Modulating Cerebral Rhythms in Parkinson's Disease: Insights on the Role of Auditory Stimulation

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Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by a slowing of brain rhythms, leading to cognitive and motor deficits. Auditory stimulation (AS) has proven to have great potential as a non-invasive approach to modulate brain activity. Nevertheless, despite promising clinical and preclinical findings, optimal AS parameters for its use in PD remain unclear. To investigate the potential therapeutic effects of AS in PD, we aimed to establish an optimal preclinical model and stimulation protocol. Two mouse strains were compared, CD1 and C57BL/6, and assessed their auditory sensitivity. 3-months C57BL/6 mice was selected as the most suitable model for auditory studies. Two literature-based AS protocols were applied, a 10 kHz carrier tone modulated with 40 Hz pulses and a 40 Hz amplitude-modulated tone. Our results demonstrate that comparing pre- and post-stimulation periods, the 10 kHz/40 Hz protocol consistently induced a reduction in delta power and an increase in gamma relative power, with persistent effects of the latter 24 hours post-stimulation. These findings suggest that this specific AS protocol holds promise for targeting abnormal brain rhythms associated with PD and may have potential therapeutic implications. Further research is needed to explore the underlying mechanisms and optimize AS parameters for clinical translation.

1 INTRODUCTION

Parkinson's disease (PD), a neurodegenerative disorder characterized by motor impairments (tremors, rigidity and akinesia), hyposmia and cognitive decline with sleep disorders, is a significant global health burden. PD has been identified as the fastest growing neurological condition affecting 11.8 million people globally in 2021, a 273.9% increase since 1990 (Steinmetz et al., 2024). The pathological hallmark of PD is the loss of dopaminergic neurons in the substantia nigra, leading to dysregulation of neuronal circuits in the basal ganglia and impairment of nigrostriatal pathway. This disruption results in abnormal brain activity, including altered neuronal oscillations.

Electroencephalography (EEG) studies in PD patients have revealed a characteristic pattern of abnormal brain waves, characterized by an early increase in delta band activity (Caviness et al., 2015; Chu et al., 2021). Additionally, there is a reduction in gamma band activity (Ahveninen et al., 2000), which

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plays a crucial role in information processing and cognitive functions. Taken together, these findings suggest that a characteristic hallmark of PD is a slowing cerebral rhythms, associated with cognitive dysfunction (Bosboom et al., 2006; Soikkeli et al., 1991) an impairment that highly correlates with motor decline (Miladinović et al., 2021).

Efforts to develop novel therapeutic strategies for PD have focused on restoring normal neuronal activity and enhancing brain plasticity. While deep brain stimulation (DBS) has shown some success, it carries risks and may not be suitable for all patients due to surgery risks (Zhang et al., 2017). Non-invasive brain stimulation techniques, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), offer alternative approaches to modulating brain activity, however, further research optimizing stimulation parameters is needed to enhance their clinical utility (Benninger et al., 2010; Goodwill et al., 2017). There are not pharmacological or non-pharmacological therapies able to slow the progression of PD.

Auditory stimulation (AS) in the audible range has emerged as a promising and non-invasive approach for modulating neuronal activity. This method involves using sound waves to modulate brain activity by a mechanism called oscillatory entrainment. This process occurs when neural intrinsic oscillations synchronize with external rhythmic stimulus (Henao et al., 2020; Ross & Lopez, 2020). This synchronization enhances neural activity at the stimulus frequency by increasing signal amplitude and/or by phase-locking, where phases of neural oscillations align with specific phases of the stimulus (Henao et al., 2020) By these synchronizations, rhythmic stimuli can modulate brain regions beyond sensory areas, reaching supramodal regions and impacting higher-level cognitive functions (Albouy et al., 2022) and influence neuroprotection and neural plasticity (Adaikkan & Tsai, 2020; Fujiki et al., 2020).

Specifically, neuronal entrainment has been observed at different frequencies, but especially in the beta-gamma range (13–44 Hz) (Will & Berg, 2007). In animal models, direct microelectrode recordings into the primary auditory cortex demonstrated an entrainment of local field potential in response to 40-click train stimuli (Li et al., 2018; Nakao & Nakazawa, 2014) Several studies in mice model have demonstrated AS potential to induce gamma entrainment, as therapy approach in various neurological diseases, including dementia (Chan et al., 2022) ischemia (Zheng et al., 2020) and Alzheimer's disease (AD) (Liu et al., 2022), were a 40 Hz audio-visual stimulation induced gamma oscillation and reduced amyloid plaque promoting glymphatic clearance (Iaccarino et al., 2016; Martorell et al., 2019; Murdock et al., 2024). These findings suggest that neuronal entrainment through visual or auditory stimulation could not only mitigate cognitive decline but also emerge as a potential treatment for neurodegenerative diseases characterized by the accumulation of pathogenic proteins.

