Neural Mechanisms of Pain Modulation by Binaural Beats

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Abstract

Binaural Beats (BB) constitute a convenient, low-cost neuromodulation approach applicable to daily life with potential analgesic effects. This study employed α-frequency band BB as stimulation and compared its effects with monaural beats (MB) and white noise in pain modulation and underlying neural mechanisms. EEG spectral analysis indicated that, compared to white noise, both BB and MB significantly reduced γ-band power, suggesting a common modulatory effect of rhythmic auditory stimulation on high-frequency neural activity. EEG microstate analysis revealed that, relative to MB and white noise, BB significantly enhanced microstate A activity while attenuating microstate C activity. Mediation analysis demonstrated that BB indirectly modulated pain-evoked P2 amplitude by reducing the transition probability between microstates C and D, thereby influencing subjective pain experience. In summary, BB may exhibit potential neuromodulatory value by reshaping dynamic functional brain networks and reducing attentional allocation to nociceptive stimuli.

Full Text

Neural Mechanisms of Binaural Beats in Pain Modulation

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Abstract

Binaural beats (BB) represent a convenient, low-cost neuromodulation approach suitable for daily applications with potential analgesic effects. This study employed alpha-frequency BB stimulation to compare its effects on pain modulation and underlying neural mechanisms against monaural beats (MB) and white noise. EEG spectral analysis revealed that, compared to white noise, both BB and MB significantly reduced gamma-band power, indicating a common modulatory effect of rhythmic auditory stimulation on high-frequency neural activity. EEG microstate analysis demonstrated that BB, relative to MB and white noise, significantly enhanced microstate A activity while attenuating microstate C activity. Mediation analysis showed that BB indirectly modulated pain-evoked P2 amplitude—and consequently subjective pain experience—by reducing the transition probability between microstate C and D. In summary, BB may exert its neuromodulatory value by reshaping dynamic functional brain networks and reducing attentional allocation to nociceptive stimuli.

Keywords: binaural beats, monaural beats, pain, EEG microstates, laser-evoked potentials

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1. Introduction

Pain is a complex experience encompassing sensory-discriminative, emotional-motivational, and cognitive-evaluative dimensions. Its essential function is to serve as an “early warning” system that helps organisms avoid potential danger. However, when pain persists beyond three months and develops into a chronic state, this adaptive mechanism can transform into a pathological process. Epidemiological data indicate that approximately 31% of the global population suffers from chronic pain, with nearly half experiencing clinically significant anxiety or depression symptoms, resulting in over $560 billion in annual healthcare expenditures and productivity losses (Cohen et al., 2021). In China, the prevalence of chronic pain reaches 30% and continues to rise annually; with population aging, this problem is expected to become more severe (樊碧发, 2020). Current clinical treatments primarily rely on opioids and nonsteroidal anti-inflammatory drugs, but long-term use may lead to tolerance, dependence, or addiction risks, and can cause serious adverse effects such as gastrointestinal bleeding and cardiovascular problems (Günther et al., 2018). Therefore, developing non-pharmacological interventions with high safety, reliable efficacy, strong compliance, and applicability in daily life has become a critical task in pain management. Such approaches would not only help alleviate patient suffering and improve quality of life but also provide important alternative pathways for reducing healthcare system burdens and medication misuse risks.

Non-invasive neuromodulation techniques (e.g., transcranial electrical stimulation and transcranial magnetic stimulation) have been widely regarded as promising pain interventions in recent years due to their unique advantages in modulating pain-related neural activity. Among them, transcranial alternating current stimulation (tACS), as a typical form of transcranial electrical stimulation, has attracted considerable attention for its ability to selectively enhance neural oscillations in specific frequency bands. tACS applies sinusoidal currents of specific frequencies to the scalp to induce synchronization of endogenous neural rhythms, thereby modulating neural activity associated with that frequency band (Herrmann et al., 2013).

Among various neural oscillation frequency bands, the alpha band (8–12 Hz) is considered closely related to perceptual suppression, attention allocation, and emotional regulation (Klimesch, 2012). EEG and MEG studies have shown that during pain states, alpha activity levels significantly decrease in the contralateral sensorimotor cortex (Hu et al., 2013; Peng et al., 2015). Conversely, higher individual alpha power before pain stimulation predicts lower subjective pain intensity, suggesting that enhanced alpha oscillations may have analgesic effects (Babiloni et al., 2006; Tu et al., 2016). Research on chronic pain patients has also found that their spontaneous alpha activity is significantly lower than healthy populations and negatively correlates with pain severity (Ahn et al., 2019), further supporting the critical role of alpha oscillations in pain modulation. Experimental evidence indicates that enhancing alpha oscillations through tACS may help alleviate pain experience (Ahn et al., 2019; Arendsen et al., 2018; Jia et al., 2025). However, the actual analgesic effects of tACS remain controversial, as some studies have failed to observe significant intervention effects (Li, Jin, et al., 2025; May, Hohn, et al., 2021; Peng et al., 2023), possibly due to individual differences, stimulation parameter settings, or non-specific neural activity changes induced by stimulation. Therefore, although tACS modulation of alpha rhythms theoretically possesses analgesic potential, its effect size may be insufficient, or it may interfere with neural oscillations in other frequency bands, affecting overall intervention efficacy. Additionally, tACS faces numerous practical limitations. Current tACS devices are typically bulky and expensive, which is not conducive to portable use in daily scenarios; their electrodes require firm attachment to the scalp, which can affect wearing comfort and user mobility, making prolonged continuous application in natural life situations difficult. These factors have limited the popularization and promotion of tACS in real-life settings, necessitating the exploration of more convenient, low-cost, and widely applicable alternative neuromodulation approaches.

