

Article

Can Soundscapes Carry 40 Hz for Gamma Entrainment?: Evidence from a Pilot EEG Study

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Featured Application

Soundscape-based auditory environments additively layered with a pure 40-Hz component may serve as a practical neuroacoustic stimulation format for exploratory brain-health applications, enabling frequency-specific gamma engagement while preserving naturalistic listening.

Abstract

This pilot EEG study examined the feasibility of a soundscape-based 40 Hz auditory stimulation format by using a soundscape-only condition as a contrast control. We tested whether a nature-based soundscape with an additively layered pure 40 Hz sine component (40 Hz ON; not amplitude-modulated) yields a more pronounced narrowband response centered at 40 Hz than the same soundscape without the 40 Hz layer (40 Hz OFF). Participants completed both conditions in a single-blind, randomized-order, within-participant crossover session with a washout interval. EEG outcomes included 40 Hz power, frequency-domain SNR around 40 Hz, scalp distribution of 40 Hz power, and phase-based connectivity in the gamma range. This study evaluates EEG-level detectability of 40 Hz-centered neural signatures and does not assess cognitive/clinical efficacy or therapeutic benefit. Across metrics, the 40 Hz ON soundscape showed a consistent ON > OFF directionality, including localized electrode-level signals and a temporal-region summary measure under nominal, uncorrected testing, accompanied by a clearer narrowband feature near 40 Hz in spectral profiles. Overall, the observed trends are consistent with the feasibility of embedding an additive 40 Hz layer into a naturalistic soundscape in a manner that yields EEG-quantifiable, 40 Hz centered signatures; however, because this is an exploratory pilot without multiplicity control, all effects should be interpreted as hypothesis-generating and warrant confirmation in larger, preregistered studies with multiplicity-aware inference.

Keywords: 40 Hz auditory stimulation; gamma-band entrainment; electroencephalography (EEG); soundscape; frequency-domain SNR; phase-locking value (PLV)



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1. Introduction

Population aging and the growing long-term burden of brain-health conditions motivate the development of low-burden, repeatedly applicable, non-invasive approaches that can be deployed in everyday contexts. Complementary stimulation paradigms have been explored, including rTMS combined with cognitive training in Alzheimer's disease [1]. Rhythmic sensory stimulation paradigms incorporating gamma-frequency components have also been presented through exploratory studies and longer-term case reports [2,3]. More recently, prospective work has directly evaluated the safety and acceptability of 40 Hz auditory stimulation, extending discussions on real-world feasibility [4]. These studies are cited here to contextualize the motivation for repeated-use-oriented designs rather than to position the present healthy-cohort pilot as a clinical efficacy trial.

Global demographic trends further underscore the importance of scalable formats that can support sustained use. According to a United Nations report, the worldwide population aged 65 years and older is projected to increase substantially by 2050 [5]. Alongside demographic aging, Alzheimer's disease and dementia remain major contributors to global disease burden [6,7]. Mental health conditions represent another major global health burden [8]. The WHO emphasizes prevention, early intervention, risk-factor management, and long-term national-level responses for dementia and related brain-health conditions [9]. Together, these trends motivate research that establishes objective neurophysiological evidence of engagement while also considering delivery formats that can realistically support repeated exposure in everyday environments.

A key scientific rationale for sensory-stimulation approaches is neural entrainment. Gamma-band activity (approximately 30–70 Hz) has been linked to higher-order cognitive processes such as perception, attention, and memory, and accumulating evidence suggests gamma-band abnormalities in neurological conditions, including Alzheimer's disease. On this basis, inducing gamma entrainment at specific frequencies—particularly around 40 Hz—has been proposed as a means to modulate neurophysiological responses and potentially influence disease-relevant processes [10,11]. Preclinical work has reported that 40 Hz acoustic stimulation may reduce pathological markers and modulate brain rhythms in an AD animal model [10], and human EEG research has suggested enhanced neural responses during 40 Hz auditory entrainment under eyes-closed conditions [11]. This line of work supports the plausibility of 40 Hz auditory stimulation as a non-invasive, sensory-based approach while also highlighting the importance of quantitative validation of the delivered stimulus in humans [10,11].

At the same time, delivery design is a practical constraint for repeated use. Presenting a standalone pure 40 Hz tone may elicit adverse perceptual experiences such as discomfort or tinnitus-like sensations, which can limit formats intended for prolonged listening [12]. Integrating 40 Hz components into existing audio content (e.g., music) has been proposed to mitigate this burden; however, music-based approaches can be preference-dependent and may introduce perceived audio-quality changes that affect listening experience. Therefore, there is a need for auditory formats that (i) embed a controlled 40 Hz component in an everyday-compatible listening context with relatively neutral content and (ii) provide objective, EEG-based evidence that the resulting composite stimulus produces detectable frequency-specific signatures around 40 Hz.

The present study is an exploratory pilot quantitative EEG investigation designed to test whether a nature-based soundscape can serve as a low-burden carrier for a frequency-specific 40 Hz component that remains EEG-quantifiable in a realistic listening context. Critically, the 40 Hz OFF (soundscape-only) condition is not positioned as a competing intervention; rather, it functions as a contrast control to isolate the incremental contribution of additively layering a pure 40 Hz sine component within an otherwise identical soundscape

experience (same content, preprocessing/mastering chain, and within-participant session structure). Within this contrast-control framework, we examine whether the 40 Hz ON condition yields stronger 40 Hz centered EEG signatures—operationalized as narrowband 40 Hz power and frequency-domain SNR around 40 Hz—and whether it is accompanied by supportive changes in phase-based synchronization indices (phase-locking value, PLV) in a narrow gamma band (35–45 Hz).

Hypotheses. Relative to the 40-Hz OFF contrast control, the 40-Hz ON condition will:

Hypothesis 1 (H1). *Increase EEG signatures centered at 40 Hz (narrowband power and SNR around 40 Hz).*

Hypothesis 2 (H2). *Show an ON > OFF tendency in phase-based synchronization (PLV) in the 35–45 Hz band.*

These hypotheses concern stimulus-locked, EEG-quantifiable signatures of the embedded 40 Hz layer, rather than clinical or behavioral efficacy. As an exploratory pilot, statistical tests are reported to flag hypothesis-generating signals rather than to support confirmatory inference.

2. Related Work

2.1. Gamma Oscillations and Network-Oriented Rationale for 40 Hz Stimulation

Recent neuroscience and cognitive-neuroscience research on cognitive decline and neurodegenerative disorders increasingly emphasizes not only regional dysfunction but also network disruption and reduced functional connectivity across brain regions. Gamma oscillations are widely regarded as a mechanism that supports inter-regional information integration and efficient cognitive processing, and disrupted gamma activity alongside network-level connectivity loss has been repeatedly reported in cognitive impairment, including Alzheimer's disease. Against this background, a growing line of work has explored whether externally delivered stimulation near 40 Hz can drive gamma entrainment and potentially support network functions.

Early human MEG evidence demonstrated coherent thalamocortical 40 Hz activity, establishing gamma oscillations as a rhythm implicated in large-scale network coordination rather than a purely local phenomenon [13]. Subsequent animal studies reported that 40 Hz visual stimulation can induce gamma entrainment and accompany changes in pathology-related markers [14], while multisensory stimulation combining visual and auditory channels has been suggested to recruit broader cortical areas and yield stronger gamma-related responses and pathology modulation than unimodal approaches [15]. Long-term gamma-frequency tACS in animal models further reported improvements in memory-related performance alongside gamma-rhythm measures [16], supporting the plausibility that gamma modulation may relate to network function. Human work has also linked gamma-band modulation to connectivity-related outcomes and cognition, reinforcing the network-level framing of gamma stimulation [1,17].

2.2. Quantitative Validation in Humans: EEG Markers of Gamma Entrainment

Human studies have reported that 40 Hz stimulation can be quantified via EEG as increased responses around 40 Hz in the power spectrum and strengthened phase-based synchronization metrics (e.g., PLV). In animal models, 40 Hz acoustic stimulation has been associated with pathology-related changes and rhythm modulation [10], and in humans, EEG evidence has reported enhanced neural responses during 40 Hz auditory entrainment [11,17]. Importantly, gamma entrainment can depend on contextual factors such as sensory state; stronger gamma power and PLV during eyes-closed conditions

highlight the importance of procedural control (e.g., eye-state standardization) for EEG-based quantification [11]. In addition, exploratory human work using gamma-frequency entrainment paradigms has discussed frequency-dependent patterns in mood, memory, and cognition outcomes, underscoring translational interest while also highlighting variability across protocols and endpoints [18].

From a translational standpoint, accumulating EEG-based evidence that a given auditory design reliably elicits measurable entrainment signatures remains essential. In particular, whether entrainment patterns remain consistent across different content types (e.g., different sound contents) and how power-based versus phase-based metrics converge or diverge are practical questions that motivate further quantitative work.

2.3. Safety/Acceptability and the Need for User-Burden-Aware Auditory Design

Beyond efficacy signals, gamma-based interventions have been evaluated for feasibility and tolerability. A pilot γ -tACS study in Alzheimer's disease, using a randomized double-blind sham-controlled crossover design, reported that exposure was feasible without major adverse events [19]. For auditory stimulation, a prospective study in healthy older adults evaluated repeated exposure to 40 Hz amplitude-modulated stimulation and reported safety and acceptability outcomes [4].

At the same time, qualitative evidence suggests that pure 40 Hz sound presented alone may be perceived as unpleasant or tinnitus-like in some listeners [12], implying a design need to reduce user burden. Although approaches that embed or transform 40 Hz components into music have been proposed, music-based delivery can be constrained by preference variability and potential changes in perceived sound quality. Therefore, it is practically important to explore auditory materials that are more broadly acceptable, integrate 40 Hz components in a low-burden manner, and confirm that the resulting stimulus still yields quantifiable EEG signatures of gamma entrainment.