In PD, rhythmic auditory stimulation (RAS) is often used in rehabilitation to engage alternative motor networks or enhances basal ganglia function, potentially improving gait, tremors and quality of life (Benoit et al., 2014; Bukowska et al., 2015; Ye et al., 2022). Our research group has previously conducted clinical studies investigating the effects of Binaural Beat Stimulation (BBs) in cognitive function and brain activity in PD patients. The results suggest that BBs can initially reduce theta power in the brain, similar effect to the normalization seen with levodopa treatment, which may lead to improvements in motor function and cognition. However, this effect appears to diminish over time due to habituation. While some patients showed sustained benefits, others exhibited variable responses, highlighting the need for further research to optimize the timing and duration of BBs to maximize therapeutic effects (Gálvez et al., 2018; González et al., 2023).

Despite these promising findings, the optimal parameters for AS in PD remain elusive. A lack of standardized AS protocols hinders cross-study comparisons and clinical translation (Ingendoh et al., 2023). Moreover, the potential therapeutic effects of AS observed in AD are uncertain in the context of PD. To fill this knowledge gap, a preclinical model of AS in PD is essential to systematically investigate the effects of different stimulation parameters, including frequency, intensity, and modulation, and to identify the most effective protocols for modulating neural activity and improving motor and cognitive function.

To advance our research on this topic, this study focused on examining the influence of AS on cerebral rhythms in the unmodified mouse brain. The goal was to determine whether an AS signal with defined parameters (see methods) could optimally induce persistent gamma entrainment and potentially counteract EEG slowing, which could have preclinical and clinical implications for the application of AS paradigms in PD.

2 MATERIALS AND METHODS

2.1 Animals

Adult male mice CD1 (outbred strain; Charles River Laboratories; RRID:MGI:5649524) and C57BL/6 Ola (inbred strain; Jackson Laboratory; RRID:MGI:2162680), both aged 3 and 6 months were used in these studies. All procedures were conducted in compliance with national ethical and legal standards and were authorized by the Ethical Committee of the Universidad Politécnica de Madrid, and the regional government of Madrid (authorization code PROEX 108.0/20). The reporting of this manuscript was conducted according to the Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines.

To minimize potential damage to the head-mounted electrode caps, the mice were housed individually in polycarbonate cages (267 × 208 mm). The animals were provided with ad libitum access to food and water, with cellulose bedding and cotton enrichment for environmental stimulation. The animals were maintained and monitored by a veterinarian and qualified personnel at the Centre for Biomedical Technology (Pozuelo de Alarcón, Spain). The housing conditions were strictly controlled, with a temperature of 21 ± 2°C, a relative humidity of 40-60%, and a 12-hour light/dark cycle. All mice were deemed healthy based on serological and PCR testing, adhering to Federation of Laboratory Animal Science Associations (FELASA) recommendations.

Daily routines were conducted between 7 AM and 4 PM, with consistent and qualified personnel assigned to each experimental procedure. To characterize auditory capacity, a preliminary test was conducted with four groups of four mice each based on both mouse strains and two ages (3 and 6 months) to select the strain with optimal hearing for subsequent AS protocols. To investigate the effect of two AS protocols in EEG, two groups of five mice each for both protocols were selected from the best hearing condition.

2.2 Electrodes Implantation

Mice underwent surgery for chronic implantation of electrodes in parietal-temporal areas to cortical auditory evoked potentials (CAEPs). We also implanted intracranial electrodes for electroencephalography (iEEG) recordings. In both procedures, the animals were anesthetized with ketamine (Imalgene, 80 mg/kg, i.p.) and xylazine (Rompun, 10 mg/kg, i.p.), and afterward by iodopovidone (Betadine, Avrio Health). Alcohol wipes were applied to the skin. Anaesthetic depth was monitored every 5 minutes by assessing pedal withdrawal reflex. Prior to surgery, mice were treated with an ophthalmic solution to prevent eye drying. The rectal temperature was maintained during surgical procedures by a heating pad (RTC-1 Thermo Controller Surgery Table, Cibertec) at a body temperature of 37 ± 0.5 °C.

Craniotomies were performed at the electrode locations specified below using a dental drill. Four stainless-steel electrodes (P1 Technologies) were implanted for CAEPs recordings on the right (AP -2.5 mm; L -4.3 mm; DV 0.0 mm, anatomic locations from bregma) and left auditory cortices (AP -2.5 mm; L +4.3 mm; DV 0.0 mm). Two additional electrodes were implanted as reference (AP 1.5 mm; L +2.0 mm; DV 0.0 mm from bregma) and ground (AP 1.5 mm; L -2.0 mm; DV 0.0 mm). For intracranial EEG (iEEG) recordings, the electrodes were implanted in the right hemisphere in frontal (AP +1.0 mm; L +10 mm; DV 0.0 mm) and parietal cortex areas (AP -3.5 mm; L +1.0 mm; DV 0.0 mm), following the same anatomic locations reported in a previous study (Lee et al., 2018). Reference (AP -5.3 mm; L 0.0 mm; DV 0.0 mm from bregma) and ground (AP -2.0 mm; L +1.5 mm; DV 0.0 mm) electrodes were also implanted (Figure 1A).

