematic review**: The authors performed a literature search using PubMed and Google Scholar to identify relevant publications and meeting abstracts. The most relevant papers identified are appropriately cited.
2. **Interpretation**: Experimental research demonstrated that sensory-evoked gamma oscillations attenuate multiple pathological mechanisms in various neurological disease models, including Alzheimer's disease (AD). Beneficial effects of sensory-evoked gamma oscillations were also reported in clinical trials in participants with prodromal, mild, and moderate AD, including reduced decline in cognitive and function abilities as well as brain volume and myelin loss. Present results showed changes in protein abundance in cerebrospinal fluid (CSF) after 8 weeks of sensory-evoked gamma oscillations. Affected proteins were primarily related to myelination, synaptic and neuroimmune functions, and regulation of cellular lipid transportation
3. **Future directions**: The CSF proteomic changes observed in this study suggest pleiotropic effects on multiple pathways involved in AD, consistent with clinical observations. Extended follow-up studies will delineate the most relevant mechanisms underlying the clinical benefits of sensory-evoked gamma oscillations.

To assess whether treatment-mediated changes in protein abundances were in the same or opposite directions to changes due to AD, CSF proteomics data of FLICKER subjects was compared to CSF reference standards pooled from biomarker-confirmed AD and control cases[6] to gain insights on how treatment impacts pathologies associated with the disease (Figure S2B). Changes in several proteins associated with myelination, including PLP1 and EVI2A, synaptic and neuroimmune functions, and regulation of cellular lipid transportation showed changes in the opposite direction of those reported in AD relative to healthy controls. For example, PLP1 protein abundance is increased in CSF in AD versus control cases, while 8 weeks of treatment with sensory stimulation lowered the protein abundance. However, there were also proteins showing concordant changes in response to treatment to those observed in AD, including mannosidase alpha class 1B member 1 and LTA4H. For example, LTA4H protein abundance is higher in AD CSF, and similarly, gamma stimulation increased the abundance of this protein compared to no stimulation.

3.3 | Analysis of biological processes

Biological pathway analysis using GO Elite software (genmapp.org) was carried out to identify additional structural and functional bio-