2.4. Soundscapes and Restorative Outcomes: Toward EEG-Grounded Extensions

Soundscape research has expanded from noise mitigation to quantitative and mixed-method investigations examining whether natural and environmental sound compositions support stress recovery and emotional well-being. Prior studies have reported faster post-stress sympathetic recovery (e.g., skin conductance) during exposure to nature sounds [20], enhanced physiological recovery in VR settings when nature sounds are integrated [21], mood recovery effects during natural sound exposure [22], and restorative outcomes measured with multiple physiological indices such as heart rate, respiration, and skin conductance [23]. Urban soundscapes have also been discussed as potentially restorative depending on context and design [24], and recent work comparing different natural soundscapes has reported differentiated psychological and physiological recovery profiles across soundscape types [25].

These findings position soundscapes as an everyday-compatible auditory context that can be repeatedly presented with relatively low cognitive demand. From the standpoint of translational auditory stimulation, this “carrier” quality is practically valuable: a soundscape format may reduce user burden compared with presenting a standalone tonal stimulus while remaining sufficiently stable and repeatable for controlled exposure. However, restorative soundscape evidence alone does not establish whether embedding a frequency-specific component (e.g., 40 Hz) yields objectively verifiable neurophysiological engagement. Therefore, an important next step is to examine whether a soundscape can function as a carrier that accommodates a controlled 40 Hz layer and produces detectable, frequency-specific EEG signatures around 40 Hz under a rigorously defined contrast-control design.

Taken together, the above strands suggest that soundscapes may serve as an everyday-compatible carrier, but they do not yet establish whether embedding a controlled 40 Hz component yields objectively verifiable, frequency-specific EEG signatures—an empirical gap addressed in Section 2.5.

2.5. Gap and Positioning of the Present Study

Prior work has advanced 40 Hz gamma stimulation from animal models to human studies under a network-oriented rationale, and human EEG studies have shown that 40 Hz stimulation can yield quantifiable signatures around 40 Hz using both power- and phase-based metrics [1,11,26]. In parallel, translational discussions increasingly emphasize feasibility considerations—safety, tolerability, and acceptability—because repeated exposure in everyday contexts is essential for any practical, long-term brain-health application [4,12,19]. Notably, qualitative evidence indicates that a standalone pure 40 Hz tone can be perceived as unpleasant or tinnitus-like by some listeners [12], underscoring that auditory delivery format is not incidental but can determine whether repeated use is viable.

Despite this progress, a practical gap remains: how to realize an everyday-compatible auditory carrier that can accommodate a controlled 40 Hz component while still yielding objectively verifiable EEG signatures. Music-based embedding has been proposed as a burden-reduction strategy, but preference dependence and perceived audio-quality changes can introduce additional variability that complicates repeated use at scale [12]. Soundscapes represent an alternative carrier because nature-based auditory materials have accumulated psychophysiological evidence related to stress recovery and emotional stabilization and are often used as low-demand listening contexts [20–25]. However, it remains underexplored whether a soundscape-based format with an additively layered 40 Hz component produces detectable, frequency-specific EEG signatures around 40 Hz when contrasted against an otherwise identical soundscape-only condition.

Accordingly, the present study is positioned as an EEG-grounded, exploratory pilot that evaluates the plausibility of a 40 Hz layered soundscape as a frequency-specific stimulation format. Importantly, the soundscape-only condition is included as a contrast control to isolate the incremental contribution of the 40 Hz layer, not to evaluate soundscapes as an intervention in their own right. We operationalize EEG signatures using 40 Hz power and frequency-domain SNR as primary metrics and examine 35–45 Hz PLV as a supportive, hypothesis-generating index of synchronization-related patterns. By emphasizing contrast-control logic and transparent reporting of analysis endpoints, this study aims to provide a defensible basis for subsequent confirmatory work on soundscape-based 40 Hz auditory delivery.

3. Materials and Methods

3.1. Study Design Overview

This pilot quantitative EEG study examined whether a nature-based soundscape combined with a pure 40 Hz sine wave via additive layering elicits stronger narrow-band gamma responses around 40 Hz than a soundscape-only contrast control. We used a single-blind, randomized-order, within-participant crossover design in which each participant completed both conditions—40 Hz OFF (contrast control) and 40 Hz ON (experimental)—within the same assigned soundscape set (Figure 1). To reduce content-specific bias, participants were assigned to one of two soundscape sets (Waves or Forest; between-participants factor), and the within-participant condition order was counterbalanced (Table 1). Figure 1 summarizes the overall workflow from recruitment and set assignment to the listening session, washout, and EEG analysis.

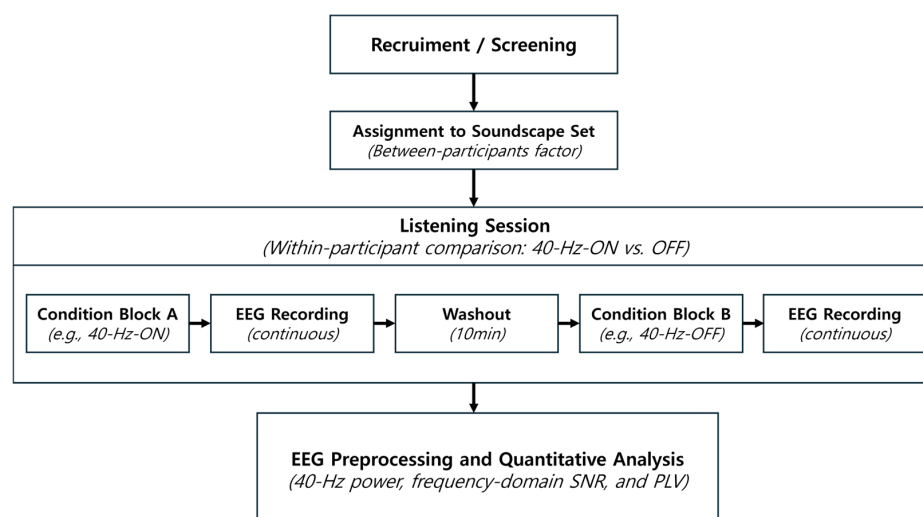


Figure 1. Experimental workflow and session timeline. Participants were recruited, screened, and assigned to one of two soundscape sets (waves vs. forest). During the listening session, each participant completed two within-participant conditions (40 Hz ON vs. OFF) in a randomized and counterbalanced order while EEG was recorded continuously. A 10 min washout period was provided between blocks. EEG analyses were conducted on playback segments only and quantified 40 Hz power, frequency-domain SNR, and phase-locking value (PLV).

Table 1. Participant characteristics, experimental allocation, and inclusion in quantitative EEG analyses. Eleven participants were enrolled. Soundscape set assignment (Waves vs. Forest) was a between-participants factor. Within each assigned set, participants completed both conditions (40 Hz OFF vs. ON) in a randomized and counterbalanced order (within-participant crossover). P01 and P04 were excluded from quantitative EEG analyses due to repeated drowsiness/sleep observed during listening blocks, indicating non-adherence to the wakefulness requirement. A' / B' denotes 40 Hz OFF; A / B denotes 40 Hz ON.

Participant	Gender	Age (Years)	Assigned Soundscape Set	Condition 1	Condition 2	Included in EEG Analysis	Exclusion Reason
P01	Male	46	Set A (Waves)	A'	A	No	Drowsiness/sleep observed during the listening block (protocol non-adherence)
P02	Male	45	Set B (Forest)	B	B'	Yes	—
P03	Male	77	Set A (Waves)	A'	A	Yes	—
P04	Male	77	Set A (Waves)	A	A'	No	Drowsiness/sleep observed during the listening block (protocol non-adherence)
P05	Female	78	Set A (Waves)	A'	A	Yes	—
P06	Female	40	Set A (Waves)	A	A'	Yes	—
P07	Female	67	Set B (Forest)	B'	B	Yes	—
P08	Male	44	Set B (Forest)	B'	B	Yes	—
P09	Male	48	Set A (Waves)	A	A'	Yes	—
P10	Female	69	Set B (Forest)	B'	B	Yes	—
P11	Male	70	Set B (Forest)	B	B'	Yes	—

3.2. Ethics

The study protocol was approved by the Institutional Review Board (IRB No. KMU-202509-HR-503). Written informed consent was obtained from all participants after they were informed of the study objectives, procedures, potential risks, and the processing and

protection of personal data. Participants were free to withdraw at any time without penalty and received compensation after completing the study.

3.3. Participants

Adults aged ≥ 40 years living in Seoul, Republic of Korea, were recruited via online channels. We restricted eligibility to adults aged ≥ 40 years to align the pilot with a midlife-to-older-adult listening context and to reduce developmental heterogeneity associated with younger adults, while maintaining recruitment feasibility for an initial within-participant EEG study. Inclusion criteria were: (1) age ≥ 40 years; (2) no substantial difficulty in everyday listening; and (3) ability to complete the listening and EEG procedures. Hearing status was screened by pure-tone audiometry; eligibility required thresholds ≤ 25 dB HL at 0.5–4 kHz in both ears, consistent with commonly used criteria for normal hearing.

Exclusion criteria included neurological disorders (e.g., epilepsy, Parkinson's disease), ongoing psychiatric treatment or psychoactive medication, severe tinnitus, implanted medical devices, pregnancy or breastfeeding, and medications known to affect brain activity.

A total of 11 participants were enrolled. Two participants (P01 and P04) were excluded from quantitative EEG analyses because the experimenter observed repeated drowsiness/sleep during the listening blocks, indicating non-adherence to the wakefulness requirement. Accordingly, quantitative EEG analyses were conducted on nine participants (Table 1). Table 1 summarizes participant characteristics, set assignment, within-participant condition order, and inclusion status (with exclusion reasons).

3.4. Conditions and Counterbalancing

Two conditions were tested within each assigned soundscape set:

- 40 Hz OFF (contrast control): soundscape-only, using an identical preprocessing/mastering chain.
- 40 Hz ON (experimental): the same soundscape with an additively layered pure 40 Hz sine component (not amplitude-modulated).

Condition order was randomized and counterbalanced across participants. Participants were not informed whether a given condition contained the 40 Hz component (single-blind).

3.5. Auditory Stimuli

3.5.1. Stimulus Structure

Stimuli followed a soundscape-by-layer structure: soundscape content (Waves vs. Forest) and presence of the 40 Hz layer (OFF vs. ON). For the Waves set, OFF and ON were denoted as A' and A, respectively; for the Forest set, OFF and ON were denoted as B' and B. Each participant was assigned to one set (between-participants factor) and completed both OFF and ON within that set (within-participant crossover).