Rhythmic auditory stimulation (such as binaural beats, BB) represents a non-invasive intervention that induces specific neural rhythms through the auditory pathway, offering advantages of low cost, simple operation, and suitability for home use. BB refers to the phenomenon where, when each ear receives pure tones of slightly different frequencies (e.g., 400 Hz and 410 Hz), although each ear alone cannot perceive a beat, the central auditory system—particularly the superior olivary complex in the brainstem—integrates these two frequencies to generate a “virtual beat” at the difference frequency (e.g., 10 Hz) at the central level. This signal is not only integrated at the brainstem level but can also be transmitted upward along the auditory pathway to induce cortical neural rhythms matching the difference frequency, thereby achieving rhythmic modulation of EEG activity (Draganova et al., 2008; Li et al., 2023; Oster, 1973; Pratt et al., 2010). In contrast, monaural beats (MB) are physical interference signals formed by mixing two different frequency pure tones in the same audio channel; their rhythmic characteristics are directly produced by external sound waves and rely more on peripheral auditory pathway processing rather than central neural integration (Draganova et al., 2008; Pratt et al., 2009; Schwarz & Taylor, 2005). Therefore, MB represents externally driven rhythmic sound stimulation, whereas BB involves more complex central neural processing mechanisms and is considered to have greater potential for inducing neural plasticity (Draganova et al., 2008). Although recent studies have preliminarily supported the analgesic potential of BB (Ecsy et al., 2017; Gkolias et al., 2020; Maddison et al., 2023; Padmanabhan et al., 2005), existing literature often compares BB with white noise (Abd Hamid et al., 2025; Ecsy et al., 2017), with few direct comparisons between BB and MB. This makes it difficult to determine whether BB’s effects stem from its unique central integration mechanism or merely reflect non-specific effects of general rhythmic sound stimulation. Based on this, our study is the first to systematically compare differences between BB and MB in pain neuromodulation, aiming to clarify whether BB achieves its modulatory effects through specific neural mechanisms, thereby providing neuroscientific evidence for BB as a viable non-pharmacological analgesic intervention.

BB may influence pain experience through frequency-specific local neural oscillation mechanisms or global brain functional state regulation mechanisms. At the local neural oscillation level, BB can induce and enhance neural activity in target frequency bands, thereby modulating sensory and cognitive processes associated with that band. Previous studies have shown that alpha-BB can enhance alpha power and phase synchronization in local brain regions (Gao et al., 2014; Ioannou et al., 2015; Kim et al., 2023; Solcà et al., 2016), thereby inhibiting the transmission and integration of nociceptive input and reducing pain perception. For example, Ioannou et al. (2015) found that alpha-BB enhanced alpha neural oscillation activity in temporal and parietal regions, which are closely related to attention allocation and distraction inhibition; therefore, alpha-BB may reduce individuals’ experience of unpleasant stimuli by improving attention and memory functions. At the global level, BB may also indirectly affect pain processing by modulating interactions between brain networks. For instance, alpha-BB has been found to enhance functional connectivity between visual, attention, and frontoparietal networks (Abd Hamid et al., 2025), while Maddison et al. (2023) proposed that BB may act on thalamocortical circuits related to pain consciousness generation. In this context, EEG microstate analysis provides a powerful tool for characterizing global dynamic brain function (e.g., Garrett et al., 2013; Preti et al., 2017). Microstates refer to stable EEG spatial patterns that persist for tens of milliseconds and constitute a limited set of functional states through rapid switching (see Custo et al., 2017; Michel & Koenig, 2018).

Research has shown that resting-state EEG can be characterized by 4–6 typical microstates, which exhibit high consistency across individuals and are associated with specific brain networks and cognitive processes (Khanna et al., 2015; Michel & Koenig, 2018). Importantly, both acute and chronic pain are accompanied by abnormalities in microstate characteristics (González-Villar et al., 2020; Jaltare & Torta, 2025; Li et al., 2022; May, Gil Ávila, et al., 2021; Qiu et al., 2023), suggesting that microstates can sensitively capture pain-related brain dynamic abnormalities. Therefore, applying microstate analysis to BB analgesia research can help reveal its underlying neuromodulatory mechanisms from the perspective of global brain functional states, providing a new theoretical framework for understanding how BB indirectly influences pain processing through dynamic network reorganization.

In summary, although preliminary studies have suggested BB's potential in modulating neural activity and subjective experience, its specific role in pain modulation and underlying neural mechanisms remain to be further validated. In particular, it remains unclear whether BB influences pain perception by inducing frequency-specific neural oscillations or through broader brain functional state regulation. Combining EEG spectral analysis with EEG microstate analysis promises to further reveal the neural basis and dynamic processes of BB-mediated pain modulation. To this end, this study aimed to systematically investigate the neural modulatory mechanisms of 10 Hz BB on pain, using MB and white noise as control conditions. By recording resting-state EEG during intervention and EEG activity during pain tasks, we compared subjective pain ratings and corresponding neural indices across the three conditions. We hypothesized that: (1) compared to MB and white noise, BB intervention would significantly reduce subjective pain intensity; (2) BB would induce more significant frequency-specific neural oscillations in the alpha band and correlate with pain relief; (3) BB might indirectly participate in pain modulation through EEG microstate activity. This study is expected to provide empirical support and theoretical foundation for the neuroscientific basis of BB as a non-pharmacological pain intervention.

