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Multisession gamma tACS modulates extracellular vesicle dynamics

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Dear Editor,

Recent literature has demonstrated alterations in gamma-band oscillations in patients with Alzheimer's disease (AD), implicated in deficits in memory, neural communication, and synaptic plasticity [1]. Dysregulation of gamma oscillations can be modulated through different approaches, including sensory and electrical stimulation [1,2]. Among these, transcranial alternating current stimulation (tACS) is a non-invasive brain stimulation technique capable of entraining endogenous oscillatory activity to an externally applied alternating current at a specific frequency [3]. In a double-blind, randomized, sham-controlled clinical trial, it was recently demonstrated that multisession gamma tACS applied over the precuneus resulted in significant clinical efficacy, cholinergic improvement, and gamma entrainment in patients with mild AD [4]. However, the biological mechanisms underlying its clinical efficacy remain largely unknown. Notably, no significant effects were observed in blood-based markers of AD pathology, including phosphorylated tau 217 (p-tau217), markers of neurodegeneration such as neurofilament light chain (NfL), or markers of astroglial activation such as glial fibrillary acidic protein (GFAP) [4]. This lack of effect may reflect the fact that these conventional biomarkers primarily index cumulative neuropathological burden, which may be insensitive to short-to medium-term functional modulation induced by tACS. Consequently, alternative biological pathways capable of capturing activity-dependent and network-level effects of neuromodulation, particularly those related to synaptic function and intercellular communication, should be considered. In this context, extracellular vesicles (EVs) represent a promising candidate. EVs are nano-sized membranous particles which are released by virtually all cell types and have been implicated in several mechanisms relevant to AD pathophysiology [5]. EVs have been shown to contribute to the dissemination of toxic proteins, promote neuronal loss, and exacerbate neuroinflammation. Nonetheless, EVs may also exert neuroprotective effects by reducing amyloid-β deposition and facilitating the intercellular transfer of protective molecules, thereby mitigating disease-related mechanisms [6]. EVs may also represent an emerging peripheral diagnostic biomarker for AD, as they reflect disease-related molecular changes occurring in the brain [7,8]. Because closely linked to cell-to-cell communication, EVs may provide a more sensitive and specific biological readout of the effects induced by neuromodulation therapies.

In this study, we explored the biological effects of gamma tACS by assessing treatment-related modifications in plasma EVs in the 50 participants enrolled in the clinical trial by Cantoni et al. [4]. Patients were randomized to receive either 40 Hz gamma tACS (5 days per week, 60 minutes per day, for 8 weeks; n = 26) or sham stimulation (n = 24). Peripheral blood samples were collected at baseline (T0) and after the 8-week intervention (T1). Detailed descriptions of the cohort, plasma EV isolation and analysis to determine EV concentration and size, and the statistical framework applied are provided in the **Supplementary Methods**.

Our results reveal, for the first time, that gamma tACS induces measurable changes in systemic EV profiles (Table S1). Repeated-measures ANOVA revealed a significant time × treatment interaction for EV concentration ($F(1,48) = 6.51$, $p = 0.014$), characterized by a significant reduction in the gamma tACS group compared with the sham tACS group (Cohen's $d = -0.44$, $p = 0.004$; Fig. 1A). No significant time × treatment interaction was observed for EV size ($F(1,48) = 0.41$, $p = 0.524$; Fig. 1B). Interestingly, changes in EV concentration positively correlated with changes in Clinical Dementia Rating Scale-Sum of Boxes (ΔCDR-SB), a measure of disease severity ($r = 0.321$, $p = 0.023$; Fig. 1C), while no association was observed between changes in CDR-SB scores and EV size ($r = -0.007$, $p = 0.961$; Fig. 1D). No significant association between EV parameters and the other cognitive assessment performed were reported.