3.5.2. Audio Preprocessing and Additive 40 Hz Layering

All auditory stimuli were rendered as stereo WAV files and followed a unified preprocessing and mastering chain across conditions. To stabilize low-frequency energy and reduce uncontrolled variability that can complicate stimulus characterization (e.g., large, content-dependent sub-bass fluctuations), we applied a steep high-pass filter (HPF; 78 Hz cutoff, 96 dB/oct) to the base soundscape signal. Because the HPF cutoff (78 Hz) strongly attenuates carrier energy around 40 Hz, intrinsic soundscape energy near 40 Hz was minimized, reducing potential masking/overlap with the added 40 Hz line component. The HPF was applied offline using a fixed, identical processing preset for all base soundscape files (both OFF and ON) to ensure consistent magnitude characteristics across stimuli. The

resulting attenuation profile was verified via file-based spectral inspection on the rendered WAV outputs (Figure 2a). The cutoff and slope were selected through iterative internal pilot checks as a pragmatic compromise: sufficiently attenuating low-frequency energy that could obscure or confound interpretation of a 40 Hz specific component while keeping the soundscape perceptually acceptable for the study's listening context. This preprocessing step was intended primarily to reduce uncontrolled low-frequency masking near 40 Hz for stimulus verification and interpretation; perceptual naturalness was informally checked in internal pilot listening.

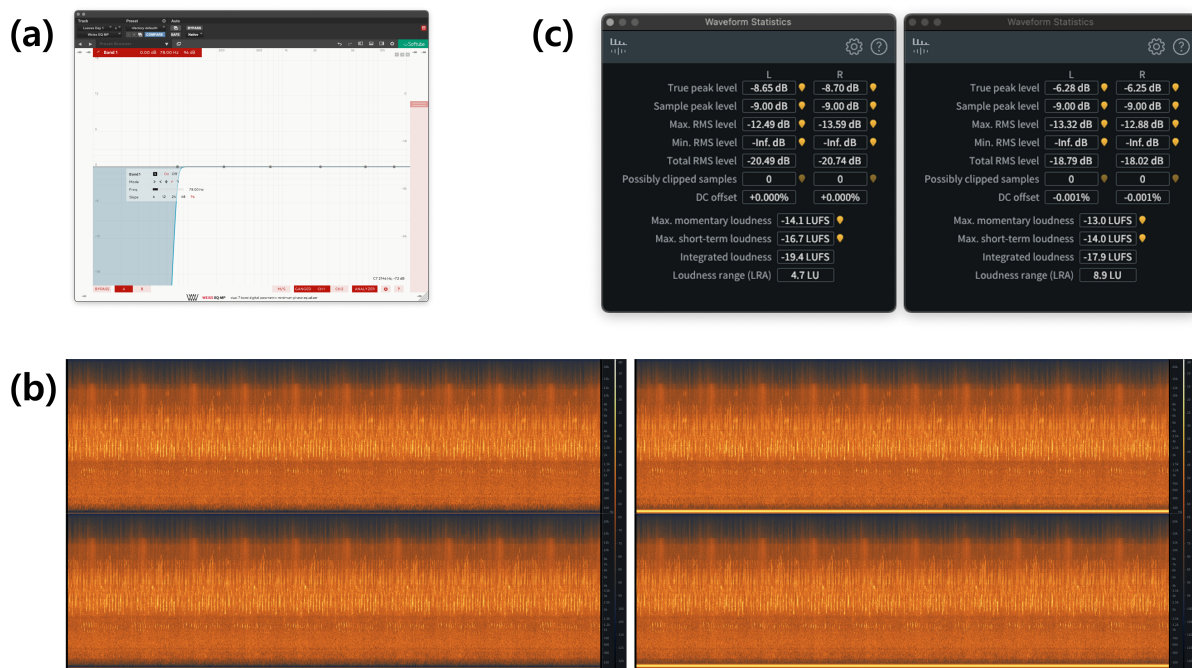


Figure 2. Audio preprocessing and stimulus verification. (a) Example magnitude response of the steep high-pass filter (HPF; cutoff 78 Hz; slope 96 dB/oct) applied to the base soundscape prior to additive 40 Hz layering. (b) Representative spectrograms of the rendered stereo WAV stimuli after preprocessing and rendering: the 40 Hz OFF stimulus (left) contains the filtered soundscape only, whereas the 40 Hz ON stimulus (right) shows an additional narrowband 40 Hz line component superimposed on the same soundscape. (c) Representative waveform statistics from the rendered files, summarizing peak/RMS and loudness metrics (LUFS/LRA) and confirming post-export quality checks, including absence of clipped samples and negligible DC offset.

We selected additive layering (rather than amplitude modulation, AM) as an engineering choice to preserve the perceptual fidelity of the naturalistic soundscape carrier. Applying AM to the entire soundscape can introduce salient envelope “pumping” artifacts and global timbral changes that may reduce naturalness and make the manipulation more perceptually salient. In contrast, additive layering keeps the carrier waveform and its dynamics intact while enabling precise control and straightforward file-level verification of the intended 40 Hz component as a narrowband line feature. Additive layering can render a tonal cue perceptually salient if the relative level is set too high; therefore, we used conservative embedding and post-export verification to minimize clipping and unintended loudness shifts (Table 2). We also did not use binaural beats because their perceptual strength depends on stable dichotic presentation and varies substantially across listeners, and the resulting “beat” is an illusory percept rather than a physically present 40 Hz line component. In addition, binaural-beat paradigms introduce dependence on stereo separation, ear-canal asymmetries, and headphone fit, complicating reproducible stimulus verification at the file level. Because our primary goal was an EEG-quantifiable, frequency-

specific 40 Hz component embedded into an everyday soundscape with straightforward stimulus verification, additive layering offered the most direct and controllable approach for this pilot.

Table 2. Post-export stimulus verification and technical quality control for the final rendered audio files. Waveform statistics were extracted from the exported stereo WAV files to document integrated loudness (LUFS), true-peak level (dBTP; maximum across channels), clipping, DC offset, and loudness range (LRA). OFF indicates the soundscape-only contrast control; ON indicates the corresponding soundscape with additive inclusion of a pure 40 Hz sine component (not AM). Loudness changes after layering were small (Waves: $\Delta + 0.1$ LUFS; Forest: $\Delta + 0.3$ LUFS, ON relative to OFF). All files showed 0 clipped samples and a negligible DC offset.

Stimulus Group	Condition Label	Integrated Loudness (LUFS)	True Peak (dBTP)	Clipped Samples (Count)	DC Offset (%)	LRA (LU)	Δ LUFS (ON–OFF)
40 Hz tone (standalone reference)	Reference	−31.3	−23.97	0	+0.000	0.0	—
Waves soundscape	40 Hz OFF (A')	−18.0	−6.36	0	−0.001	9.7	—
Waves soundscape + 40 Hz	40 Hz ON (A)	−17.9	−6.28	0	−0.001	8.9	+0.1
Forest soundscape	40 Hz OFF (B')	−19.7	−8.57	0	+0.000	5.1	—
Forest soundscape + 40 Hz	40 Hz ON (B)	−19.4	−8.65	0	+0.000	4.7	+0.3

For the 40 Hz ON condition, a pure 40 Hz sine component was additively layered onto the HPF-processed soundscape. Importantly, the HPF was applied only to the base soundscape signal, and the 40 Hz sine component was generated and added after the HPF stage. Let the left and right HPF-processed soundscape channels be $s_L(t)$ and $s_R(t)$. The additively layered 40 Hz component was defined as

$$x_{40}(t) = A \sin(2\pi \cdot 40t), \quad (1)$$

and the final stereo outputs are given by

$$y_L(t) = s_L(t) + x_{40}(t), y_R(t) = s_R(t) + x_{40}(t). \quad (2)$$

Here, A is a predefined mixing gain applied to the 40 Hz sine component to keep the added component at a consistent, conservative level relative to the carrier soundscape at the file level. The same predefined value of A and the same rendering chain were applied consistently when producing the ON stimuli, thereby fixing the soundscape-to-40 Hz level relationship across files. Spectrogram examples confirm that the 40 Hz OFF stimuli do not show a narrowband 40 Hz line component, whereas the corresponding 40 Hz ON stimuli exhibit a distinct narrowband line at 40 Hz (Figure 2b). Post-export file-based verification further documented integrated loudness (LUFS), true peak level (dBTP), DC offset, clipping, and loudness range (LRA), indicating small loudness shifts after layering and no clipping across files (Table 2). Together, these steps support that OFF/ON stimuli differed primarily by the intended additive 40 Hz layer under an otherwise consistent production chain.

3.5.3. Block Structure and Duration

Each condition comprised seven cycles of 50 s playback followed by 10 s silence, yielding 420 s (~7 min) per condition. Quantitative EEG analyses included playback segments only (50 s) and excluded the silence intervals (10 s).

3.5.4. Post-Export File Verification and Loudness Consistency Checks

Waveform statistics were extracted from the final rendered WAV files to document integrated loudness (LUFS), true-peak level (dBTP), clipping, DC offset, and loudness range (LRA). Overall loudness changes after layering were small (Waves: +0.1 LUFS; Forest: +0.3 LUFS, ON relative to OFF), and no clipping was detected in any file (Table 2). These file-level checks document consistency of the rendered stimuli (e.g., integrated loudness/true peak/no clipping) but do not substitute for participant-level ear-canal SPL calibration. Table 2 retains the same underlying values but is presented with a slightly revised layout to facilitate EEG-oriented interpretation, including condition-wise comparisons.