2. Methods

2.1 Participants

Sample size estimation was conducted using G*Power software (Faul et al., 2007), setting a medium effect size ($f = 0.25$), desired statistical power ($1-\beta = 0.95$), and significance level ($\alpha = 0.05$), which indicated a minimum required sample size of 43 participants. Ultimately, 50 healthy university students were recruited (24 males, age: $M \pm SE = 20.24 \pm 0.27$ years). All participants were non-psychology or music majors, right-handed, had normal or corrected-to-normal vision, and had no history of acute/chronic pain, neuropsychiatric disorders, or cardiovascular diseases. Due to 6 participants being unable to complete the experimental task and 2 experiencing equipment failure, the final sample included in data analysis comprised 42 participants (21 males, age: $M \pm SE = 20.41 \pm 0.31$ years). This study protocol was approved by the Human Research Ethics Committee of the Non-Clinical Department of the School of Psychology, Shenzhen University (approval number: SZU_PSY_2023_042). All participants signed informed consent forms before the experiment and were informed about relevant experimental procedures and their rights.

2.2 Research Design and Experimental Procedure

This study employed a single-factor three-level within-subjects design, with auditory intervention type (BB, MB, white noise) as the independent variable. The main dependent variables included pain intensity and unpleasantness ratings from the pain rating task, laser-evoked EEG responses, and characteristics of spontaneous brain activity during auditory intervention.

The experimental procedure is illustrated in Figure 1A. Upon arrival at the laboratory, participants first underwent pain threshold calibration to determine individualized laser stimulation intensity. Subsequently, participants received three auditory interventions (BB, MB, white noise) in random order, each lasting 10 minutes. Following each intervention, participants completed a pain rating task lasting approximately 7.5 minutes to assess the impact of different auditory interventions on pain perception. To avoid carryover effects between interventions, a 15-minute rest interval was provided after each task.

[Figure 1: see original paper] Research design and experimental procedure. (A) Schematic diagram of the overall experimental procedure. (B) Three auditory stimulation conditions: binaural beats (BB), monaural beats (MB), and white noise (WN). (C) Single-trial procedure for the pain rating task.

2.3 Pain Stimulus Intensity Calibration

This study used a laser pulse pain stimulator (Nd:YAP, Electronical Engineering, Italy) to generate brief thermal pulse stimuli. Laser pulses were delivered through an optical fiber with a wavelength of 1.34 μm, spot diameter of approximately 7 mm, and pulse duration of 4 ms. Before the formal experiment, all participants completed individualized calibration of laser stimulation intensity to determine the moderate-intensity pain stimulation energy used in the experiment. Calibration employed an ascending method, with initial laser energy set at 2 J and gradually increased in 0.25 J steps, with inter-stimulus intervals controlled between 8000–10000 ms. After each laser stimulation, participants immediately rated the perceived pain intensity on a scale of 0–10, where 0 indicated "no pain" and 10 indicated "unbearable pain." Each participant completed three rounds of calibration tests. Based on the average curve of the energy-rating relationship from the last two rounds, the laser energy that consistently evoked a pain rating of 6 was determined as the moderate-intensity stimulation level used in the formal experiment.

2.4 Auditory Stimulus Materials

All auditory stimuli in this study were generated offline using MATLAB 2021a and presented binaurally through headphones. To induce cortical neural synchronization in the alpha band, the BB condition presented pure tones of 445 Hz and 455 Hz to the left and right ears, respectively, with consistent starting phases. Their frequency difference (10 Hz) was subjectively perceived as the beat frequency (Draganova et al., 2008). This rhythmic perception is believed to originate in the medial nucleus of the superior olivary complex in the brainstem, even though the stimulus signal itself contains no physical energy at 10 Hz in either spectrum or envelope (Wernick & Starr, 1968). To control for potential confounding effects of rhythmicity, two control conditions were included: MB and white noise. In the MB condition, 445 Hz and 455 Hz pure tones were linearly superimposed in the digital domain, with amplitude divided by 2 to control loudness; the resulting monaural signal was then presented synchronously to both ears (Orozco Perez et al., 2020). This stimulus retained the 10 Hz rhythmicity but eliminated the binaural integration process, allowing examination of rhythmic effects on emotion and cognition independent of binaural processing. In the white noise condition, a white noise signal with a flat power spectrum was randomly generated using MATLAB as a non-rhythmic control. All auditory stimuli were adjusted to a comfortable listening intensity level as subjectively determined by each participant.

2.5 Pain Rating Task

The single-trial procedure for the pain rating task is illustrated in Figure 1C. Each trial began with a fixation cross ("+") presented at the center of the screen for 2000 ms, followed by a blank screen lasting 4000 ms. Subsequently, a moderate-intensity laser stimulus was delivered to the dorsum of the participant's left hand. After an interval of 4000–5000 ms following stimulation, participants were asked to verbally rate the intensity and unpleasantness of the laser-evoked pain they experienced. Ratings ranged from 0 to 10, where 0 indicated "no pain/unpleasantness" and 10 indicated "unbearable pain/unpleasantness." To avoid local skin sensitization or habituation of pain perception, the target point for each laser stimulus was manually moved at least 1 cm randomly. Each pain rating task consisted of 20 trials, with inter-trial intervals of 2000–4000 ms. During the experiment, E-Prime 3.0 software was used to record behavioral data, while EEG signals were continuously recorded for subsequent analysis.

