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Brain wave modulation and EEG power changes during auditory beats stimulation

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Abstract

Auditory beats stimulation (ABS) has received increased attention for its potential to modulate neural oscillations through a phenomenon described as brain entrainment (i.e., synchronization of the brain's electrocortical activity to external stimuli at a specific frequency). Recently, a new form of ABS has emerged, inspired by isochronic tones stimulation (ITd). This study investigated neural oscillatory responses induced by ITd in comparison with formerly well-established ABS protocols, such as gamma-binaural beats (BB) and white noise (WN). We recorded the electroencephalographic brain activity in 28 participants during 4 minutes of BB, ITd, and WN presentation. Data demonstrated that while both BB and WN enhanced oscillatory power in the EEG gamma band, consistently with the expected brain entrainment effect, ITd yielded greater changes in EEG power ($p < 0.001$). This was confirmed by time-based analysis, which showed a progressive increase in normalized EEG power within the ITd window compared to BB ($p < 0.05$). Findings also revealed that ITd elicited acute changes in the alpha band of EEG oscillations, through a progressive decrease in power over time, which was distinctly different from the pattern observed while listening to BB and WN. Such dual alpha-gamma effects underline the promising and unique potential of ITd to modulate neural oscillations, which selectively differ from BB and WN. This study contributes to the evolution of ABS research, highlighting the promise of ITd for cognitive enhancement and clinical applications.

Keywords: Auditory beats stimulation, Binaural beats, Intermittent pure tone, EEG, Brainwave entertainment

Introduction

The human brain is characterized by endogenous oscillatory activity, i.e., rhythmic fluctuations stemming from electrical potentials spontaneously generated by neurons. Such oscillations are the result of synchronized and coordinated electrical activities that describe functional networks across large populations of neurons. It is generally accepted that brain oscillations reflect neural communication and information processing within neural networks Cole and Voytek (2017), resulting in distinct patterns of brain wave frequencies. Natural brain frequencies are classified into five bands: alpha (8–12 Hz), beta (15–29 Hz), theta (3.5–7 Hz), delta (0.5–3.5 Hz), and gamma (30–90 Hz). Interestingly, each phase and power of each endogenous frequency range of neural activity was assigned to a functional role in behavior and cognition Buzsaki (2004); Van Wassenhove (2016). The close relationship between changes in brain oscillations and behavior suggests that targeting oscillations could improve cognitive functions. Accordingly, a significant number of studies focusing on brain-behavior relationships were published in the scientific literature. As a result, there has been extensive research into the use of brain stimulation techniques for therapeutic purposes, with the goal of inducing specific frequencies to guide the brain into desired states Polanía et al. (2018). Despite the promise of non-invasive methods to induce modulation of brain activity, some of them require expensive equipment (i.e., transcranial magnetic stimulation, transcranial direct current stimulation, transcranial alternating current stimulation). Interestingly, auditory beats stimulation (ABS), which requires only an intact auditory pathway, is an emerging non-invasive neuromodulatory technique providing promising effects on oscillatory waves by synchronizing endogenous brain activity with audible stimuli Pastor et al. (2002); Schwarz and Taylor (2005); Chaieb et al. (2015).

The most studied form of ABS is binaural beats (BB), which are characterized by auditory illusions spontaneously generated by the brain when two tones of slightly different frequencies are presented to each ear Chaieb et al. (2015); Avan et al. (2015). Numerous studies emphasized the potential of BB to influence behavior and cognition, particularly in the context of cognitive processing of mood states and vigilance, as well as psychiatric disorders such as anxiety Katmah et al. (2023); Leistiko et al. (2024); Chaieb et al. (2015); Garcia-Argibay et al. (2019b); Basu and Banerjee (2023); Ingendoh et al. (2023). However, other reports investigating the effects of BB yielded inconsistent cognitive modulations Gao et al. (2014); López-Caballero and Escera (2017), attributing this variability to factors such as carrier tone frequency, beat frequency, exposure duration, and recording methods Jirakittayakorn and Wongsawat (2017); Garcia-Argibay et al. (2019a). For example, researchers reported that carrier tones in the frequency range of 200–900 Hz, and most especially around 400 Hz, appeared to be more effective in eliciting BB-related auditory illusions Wahbeh et al. (2007); Pratt et al. (2010); Goodin et al. (2012). However, there is no consensus on the frequency of the carrier tone. Fundamentally, the cognitive effects of BB are attributed to the brainwave entrainment hypothesis, which suggests that the brain’s electrocortical activity tends to oscillate in the direction of the external signal frequency Huang and Charyton (2008). Such an entrainment effect was observed in several studies using electroencephalography (EEG) through various parameters including absolute power Wahbeh et al. (2007); Jirakittayakorn and Wongsawat (2017), relative power Gao et al. (2014); Corona-Gonzalez et al. (2021), normalized power measures López-Caballero and Escera (2017), as well as auditory steady-state responses and frequency-following response measures Orozco Perez et al. (2020). Nevertheless,

phase synchronization between pairs of brain channels in response to auditory BB was only reported in some frequency bands, including theta Orozco Perez et al. (2020), alpha Puzi et al. (2013), and gamma Jirakittayakorn and Wongsawat (2017). Specifically, BB frequencies in the gamma and theta ranges showed effects on cortical activity, leading to increased functional connectivity and cross-frequency interactions Orozco Perez et al. (2020).

Conversely, the auditory entrainment of BB yielded inconclusive findings in numerous studies, prompting various hypotheses, such as the possibility of BB as being a resonance phenomenon Oster (1973); Solcà et al. (2016). Interestingly, the phenomenon of stochastic resonance, which refers to the situation where the addition of random noise is presumed to enhance information transfer and processing, was originally associated with another type of ABS: listening to white noise (WN) Ward et al. (2010). The effect of WN (i.e., a sound containing all frequencies equally) can also be quantified by EEG, showing increased synchronization between brain regions, particularly in the alpha and gamma frequency bands Ward et al. (2010). Note that the limited facilitating effects of WN on higher cognitive functions, compared to BB, led to its widespread use as a controlled stimulation in auditory stimulation research Engelbregt et al. (2019). Overall, the existing literature primarily emphasized the effects on BB, yielding various outcomes. These mixed results raise concerns about the practical implications and suitability of BB as a preferred choice for ABS applications Basu and Banerjee (2023).