3.6. Procedure

Sessions were conducted in a quiet indoor room. After fitting the EEG cap and adjusting electrode impedances, participants listened to the two conditions within the assigned soundscape set in a randomized and counterbalanced order. Each condition lasted ~7 min, and a 10 min washout period was provided between conditions (Figure 1). A 10 min washout was used to minimize potential carryover related to short-term sensory adaptation, fatigue, or state drift between blocks. Our primary EEG endpoints quantify stimulus-locked steady-state-like signatures during playback; ASSR/steady-state responses are elicited by temporally structured stimulation and typically track the frequency/phase of the ongoing stimulus [27]. Although post-stimulus (“OFF”) responses have been reported in some paradigms, a 10 min interval is expected to substantially reduce immediate carryover in this context [28]. During listening, participants were instructed to keep their eyes closed, minimize movement (particularly jaw and facial muscle tension), and maintain a stable seated posture. During washout, participants were allowed to open their eyes and were reminded to remain awake. Throughout the session, the experimenter monitored participants’ vigilance (e.g., head nodding and reduced responsiveness) and provided verbal reminders when drowsiness was suspected. In addition, session video recordings (collected with participant consent for monitoring purposes) were reviewed to corroborate drowsiness-related observations; this served as supplementary verification rather than an objective physiological vigilance measure. No objective vigilance or eye-state channels (e.g., EOG) or standardized behavioral probes were implemented in this pilot. Nevertheless, clear drowsiness was observed during the listening blocks for P01 and P04, and their concurrent EEG traces were deemed unsuitable for reliable estimation of narrowband 40 Hz signatures. These datasets were therefore excluded from the quantitative EEG analyses according to the predefined quality-control criteria.

3.7. Playback Equipment

Stimuli were presented from a laptop computer positioned approximately 3 m away from the EEG system to reduce potential electromagnetic interference. Audio was delivered via wired in-ear earphones (XBA-A2; Sony Corporation, Tokyo, Japan). Individual ear-canal sound pressure level (SPL) was not measured in this pilot study. However, the rendered stimulus files used a fixed file-level embedding ratio between the carrier soundscape and the 40 Hz component, controlled by a predefined mixing gain (A) applied consistently during stimulus rendering (Section 3.5.2; Table 2). During data collection, the same playback device, earphones, and player/software settings were used for all participants; Windows 11 system volume was fixed at 90/100 (software scale), and the media player volume was fixed at 60/100 (software scale), and these settings were kept unchanged across participants and across both conditions. We did not perform individual loudness normalization or coupler/ear-canal SPL calibration; thus, between-participant differences in delivered ear-canal SPL may remain due to insertion depth, ear-canal acoustics, and sealing. Given the

within-participant crossover design and the contrast-control pairing of OFF vs. ON within the same soundscape set, unmeasured between-participant SPL variability is unlikely to explain ON–OFF directionality, but it may contribute to inter-subject variability in response magnitude.

3.8. EEG Acquisition

EEG was recorded using a multichannel system (BIOS-S series, BioBrain Inc., Daejeon, Republic of Korea). Twenty-one electrodes were placed according to the international 10–20 system (Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, Oz, and O2). Signals were sampled at 250 Hz, impedances were kept below 10 k Ω , and the system's default reference/ground configuration was used.

Although O1 was recorded in the 10–20 montage, persistent noise/unstable contact was repeatedly observed at this channel; therefore, O1 was excluded from quantitative analyses. Accordingly, all EEG metrics (power, SNR, and PLV) were computed using the remaining 20 electrodes.

3.9. EEG Preprocessing

All EEG signals were processed in MATLAB (R2024b). Channel-wise mean removal was first applied to correct DC offsets. To attenuate 60 Hz power-line interference, a zero-phase two-pass IIR notch filter was applied. For spectral analyses (40 Hz power and SNR), a 4th-order two-pass IIR band-pass filter (0.5–50 Hz) was used. For PLV analyses, a 4th-order two-pass IIR band-pass filter (35–45 Hz) was applied, and the instantaneous phase was computed using the Hilbert transform.

Automated component-level cleaning (e.g., ICA) and formal epoch-rejection pipelines were not applied in this pilot because the primary goal was feasibility-oriented detection of stimulus-locked, narrowband signatures under a constrained sample size and a controlled listening procedure. We prioritized procedural control and conservative quality assurance: participants were instructed to keep their eyes closed and minimize movement, vigilance was monitored during the session, and participants with clear noncompliance (repeated drowsiness/sleep; P01, P04) were excluded (Section 4.1). In addition, raw EEG traces were visually checked for gross recording failures (e.g., persistent saturation or disconnection), and an electrode with persistent instability (O1) was excluded from quantitative analyses (Section 3.8). Under the within-participant contrast-control design (ON vs. OFF) with identical procedures and preprocessing across conditions, broadband artifacts such as motion/EMG would likely affect wider frequency ranges and both conditions similarly; therefore, the primary interpretation is restricted to candidate stimulus-locked, frequency-specific differences around 40 Hz rather than to generalized changes in signal quality. This consideration is particularly important for phase-based connectivity (e.g., PLV), which can be more sensitive to residual EMG, common-source effects, and referencing choices than narrowband power alone. Nevertheless, we acknowledge that ICA- or epoch-based cleaning could further improve robustness and is recommended for future confirmatory studies with larger samples and preregistered preprocessing plans.

3.10. Outcome Measures

3.10.1. 40Hz Power Quantification

The 40 Hz response was quantified from the power spectral density (PSD) as narrowband 40 Hz power, defined as the PSD value at the frequency bin nearest to 40 Hz (in $\mu\text{V}^2/\text{Hz}$). Channel-level outcomes were computed for all valid electrodes that passed quality control (O1 excluded as described in Section 3.8), and region-level summaries (frontal, temporal, central, and parietal–occipital) were calculated for descriptive comparison.

This narrowband metric was intended to reflect frequency-specific responses centered at 40 Hz rather than broadband power differences.

3.10.2. Frequency-Domain SNR

We quantified frequency-domain signal-to-noise ratio (SNR) around 40 Hz from the power spectral density (PSD) by comparing the PSD at the target frequency bin to the mean PSD of neighboring bins. Specifically, for each channel, SNR at frequency f was computed as:

$$\text{SNR}(f) = 10 \log_{10} \left(\frac{P(f)}{\bar{P}_{\text{neighbor}}(f) + \epsilon} \right), \quad (3)$$

where $P(f)$ denotes the PSD value at the FFT bin closest to f , and $\bar{P}_{\text{neighbor}}(f)$ denotes the mean PSD of the ± 5 neighboring bins around the center bin (i.e., 10 bins total), excluding the center bin itself. A small constant ϵ was added to the denominator to avoid numerical instability when the neighborhood power approached zero. For the main analysis, SNR was evaluated at $f = 40$ Hz.

3.10.3. Topographic Mapping

Condition-wise, channel-averaged 40 Hz power was visualized as scalp topographies using standard 10–20 coordinates with interpolation. Identical color scales were applied across conditions.

3.10.4. Phase-Locking Value (PLV)

Phase-based connectivity was quantified using the phase-locking value (PLV) in a narrow gamma band centered on the stimulation frequency. For each electrode pair, EEG signals were bandpass-filtered to 35–45 Hz (4th-order zero-phase bidirectional IIR), and the instantaneous phase was obtained via the Hilbert transform. PLV was computed for all unique channel pairs among the analyzed electrodes as:

$$\text{PLV} = \left| \frac{1}{T} \sum_{t=1}^T e^{j\Delta\phi(t)} \right|, \quad (4)$$

where T denotes the number of time samples and $\Delta\phi(t)$ is the instantaneous phase difference between the two channels at time t . The 35–45 Hz band was selected to center on 40 Hz while allowing a margin for inter-individual variability in how narrowband phase synchronization expresses around the target frequency. PLV was computed over the concatenated playback samples within each condition (seven 50 s playback segments per condition; total 350 s), which provides a longer effective estimation window to reduce instability associated with short segments. Because the O1 electrode was excluded due to persistent noise/artifacts, PLV analyses were conducted on the remaining 20 electrodes, yielding 190 unique pairs ($20 \times 19/2$).

3.11. Statistical Analysis and Multiple Testing Considerations

This study was designed as an exploratory pilot investigation to assess whether a nature-based soundscape can serve as a low-burden carrier for a frequency-specific 40 Hz component that remains EEG-quantifiable in a realistic listening context. Importantly, the 40 Hz OFF condition (soundscape-only) was treated as a contrast control to isolate the incremental contribution of the additively layered 40 Hz sine component within an otherwise identical listening context (same content set, preprocessing/mastering chain, playback procedure, and within-participant block structure). Accordingly, the primary analytic goal was to examine detectability, directionality, and spatial tendencies of 40 Hz–

centered EEG signatures in 40 Hz ON relative to 40 Hz OFF, rather than to make competitive efficacy claims about the soundscape-only condition.

Planned analyses. The primary planned endpoints were 40 Hz-centered narrow-band power and frequency-domain SNR around 40 Hz (OFF vs. ON) computed during playback epochs, summarized at both electrode and region levels (including grand average). Supportive analyses included descriptive scalp topographies of 40 Hz power/SNR and a narrow gamma-band (35–45 Hz) PLV analysis, which are explicitly interpreted as exploratory/hypothesis-generating without multiplicity-aware inference.

Post hoc/exploratory analyses. Any additional inspections beyond these endpoints (e.g., additional descriptive checks for interpretation) were conducted post hoc and are labeled as exploratory where reported.

Within-participant condition differences (40 Hz ON vs. 40 Hz OFF) were evaluated using two-sided Wilcoxon signed-rank tests. For channel-wise outcomes (40 Hz power and frequency-domain SNR), tests were conducted across the analyzed electrodes; because the O1 electrode (left occipital) was excluded due to persistent noise, channel-wise inference was based on the remaining 20 electrodes. For phase-based connectivity, PLV was computed in the 35–45 Hz band for all unique channel pairs among the analyzed electrodes (20 electrodes, 190 unique pairs), and condition differences were evaluated per pair using the same two-sided nonparametric framework.

Given the exploratory nature of this pilot study, no multiplicity control (e.g., FDR) was applied across electrodes or channel pairs. Therefore, all reported *p*-values are uncorrected and are presented to indicate where signals may concentrate as hypothesis-generating evidence, not to support confirmatory inference. Consistent with this rationale, any visual markers or maps derived from nominal *p*-values (e.g., asterisks, $-\log_{10}(p)$ panels, or $p < 0.05$ masks) should be interpreted as uncorrected reference cues only and not as multiplicity-validated findings. Future confirmatory work should preregister primary endpoints, incorporate multiplicity-aware inference (or cluster-based/permutation approaches for spatial/connectivity structure), and include calibrated playback-level documentation (e.g., coupler-based SPL estimation) to strengthen reproducibility and external validity.