2.6 EEG Data Acquisition and Analysis

This study used an ERP recording system from Brain Products GmbH, Germany, equipped with a 64-channel Ag/AgCl electrode cap based on the extended international 10–20 system, to collect EEG signals from participants during the pain rating task and auditory intervention. Recording parameters were set with a bandpass filter of 0.01–100 Hz, sampling rate of 1000 Hz, and electrode-scalp impedance maintained below 10 kΩ, with the FCz electrode serving as the online reference.

EEG data preprocessing and analysis were completed using the EEGLAB v21.0 toolbox (Delorme & Makeig, 2004) in the MATLAB 2021a environment. During offline analysis, EEG signals were first bandpass-filtered at 1–100 Hz and notch-filtered at 49–51 Hz to remove power line noise, then converted to average reference. For EEG response analysis of the pain rating task, data epochs from 1000 ms before to 2000 ms after laser stimulus presentation were extracted, with the 1000 ms pre-stimulus period used as baseline for correction. For EEG data during the auditory intervention phase, continuous signals were segmented into independent epochs of 2000 ms length for analysis. To remove ocular and muscle artifacts, independent component analysis was applied for correction (Jung et al., 2001), after which processed data underwent further baseline correction.

2.6.1 Event-Related Potential Analysis Laser pain stimulation selectively activates peripheral $A\delta$ and C nerve fibers and elicits laser-evoked potentials (LEPs) in the brain (Iannetti et al., 2003). In this study's pain rating task, we focused on analyzing the N2 and P2 components of LEPs. The specific analysis procedure was as follows: First, single-trial LEP waveforms obtained under each of the three experimental conditions (BB, MB, white noise) were averaged for each participant to obtain individual average waveforms for each condition. Subsequently, these individual average waveforms were averaged across participants to obtain group-level LEP waveforms for each condition. Based on group-level waveform maps, topographic distributions, and previous literature (Tarkka & Treede, 1993), electrode sites and time windows for component extraction were determined. N2 and P2 components are typically most prominent at central electrode sites (Hu & Iannetti, 2019; Iannetti et al., 2008; Mouraux & Iannetti, 2009; Zhang et al., 2022). In this study, we examined waveforms at three central electrodes (C1, Cz, C2) and used their average amplitude as the measurement for N2 and P2 to improve robustness and reduce noise or individual differences from single electrodes. Analysis time windows were set at 210–260 ms post-stimulation (N2) and 330–390 ms (P2), with mean amplitudes calculated within each window for statistical analysis.

2.6.2 EEG Spectral Analysis To examine the effects of alpha-band BB sound on local neural oscillations, spectral analysis was performed on resting-state EEG data collected during the auditory intervention phase. Considering that transient neural activity changes may occur at stimulation onset and offset, to obtain more stable neural oscillation characteristics, we selected the middle 6 minutes of data (excluding the first and last 2 minutes) for analysis. Spectral analysis employed Welch's method, computing spectrograms for each electrode and time period for each participant, then averaging spectrograms across the same experimental condition. To standardize power spectral intensity, logarithmic values of power spectra were calculated to obtain absolute power spectral density in decibels (dB). Frequency bands were defined as: δ (1–3 Hz), θ (3–8 Hz), α (8–13 Hz), β (13–30 Hz), γ (30–90 Hz). In each frequency band, the following key brain region electrodes were selected for analysis: left sensorimotor region electrodes (C3, CP1, CP5), right sensorimotor region electrodes (C4, CP2, CP6), frontal electrodes (Fz, FC1, FC2), and parietal electrodes (Pz, P3, P4, POz).

2.6.3 Microstate Analysis To investigate the effects of alpha-band BB sound on global neural network activity, microstate analysis was performed on the middle 6 minutes of resting-state EEG data during the auditory intervention phase. Following the procedure proposed by Brodbeck et al. (2012): preprocessed EEG data were bandpass-filtered at 2–20 Hz and downsampled to 250 Hz to improve spatiotemporal precision and reduce computational burden (Qiu et al., 2023). Subsequently, Global Field Power (GFP) was calculated as the standard deviation of signals across all electrodes at each time point, measuring the instantaneous intensity of EEG activity across the entire scalp. Considering that topographic distributions near GFP peaks exhibit high temporal stability, EEG topographies corresponding to GFP peaks were selected for subsequent cluster analysis. The clustering method employed T-AAHC (Topographic Atomize and Agglomerate Hierarchical Clustering), an optimization of K-means clustering that effectively ignores polarity differences to more accurately capture topographic features. Clustering proceeded in two stages: (1) Individual-level clustering: for each participant, initial clustering was performed across cluster numbers $k = 2$–8 to obtain multiple candidate topographies; (2) Group-level clustering: ignoring polarity, all individual clustering results were clustered again across $k = 2$–8 to generate group-level microstate topographies. The optimal cluster number was finally determined using the Krzanowski-Lai criterion, which indicated that four microstate prototypes most stably explained EEG topographic distributions across all experimental conditions.

Using a "back-fitting procedure," microstate time series were constructed for each participant under each experimental condition by calculating correlation coefficients between EEG topographies at each time point and the identified group-level microstate topographies. Specifically, the system assigned each time point's EEG distribution to the microstate category with which it was most correlated, thereby forming a complete microstate sequence. Based on this microstate time series, three commonly used microstate characteristic parameters were extracted: (1) Mean duration: the average time length that each microstate remains in a single occurrence, reflecting its neural stability; (2) Occurrence rate: the number of times the microstate appears per unit time, characterizing the trend of neural activity; (3) Time coverage: the proportion of total time during which the microstate dominates, reflecting its dominance in overall neural dynamics. Additionally, transition probability matrices between microstates were calculated to assess dynamic transformation patterns between different brain states, providing a basis for further understanding the dynamic regulatory mechanisms of brain networks under BB stimulation.