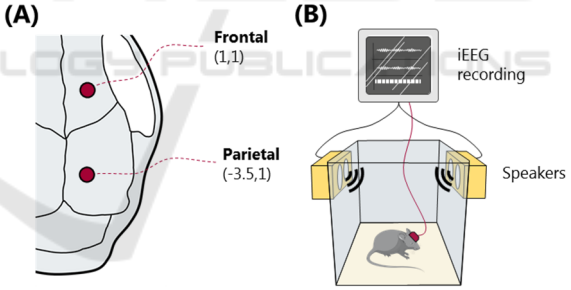

Figure 1: Experimental Setup. (A) Electrode placement for frontal and parietal intracranial EEG (iEEG) recording. (B) Experimental setup for simultaneous auditory stimulation and iEEG recording.

After the electrode's implantation, the skull was dried, the coated portions of the wires were secured with gel glue (Henkel, Loctite 454) and covered with dental cement (DuraLay, Inlay Pattern Resin Powder and Liquid). A header (P1 Technologies) was connected to the wires and angled upwards.

For pain management, buprenorphine (Buprex, 0.05 mg/kg) was administered subcutaneously before and 8 hr after surgery. The animals were allowed to recover for 1 hr in a warm environment before returning to the standard housing environment.

2.3 CAEPs Recordings

Mice hearing thresholds and auditory discrimination were study by CAEPs recordings following Mei et al. (2021) and Martorell et al. (2019) methodologies and our previous knowledge on evoked potentials recordings (Barios et al., 2016; Fernández-García et al., 2016, 2018; Fernández-Serra et al., 2022) Animals recovered for at least 1 week after electrodes implantation. For evoked potentials recordings, mice were first anaesthetized with ketamine and xylazine and treated with an ophthalmic solution to prevent eye drying. During auditory stimulation, anaesthetic depth and body temperature were maintained. Two speakers (Logitech Multimedia Speakers Z200) were place facing each other and 25 cm away from left and right mice ears. Stimulus consisted of a series of 50 ms tones of 0.5 and 1 kHz with a stimulating rate of 1 Hz, from a level of sound pressure level (SLP; SC310 sound level meter, CESVA) of 100 dB decreasing in 10 dB steps until no response was detected (hearing threshold) or sound intensity level reached the ambient noise level (~54.3 dB). The recording time window was 500 ms, starting 50 ms before the beginning of the stimulation, with 4,096 Hz sampling rate. The signal was bandpass filtered at 0.2–2000 Hz and averaged 300 times. Signals were recorded in an isolated chamber by using a portable electromyography (EMG)-evoked potentials (EP) device (Micromed, Italy) and SystemPLUS Evolution software (v.1.4; Micromed). Peak amplitudes of the main potential wave (N1 component) were measured in relation to the first 50 s as baseline.

2.4 iEEG Recordings and Analysis

For recovery and habituation, the mice were moved to the recording room, 1 week prior to the start of the recording sessions. iEEG signals were acquired in mice with freedom of movement in a 30 × 30 × 30 cm cage during 3-hr sessions (Figure 1B), following previous methodologies of our group (García-Peña et al., 2023; Herrero et al., 2021)The header was attached to a flexible cable (P1 Technologies) and connected to single-channel AC amplifiers (Grass, 78D). The power line frequency was removed using a 50 Hz notch filter. The cortex signals were amplified by 8,000x, bandpass filtered (CyberAmp, Axon Instruments, 380) at 0.3–100 Hz. Signals were then digitally converted (National Instruments, BNC-2090A) at a sampling frequency of 500 Hz and recorded using the LabVIEW Biomedical Toolkit software (v.2012; National Instruments; RRID:SCR_014325).

For spectral analysis, the power in the range of 0–100 Hz with a 1024-bin size was calculated using a customized code from MATLAB software (version 2022b). EEG frequency bands were classified into delta (δ; 0.5–4 Hz), theta (θ; 4–8 Hz), alpha (α; 8–12 Hz), beta (β; 14–30 Hz) and gamma (γ; 30–100 Hz). Before (PRE) and after (POST) relative band power change was calculated subtracting POST to PRE relative power for each band power studied.