For planning future confirmatory work, paired-sample effect sizes (e.g., Cohen's d_z for ON–OFF differences on a prespecified primary endpoint) can be used to estimate the required sample size; we provide a brief planning reference in the Discussion.

4. Results

4.1. EEG Data Quality Control and Analyzed Sample

A total of 11 participants completed the study. Two participants (P01 and P04) exhibited clear drowsiness during the listening blocks as observed by the experimenter, and their concurrent EEG traces were deemed unsuitable for reliable estimation of narrowband 40 Hz signatures. These datasets were therefore excluded from the quantitative EEG analyses. The final quantitative EEG results are based on nine participants.

In addition, the O1 electrode (left occipital) showed persistent noise and was excluded from quantitative channel-wise analyses. For scalp-topography visualization, the O1 location is displayed via interpolation from neighboring electrodes; this is noted in the relevant figure captions.

4.2. 40 Hz Power

Table 3 reports electrode-wise comparisons of 40 Hz power between conditions, and provides region-wise summaries (frontal/temporal/central/parietal-occipital). Overall, 40 Hz power tended to be higher in 40 Hz ON (soundscape with an additively layered pure 40 Hz sine component) than in 40 Hz OFF (soundscape-only). At the electrode level,

Cz, C4, T4, and T6 showed ON > OFF differences with nominal (uncorrected) $p < 0.05$. In the region-wise summaries, the temporal region showed an ON > OFF pattern with nominal (uncorrected) $p = 0.039$, whereas the frontal region and the grand average exhibited trend-level differences ($p = 0.055$ and $p = 0.074$, respectively). Because this pilot study did not apply multiple-comparison correction, the p -values in Table 3 should be interpreted as exploratory, hypothesis-generating signals rather than confirmatory significance (see Section 3.11 for the statistical rationale).

Table 3. Channel- and region-level 40-Hz power (40-Hz ON vs. 40-Hz OFF). Channel-wise 40-Hz power for 40-Hz ON versus 40-Hz OFF is shown first, followed by region-wise summaries (frontal/temporal/central/parietal-occipital). p -values are two-sided and uncorrected, intended for exploratory, hypothesis-generating interpretation (no multiplicity control). The O1 electrode (left occipital) was excluded due to persistent noise. Region-wise summaries (frontal/temporal/central/parietal-occipital) of 40 Hz power for 40 Hz ON versus 40 Hz OFF. p -values are two-sided and uncorrected (no multiplicity control).

Channel-Level Results (Electrode-Wise)			
Channel	40 Hz ON	40 Hz OFF	p -Value (Two-Sided)
Fp1	1.284	0.988	0.055
Fpz	0.945	0.816	0.098
Fp2	1.289	1.171	0.359
F7	1.614	1.415	0.301
F3	0.804	0.634	0.074
Fz	0.406	0.367	1.000
F4	0.807	0.659	0.129
F8	1.431	1.241	0.359
T3	1.571	1.362	0.098
C3	0.431	0.286	0.301
Cz	0.425	0.152	0.039
C4	0.423	0.290	0.039
T4	1.351	1.044	0.039
T5	0.356	0.203	0.301
P3	0.625	0.484	0.098
Pz	0.550	0.452	0.301
P4	0.580	0.455	0.250
T6	0.469	0.297	0.039
Oz	0.249	0.202	0.164
O2	0.509	0.389	0.496
Region-Level Summaries			
Region	40 Hz ON	40 Hz OFF	p -Value (Two-Sided)
Frontal	1.073	0.911	0.055
Temporal	0.937	0.726	0.039
Central	0.426	0.242	0.098
Parietal-Occipital	0.503	0.397	0.203
Grand average	0.806	0.645	0.074

Figure 3 visually summarizes the quantitative results in Table 3. The top panel shows electrode-wise 40 Hz power (ON vs. OFF), the middle panel shows region-wise summaries, and the bottom panel shows the grand average. Across panels, ON values are generally

higher than OFF, with a more noticeable ON > OFF pattern around central–temporal adjacent channels (Figure 3).

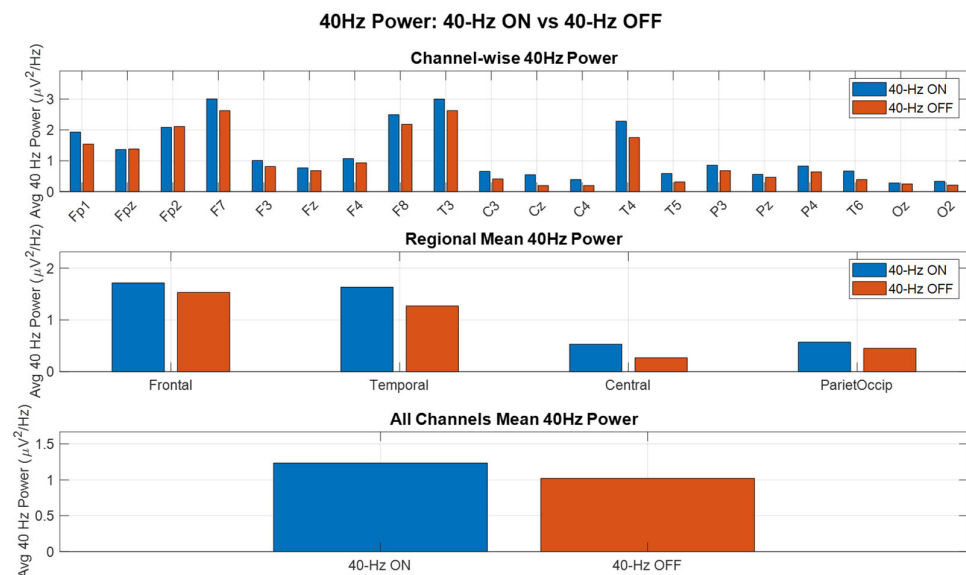


Figure 3. A 40 Hz power comparison (40 Hz ON vs. 40 Hz OFF). Top: channel-wise mean 40 Hz power across electrodes. Middle: region-wise means (frontal, temporal, central, and parietal–occipital). Bottom: grand average 40 Hz power across all included electrodes. Asterisks (where shown) denote nominal, uncorrected two-sided $p < 0.05$ and are provided for exploratory reference only; no multiple-comparison correction was applied.

Taken together, Table 3 and Figure 3 suggest that additive 40 Hz layering onto a natural soundscape may increase narrowband responses around 40 Hz relative to the soundscape-only contrast control. Given the exploratory nature and the lack of multiplicity control, these findings should be viewed as hypothesis-generating support for follow-up confirmatory studies rather than definitive conclusions. The scalp-level spatial tendencies should be interpreted as descriptive patterns rather than confirmatory localization, given the small sample size and uncorrected, multiplicity-unaware channel-wise testing.

Because this pilot was not powered for covariate modeling, we did not perform age-stratified analyses or formal age-adjusted inference. Visual inspection of participant-level ON–OFF differences did not suggest a pronounced age-graded pattern within the enrolled range; however, this observation is descriptive and should not be interpreted as evidence of no age effect.

4.3. Frequency-Domain SNR (30–50 Hz)

The frequency-domain SNR spectrum computed over 30–50 Hz showed a more pronounced narrowband feature around 40 Hz in the 40 Hz ON condition (Figure 4). Spatial patterns differed by electrode location. To enhance transparency regarding individual-level variability in this small pilot, participant-level visualizations are provided in Appendix A. Figure A5 shows paired OFF→ON changes in a predefined ROI-averaged SNR@40 metric (temporal-region summary) for each participant, and Figure A6 shows participant-specific ON/OFF SNR spectra (30–50 Hz) to visualize heterogeneity and the presence/absence of a narrowband peak near 40 Hz. Consistent with the exploratory aim of this pilot study, Figure 4 is presented as a descriptive comparison of spectral shape and directionality near 40 Hz (ON > OFF), rather than as a multiple-comparison-controlled significance map.

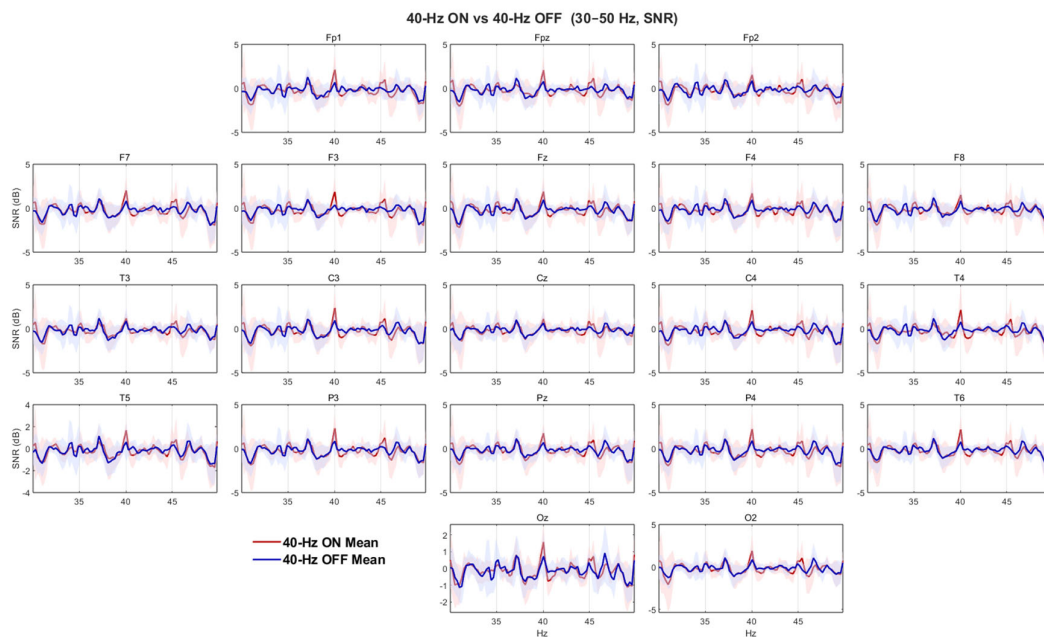


Figure 4. Frequency-domain SNR spectra (30–50 Hz) for 40 Hz ON vs. 40 Hz OFF. Panels show electrode-wise SNR spectra over 30–50 Hz, comparing 40 Hz ON (soundscape + 40 Hz sine layering) against 40 Hz OFF (soundscape-only). Curves are displayed to illustrate the spectral shape and the narrowband feature around 40 Hz. This figure is intended as a descriptive comparison; it is not a multiple-comparison-controlled significance map.