2.7 Statistical Analysis

This study used statistical toolboxes in MATLAB and SPSS 26 for statistical analysis. First, one-way repeated measures ANOVA was performed separately on pain intensity and unpleasantness ratings from the pain rating task, mean amplitudes of LEP components, power spectral density across different frequency bands during the auditory intervention phase, and microstate parameters (such as mean duration, occurrence rate, and time coverage) to test whether experimental conditions (BB vs. MB vs. white noise) had significant effects on these variables. If data violated sphericity assumptions, Greenhouse–Geisser correction was applied to adjust degrees of freedom. If main effects were significant, post-hoc pairwise comparisons were conducted using Bonferroni correction to control for Type I error rate inflation from multiple comparisons. After identifying significant modulatory effects of BB intervention on pain experience and neural indices, Pearson correlation analysis was further conducted to explore associations between these subjective and objective variables. For example, whether changes in power spectral density or microstate features showed significant correlations with changes in pain-evoked responses, providing a basis for subsequent mechanism analysis. To further verify whether neural activity changes mediated the relationship between BB and pain experience, this study employed the MEMORE 2.1 macro (Montoya & Hayes, 2017) for mediation analysis. Specifically, the bootstrap percentile method was used to estimate the stability of mediation effects, with 5000 resampling iterations to obtain confidence intervals (CI). If the 95% CI did not include 0, the mediation effect was considered significant, indicating that a particular neural index might explain BB's effect on pain experience; conversely, it suggested that the mediation effect was not established.

3. Results

3.1 Effects of Alpha-Band Binaural Beats on Pain-Evoked Responses

As shown in Figure 2A, mean values of laser-evoked pain intensity and unpleasantness ratings were relatively similar across auditory intervention conditions. One-way repeated measures ANOVA on behavioral indices revealed no significant differences in pain intensity ratings across the three conditions

($F(2,82) = 0.83, p = 0.432, \eta_p^2 = 0.02$), and no significant main effect was observed for unpleasantness ratings ($F(2,82) = 0.74, p = 0.465, \eta_p^2 = 0.02$). These results indicate that alpha-band binaural beats (BB) did not produce significant changes in subjective pain perception.

At the neurophysiological level, Figure 2B shows waveform and topographic maps of LEPs under each condition. Further one-way repeated measures ANOVA on amplitudes of N2 and P2 components similarly revealed no significant differences (see Figure 2C): no significant main effect was found for mean amplitude of the N2 component across conditions ($F(2,82) = 0.47, p = 0.624, \eta_p^2 = 0.01$), nor for the P2 component ($F(2,82) = 0.48, p = 0.613, \eta_p^2 = 0.01$).

In summary, alpha-band binaural beats showed no significant analgesic effects compared to rhythm-matched MB or white noise, either in subjective ratings or LEP neural responses. This suggests that under our experimental settings, alpha-BB did not effectively modulate perceptual experience or cortical responses during pain processing.

[Figure 2: see original paper] Laser-evoked pain ratings and brain responses. (A) Laser-evoked pain intensity and unpleasantness ratings. (B) Laser-evoked potential (LEP) waveforms and topographic maps. Green markers indicate electrode regions included in the component analysis; gray shaded areas in waveforms indicate time windows included in the analysis. (C) Laser-evoked N2 and P2 amplitudes. Bar graphs show mean amplitudes across conditions, with data presented as $M \pm SE$.

3.2 Effects of Alpha-BB on Neural Oscillations

Figure 3A shows grand-average power spectra across the three auditory intervention conditions (BB, MB, white noise) and topographic distributions of energy across frequency bands. We selected gamma-band power in regions of interest and performed one-way repeated measures ANOVA separately for each region.

Results showed significant differences in gamma oscillation power across intervention conditions in the right sensorimotor region ($F(2,82) = 3.96, p = 0.029, \eta_p^2 = 0.09$) and parietal region ($F(2,82) = 4.33, p = 0.018, \eta_p^2 = 0.10$) (see Figure 3B). Further simple effects analysis indicated that, compared to the white noise condition, both BB and MB interventions trended toward reducing gamma power in the right sensorimotor region (BB: -19.36 ± 0.32 dB; MB: -19.17 ± 0.36 dB; white noise: -18.64 ± 0.39 dB; BB vs. white noise: $p = 0.077$; MB vs. white noise: $p = 0.061$) and significantly reduced gamma power in the parietal region (BB: -21.09 ± 0.41 dB; MB: -20.93 ± 0.46 dB; white noise: -20.27 ± 0.44 dB; BB vs. white noise: $p = 0.033$; MB vs. white noise: $p = 0.047$). Main effects for other brain regions and frequency bands were not significant (all $p > 0.05$). These results indicate that MB/BB in the alpha band can reduce gamma oscillations in some brain regions, particularly parietal and right sensorimotor areas, suggesting that low-frequency rhythmic auditory stimulation may regulate neural state by modulating local high-frequency neural activity, but BB's modulatory effect was not significantly superior to MB.