Recent clinical studies addressed the therapeutic efficacy of a novel form of ABS derived from isochronic tones, which could represent a novel form of neuromotor treatment in rehabilitation Colombié and Ladoucette (2023); ?. Initially, isochronic tones were developed under the central assumption that they could elicit brainwave entrainment by rapidly pulsing a single frequency on and off in both ears, the frequency of auditory stimulation corresponding to the desired brainwave frequency. Isochronic tones demonstrated encouraging attention-enhancing effects, yielding a 15% increase in the root mean square values of electric potentials recorded at the anterior frontal electrode of the scalp, compared to BB Deraman and David (2017). There has been, since then, a very limited interest in exploring the effects of isochronic tones, resulting in neglect or conflation with other ABS modalities Aparecido-Kanzler et al. (2021). Contrary to traditional isochronic tones, ABS used in recent clinical studies did not involve pulsing a single frequency throughout the stimulation, but rather a frequency that alternates after each time interval within a predetermined auditory range. This form of ABS can be considered a derivative of isochronic tones (ITd). ITd frequencies are typically centered around 250 Hz, thus encompassing a series of low-frequency sounds. As highlighted by Dimitrijevic et al. Dimitrijevic et al. (2008), the use of frequencies around 250 Hz is promising for brain stimulation due to the larger population of neurons engaged while processing auditory stimuli compared to higher frequency sounds (e.g. 4000 Hz). Nevertheless, the neurophysiological correlates of ITd remain unclear and warrant further experimental investigation.

The aim of the present study was therefore to elucidate the mechanisms underlying the effects of ITd, based on EEG recordings of ongoing brain oscillations. Particularly, we investigated whether ITd is more consistent with the neural mechanisms observed in WN or BB, and assessed its potential as a brainwave entrainment tool. Despite the exploratory nature of the present study, closer patterns of brain connectivity between ITd and BB were expected as both forms of ABS use a limited set of single frequency tones that are easily perceived by the brain.

Methods

Participants

Twenty-eight participants (14 females, 14 males; mean age = 32.42 years \pm 13.68) volunteered to participate in the experiment. Individuals with a history of epilepsy, psychological or psychiatric disorders, or who were under medication at the time of the experiment, were excluded from the study. A pre-experimental hearing test was conducted to determine participants' individual absolute hearing thresholds, ensuring their mean hearing thresholds were less than 20 dB for each ear. Seven participants reported being familiar with playing an instrument and had started their practice during adolescence (mean age = 10.00 years \pm 2.77). Except for 5 participants, all reported music listening habits ranging from 0.5 to 4 hours per day (mean = 1.59 \pm 1.14 hours). All participants signed a written informed consent for the study, which presented no major risks, and the study received approval from the Research Ethics Committee of the University of Lyon 1 (2022-02-10-002).

Auditory stimuli

All stimuli were calibrated to be presented at 40 dB through noise-cancelling earphones (1MORE USA Inc.) using software that calibrates sound presentation based on individual absolute thresholds (Presentation[®], Version 18.0, Neurobehavioral Systems, Inc., Berkeley, CA, USA, <https://www.neurobs.com>). BB and WN recordings were made using the freeware audio editing program Audacity, version 3.1.3 (<https://www.audacityteam.org/download/>). The gamma BB recording contained two sinusoidal tones, with the left at 200 Hz and the right at 250 Hz, to create a 50 Hz gamma BB (see Fig. 1A). ITd involves alternating single-frequency tones bound within a predetermined auditory range (200–400 Hz), typically with a 50 Hz interval. The choice of a 50 Hz BB ensured homogeneity of methods to enable comparisons with ITd. The ITd sequence was delivered using the medical device Alphabox[®] (Allyane, France), which had been used in previous clinical trials for neuromotor programming applications Colombié and Ladoucette (2023); Dos Anjos et al. (2023). For each ABS condition (i.e., BB, ITd, WN), auditory stimuli were delivered stimulations were presented under the form of one single block of 12 sequences of 9.4 s, interspersed with rest sequences of 10 s, for a total block duration of 4 min per experimental condition (Fig. 1B). The beginning and end of the stimuli were attenuated for 1.2 s to facilitate smooth listening transitions between rest periods.