4.4. Scalp Topography of 40 Hz Power

Condition-wise, scalp topographies were generated to examine the spatial distribution of 40 Hz power (Figure 5). Identical color scales were used across conditions to support direct comparison. Overall, the 40 Hz ON condition showed a distribution consistent with the directionality observed in Figure 3 and Table 3. Because O1 was excluded from channel-wise comparisons due to noise, the O1 location in the topography should be interpreted cautiously as an interpolated (not directly measured) value.

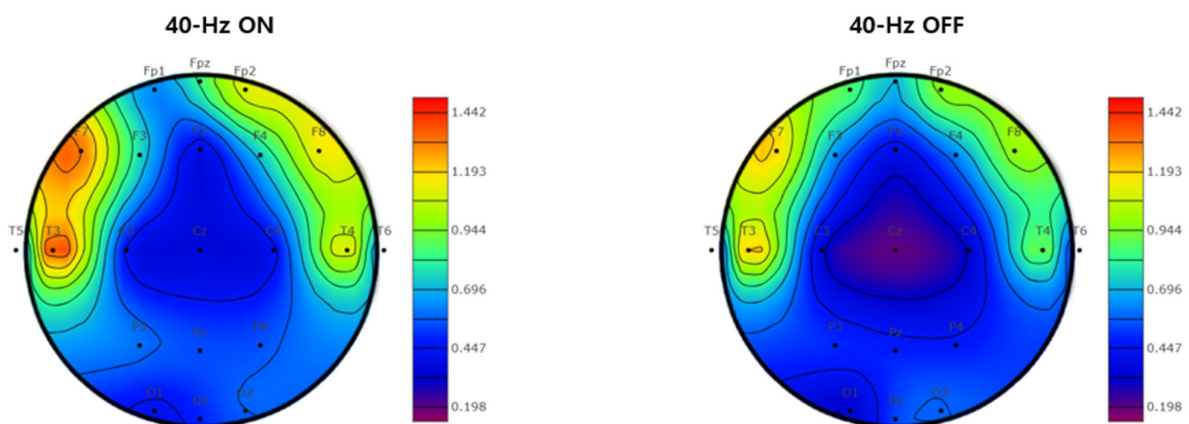


Figure 5. Scalp topographies of 40 Hz power (40 Hz ON vs. 40 Hz OFF). Condition-wise, scalp maps visualize the spatial distribution of 40 Hz power using identical color scales to enable direct comparison. The O1 electrode (left occipital) showed persistent noise and was excluded from channel-wise comparisons; the O1 location in the topography reflects interpolation from neighboring electrodes and should be interpreted cautiously.

4.5. Descriptive Phase-Based Connectivity (PLV) in the 35–45 Hz Band

Given the large number of pairwise comparisons (190 channel pairs) and the pilot nature of this study, PLV results are reported as descriptive, hypothesis-generating

patterns based on uncorrected tests. Future confirmatory work should apply preregistered, multiplicity-aware inference (e.g., FDR, permutation/cluster-based approaches) and network-level statistics.

PLV was computed in the 35–45 Hz band for all unique channel pairs. Because the O1 electrode exhibited persistent noise/artifacts and was excluded from downstream analyses, connectivity analyses were performed on the remaining 20 electrodes, yielding 190 unique pairs. For each pair, participant-level PLV was computed for each condition, and the condition contrast was defined as $\Delta\text{PLV} = \text{PLV}_{\{40 \text{ Hz ON}\}} - \text{PLV}_{\{40 \text{ Hz OFF}\}}$. Given the exploratory nature of this pilot study and the large number of pairwise comparisons (190 channel pairs), the PLV results are presented strictly as descriptive, hypothesis-generating visualizations; inferential conclusions are not warranted.

To address the interpretability limitations of dense network (“spaghetti”) plots and to avoid arbitrary edge selection, we replaced the prior connectivity visualization with complementary summaries centered on an all-pairs matrix view (Figure 6). The top-right panel presents a ΔPLV heatmap for all channel pairs (20 electrodes; 190 pairs), showing the full distribution and where ΔPLV differences concentrate across the channel-pair matrix without any edge selection. The top-left panel provides a schematic network view restricted to nominal $p < 0.05$ pairs (two-sided Wilcoxon signed-rank test, uncorrected), shown only as a visual cue to reduce visual clutter; edge color encodes the direction and magnitude of ΔPLV . To contextualize magnitude versus nominal consistency, the bottom-left panel shows a $-\log_{10}(p)$ heatmap for all pairs (nominal, uncorrected), and the bottom-right panel provides a corresponding binary $p < 0.05$ mask indicating the locations of nominally different pairs. Consistent with the exploratory nature of this pilot and the absence of multiplicity control across 190 pairwise tests, these visualizations are intended strictly for descriptive, hypothesis-generating interpretation rather than confirmatory inference.

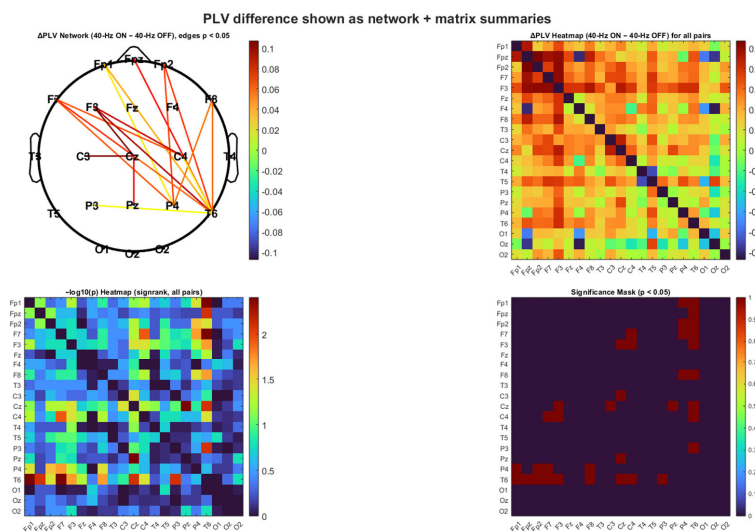


Figure 6. Pairwise PLV differences (35–45 Hz) between conditions and their distribution across all channel pairs (40 Hz ON vs. 40 Hz OFF). PLV was computed for all unique channel pairs after excluding the O1 electrode due to persistent noise/artifacts (20 electrodes; 190 pairs). ΔPLV was defined as $\Delta\text{PLV} = \text{PLV}_{\{40 \text{ Hz ON}\}} - \text{PLV}_{\{40 \text{ Hz OFF}\}}$. Top-left: schematic ΔPLV network visualization restricted to nominal $p < 0.05$ pairs (two-sided Wilcoxon signed-rank test, uncorrected) shown only as a visual cue; edge color encodes ΔPLV . Top-right: ΔPLV heatmap across all channel pairs (no edge selection), showing the full distribution and where differences concentrate within the channel-pair matrix. Bottom-left: heatmap of $-\log_{10}(p)$ values for all pairs (nominal, uncorrected). Bottom-right: corresponding binary significance mask ($p < 0.05$, uncorrected). Because connectivity was evaluated across 190 pairs and no multiple-comparison correction was applied, these visualizations are intended for descriptive, hypothesis-generating interpretation rather than confirmatory inference.

5. Discussion and Conclusions

This pilot EEG study evaluated the plausibility of a soundscape-based auditory format as a practical carrier for a frequency-specific 40 Hz component that is EEG-quantifiable in a realistic listening context. The central contribution is not a competitive comparison between “soundscape-only” and “soundscape-plus,” but a contrast-control test that isolates the incremental contribution of an additively layered 40 Hz sine component within an otherwise identical soundscape experience. From this perspective, the observed ON > OFF directionality across multiple EEG readouts suggests a cautious, design-oriented interpretation: a naturalistic soundscape can accommodate a controlled 40 Hz layer in a way that yields detectable 40 Hz centered EEG signatures, thereby motivating more rigorous confirmatory work. The stimulus-production chain also reflected a pragmatic trade-off between suppressing low-frequency variability that could mask a 40 Hz-centered signal and preserving perceived soundscape naturalness, as described in Section 3.5.2.

Across complementary EEG readouts, the 40 Hz ON soundscape showed consistent ON > OFF directionality in 40 Hz centered signatures. This convergence is important from an applied-science perspective because it suggests a design-oriented interpretation: a naturalistic soundscape can accommodate a controlled 40 Hz component while preserving an everyday-compatible listening context, and the embedded component remains detectable at the EEG level. The appropriate inference is therefore about the feasibility of EEG quantification and pattern plausibility—not definitive localization, generalizable effect sizes, or functional/clinical benefit. Any apparent scalp localization should be treated as exploratory and may not generalize without multiplicity-aware replication and uncertainty quantification (e.g., bootstrap-based topographies).

The exploratory connectivity (PLV) findings should be interpreted with particular caution. Pairwise connectivity involves many simultaneous tests; without multiplicity-aware inference, connectivity summaries can be over-interpreted. In this study, PLV results are best treated as a hypothesis-generating map of where larger ON–OFF phase-consistency differences may concentrate, motivating preregistered, multiplicity-aware replication and network-level statistical approaches. Moreover, sensor-level PLV can be inflated by volume conduction and other common-source effects and is sensitive to residual noise and referencing choices; therefore, it should not be interpreted as evidence of true inter-regional communication in this pilot. Future work should consider connectivity metrics designed to mitigate zero-lag coupling (e.g., imaginary coherence, PLI/wPLI, and/or source-space connectivity), together with preregistered, multiplicity-aware inference.

Several limitations delimit interpretation and define next steps. As a transparency note, this pilot study was not preregistered; therefore, Section 3.11 explicitly distinguishes prespecified primary endpoints from supportive/exploratory analyses. First, the study was not powered for confirmatory inference; future work should increase sample size, preregister primary endpoints, and adopt multiplicity-aware (or cluster-/permutation-based) inference for spatial and connectivity structure.