[Figure 3: see original paper] EEG spectral analysis results during auditory intervention. (A) Grand-average spectra across different intervention conditions and topographic distributions of energy across frequency bands. (B) Effects on spectral power in the gamma band across different brain regions. Bar graphs show mean power across conditions, with data presented as $M \pm SE$. #: $p < 0.10$; *: $p < 0.05$; **: $p < 0.01$.

3.3 Effects of Alpha-BB on Microstates

Figure 4A shows the four dominant microstate topographies identified under the three auditory intervention conditions (BB, MB, white noise), with distribution patterns consistent with previous research (Tarailis et al., 2024). Specifically, microstate A showed an opposite-polarity distribution between right frontal and left occipital regions, microstate B exhibited left frontal-right occipital opposition, microstate C was located between frontal and occipital regions, and microstate D concentrated between frontocentral and occipital regions. Microstate topographies obtained under the three intervention conditions were highly similar to each other, with very high correlations between topographies of each microstate (all $r > 0.93, p < 0.001$), indicating that microstate spatial patterns remained stable across different intervention conditions. To assess the goodness-of-fit and stability of the microstate model, we compared Global Explained Variance (GEV) across the three auditory intervention conditions. Results showed that GEV was high under all conditions (BB: 76.38% ± 0.84%; MB: 76.65% ± 0.68%; white noise: 76.41% ± 0.80%). One-way ANOVA revealed no significant differences in GEV across conditions ($F(2, 82) = 0.57, p = 0.56, \eta_p^2 = 0.01$), indicating that microstate templates extracted under the three conditions all had good stability and comparability.

Figures 4B–D show mean duration, occurrence rate, and time coverage of each microstate under the three conditions. Two-way repeated measures ANOVA with microstate type (A, B, C, D) × intervention condition (BB, MB, white noise) was performed on these three features, with statistical results shown in Table 1. Analysis of mean duration (Figure 4B) revealed a significant interaction between microstate type and intervention condition ($F(6, 246) = 7.63, p < 0.001, \eta_p^2 = 0.16$). Simple effects analysis showed that under the BB condition, mean duration of microstate A (68 ± 1 ms) was significantly longer than under MB (66 ± 1 ms, $p = 0.009$) and white noise (65 ± 1 ms, $p < 0.001$); duration of microstate C under BB (63 ± 2 ms) was significantly shorter than under MB (68 ± 3 ms, $p = 0.002$) and white noise (69 ± 3 ms, $p = 0.019$).

Statistical results of dynamic characteristics of EEG microstates during auditory intervention

Analysis of occurrence rate (Figure 4C) showed significant main effects of microstate type ($F(3, 123) = 7.18, p < 0.001, \eta_p^2 = 0.15$) and its interaction with intervention condition ($F(6, 246) = 25.08, p < 0.001, \eta_p^2 = 0.38$). Specifically: microstate A occurrence rate under BB (4.22 ± 0.09 microstate/s) was significantly higher than under MB (3.89 ± 0.09 microstate/s) and white noise (3.86 ± 0.09 microstate/s, both $p < 0.001$); microstate B occurrence rate under BB (3.94 ± 0.08 microstate/s) was higher than under MB (3.76 ± 0.07 microstate/s, $p = 0.001$); microstate C occurrence rate was lowest under BB (3.35 ± 0.09 microstate/s), significantly lower than under MB (3.72 ± 0.09 microstate/s) and white noise (3.88 ± 0.10 microstate/s, both $p < 0.001$); microstate D occurrence rate under MB (4.20 ± 0.11 microstate/s) was higher than under white noise (4.07 ± 0.11 microstate/s, $p = 0.049$).

Analysis of time coverage (Figure 4D) revealed a significant interaction between microstate type and intervention condition ($F(6, 246) = 18.78, p < 0.001, \eta_p^2 = 0.31$). Results showed that under BB, time coverage of microstate A ($28.29 \pm 7.22\%$) was significantly higher than under MB ($25.29 \pm 7.32\%$) and white noise ($24.55 \pm 7.15\%$, all $p < 0.001$); microstate B under BB ($24.45 \pm 5.94\%$) was higher than under MB ($23.24 \pm 5.16\%$, $p = 0.005$); microstate C under BB ($20.90 \pm 8.60\%$) was significantly lower than under MB ($24.65 \pm 10.38\%$) and white noise ($25.93 \pm 11.06\%$, both $p < 0.001$). These results indicate that BB significantly modulated brain microstate dynamics during resting state, manifested as increased activity of microstate A (increased duration, occurrence rate, and coverage) and decreased activity of microstate C.

[Figure 4: see original paper] EEG microstate characteristics during auditory intervention. (A) Microstate topographies under different auditory intervention conditions. (B) Mean microstate duration. (C) Microstate occurrence rate. (D) Microstate time coverage. Note: Bar graphs show microstate features across intervention conditions, with data presented as $M \pm SE$. $: p < 0.05$; $: p < 0.01$; $: p < 0.001$.