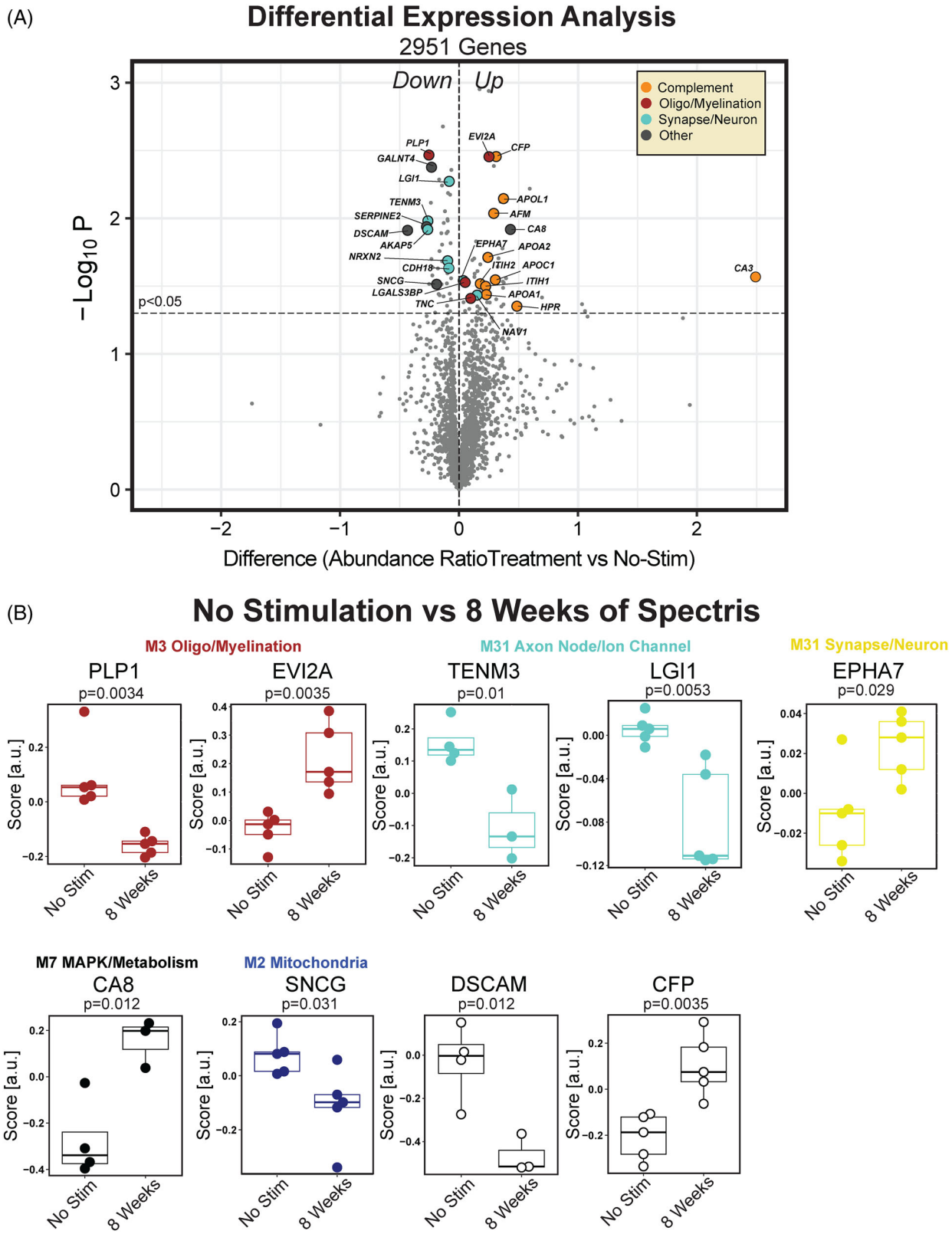

FIGURE 1 Identification of proteins differentially impacted by sensory evoked gamma oscillation. A, Volcano plot displaying the log2-transformed difference in abundance ratio (*x* axis) against the −log10 statistical *P* value (*y* axis) for all proteins demonstrating differential responses after no stimulation (No-Stim) or 8 weeks of gamma sensory stimulation (Spectris) treatment. Proteins above dashed line ($P < 0.05$, no FDR) indicate significantly different abundance. Modulated proteins closely linked to previous clinical outcome results, such as proteins belonging to oligodendrocyte/myelination (red), synapse/neuron (teal), and complement (orange) modules are indicated with colored data points. B, Box plots demonstrate the log2-transformed ratio of post-treatment panel abundance levels relative to pre-treatment (*Z* score) in no stimulation (No-Stim) or 8 weeks of gamma sensory stimulation (Spectris) treatment groups. Significant differences ($p < 0.05$) in abundance after 8 weeks of gamma

logical pathways beyond those revealed by brain co-expression mapping (Figure 2B). Biological pathways that were most significantly altered included mechanisms associated with lipoproteins, cholesterol, phospholipids processing, and phosphatidylcholine biosynthesis. Furthermore, synaptic and immune functions were also impacted, such as negative regulation of cytokine production, regulation of synaptic transmission, leukocyte-mediated immunity, response to growth factor stimulation, cell activation involved in immune response, and glial cell differentiation.

4 | DISCUSSION

The present biomarker findings from the FLICKER study demonstrate significant alterations in the CSF proteome after 8 weeks of Spectris treatment in participants with MCI due to AD. Several proteins previously linked to AD pathophysiology were altered by the treatment, including proteins associated with myelin biology, synaptic function, and the complement system. The observed protein modifications are consistent with treatment effects on brain functional connectivity, MRI brain structure, and myelin preservation and should be confirmed and extended in larger future studies of Spectris treatment, such as the HOPE pivotal trial in mild–moderate AD (NCT05637801).