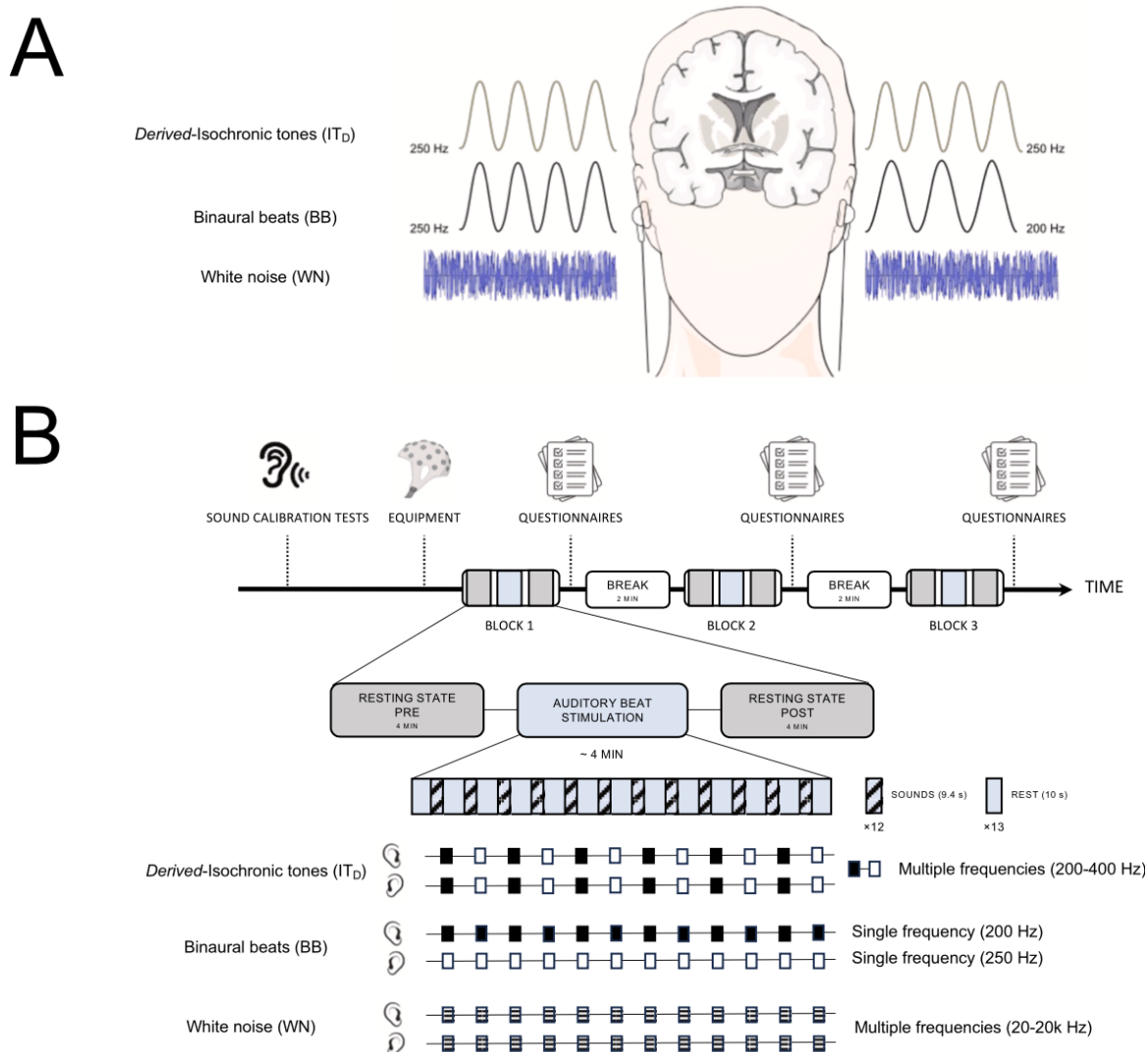


Figure 1: Illustration of the auditory beats stimulation condition during the experimental protocol. (a) IT_D was characterized by alternating pure tones in the 200–400 Hz frequency range, presented simultaneously to both ears. For BB, two pure tones, with frequencies of 200 Hz and 250 Hz, were presented concomitantly to each ear, resulting in the generation of a beat with a frequency equal to the difference between these tones, specifically 50 Hz. WN was defined as a sound that covered the entire human hearing range (20 Hz–20 kHz) at uniform intensities. (b) Experimental protocol and time windows recorded by electroencephalography. The experimental "auditory beats stimulation" block consisted of 12 sequences of 9.4 s of auditory beats stimulation ("sounds") followed by 10 s of rest. Each block was associated with one ABS condition. ABS conditions were counterbalanced across participants. IT_D: derived-IT, BB: Binaural beats, WN: White noise.

Experimental design

The EEG experiments took place in a dimly lit room with participants comfortably seated in a chair. Preparation time included measuring head circumference to place the EEG cap that best fitted the participants' head shape and volume, positioning EEG references, and filling the contact between the EEG electrodes and the scalp with conductive gel.

Participants were then instructed to close their eyes, remain awake, listen passively to the sounds through noise-canceling headphones (1MORE USA Inc.), and avoid any movement. They completed each ABS condition in a seated position. ABS conditions were separated from each other by a 2-minute break. During the breaks, participants received no specific instructions and could freely stretch and move their limbs as long as they remained in a seated position. Time breaks were expected to mitigate fatigability and facilitate participants' return to a baseline resting state, hence limiting the risk of contamination across ABS conditions due to carryover effects. Each ABS condition involved one block of auditory stimulation (BB, ITd or WN), preceded and followed by 4-min of resting state EEG (Fig. 1B). Before each break, they rated their perceived relaxation and absorption using visual analog scales Orozco Perez et al. (2020). The order of the blocks was counterbalanced across participants, so that each of the six possible orders for ABS condition was assigned to 4–6 participants. The entire experiment was conducted within a single 1-hour session, including preparation time.

Measurements

EEG Acquisition

Spontaneous brain electrical activity was recorded using a 10/20 system of 32 Ag-AgCl sintered electrodes (MLAEC2 Electro-cap system 2, ADInstrument®[®], Dunedin, New Zealand). The ground electrode was placed on the right acromion, and the reference electrode was placed outside of the cap, on the right side of the nose, following the standard procedure for EEG recordings using the ADInstrument®[®] system. The EEG signal was amplified using a PowerLab system (PL3516, Power Lab 16/35, ADInstrument®[®]) with two amplifier systems (FE238, Octal Bio Amp, ADInstrument®[®]). During acquisition, all electrode impedances were kept below 5 k. The raw EEG signal was filtered at 0.5–200 Hz, with an additional 50 Hz hardware notch filter to avoid power line contamination Ladrova et al. (2019). The 50 Hz hardware notch filter, commonly used to prevent interference from the electric grid, was applied based on its effectiveness in regions with high artifact prevalence Widmann et al. (2015).

EEG Data Processing

Data from 32 scalp electrodes (FP1, FPZ, FP2, FZ, AFZ, F3, F4, F7, F8, FT7, FT8, FCZ, FC3, FC4, CZ, C3, C4, CPZ, CP3, CP4, PZ, P3, P4, T3, T4, TP7, TP8, T5, T6, OZ, O1, O2) were analyzed using Brainstorm Tadel et al. (2011) running on MATLAB®[®]. The EEG signals were re-referenced to the average of all scalp electrodes and corrected for heartbeat and muscle artifacts using a signal-space projection algorithm Uusitalo and Ilmoniemi (1997). Any remaining artifacts were identified through visual inspection, and 3.29% of the channels were rejected. A bandpass filter (1 Hz–200 Hz) was applied, and EEG signals were epoched in two time windows: [0, 233 s] for resting state and ABS blocks and [0, 9.4 s] for stimulus epochs within the ABS block.

For each time window, power spectrum densities were calculated using Welch's method with a fixed time window (1 s, 50% overlap) for low (30–59 Hz) and high gamma frequency bands (60–90 Hz). Analysis was also conducted for alpha (8–12 Hz) and beta (15–29 Hz) frequency bands due to their relevance in ABS studies Jirakittayakorn and Wong-sawat (2017); Orozco Perez et al. (2020) and motor recovery interventions Colombié and Ladoucette (2023); Dos Anjos et al. (2023). Power spectrum densities during auditory

stimulation were averaged for each condition and normalized relative to baseline resting state (PSD_{RESTING-PRE}) using the following formula:

$$\text{PSD}_{\text{STIM-NORM}} = \frac{(\text{PSD}_{\text{ABS}}(\textit{condition time window}) - \text{PSD}_{\text{RESTING-PRE}}(\textit{baseline time window}))}{(\text{PSD}_{\text{ABS}}(\textit{condition time window}) + \text{PSD}_{\text{RESTING-PRE}}(\textit{baseline time window}))}$$