Spectrograms representing powers at frequencies up to 100 Hz were obtained using a Hamming window with a block size of 512 and 2048 for the 0.5-4 Hz and the 30-100 Hz spectrograms, respectively, calculated using Spike2 (v. 6.18, Cambridge Electronic Design; RRID:SCR_000903).

2.5 Auditory Stimulation Experimental Designs

All AS protocols were generated by custom-built program under LabVIEW Virtual Instrument and delivered via two opposite speakers (Logitech Multimedia Speakers Z200) placed 20 cm above the ground of the cage (Figure 1B).

2.5.1 AS-Protocol 1

The proposed protocol adapts Lee et al. (2018) auditory stimulation protocol. Briefly, mice underwent a 1-day duration auditory stimulation of 1-hr session accompanied by 1-hr iEEG recordings immediately before (PRE) and after (POST) stimulation. One day after starting the protocol (basal; B; D0) and in non-stimulation days iEEG recordings were performed but no stimulation (Figure 2).

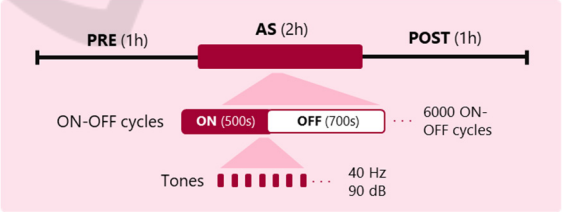

Figure 2: AS-protocol 1 adapted from Lee et al. (2018). 2-h auditory stimulation (AS) of 500-s ON (40 Hz tones) and 700-s OFF periods was preceded and followed by 1 h of iEEG recordings, PRE and POST respectively.

Mice were exposed to tones of 40 Hz. 20 pulses of 10 ms delivered at a SPL of 90 dB were played. Animals were presented with 1.2-s cycles alternating 500 ms of audio stimulation (ON period) interleaved with 700 ms of no tones (OFF period). Stimuli were presented in this manner for 2-hr sessions for 7200 ON-OFF cycles according to protocol (Table 1).

Table 1: Comparison of AS protocols.

	As-protocol 1	AS-protocol 2
Tones	40 Hz	10 kHz
Modulation	None	40 Hz
Cycles	500 ms ON 700 ms OFF	10 s ON 10 s OFF
SLP	90 dB	90 dB
Sessions	2 h	1 h
Adapted from	(Lee et al., 2018)	Martorell et al. (2019) and Lee et al. (2018)

2.5.2 AS-Protocol 2

The proposed protocol adapts Martorell et al. (2019) and Lee et al. (2018) auditory stimulation protocols. Briefly, mice underwent a 5-day duration with alternating days auditory stimulation of 1-hr session (AS; D1, D3 and D5) interleaved with non-stimulating days (resting period; RP; D2 and D4) accompanied by 1-hr iEEG recordings immediately before (PRE) and after (POST) stimulation. One day after starting the protocol (basal; B; D0) and in non-stimulation days iEEG recordings were performed but no stimulation (Figure 3).

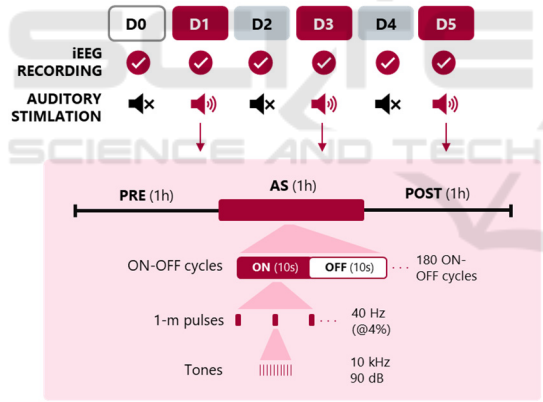

Figure 3: AS-protocol 2 adapted from Martorell et al. (2019) and Lee et al. (2018). 1-h auditory stimulation (AS) of 10-s ON (40 Hz pulses modulating 10 kHz tones) and 10-s OFF periods was preceded and followed by 1 h of iEEG recordings, PRE and POST respectively, in alternating days (D1, D3 and D5). Basal day (D0) and resting days (D2 and D4), iEEG recording was performed but no stimulation was not.

Mice were exposed to 1-ms pulses of 10 kHz tones presented at 40 Hz. Pulses were delivered at 25 ms intervals (40 Hz with a 4% duty cycle) and at SPL of 90 dB. Animals were subjected to 20-s cycles alternating between 10-s periods of audio stimulation (ON periods) interleaved with 10-s periods without stimulation (OFF periods). These stimuli were presented during 1-hr sessions, each consisting of 180 ON-OFF cycles, administered on AS days in accordance with the protocol (Table 1).