Previous work, applying unbiased integrative proteomics analyses to the AD brain and CSF, identified network-based CSF proteins associated with a broad range of brain pathologies, including altered synaptic, vascular, myelin, immunological, and metabolic functions, grouped into protein modules.[9] Nearly half of the proteins altered by Spectris treatment were mapped to the established AD-associated brain modules, indicating an impact on pathways relevant to AD pathophysiology. Significant changes in the abundance of four proteins of the oligodendrocyte/myelination module were detected, potentially linked to the preservation of brain white matter and myelination.[10] Furthermore, 12 of the 19 most significantly impacted biological processes analyzed with Gene Ontology were linked to lipoproteins, phospholipids, and cholesterol processing, which are connected to myelin biology.[11] Most recently, two preclinical studies demonstrated attenuated myelin loss in response to sensory gamma stimulation in the cuprizone- and chemotherapy-induced demyelination mouse models, via mechanisms of oligodendrocyte activation and diminished neuroinflammation.[12,13] The current proteomics data also showed that most of the affected proteins belonged to the complement/acute phase AD module, consistent with the previously reported effect on cytokines and neuroinflammatory markers.[2] Neuroimmune dysfunction is a critical pathophysiological mechanism in AD,[14] therefore downregulation of immune factors and alterations of the neuroimmune system after 8 weeks of Spectris treatment could potentially contribute to the observed clinical effects.

Changes were observed in other proteins regulating synaptic function, synaptic plasticity, and neuronal network development, including LGI1, TENM3, EPHA7, DSCAM, and phosphatidylcholine biosynthesis relating to synaptic preservation by gamma sensory stimulation in preclinical animal models[15–18] and clinical findings showing cognitive benefits.[3,4] Interestingly, we observed changes in LTA4H and CA8, proteins in the MAPK module. In the brain, the MAPK M7 module is the most strongly associated with cognition.[7] The MAPK pathway is activated by synaptic activity, is known to affect both synaptic plasticity and immune function, and is rapidly activated by gamma sensory stimulation in mice.[18] Last, synuclein gamma (SNCG), a protein linked to neurodegeneration, exhibited significant decreases in abundance in the CSF after 8 weeks of treatment.

Our results reveal that sensory-evoked gamma oscillations may influence myelin biology, synaptic function, and the complement system, suggesting that the therapy may be affecting several pathways linked to AD cellular phenotypes. These findings also align with experimental results demonstrating that the treatment modulates multiple genes and proteins involved in synaptic function, myelin integrity, and neuroimmune biology across various preclinical neurodegenerative disease models.[15–20] Study limitations include a small sample size and short treatment time. Nevertheless, this unbiased exploratory proteomics analysis identified 110 proteins, out of 2785 analyzed, that exhibited nominally significant changes after the treatment. Further investigations are warranted with targeted analysis of selected protein candidates in trials with extended treatment durations.

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CONFLICT OF INTEREST STATEMENT

Mihály Hajós, Monika Shpokayte, Zach Malchano, and, Ralph Kern are/were employees and own stock options in Cognito Therapeutics, Inc. Mihály Hajós, Zach Malchano, and Ralph Kern have patent applications assigned to Cognito Therapeutics, Inc. Annabelle C. Singer owns shares in Cognito Therapeutics, which aims to develop gamma stimulation-related products; these conflicts of interest have been disclosed to and are managed by the Georgia Institute of Technology

sensory stimulation compared to No-Stim were identified via Student *t* test (*P* values shown above each plot). Significant differences in the abundance of selected proteins are shown, including proteins associated with myelination (PLP1 and EVI2A), axon node/ion channel, neuronal and synaptic function and plasticity (LGL1, TENM3, DSCAM), immune function (CFP), MAPK pathway (CA8) and SNCG. CA8, carbonic anhydrase-related protein 8; CFP, complement factor properdin; DSCAM, Down syndrome cell adhesion molecule; EVI2A, ecotropic viral integration site 2A; FDR, false discovery rate; LGL1, lethal giant larvae 1; MAPK, mitogen-activated protein kinase; PLP1, proteolipid protein 1; SNCG, synuclein gamma; TENM3, teneurin transmembrane protein 3.