We also calculated the pretest resting PSD vs. posttest resting PSD (PSD_{POST-NORM}) using the same formulae. The ABS time window was replaced by the resting state post-condition to assess the short-term effects of ABS. Normalized power spectrum densities were then extracted for further statistical analyses (see below). We further explored the short-term effects of different ABS on the interaction between different brain regions by using imaginary coherence (iCOH; ?). This method, which assesses the linear relationship (i.e., correlation) between two signals (in this case, electrodes) at specific frequencies (alpha, beta, low gamma, and high gamma), can be used as a long-distance synchronization index ?. We computed the $N \times N$ (EEG sensors) imaginary coherence matrices on EEG data recorded during the resting state blocks (iCOH_{RESTING-PRE}, iCOH_{RESTING-POST}), coherence matrices were computed using a 1-s sliding Hanning window with a 50% overlap (<https://neuroimage.usc.edu/brainstorm/Tutorials/Connectivity>, accessed in December 2022). Coherence matrices were averaged for each experimental condition, extracted for statistical tests, and normalized relative to the baseline resting state as described above (iCOH_{POST-NORM}).

Self-report Ratings

Participants rated their mental relaxation and depth of absorption Orozco Perez et al. (2020) on 5-point Likert-type scales after each experimental condition. Mental relaxation was measured on a scale from 1 (“Extremely aroused”) to 5 (“Completely relaxed”). Absorption was self-reported on a scale from 1 (“Very unabsorbed”) to 5 (“Very absorbed”).

Statistical Analysis

We conducted statistical analysis of dependent variables using linear mixed-effects analyses with random intercepts by subject, using the R programming environment ? and the nlme package Pinheiro et al. (2014). First, we analyzed the PSD_{RESTING-PRE} recorded during the pre-experimental resting state, using the fixed effects of CONDITION (ITd, BB, WN), FREQUENCY BAND (alpha, beta, low gamma, high gamma), and SENSOR (all electrodes) to confirm the absence of pre-existing effects. We then analyzed PSD_{STIM-NORM} and PSD_{POST-NORM} using SENSOR, FREQUENCY BAND, and CONDITION as fixed effects with interaction terms.

The predictive relationships between PSD_{STIM-NORM} time windows were analyzed using a model including TRIAL (1–12) and WINDOW (sound, rest) as fixed effects. According to the Shapiro-Wilk test for normality, connectivity data (i.e., iCOH) were confirmed to be normally distributed Shapiro and Wilk (1965). For each electrode and frequency band (alpha, beta, low gamma, high gamma), paired t-tests were conducted on iCOH_{RESTING-PRE} and iCOH_{RESTING-POST}. The electrode most frequently involved in significant differences was selected for further analysis.

Self-reported relaxation and absorption scores were analyzed using one-way ANOVA models with CONDITION as the fixed factor. Residual plots were inspected to ensure no deviations from homoscedasticity or normality. The type 1 error rate was set at =

5%. Effect sizes were calculated using partial Cohen’s f -statistic from the `effectsize` package Ben-Shachar et al. (2020), with $f \geq 0.10$ considered small, $f \geq 0.25$ medium, and $f \geq 0.40$ large Selya et al. (2012). Post-hoc comparisons were conducted using general linear hypotheses testing of planned contrasts from the `multcomp` package Hothorn et al. (2008); Bretz and Hothorn (2016), with Holm’s sequential Bonferroni corrections applied to control the false discovery rate.

Results

Baseline resting state power levels

The experimental condition did not affect the $\text{PSD}_{\text{RESTING-PRE}}$ recorded during the resting state time window ($f = 0.02$, $F(2, 7594) = 1.03$, $p = 0.36$). We observed no interaction effect between `SENSOR` and `CONDITION` ($f = 0.04$, $F(62, 7594) = 0.22$, $p = 1.00$), nor between `FREQUENCY BAND` and `CONDITION` ($f = 0.03$, $F(6, 7594) = 1.26$, $p = 0.27$, Table 1), on $\text{PSD}_{\text{RESTING-PRE}}$.

Frequency Band	ITd [95% CI]	BB [95% CI]	WN [95% CI]
Alpha (8–12 Hz)	1.68 [1.49, 3.34]	1.69 [1.43, 3.29]	1.64 [1.39, 3.24]
Beta (15–29 Hz)	5.52 [4.60, 6.45]	5.47 [4.55, 6.40]	5.45 [4.52, 6.38]
Low Gamma (30–59 Hz)	3.47 [2.54, 4.39]	3.92 [3.00, 4.85]	3.73 [2.80, 4.65]
High Gamma (60–90 Hz)	2.41 [1.59, 1.78]	2.36 [1.59, 1.78]	2.32 [1.55, 1.74]

Table 1: Mean [95% confidence intervals] of power spectral densities relative to the baseline resting state ($\text{PSD}_{\text{RESTING-PRE}}$) for ITd, BB, and WN in different frequency bands (in $\text{W} \cdot \text{Hz} \cdot 10^{-13}$). ITd: derived-IT, BB: Binaural beats, WN: White noise.

Self-report ratings

Self-reports of depth absorption and mental relaxation during the experimental sessions were not affected by `CONDITION` ($f = 0.22$, $F(2, 54) = 1.31$, $p = 0.28$; $f = 0.23$, $F(2, 54) = 1.44$, $p = 0.25$, respectively, Fig. 2).

Power during ABS blocks

The linear mixed-effect analysis conducted on $\text{PSD}_{\text{STIM-NORM}}$ revealed a two-way interaction between `CONDITION` and `FREQUENCY BAND` ($f = 0.06$, $F(6, 7124) = 4.62$, $p < 0.001$). Post-hoc contrasts revealed a reduction in $\text{PSD}_{\text{STIM-NORM}}$ during BB and WN (−14%, 95% CI [−0.19, −0.09], $p < 0.05$ for both) compared to ITd (−11%, 95% CI [−0.16, −0.06]) within the alpha band. As shown by Fig. 3A, comparable results were found for the low gamma band (BB: 5%, 95% CI [0.005, 0.10]; WN: 6%, 95% CI [0.02, 0.11]; ITd: 9%, 95% CI [0.05, 0.15], $p < 0.001$).