For statistical analysis of microstate transition rates, Bonferroni correction was applied to control for multiple comparisons bias, with specific results shown in Table 2. As illustrated in Figure 5A, transition rates for $A \leftrightarrow D$ (including $A \to D$ and $D \to A$), $A \leftrightarrow B$ (including $A \to B$ and $B \to A$), $C \leftrightarrow B$ (including $C \to B$ and $B \to C$), $C \leftrightarrow D$ (including $C \to D$ and $D \to C$), and $A \to C$ all showed significant intervention effects. To more intuitively present the overall interaction strength between two microstates and highlight their coupling degree, we combined unidirectional transition rates between two microstates. As shown in Figures 5B–E, compared to white noise, both MB and BB increased $A \leftrightarrow D$ transition rates (MB: $p = 0.005$; BB: $p < 0.001$); relative to MB and white noise, BB also demonstrated more specific neural effects, increasing $A \leftrightarrow B$ and $A \leftrightarrow D$ transition rates (both $p < 0.001$) and significantly decreasing $C \leftrightarrow B$ and $C \leftrightarrow D$ transition rates (both $p < 0.001$). Additionally, compared to white noise, BB further reduced $A \to C$ transitions ($p = 0.001$). These findings suggest that BB may reshape brain functional architecture by promoting dynamic interactions between microstate A and B and D, while simultaneously inhibiting transition frequencies between microstate

C and B and D, thereby enhancing the brain's information integration and response capabilities to external stimuli.

Statistical results of EEG microstate transition rates during auditory intervention

[Figure 5: see original paper] Transition probabilities between EEG microstates during auditory intervention. (A) Comparison of microstate transition probabilities across binaural beats (BB), monaural beats (MB), and white noise (WN) conditions. (B) Comparison of microstate transition probabilities between BB and MB conditions. (C) Comparison between BB and WN conditions. (D) Comparison between MB and WN conditions. (E) Illustration of transition probabilities between microstates under different auditory intervention conditions. Note: Orange and purple arrows indicate significantly increased or decreased transition probabilities between microstates under two intervention conditions, respectively; gray dashed arrows indicate no significant difference in transition probability between conditions. Mean transition probability differences between each microstate pair are shown in bar graphs, with data presented as $M \pm SE$. : $p < 0.05$; : $p < 0.01$; : $p < 0.001$.

3.4 Mediation Analysis

Pearson correlation analysis results indicated that in the BB vs. MB comparison, the degree of reduction in microstate $C \leftrightarrow D$ transition probability under BB was significantly positively correlated with reduction in laser-evoked P2 amplitude ($r(42) = 0.38, p = 0.014$; see Figure 6A). This suggests a potential intrinsic association and that microstate dynamic changes may play a role in BB's analgesic mechanism. Based on this, we further constructed a serial mediation model for validation. In model design, we selected BB vs. MB as the comparison pair because they are similar in physical properties and rhythmic structure, but differ fundamentally in that BB relies on central auditory system integration of binaural inputs to generate a virtual rhythm, whereas MB depends only on physical interference of sound waves to form an external rhythm (Draganova et al., 2008; Pratt et al., 2009, 2010). Therefore, the BB vs. MB comparison better focuses on the unique mechanism of centrally synthesized rhythm while avoiding non-specific effects introduced by large differences in sound properties when comparing with white noise.

Specifically, the mediation model set auditory intervention type (BB vs. MB) as the independent variable, microstate $C \leftrightarrow D$ transition probability and P2 amplitude as mediators, and pain intensity rating as the dependent variable. Results (Figure 6B) showed that alpha-BB intervention indirectly reduced P2 amplitude by decreasing transition probability between microstates C and D, ultimately reducing pain perception (indirect effect: $a \times b \times c = -0.41, SE = 0.24, 95\% CI = [-1.05, -0.11]$, which does not include 0, indicating a significant mediation effect). Therefore, BB influences individual subjective pain experience by modulating the transformation pattern between microstates C and D, affecting pain-related EEG responses (P2).

[Figure 6: see original paper] Correlation between EEG microstates and pain-evoked responses and mediation effect analysis. (A) Correlation between microstate $C \leftrightarrow D$ transition probability and laser-evoked P2 amplitude. Compared to the MB condition, the reduction in microstate $C \leftrightarrow D$ transition probability under BB was significantly positively correlated with reduction in P2 amplitude. Scatter plot shows individual data and regression line, with gray shaded area indicating 95% confidence interval. (B) Mediation model results. Auditory intervention type (BB vs. MB) indirectly reduced subjective pain ratings by decreasing transition probability between microstates C and D, which reduced P2 amplitude. Path coefficients and significance levels are marked in the figure. : $p < 0.05$; : $p < 0.01$; : $p < 0.001$.

4. Discussion

This study aimed to investigate the modulatory effects of BB on experimental short-duration pain and its underlying neural mechanisms. Although no significant direct intervention effects were observed on subjective pain ratings (including pain intensity and unpleasantness), EEG spectral and microstate analyses provided supportive evidence for BB's neuromodulatory effects. Specifically, compared to white noise, both BB and MB interventions significantly reduced gamma-band energy during the intervention period, suggesting a common effect of rhythmic auditory stimulation on high-frequency neural activity. However, microstate analysis further revealed unique modulatory effects of BB: compared to MB and white noise, BB significantly enhanced microstate A activity, which is typically associated with early auditory information processing and primary sensory processing, while significantly attenuating microstate C activity, which is believed to be related to default mode network function and involves inward thinking and self-related processing. More critically, mediation analysis revealed that BB indirectly influenced pain-evoked P2 amplitude—and consequently subjective pain experience—by reducing transition probability between microstates C and D. Therefore, although BB did not directly reduce pain perception at the behavioral level, it may influence the way pain information is processed by altering brain functional dynamics.