To make the pilot nature of this work more actionable for future confirmatory studies, we provide a simple sample-size planning reference based on paired-sample effect sizes (Cohen’s d_z) from an ON–OFF within-participant design. These values are provided solely for rough planning and should not be treated as definitive requirements. For a two-sided $\alpha = 0.05$ paired t -test (a common approximation for planning even when nonparametric tests are used in analysis), achieving 80% power requires approximately $n = 34$ for $d_z = 0.5$ (moderate), $n = 24$ for $d_z = 0.6$, $n = 19$ for $d_z = 0.7$, and $n = 15$ for $d_z = 0.8$ (large); for 90% power, the corresponding values are approximately $n = 44, 32, 24$, and 19, respectively. Because effect sizes estimated from small pilots can be unstable, future studies should select a single prespecified primary endpoint (e.g., an ROI summary SNR@40 metric), estimate

d_z from the pilot cautiously, and power the confirmatory study toward the conservative end of the plausible range.

The age range was intentionally broad within a ≥ 40 years eligibility window to support recruitment feasibility; however, age-related heterogeneity could have contributed to between-participant variability in EEG responsiveness and listening-related factors. With $n = 9$, we were not positioned to conduct age-stratified analyses or robust covariate modeling. Future confirmatory studies should either narrow the age band or prespecify age-adjusted/stratified analyses to quantify and control potential age effects.

Generalizability is also restricted by the participant profile: this pilot enrolled middle-aged to older adults with normal hearing, and the findings may not extend to younger listeners, individuals with hearing loss, or clinical populations with cognitive impairment. Auditory aging and hearing status can alter effective stimulus delivery and neural responsiveness (e.g., audibility, salience, and cortical synchrony), and cognitive status may further modulate state dependence and variability in gamma-band signatures. Future confirmatory studies should explicitly include broader demographic and clinical strata (including hearing-screened younger adults and target populations such as MCI) and examine whether calibration and embedding parameters require adaptation across groups.

An additional limitation is the absence of objective vigilance/eye-state monitoring. Although drowsiness-related exclusions were based on direct behavioral observation and corroborated by post-session video review, this does not replace objective physiological vigilance measures, and residual state fluctuations may have affected EEG responsiveness. Future confirmatory studies should incorporate standardized vigilance control (e.g., EOG-based eye-state verification and/or brief behavioral probes) and prespecify exclusion criteria to minimize subjective decisions.

Second, we did not implement ICA- or epoch-based artifact-rejection pipelines in this pilot. While we emphasized procedural control and conservative quality checks (eyes-closed, minimal-movement instructions; vigilance monitoring; exclusion of participants with repeated drowsiness/sleep; visual screening for gross recording failures; and exclusion of an unstable channel), residual physiological and environmental noise may remain and could influence effect estimates. Future confirmatory studies should prespecify and apply standardized artifact-cleaning procedures (including ICA and/or epoch rejection) and report their impact on the primary 40 Hz centered endpoints.

Third, individual ear-canal SPL was not calibrated in this pilot. Because ASSR-like steady-state responses can depend on stimulus intensity, the absence of calibration may have introduced uncontrolled between-participant heterogeneity in the effective “dose” of the 40 Hz component, which could inflate inter-subject variability in SNR and attenuate apparent effect sizes. In practice, even when the file-level embedding ratio is fixed (via a predefined A), the delivered ear-canal SPL can vary with insertion depth, ear-canal acoustics, sealing/attenuation, and individual loudness preference. Importantly, the within-participant contrast-control design reduces the likelihood that such variability alone explains ON–OFF directionality; however, calibrated SPL documentation (e.g., coupler-based estimation and explicit gain-setting records) will be essential for reproducibility, cross-study comparability, and interpretation of individual differences in response magnitude.

Fourth, the present outcomes quantify EEG-level signatures and do not establish cognitive or clinical benefit; integrating behavioral proxies and longer-term feasibility protocols will be essential to connect EEG detectability to functional relevance. In addition, we did not collect concurrent subjective measures such as listening comfort, perceived naturalness, or perceptual detectability of the embedded 40 Hz layer. Future confirmatory studies should incorporate standardized self-reports and/or brief in-session checks along-

side EEG to evaluate long-term real-world feasibility and to relate subjective acceptability to EEG detectability.

Finally, future studies should systematically parameterize the embedding strategy (e.g., relative 40 Hz level, personalization, audibility/comfort constraints) to derive robust design rules that maximize EEG detectability while maintaining a natural listening experience.

In conclusion, using a soundscape-only contrast control to isolate the incremental contribution of a 40 Hz layer, this pilot EEG study provides hypothesis-generating evidence that a soundscape can serve as a feasible carrier for an embedded 40 Hz component that remains EEG-quantifiable; it does not evaluate therapeutic efficacy.

Author Contributions: Conceptualization, K.N., K.L., J.S. and M.L.; methodology, K.N., K.L. and K.K.; software, D.Y. and K.N.; validation, K.N., K.K. and H.K.; formal analysis, K.N., K.K. and D.Y.; investigation, K.N., H.K., S.Y., Y.L. and J.Y.; resources, K.N. and S.J.; data curation, K.N., D.Y. and H.K.; writing—original draft preparation, K.N. and H.K.; writing—review and editing, K.N., K.K., K.L., S.J. and M.L.; visualization, D.Y. and K.N.; supervision, K.N. and K.K.; project administration, K.N.; funding acquisition, K.N. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Kookmin University (protocol code KMU-202509-HR-503, date of approval: 30 September 2025).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The EEG data generated and analyzed in this study contain potentially identifiable information and are therefore not publicly available due to Institutional Review Board (IRB) and privacy/ethical restrictions. De-identified data may be made available from the corresponding author upon reasonable request, subject to IRB approval and appropriate data-use agreements.

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Conflicts of Interest: Kiechan Namkung, Kanghyun Lee, and Junghun Shin are affiliated with AUDIAS Co., Ltd.; Kiseong Kim and Dongjune Yeo are affiliated with BioBrain Inc.; and Sumin Jeon is affiliated with JS Sound Co., Ltd. These commercial entities had no role in the study design; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The remaining authors declare no conflicts of interest.

Appendix A

To examine whether the ON > OFF directionality is robust across different background contents, we present supplementary EEG results separately for the Waves set (Set A) and the Forest set (Set B). Because the per-set sample size is limited and these analyses involve multiple channels/metrics, results are provided for descriptive, hypothesis-generating purposes only and were not used to derive primary conclusions. Figures A1–A4 display set-wise summaries of 40 Hz power and 30–50 Hz SNR spectra using the same outcome definitions as in the main text.

Appendix A.1 Set-Wise 40 Hz Power (40 Hz ON vs. 40 Hz OFF)

Figures A1 and A2 visualize the channel-wise, region-wise, and grand-average 40 Hz power comparisons within each soundscape set. Across both sets, the plotted summaries provide a descriptive check on whether the ON > OFF directionality observed in the main analysis appears similarly when Waves and Forest are inspected separately. Any asterisks or nominal *p*-value markings (if shown in the plots) should be interpreted strictly as uncorrected, exploratory references.

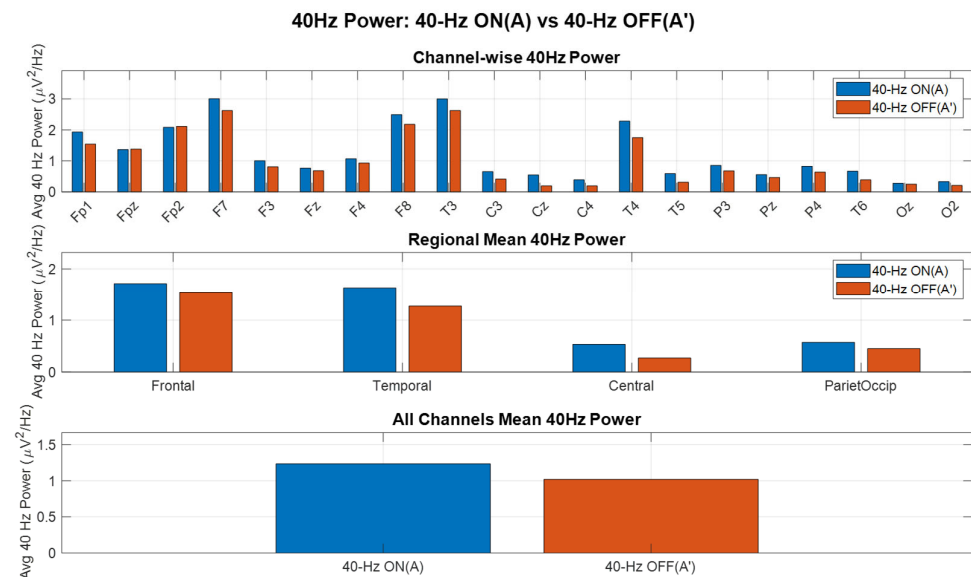


Figure A1. Set-wise 40 Hz power comparison for the Waves set (Set A; 40 Hz ON vs. 40 Hz OFF). Top: channel-wise mean 40 Hz power across electrodes. Middle: region-wise means (frontal, temporal, central, and parietal–occipital). Bottom: grand-average 40 Hz power across all included electrodes. This figure is provided as a descriptive set-wise summary; any significance markers, if present, reflect nominal uncorrected testing and do not imply confirmatory inference.