Within the high gamma band, $\text{PSD}_{\text{STIM-NORM}}$ during WN (12%, 95% CI [0.07, 0.16]) was greater than during BB (9%, 95% CI [0.04, 0.14], $p < 0.01$) and ITd (9%, 95% CI [0.04, 0.14], $p < 0.01$). However, there was no difference between $\text{PSD}_{\text{STIM-NORM}}$ across `CONDITION` for beta oscillations. $\text{PSD}_{\text{STIM-NORM}}$ recorded during the experimental condition was not affected by either the `SENSOR` × `CONDITION` interaction ($f = 0.05$, $F(62, 7124) = 0.30$, $p = 1.00$) or the main `SENSOR` effect ($f = 0.07$, $F(31, 7124) = 1.23$,

$p = 0.18$). This phenomenon can be attributed to the uniform spatial power distribution of the sensors (Fig. 3B).

Power during sound stimulation time windows within the ABS block

The linear mixed-effects analysis performed on $\text{PSD}_{\text{STIM-NORM}}$ revealed no main effect of time windows ($f = 0.001$, $F(1, 207447) = 0.44$, $p = 0.51$), suggesting a constant level of power between sound and rest time windows. However, we found that $\text{PSD}_{\text{STIM-NORM}}$ was influenced by the $\text{CONDITION} \times \text{FREQUENCY BAND} \times \text{TRIAL}$ interaction ($f = 0.03$, $F(6, 32245) = 5.23$, $p < 0.001$) for alpha and low gamma bands (Fig. 4). Post-hoc comparisons revealed a negative relationship that gradually decreased across trials for ITd in the alpha band (fitted estimate: $-0.003\% \pm 0.001$, $p = 0.05$), which was distinct from the positive relationships observed for WN (fitted estimate: $0.002\% \pm 0.001$, fitted difference: 0.005 , $p < 0.001$, Fig. 4A). Conversely, there was a main TRIAL effect on $\text{PSD}_{\text{STIM-NORM}}$ within the low gamma band during ITd (fitted estimate: $0.013\% \pm 0.0008$, $p < 0.001$), BB (fitted estimate: $0.010\% \pm 0.0009$, $p < 0.001$) and WN (fitted estimate: $0.015\% \pm 0.0008$, $p < 0.001$). Post-hoc contrasts revealed a greater increase in $\text{PSD}_{\text{STIM-NORM}}$ across TRIAL during WN and ITd compared to BB ($p < 0.001$, $p < 0.05$, respectively). The topographical distribution of $\text{PSD}_{\text{STIM-NORM}}$ values in the EEG sensor space across trials within the alpha bands showed a gradual decrease around CZ during WN and BB, while a more pronounced decrease was observed during ITd, with the lowest values around CZ reached between trials 9 and 12 (Q3, Fig. 4B). Within the low gamma band, there was a strong increase in the normalized power measure in all conditions since the first trials. Interestingly, as shown in Fig. 4B, the increase in $\text{PSD}_{\text{STIM-NORM}}$ was homogeneously distributed across the sensor-space.

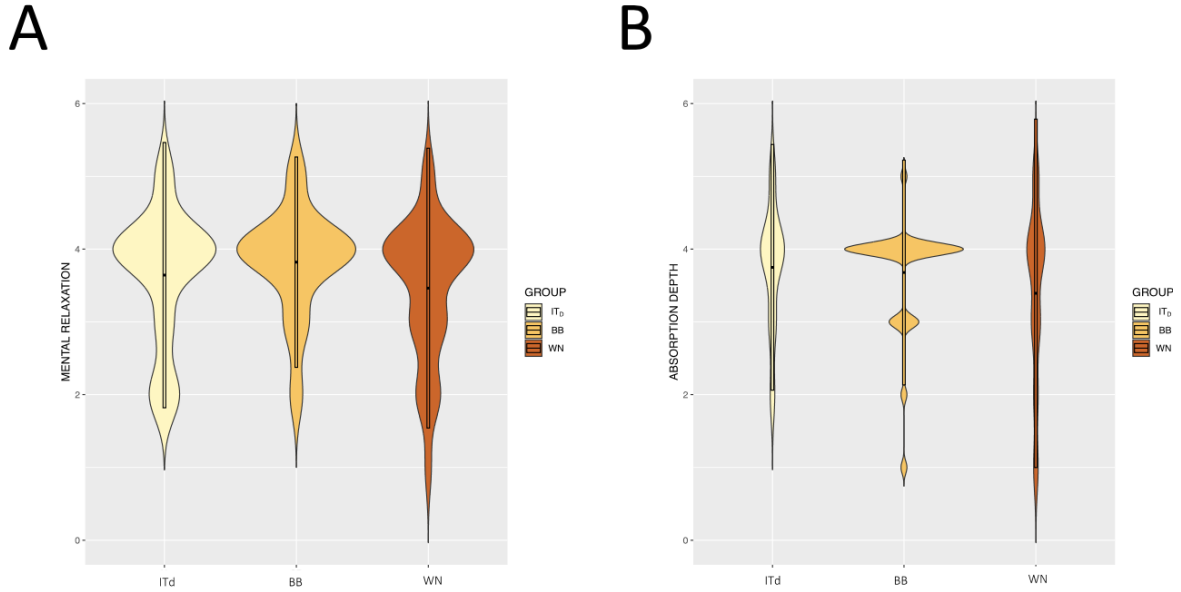


Figure 2: Violin plots obtained from participants’ self-report on the 5-points Likert scale. (a) Mental relaxation across experimental conditions. (b) Absorption depth across experimental conditions. In each plot, the median is represented by the horizontal black line inside the box, while the first and third quartiles are shown by the hinges. The width of the shaded regions reflects the data distribution.

Power during resting state post-experimental condition

The $PSD_{POST-NORM}$ was influenced by the FREQUENCY BAND \times CONDITION interaction ($f = 0.07$, $F(6, 7470) = 5.81$, $p < 0.001$). Table 2 summarizes the negative $PSD_{POST-NORM}$ values across conditions, except for ITd in the low gamma band, which showed statistically significant differences in post-hoc comparisons. Post-hoc tests revealed increased $PSD_{POST-NORM}$ within the low gamma band during ITd at the electrode CPZ, suggesting better connectivity in centro-parietal regions (10%, 95%CI [0.07, 0.16]). These results were not similar for BB and WN conditions, as BB did not reveal a different connectivity from the resting state period after listening, and WN demonstrated mainly negative connectivity values near the fronto-temporal region (14.6%, 95%CI [-0.11, 0.14]).