Regarding EEG activity during sound intervention, results showed that alpha-band BB did not significantly increase alpha power compared to MB and white noise. This finding is consistent with a meta-analysis on binaural beats regulating neural oscillations conducted by Ingendoh et al. (2023), which found that BB's modulatory effects on neural oscillations across frequency bands exhibited substantial heterogeneity, with only 5 out of 14 studies supporting the rhythmic synchronization hypothesis. This hypothesis posits that external rhythmic stimulation can regulate neural oscillation activity through neural synchronization that phase-locks with endogenous rhythms in specific frequency bands within the brain (Thut et al., 2011). The manifestation of rhythmic synchronization effects is influenced by multiple factors, such as stimulation frequency band, stimulation duration, and individual differences. In this study, alpha-band BB intervention lasted only 10 minutes, which may have been insufficient to induce significant neural synchronization effects. In contrast, Jirakittayakorn and Wongsawat (2017) found in a study using 30-minute gamma-band BB intervention that EEG gamma power only began to show significant increases after 15 minutes, suggesting that longer continuous stimulation may be one of the key conditions for generating rhythmic synchronization. Although no significant energy changes were observed in the alpha band, both BB and MB significantly reduced gamma-band energy during the intervention period. This result may not reflect activation of rhythmic synchronization mechanisms but rather demonstrate an inhibitory effect of rhythmic sounds on neural arousal levels. Previous research has shown that gamma neural oscillations are closely related to high-level attention control and alertness (Herrmann & Knight, 2001; Jensen et al., 2007; Tiitinen et al., 1993). Compared to white noise, BB and MB as structured, periodic auditory inputs may possess certain relaxing regulatory effects, promoting a transition in brain state from high alertness to a more relaxed, low-arousal state. Conversely, the unstructured nature of white noise may lack this rhythmic regulatory effect, resulting in maintained gamma energy at relatively higher levels. Therefore, alpha-band BB applied for a short duration failed to effectively induce rhythmic synchronization effects, but rhythmic sounds may regulate the nervous system's arousal state by reducing gamma-band activity.

Compared to white noise, both MB and BB reduced microstate C occurrence rate, reflecting the general regulatory potential of beat stimulation. However, BB exhibited specific neuromodulatory effects on EEG microstates: compared to MB and white noise, BB significantly enhanced activity features of microstate A while significantly suppressing activity of microstate C. Previous research has attempted to map different microstates to brain functional networks (Custo et al., 2017; Michel & Koenig, 2018; Tarailis et al., 2024), but these conclusions mostly rely on indirect evidence from functional imaging and source localization, with their causality and specificity still uncertain.

For example, microstate A is generally considered related to the auditory network (Tarailis et al., 2024), particularly involving neural activity in the superior temporal gyrus (Custo et al., 2017), and may play an important role in processing sound features (such as frequency, rhythm, and pitch) and semantic decoding. The enhancement of microstate A may reflect BB's ability to improve the brain's encoding efficiency and perceptual clarity for beat inputs, thereby promoting integration of auditory information at peripheral and central levels. This is also consistent with previous research showing that BB can enhance central-cortical coupling in auditory pathways and improve rhythm perception ability (Gao et al., 2014). In contrast, microstate C is typically closely associated with the default mode network (DMN) (Tarailis et al., 2024), involving regions such as the medial prefrontal cortex, cingulate cortex, and precuneus (Custo et al., 2017), and primarily participates in endogenous psychological processes such as introspection and self-related thinking. BB's significant suppression of microstate C activity may reflect its inhibitory effect on the DMN, promoting a shift in brain state from an "inward-oriented" processing mode centered on self-referential processing to an "outward-oriented" state more sensitive to the external environment. In summary, BB's enhancement of microstate A and suppression of microstate C suggest that it may achieve dynamic reorganization of brain functional networks by promoting external perceptual processing and inhibiting internal self-referential processing. This "perceptual enhancement—introspection reduction" pattern indicates that individuals' neural activity states gradually shift from endogenous, self-oriented processing to a state more responsive to external information, which may help reduce focus on internal emotions and pain experiences.

EEG microstate transition analysis further revealed that, compared to white noise and MB, BB intervention significantly altered dynamic switching patterns between brain functional states. Specifically, BB enhanced transition probabilities between microstates $A \leftrightarrow B$ and $A \leftrightarrow D$, while significantly reducing transition probabilities between $B \leftrightarrow C$ and $C \leftrightarrow D$. Among these, microstates A and B represent activities related to auditory and visual networks, respectively (Custo et al., 2017; Tarailis et al., 2024). Increased $A \leftrightarrow B$ transition probability may reflect BB's enhancement of the brain's dynamic switching ability between different sensory pathways, thereby improving flexibility of multisensory integration and efficiency of environmental response. Meanwhile, microstate D is closely related to the dorsal attention network (DAN) (Custo et al., 2017; Tarailis et al., 2024), primarily involved in goal-directed attention allocation and executive control processes. Increased $A \leftrightarrow D$ transition probability suggests that BB may enhance the driving effect of auditory input on the attention system, helping maintain attention to task-relevant stimuli and improve information processing efficiency. It is worth noting that compared to white noise, enhanced $A \leftrightarrow D$ transitions were observed not only under BB but also under MB, suggesting that rhythmic auditory stimulation generally has the function of promoting perception-attention coupling. In contrast, BB significantly suppressed $B \leftrightarrow C$ and $C \leftrightarrow D$ transitions. Considering that microstate C is generally considered related to the DMN (Tarailis et al., 2024), this suppression may indicate that BB weakened functional coupling between the DMN and visual network and DAN. This "decoupling" characteristic suggests that BB may reduce frequent switching between introspective and external attention states,

Note: Figure translations are in progress. See original paper for figures.

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