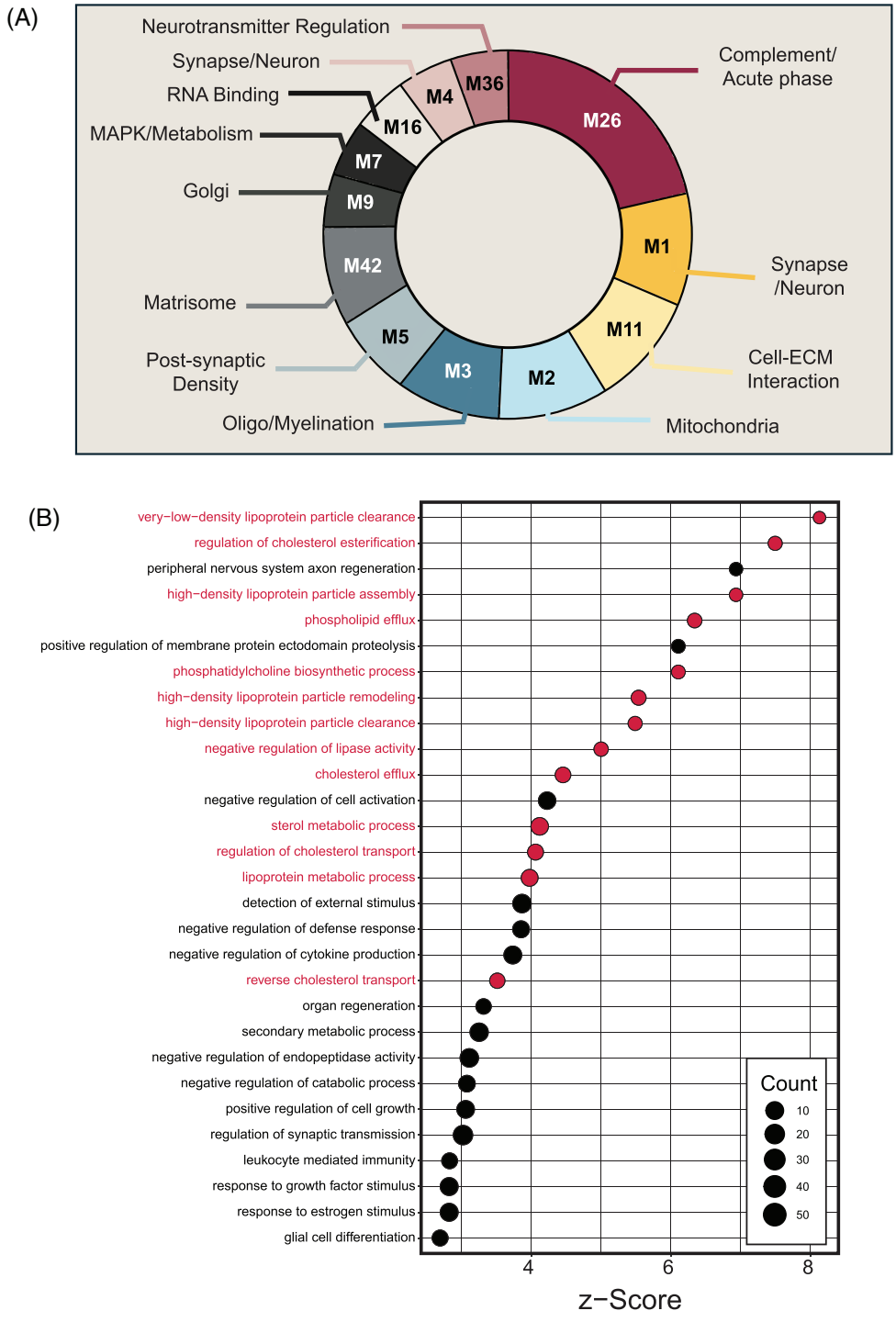

FIGURE 2 Proteins and biological processes impacted by sensory-evoked gamma oscillation. A, Proteins with significantly ($P \leq 0.05$) different abundance after treatment compared to no stimulation were mapped to previously established AD co-expression modules.[8] The related categories for each module are shown in corresponding colors on the pie chart. The representation of the number of proteins ascribed to the module is reflected by the relative size of the slice of pie. B, Biological pathway analysis using GO Elite software revealed that the biological processes most impacted by gamma sensory stimulation included 13 mechanisms (in red) which are related to myelination biology. AD, Alzheimer's disease; ECM, extracellular matrix; MAPK, mitogen-activated protein kinase.

Office of Research Integrity Assurance. Allan I. Levey serves as a consultant to Cognito Therapeutics. Kiran Pandey, Duc Duong, Allan I. Levey, Nicholas T. Seyfried are co-founders, employees, consultants, and/or shareholders of EmTheraPro. The authors declare no other competing financial interests. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All human subjects provided written informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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