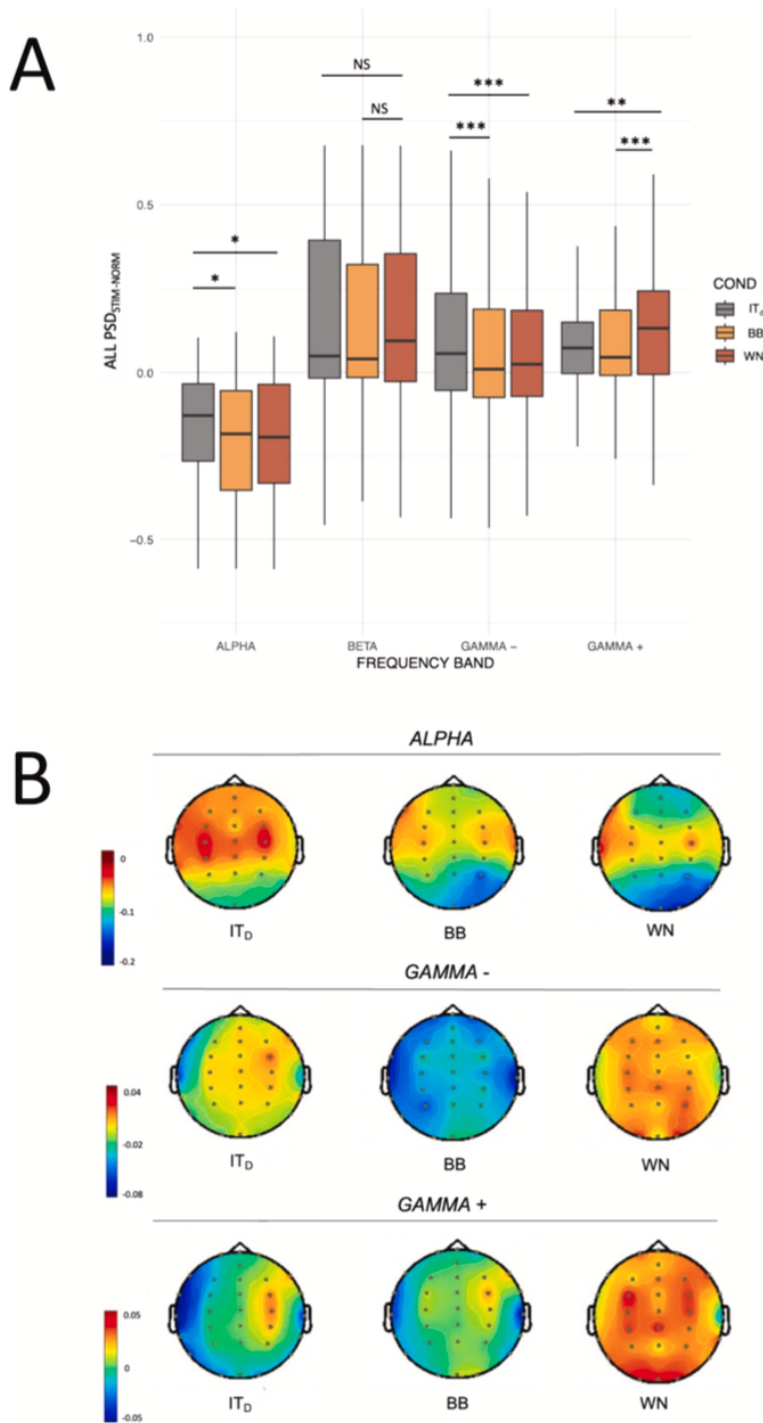


Figure 3: EEG power spectrum density during experimental conditions normalized against the baseline. (a) PSD_{STIM-NORM} values for the alpha (8–12 Hz), beta (15–29 Hz), low gamma (30–59 Hz) and high gamma (60–90 Hz) frequency range for all conditions (IT_d, BB, WN). (b) Topographic EEG maps of spectral power for alpha, low gamma, and high gamma frequency bands for all conditions. IT_d: derived-IT. BB: Binaural beats. WN: White noise. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NS: Not statistically significant.

Functional connectivity

For each condition, the sensors of interest demonstrated statistically significant differences between $iCOH_{RESTING-PRE}$ and $iCOH_{RESTING-POST}$ in the alpha ($p < 0.05$) and the low gamma ($p < 0.05$) frequencies, as revealed by a series of uncorrected paired t-tests. Fig. 5 presents the electrodes with significant maximum $iCOH_{POST-NORM}$ for ITd, BB, and WN conditions. Strong negative connectivity values after listening in the alpha bands (Fig. 5A) were located around central areas for ITd (C3 electrode, 13%, 95%CI $[-0.09, -0.16]$), compared to parietal areas for both BB and WN (PZ electrode, BB: 11.5%, 95%CI $[-0.08, -0.10]$; WN: 12.4%, 95%CI $[-0.10, -0.14]$). In the low gamma band (Fig. 5B), maximum $iCOH_{POST-NORM}$ was associated with positive values around the electrode CPZ for ITd, suggesting better connectivity in centro-parietal regions (10%, 95%CI $[0.07, 0.16]$). These results were not similar for BB and WN conditions, as BB did not reveal different connectivity from the resting state period after listening, and WN demonstrated mainly negative connectivity values near the fronto-temporal region (14.6%, 95%CI $[-0.11, 0.14]$).