In this study, we provide novel evidence that multisession gamma tACS exert measurable biological effects beyond its previously demonstrated clinical efficacy [4]. To the best of our knowledge, this is the first study linking a non-invasive brain stimulation intervention to systemic changes in EV levels. EVs are increasingly recognized as key contributors to AD pathophysiology, mediating both the dissemination of pathogenic proteins, as well as the intercellular transfer of neuroprotective factors that may counteract disease progression [6]. Alterations in EV profiles have been consistently reported in AD patients compared with cognitively healthy individuals at both the peripheral (plasma) and central (CSF) levels [8,9]. Importantly, these EV alterations have been shown to

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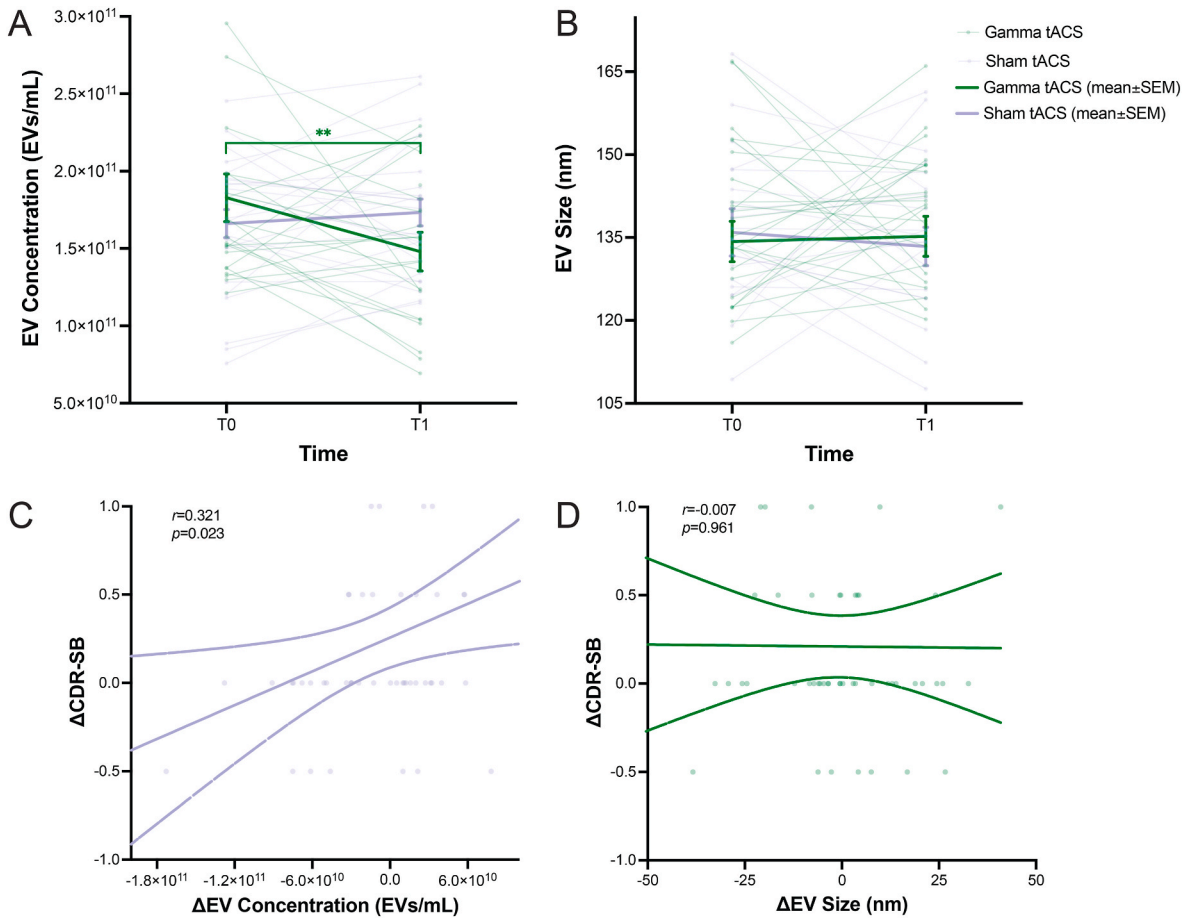

Fig. 1. Extracellular vesicle (EV) measures at baseline (T0) and post-intervention (T1) and their associations with clinical stage, as measured by the Clinical Dementia Rating–Sum of Boxes (ΔCDR-SB).