2.6 Statistical Analysis

All statistical analyses were performed using SigmaPlot v.12.0 (Systat, Germany). Unless otherwise indicated, all statistical data are presented as mean ± standard error of the mean (SEM). Outliers were detected and removed using the z-score method with a threshold of 2.0. Removal of outliers had no significant impact on the results of the analysis. Shapiro-Wilk normality test and the test for equality of variance were performed. For normally distributed data, an analysis of variance (one-way ANOVA) followed by Bonferroni multiple comparison post-hoc test were applied to determine significant differences in the POST–PRE relative band power. For not-normally distributed data, Kruskal-Wallis *H* test followed by Dunn multiple comparison post-hoc test were applied. Statistical significance was defined as a p-value less than .05 ($p < .05$). Statistical values were expressed as: *, if $.05 > p > .005$; **, if $.005 > p > .001$; and ***, if $p > .001$.

3 RESULTS

To establish optimal experimental conditions for investigating auditory-evoked neuronal oscillations, we first conducted a comparative analysis of the auditory capacity in CD1 and C57BL/6 mice, to select the more suitable mouse strain where to test the effect of auditory stimulation on spontaneous EEG. The choice of CD1 and C57BL/6 mice strains was based on their distinct genetic profiles, allowing for a comprehensive evaluation of AS efficacy across diverse genetic backgrounds, thereby increasing the generalizability of the findings to a broader population.

CAEPs recordings revealed a pronounced age-dependent decline in hearing sensitivity in CD1 mice, with complete auditory loss observed at 3 and 6 months of age at 0.5 and 5 kHz (0 of 8 CD1 mice of both ages periods responded at the maximum of 100 dB; data not shown). In contrast, C57BL/6 mice aged 3 months maintained auditory function for 5 kHz (41.57 ± 10.005 mV of N1 peak at 90 dB in the right hemisphere) and 0.5 kHz (33.98 ± 9.067 mV) (Figure 4). Also, C57BL/6 exhibited a notable reduction in hearing sensitivity at 6 months with no detectable evoked responses in 2 of 4 mice studied at ~60 dB for 5 kHz and at ~70 dB for 0.5 kHz stimulation in the

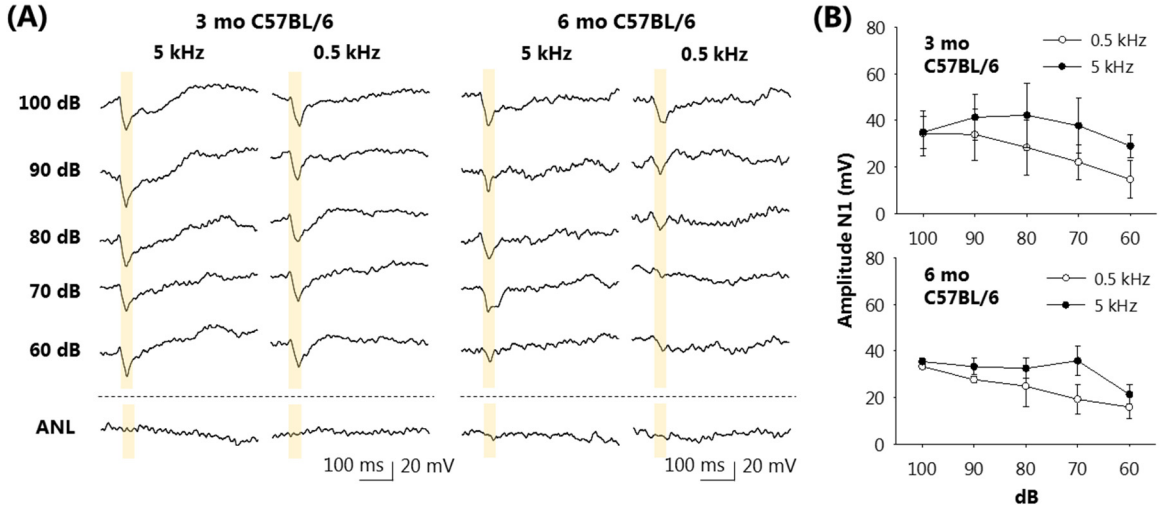

Figure 4: C57BL/6 mice auditory capacity evaluation by CAEPs. (A) CAEPs recordings of 3 (3 mo) and 6 (6 mo) months old C57BL/6 mice at 5 and 0.5 kHz stimuli from 100 to 60 dB SPL dB. ANL means ambient noise level. (B) N1-peak amplitude from first 50-ms baseline of 3 (upper graph) and 6 months old (lower graph) C57BL/6 mice at 5 and 0.5 kHz. Recordings without an identifiable N1-peak were removed from the mean. Data is shown as means ± SEM analysed from four recordings from each condition. No statistical analysis was performed.

right hemisphere. Among 6-months C57BL/6 mice with a detected N1-wave, a reduction auditory function at 5 kHz (33.52 ± 3.55 mV of N1 peak at 90 dB in the right hemisphere) and 0.5 kHz (27.70 ± 1.21 mV) was observed compared with 3-months C57BL/6 (Figure 4). N1 amplitude showed no significant differences between the left and right hemispheres in CD-1 and C57BL/6 mice with all the parameters studied. These findings indicate how crucial is to select an adequate mouse strain in auditory research and suggest that C57BL/6 mice at 3 months might represent an appropriate model for studying auditory-driven neural activity.