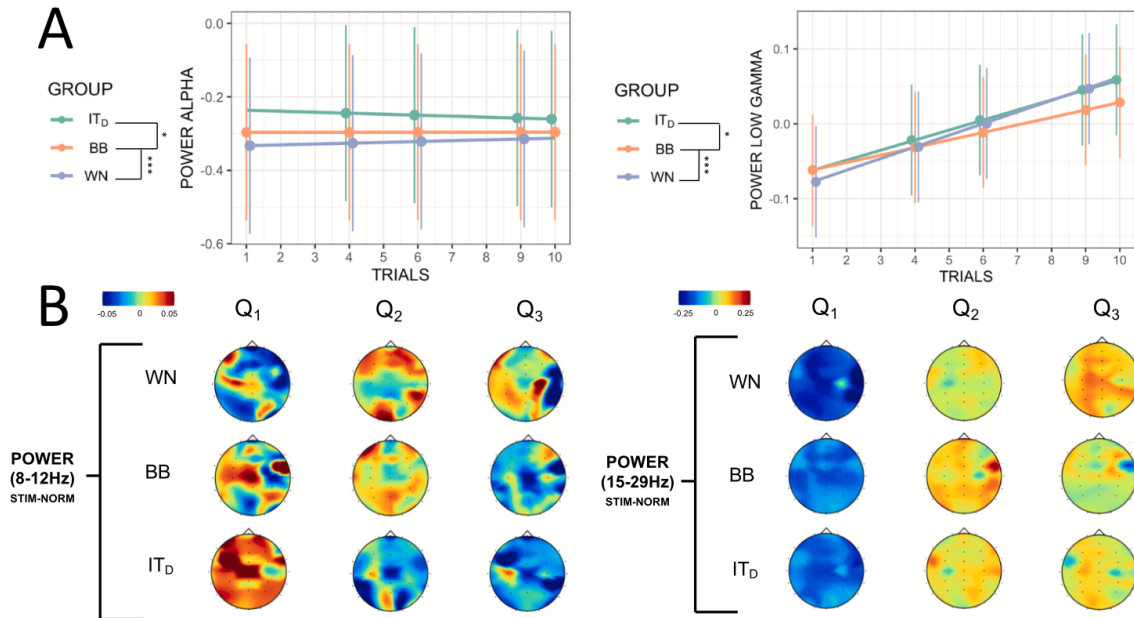


Figure 4: Main effect of TRIAL on the power spectrum density normalized to the baseline for the alpha (8–12 Hz) and low gamma (30–59 Hz) bands. (a) Regression slopes between PSDSTIM-NORM and TRIAL for each CONDITION. (b) Evolution of PSDSTIM-NORM topographies in the EEG sensor space for all conditions (ITd, BB, WN) according to TRIAL (Q1: Trial 1 to 4, Q2: Trial 5 to 8, Q3: Trial 9 to 12). ITd: derived-IT. BB: Binaural beats. WN: White noise. * $p < 0.05$, *** $p < 0.001$.

Discussion

The aim of the present study was to expand current knowledge regarding the effect of ITd on EEG oscillations, with a particular emphasis on power and coherence measures within the sensor space. Our investigation included a comparative study of the effects of ITd on

brain activity compared to other ABS approaches, such as WN and BB. In addition to objective measurements, we implemented subjective measures of depth absorption and mental relaxation induced by the various forms of ABS. Results demonstrated the selective influence of BB, ITd, and WN on EEG power spectrum densities, particularly within the low gamma and alpha frequency ranges. ITd showed a unique and distinct EEG pattern of power and coherence across alpha and gamma frequencies that differed from those observed during WN and BB. This original finding is the first experimental investigation and comparison of ITd with other ABS methods on brain activity within the same experimental design. We implemented a within-subjects experimental design, allowing detection of low effect sizes with high statistical power. Indeed, the primary purpose of the study was to detect oscillatory changes in response to different types of ABS at the single-session level. Although we measured small effect sizes, expecting large effects in the present design would be unrealistic, since experience-based neuroplasticity at the network level typically requires multiple days of stimulation.

While a priori selection of specific electrodes could have increased the magnitude of effect sizes, such an approach would be blind to events occurring at other scalp locations. EEG analyses can involve a preliminary selection of specific electrodes, as illustrated in past studies evaluating relaxation from a restricted number of scalp sensors (e.g., F3, F4, CZ) Krasnoff and Chevalier (2023). This could reduce the degrees of freedom and potentially reveal higher effect sizes. Here, we prioritized a data-driven and reproducible approach rather than arbitrarily attempting to report larger effect sizes. We are convinced that small effect sizes in fundamental research can be a starting point for future investigations with important theoretical and practical implications. The present study could thus serve as a basis for future investigations of ITd use as a method of inducing brainwave entrainment, leading to multiple applications, including novel therapeutic approaches.

Self-report ratings initially revealed no differences in depth absorption or mental relaxation between the ABS conditions. This confirms previous observations by Orozco Perez et al. (2023), who showed that the subjective experience of binaural and monaural auditory exposure do not differ from each other. Such a lack of difference attests that the subjective experience of the different types of auditory stimulation cannot be considered a confounding factor for the patterns of results, especially with regard to depth absorption and mental relaxation. The importance of investigating the effects of ABS on brain activity stems from the brainwave entrainment hypothesis, which provides a reliable neuroscientific framework for interpreting the neural synchronization response in human brain activity during ABS (for a review, see Ingendoh et al., 2023). The postulate of the entrainment hypothesis would predict increased EEG power during BB exposure compared to control conditions or baseline recordings in the frequency corresponding to the external signal. With respect to binaural gamma beats, these predictions are consistent with previous results (Jirakittayakorn and Wongsawat (2017)), thus providing experimental evidence for this theoretical framework. However, as reported in a recent systematic review of BB stimulation effects, more than half of the studies showed partially inconsistent results with the brainwave entrainment hypothesis (Ingendoh et al. (2023)). This could be attributed to small oscillatory changes in response to BB stimulation at the network level, especially after a single session, resulting in a small effect size [e.g. López-Caballero and Escera (2017); Ross and Lopez (2020)]. Here, we observed mixed results in validating the entrainment hypothesis, characterized by (i) an increase in EEG power during BB exposure compared to baseline, and (ii) a clear decrease in EEG power immediately after initial BB exposure, followed by a gradual increase throughout the BB stimulation

window. At the session level, although these findings were in line with the expected enhancement in neural activity, data did not reveal any differences in EEG power compared to WN. This observation is consistent with the work by López-Caballero López-Caballero and Escera (2017) who found no change in the power of rhythmic brain activity when participants were exposed to pink noise and BB stimulation. The lack of significant differences between WN and BB does not support ITd as being predominantly mediated by the brainwave entrainment or the stochastic resonance hypothesis, raising questions about the clear boundaries between these two phenomena. Interestingly, considering the assumption of EEG power enhancement through brainwave entrainment, ITd stimulation appeared to be more effective than BB and WN in eliciting synchronization of oscillations within the gamma frequency than WN or BB, as evidenced by the greater increase in EEG power relative to baseline. Furthermore, when all frequencies were examined, ITd elicited cross-frequency responses characterized by an increase in power within the alpha frequencies and a decrease in power within the gamma frequencies that were different from those elicited by WN and BB exposure.