Panel A. EV concentration (EVs/mL), showing a significant time × treatment interaction ($F(1,48) = 6.51$, $p = 0.014$). ***Panel B.*** EV size (nm), showing no significant time × treatment interaction ($F(1,48) = 0.41$, $p = 0.524$). ***Panel C.*** Pearson's correlation between ΔCDR-SB (T1 − T0) and ΔEV concentration (EVs/mL; $r = 0.321$, $p = 0.023$). ***Panel D.*** Pearson's correlation between ΔCDR-SB (T1 − T0) and ΔEV size (nm; $r = -0.007$, $p = 0.961$). Error bars represent the standard error of the mean (SEM). Variability ranges are 95% confidence interval. EV = extracellular vesicle; tACS = transcranial alternating current stimulation; CDR-SB = Clinical Dementia Rating–Sum of Boxes. Statistics from repeated-measures ANOVA and Pearson's correlation, $^{**}p < 0.01$.

emerge during the early stages of the disease, highlighting the sensitivity of EVs in detecting early pathological changes [7].

In the present study, the reduction in EV concentration observed after gamma tACS stimulation may reflect a modulation of pathological EV dynamics. One possible interpretation is a reduction in the release or systemic dissemination of disease-associated EVs. Alternatively, these changes may reflect increased central uptake of EVs as part of a neuroprotective response. In support of this interpretation, previous studies in AD mouse models have demonstrated that gamma tACS stimulation enhances microglial activation and promotes the endocytosis and clearance of amyloid-β and phosphorylated tau protein [10]. Given the central role of microglia in EV uptake, processing, and signaling, gamma-induced microglial engagement may indirectly influence EV trafficking and systemic EV levels. While speculative, this mechanism provides a plausible link between gamma tACS and the observed EV changes. We also observed a significant association between changes in EV concentration and changes in disease severity, as measured by the CDR-SB. Patients exhibiting the largest modulation of EV levels also showed the greatest clinical improvement, supporting the biological relevance of our findings and suggesting that EV changes may reflect functional recovery rather than non-specific peripheral effects.

Validation in larger cohorts is required to address firstly our limited sample size and secondly the heterogeneous origin of plasma EVs, further clarifying the mechanisms behind the observed effects.

In conclusion, while the mechanisms of gamma-tACS on EV trafficking require further elucidation, our findings demonstrate a significant modulation of EV dynamics in AD, suggesting plasma EV profiling as a feasible and accessible biomarker for monitoring therapeutic efficacy.

CRediT authorship contribution statement

Sonia Bellini: Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Valentina Cantoni:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Antonio Longobardi:** Writing – original draft, Investigation, Data curation, Conceptualization. **Chiara Cupidi:** Writing – review & editing, Resources, Investigation. **Valeria Bracca:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Andrea Geviti:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Elisa Zummo:** Writing – review & editing, Resources, Investigation, Conceptualization. **Maria Sofia Cotelli:** Writing – review & editing, Resources, Investigation. **Enrico Premi:** Writing – review & editing, Resources, Investigation. **Alberto Benussi:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Roberta Ghidoni:** Writing – original draft, Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Barbara Borroni:** Writing – original draft,

Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Ethics statement

All participants provided written informed consent in accordance with the Declaration of Helsinki. The study was approved by the local ethics committee (NP5395).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2026.103085.

Data availability

The data presented in this study are available in the Zenodo Data Repository at doi: 10.5281/zenodo.18173865.

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