To investigate the impact of 40 Hz auditory stimulation on iEEG activity, studies in 3-month-old C57BL/6 mice were conducted following AS-protocol 1 and 2 detailed in 2.4 subsection of Materials and Methods, that include frequencies higher than 0.5 kHz and SPL above 60 dB.

AS-protocol 1 adapted from Lee *et al.* (2018) with 40 Hz auditory pulses without high-frequency carrier tone, showed no effects of auditory stimulation in EEG power (data not shown). No longitudinal study was performed.

AS-protocol 2 adapted from Martorell et al. (2019) and Lee et al. (2018) with 10 kHz carrier tone modulated by 40 Hz auditory pulses, showed EEG changes produced by AS (Figure 5). iEEG recordings were stable during 1-hr recording and total power showed no statistical differences between days (data not shown). Baseline-day (B) recordings revealed no significant differences in POST–PRE relative

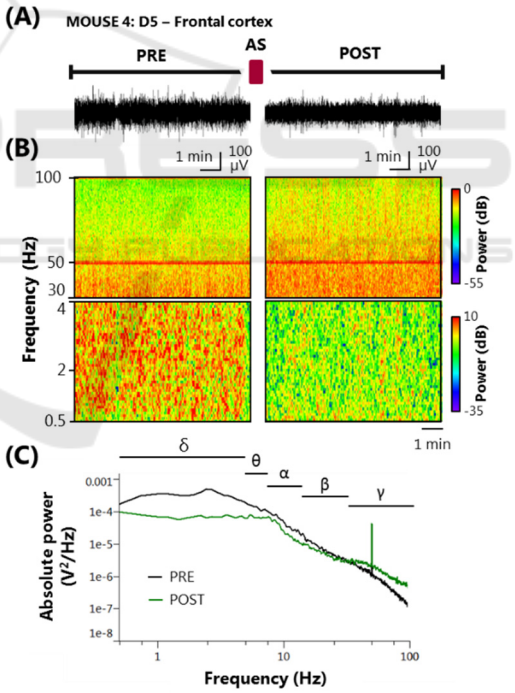

Figure 5: Spectral analysis of pre- (PRE) and post- (POST) stimulation periods at day 5 from AS-protocol 2. (A) Representative 10-min intracranial electroencephalography (iEEG) recordings of D5 PRE and POST periods. (B) Spectrograms (0.5–4 Hz, delta, upper panel; 30–100 Hz, gamma, lower panel) obtained from the two same PRE and POST regions showed in panel A. (C) Absolute power iEEG of same PRE and POST regions showed in panel A showing delta (δ) and theta (θ), and alpha (α), beta (β), and gamma (γ) bands.

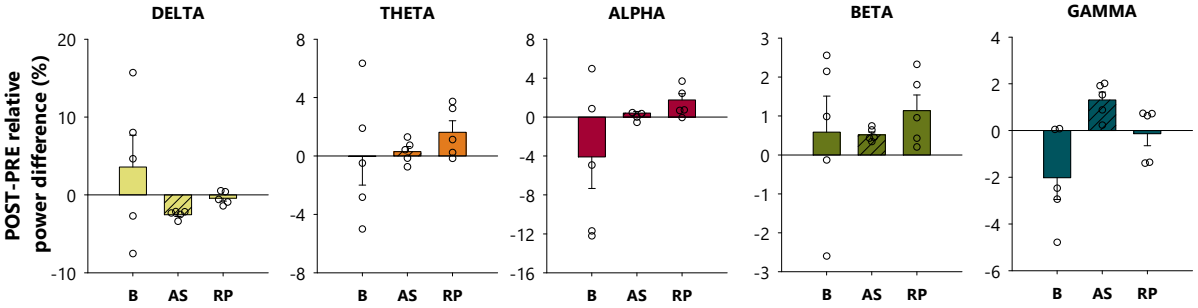

Figure 6: Relative power bands difference between pre- (PRE) or post-stimulation (POST) during basal day (B), auditory stimulation days (D1, D3 and D5) and resting period days (D2 and D4) obtained from iEEG recordings of five mice at AS-protocol 2. Data is shown as means ± SEM analyzed from five mice. No statistical test were performed.

potentials and in all frequency bands studied (Figure 6). However, during AS, frontal cortex showed a consistent increase in gamma POST–PRE relative power (+1.31 ± 0.38 %) accompanied by a marked reduction in delta POST–PRE relative power (-2.54 ± 0.25 %), compared to their basal. The parietal cortex showed the same observations in delta and gamma POST–PRE relative powers (data not shown).