During the auditory stimulation periods, the EEG power spectrum densities within the gamma band exhibited a gradual increase over time in all ABS conditions. However, the increase was reduced during BB compared to ITd and WN. Particularly, we observed negative values in PSDSTIM-NORM during the first six stimulation epochs before reaching positive values within the gamma band. These findings suggest that the impact of all types of ABS on EEG power may not be uniform throughout the duration of stimulation, which could contribute to the discrepancies in results observed in past studies. Indeed, previous investigations of continuous BB stimulation reported EEG findings supporting the brainwave entrainment hypothesis, by analyzing 5 min of continuous BB stimulation ?. In contrast, studies using shorter durations of BB tone presentation, ranging from 1 to 3 min López-Caballero and Escera (2017); Bazanova and Vernon (2014), or longer durations exceeding 20 min Wahbeh et al. (2007); Goodin et al. (2012); Gao et al. (2014), failed to detect observable changes in EEG activity. These discrepancies in the scientific literature across varying timings of BB-induced brain entrainment effects spurred the exploration of alternative auditory stimulation paradigms. For instance, several experiments examined evoked responses to short bursts of stimulation, and the resulting frequency-specific changes in EEG oscillations Bazanova and Vernon (2014); Becher et al. (2015). This approach was discussed in numerous studies, suggesting that presenting BB in short bursts might offer a more robust auditory stimulus, enhancing its ability to evoke EEG responses Goodin et al. (2012); Bazanova and Vernon (2014); Becher et al. (2015); López-Caballero and Escera (2017). The potential efficacy of the sound bursts as a more potent stimulus can be attributed to a stimulant effect that may mitigate the effects of habituation. Although the present study did not directly compare the effectiveness of continuous BB vs. short bursts, the ITd condition involved intermittent auditory stimulation. Henceforth, our findings support the modulatory influence of short stimulus durations on brain activity, as evidenced by the positive relationship observed between ABS exposure and EEG power spectrum density of low-gamma frequencies. Numerous studies documented the effect of gamma-BB on neural activity within temporal, frontal, and central brain regions Schwarz and Taylor (2005); Jirakittayakorn and Wongsawat (2017). Interestingly, Schwartz et al. Schwarz and Taylor (2005) reported that the maximal steady-state responses for both acoustic and BB burst stimulation were observed at CZ. While we failed to observe significant changes in EEG power amplitude within the gamma band at CZ, we detected changes around this electrode within the alpha frequency band. Notably, the

spatial distribution of PSDSTIM-NORM values across EEG sensors during trials revealed a gradual decrease around CZ, with the most pronounced effect observed during ITd compared to BB. These findings corroborate earlier observations regarding the ability of ITd to induce cross-frequency responses, through the decrease in the distribution of PSD in the alpha band over repeated stimulation. Furthermore, these changes near the central regions seemed to be reflected post-listening to ITd, as the analysis of iCOH revealed significant connections (either increase or decrease) at C3 and CPZ within the alpha and low gamma bands, respectively. The gamma connectivity, which decreased mainly at the PZ electrode after listening to WN and BB, further demonstrates the unique character of ITd in terms of brain responses in alpha and gamma frequencies compared to WN and BB. From a fundamental standpoint, the modulation of alpha amplitude appears to be related to distinct cognitive processes. Particularly, accumulated evidence supported that the decrease in alpha synchronization is correlated with enhanced attention Foxe et al. (1998); Feng et al. (2017); Bagherzadeh et al. (2020). In addition, the inverse temporal EEG pattern in alpha and gamma frequencies induced by ITd exposure supports existing evidence suggesting that these cross-frequency interactions may play a role in the maintenance of sensory-spatial working memory Roux and Uhlhaas (2014).

Another noteworthy critical result is the persistence of brain activity modulation, particularly in the low gamma band, after completion of the ABS paradigm under ITd. These observations of long-term effects seem specific to ITd, in contrast to BB exposure López-Caballero and Escera (2017). Our findings showed no long-term effects following BB exposure (neither in EEG power nor in EEG coherence), challenging previous research reporting such lasting effects Jirakittayakorn and Wongsawat (2017). However, comparisons of the present results with earlier research must be made with caution, since we administered short bursts of BB under ITd, whereas past intermittent auditory stimulation investigations did not explore post-listening brain activity Schwarz and Taylor (2005); Draganova et al. (2008). Another plausible factor contributing to the mixed patterns of findings is the variability in individual response patterns to ABS. As underlined by Kraus Kraus (2021), and Krasnoff and Chevalier Krasnoff and Chevalier (2023), individual variability may lead to important variations in EEG results. Although we included a large sample of participants in a within-subjects design to enhance the statistical power, future research on ABS should consider clustering or correlation analyses. Understanding individual variability in neurophysiological responses to ABS could strengthen the potential for ABS use in healthcare Ingendoh et al. (2023). For instance, a replication study would be essential to attest that the brainwave entrainment effect is more pronounced with ITd than with BB exposure. Additionally, our present assessment of the subjective effect of ABS on relaxation, and potentially absorption, might have been insufficient. First, we only collected subjective ratings after ABS, which prevented test–retest investigations of the different ABS conditions. Second, using a 5-point Likert-type scale on perceived relaxation may not be precise enough compared to standardized questionnaires. This might account for the fact that different EEG profiles between ABS conditions were not mirrored in subjective ratings, which were high and comparable across experimental conditions. Such discrepancies may be addressed in future research with more sensitive assessment tools, such as standardized questionnaires (e.g., the Relaxation State Questionnaire Steghaus and Poth (2022)) and additional objective markers, e.g., cardiac activity or galvanic skin resistance activity Masri et al. (2024). Eventually, future research could consider including the analysis of theta and delta waves, as documented in the context of relaxation and meditation Dobrakowski et al. (2020). There are indeed potential cross-frequency

effects with gamma BB Roux and Uhlhaas (2014). Such analyses could provide a better understanding of the complex neurophysiological processes underlying ABS, allowing for more conclusive results regarding brainwave entrainment effects.

The specific EEG signature in response to ITd might open promising avenues of research in neurocognition and consider ITd as a novel relevant form of ABS. Given that alpha and gamma oscillations are involved in distinct cognitive processes (e.g., attentional focus, working memory maintenance), the cross-frequency interaction induced by ITd may have the potential to enhance mental performance, such as motor imagery. ITd could thus represent an emerging paradigm for harnessing experience-based plasticity in conjunction with endogenous forms Colombié and Ladoucette (2023); Dos Anjos et al. (2023).

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CRedit authorship contribution statement

Typhanie Dos Anjos: Conceptualization, Formal analysis, Investigation, Project administration, Supervision, Visualization, Writing–original draft. **Franck Di Rienzo:** Conceptualization, Formal analysis, Methodology, Software, Supervision, Validation, Writing–review & editing. **Charles-Etienne Benoit:** Writing–review & editing. **Sebastien Daligault:** Data curation, Formal analysis, Software. **Aymeric Guillot:** Conceptualization, Methodology, Project administration, Supervision, Validation, Writing–review & editing.

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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