A longitudinal analysis of the data revealed that delta relative power decreased during the post-stimulation period compared to the pre-stimulation period (Figure 7). However, subsequent resting days (D2 and D4) showed a recovery of delta power to baseline levels. Gamma relative power, on the other hand, was increased during the post-stimulation period and this effect persisted for at least one day post-stimulation, as indicated in resting days (D2 and D4). However, the effect of gamma power enhancement appeared to dissipate by the second resting day, as the PRE period of the subsequent stimulation day (D3-PRE and D5-PRE) returned to baseline levels. There were not statistically significant differences in one-way ANOVA analysis.

4 DISCUSSION

The present study aims to establish a preclinical model of AS to investigate its potential to modulate aberrant neural oscillations in PD. Despite promising findings in human studies demonstrating the capacity of AS to normalize EEG power and enhance functional connectivity (Gálvez et al., 2018; González et al., 2023) and promising mice studies in AD (Lee et al., 2018; Martorell et al., 2019; Murdock et al., 2024), the optimal parameters for AS in PD remain elusive. A standardized preclinical model is crucial to systematically explore the effects of various stimulation parameters to ensure accurate, reproducible, and translatable results into effective clinical interventions. Even though PD mice models do not fully replicate all features of human pathology, PD mice models are useful for studying specific aspects of the disease, performing moderate invasive procedures or chronic recordings and stimulations that are unfeasible in humans (Blandini & Armentero, 2012; Chesselet & Richter, 2011; Meredith & Rademacher, 2011).

In this study, we compared two distinct bibliography-based AS protocols into the best hearing mouse strain. The choice of CD1 and C57BL/6 mice was strategic, as these strains exhibit contrasting genetic profiles. CD1 mice is a nonconsanguineous mouse that aimed to mimic the genetic diversity of the human population.

Despite of that, these distinct genetic characteristics can influence auditory sensitivity as CD1 mice exhibited an age-related decline in auditory sensitivity that has also observed in some studies (Shone et al., 1991; Wu & Marcus, 2003). On the other hand, C57BL/6, which provides a more homogenous genetic background, maintained their auditory function until 6 months of age. These results should be treated with caution as other studies have found C57BL/6 to develops progressive age-related sensorineural hearing loss (Walton et al., 1995; Willott, 1986). Perhaps the normal hearing of C57BL/6 reported in our hands is related to the specific substrain used. The “Ola” C57BL/6 substrain was studied in our work whereas, unfortunately, most studies do not report the specific examined substrains, which as we have demonstrated here, can influence hearing outcomes (Mekada et al., 2009).

Our findings also underscore the critical importance of considering age and strain-specific differences in auditory processing when designing preclinical studies. A comprehensive characterization of mice using objective auditory tests, such as CAEPs, prior to initiating any auditory stimulation protocol is essential.

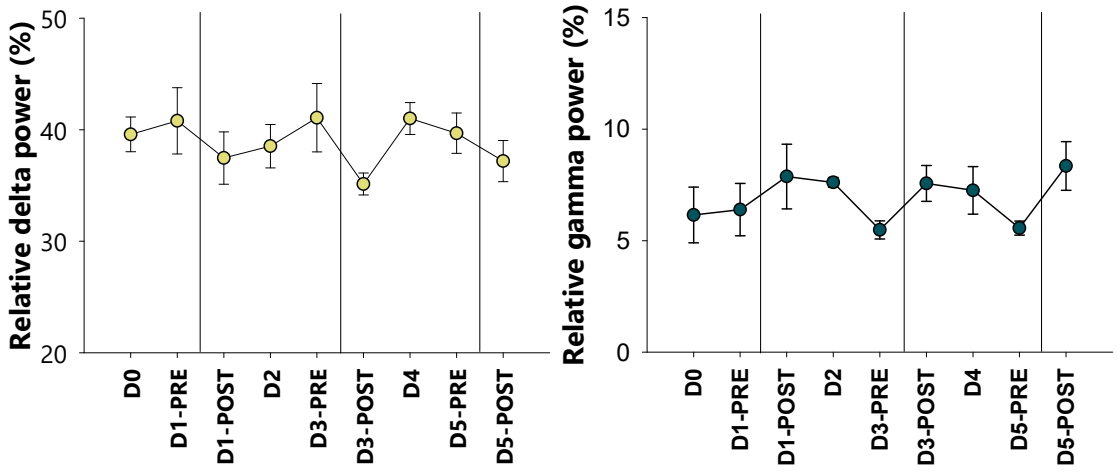

Figure 7: Relative delta (left panel) and gamma (right panel) power at baseline day (D0), pre- (PRE) and post-stimulation (POST) periods days (D1, D3 and D5) and resting days (D2 and D4) obtained from iEEG recordings of five mice at AS-protocol 2. Vertical black lines represent auditory stimulation (AS) periods. Data is shown as means ± SEM analyzed from five mice. One-way analysis of variance (ANOVA) was performed.

Previous studies have demonstrated the efficacy of prolonged auditory stimulation protocols, lasting up to two weeks, in inducing long-lasting changes in brain oscillations (Lee et al., 2018). However, our study provides a novel perspective by evaluating the effects of a shorter, more focused stimulation protocol. By focusing on a shorter stimulation period, we have been able to determine whether a more concise intervention is enough to induce the desired neuronal entrainment. This strategy has several significant advantages as it allows for a reduction in the time of exposure to stimulation, minimizing the risk of adverse effects and facilitating patient adherence to long-term treatment.

Surprisingly, when faithfully replicating the AS protocol described Lee et al. (2018) in a mouse model of AD, we did not observe the same effects in reducing gamma power in the short-term. In particular, the claim that 40 Hz pulses were used to stimulate the auditory system of mice raises questions. Considering the auditory range of rodents, stimuli of this frequency are likely not optimal for inducing significant changes in neuronal activity (Heffner & Heffner, 2007; Naff et al., 2007; Ohlemiller et al., 2016). Previous studies have shown that the use of tones within the auditory range of mice, combined with frequency modulations, is a more effective strategy for modulating neuronal activity (Kilgard et al., 2001; Martorell et al., 2019). Consequently, we modified AS-protocol 1 exploring the use of audible tones following with the aim of optimizing the efficacy of stimulation and replicating the results reported in peer-reviewed publications.

In contrast, the AS-protocol 2, incorporating a 10 kHz tone presented in 40 Hz auditory pulses, induced notable changes in EEG power. Specifically, we observed a significant increase in gamma band power and a decrease in delta band power that is consistent with previous studies demonstrating the potential of AS to enhance cortical excitability and promote neural synchrony (Gálvez et al., 2018; González et al., 2023).

The observed increase in gamma power is particularly intriguing, as gamma oscillations are implicated in cognitive processes, including attention, memory, and sensory perception. The enhancement of gamma power may counteract the slowing of neural oscillations, a hallmark of PD (Soikkeli et al., 1991) Furthermore, the persistence of gamma power enhancement for at least one day post-stimulation suggests a potential, although reversible, long-lasting impact of AS on neural networks. However, further longitudinal studies with continuous AS are needed to fully elucidate the time course of these effects and to determine the optimal stimulation parameters for maximizing therapeutic benefits.

The reduction in delta power because of AS and its subsequent recovery to baseline levels is also noteworthy. Increased delta band power has been identified as a potential early PD biomarker (Caviness et al., 2015; Chu et al., 2021) that also associates with dementia in PD (Bosboom et al., 2006). A study from Stanley et al. (2019) in rhesus macaque monkey examined the influence of gamma (40 Hz) click trains on delta transient response followed finding an entrainment of gamma and suppression of delta. Also,

a construction of a computational model determined that long duration gamma-rhythmic input stimuli induce a steady-state containing entrainment at gamma, and suppression of delta oscillations. This suppression is achieved in the model by an action driven by the thalamus.

Several limitations in this study warrant careful attention. First, SPL used in AS protocols may be high. Consequently, assessing auditory thresholds before starting AS will improve animal welfare. Second, given the potential for distortion in conventional audio equipment, future studies should rigorously control frequency behavior. Future research will also refine AS-protocol 2 and investigate the neural mechanisms modulated by AS during the ON period. Further investigation should focus on the therapeutic potential of the AS protocol in PD models, particularly targeting early pathological EEG biomarkers for timely intervention.

In conclusion, while our findings offer compelling evidence supporting the capacity of AS to modulate neural oscillatory activity in a preclinical PD model, additional studies are imperative to delineate the specific neural dynamics induced by AS and to evaluate its long-term therapeutic efficacy.

5 CONCLUSIONS

In conclusion, this study represents a significant step in elucidating and detailing an effective AS protocol for PD. Our findings demonstrate that AS with 10 kHz tones presented with 40 Hz pulses can induce a reduction in delta power and an increase in gamma power, in the unmodified brain. AS-based technologies could theoretically counteract EEG changes linked with injury and neurodegeneration. Our results reinforce the pathway toward the identification of specific, increasingly optimized AS paradigms for the treatment of PD.

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