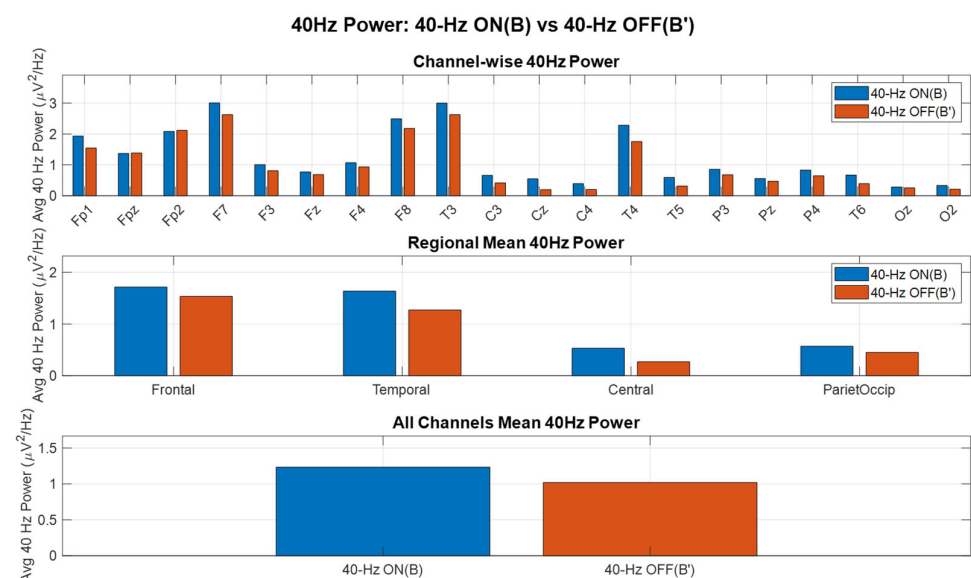


Figure A2. Set-wise 40 Hz power comparison for the Forest set (Set B; 40 Hz ON vs. 40 Hz OFF). Top: channel-wise mean 40 Hz power across electrodes. Middle: region-wise means (frontal, temporal, central, and parietal–occipital). Bottom: grand-average 40 Hz power across all included electrodes. This figure is provided as a descriptive set-wise summary; any significance markers, if present, reflect nominal uncorrected testing and do not imply confirmatory inference.

Appendix A.2 Set-Wise Frequency-Domain SNR (30–50 Hz)

Figures A3 and A4 present the 30–50 Hz SNR spectra separately for the Waves and Forest sets. Each panel shows an electrode-wise mean SNR (dB) spectrum for 40 Hz ON vs. 40 Hz OFF, with shaded bands indicating between-participant variability. These spectra are intended to support a descriptive inspection of whether the narrowband feature around 40 Hz appears more pronounced in the ON condition within each set, consistent with the stimulus definition.

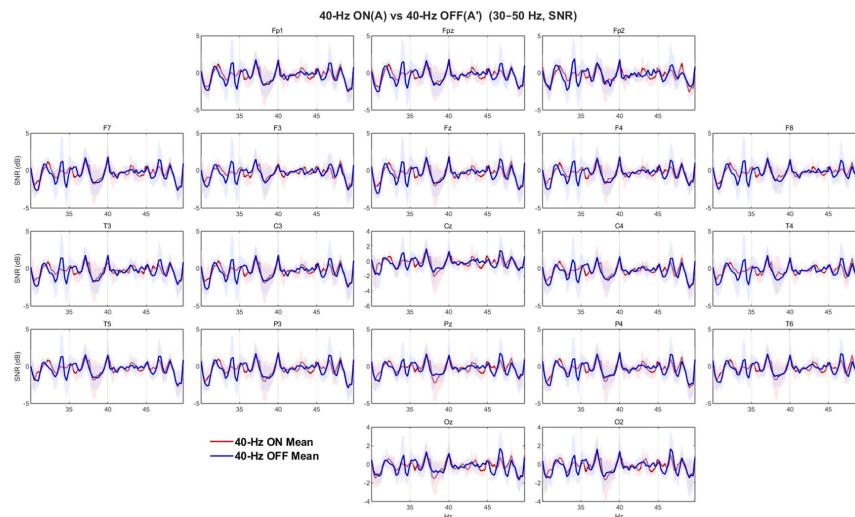


Figure A3. Set-wise frequency-domain SNR spectra (30–50 Hz) for the Waves set (Set A; 40 Hz ON vs. 40 Hz OFF). Panels show electrode-wise mean SNR (dB) over 30–50 Hz for the two conditions (see plot legend). Shaded bands indicate mean $\pm$ SD across participants within the set. SNR was computed from Welch PSD estimates by normalizing each frequency-bin power by the mean of neighboring bins (excluding the center bin) and converting to dB. This figure is descriptive and is not a multiple-comparison-controlled significance map.

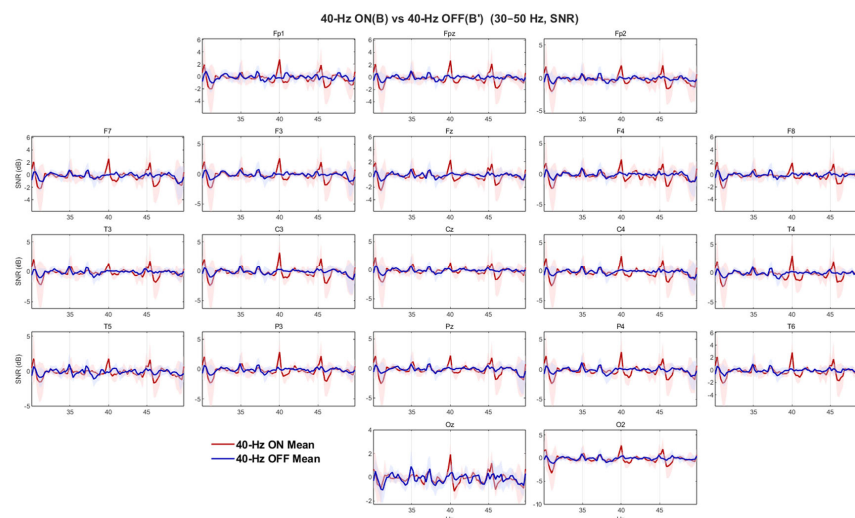


Figure A4. Set-wise frequency-domain SNR spectra (30–50 Hz) for the Forest set (Set B; 40 Hz ON vs. 40 Hz OFF). Panels show electrode-wise mean SNR (dB) over 30–50 Hz for the two conditions (see plot legend). Shaded bands indicate mean $\pm$ SD across participants within the set. SNR was computed from Welch PSD estimates by normalizing each frequency-bin power by the mean of neighboring bins (excluding the center bin) and converting to dB. This figure is descriptive and is not a multiple-comparison-controlled significance map.

Appendix A.3 Individual-Level Variability in SNR@40 and Spectral Profiles

To improve transparency regarding between-participant heterogeneity in this small pilot, we provide participant-level OFF→ON trajectories and participant-specific ON/OFF spectral overlays.

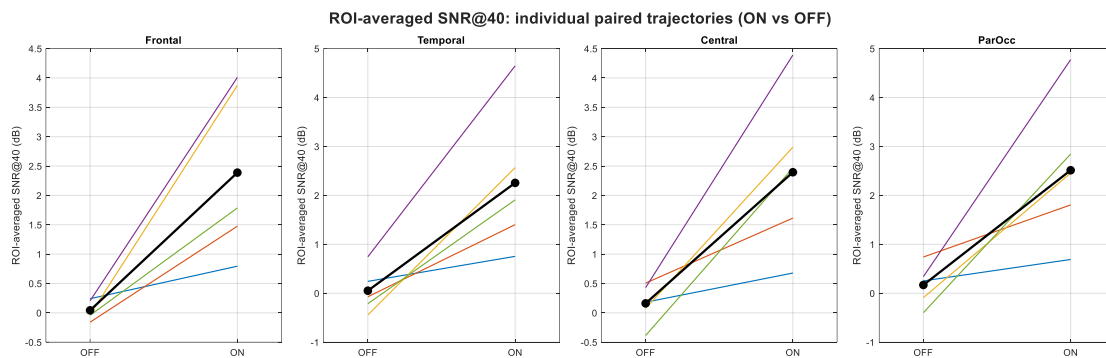


Figure A5. Individual-level paired changes in ROI-averaged SNR@40 (40 Hz OFF→40 Hz ON). Each line represents one participant's change from the OFF condition to the ON condition using a predefined ROI-averaged SNR@40 metric (temporal-region summary). Upward lines indicate ON > OFF, whereas downward lines indicate ON < OFF. The thick black line/markers denote the group means across participants.

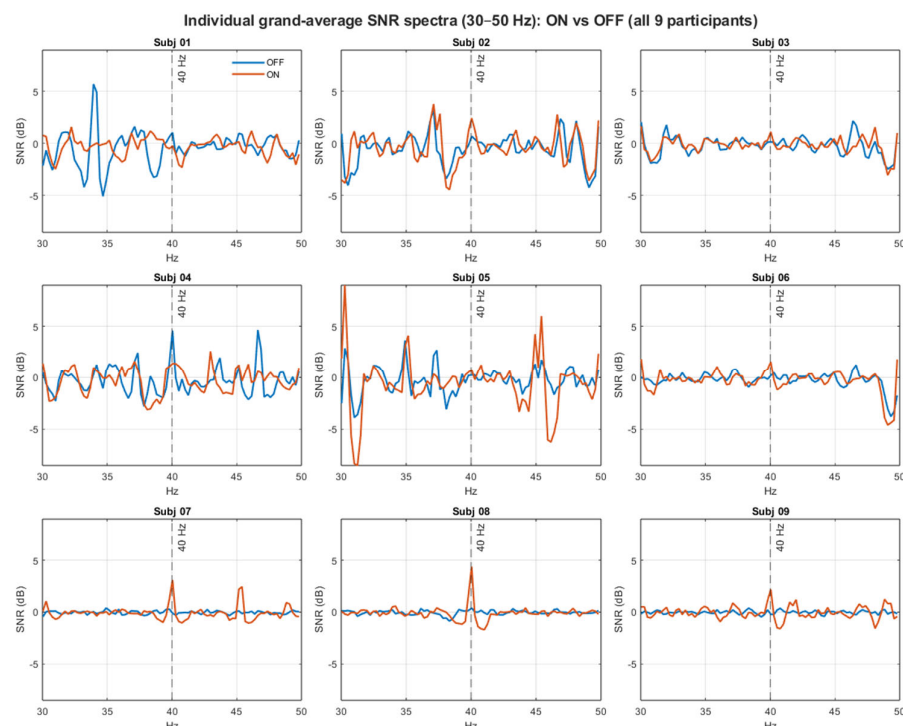


Figure A6. Participant-specific grand-average SNR spectra (30–50 Hz) with ON/OFF overlays. Each panel corresponds to one participant and shows the 40 Hz OFF and 40 Hz ON spectra over 30–50 Hz (see legend). The vertical dashed line marks 40 Hz. These overlays visualize whether a narrowband feature near 40 Hz is more pronounced under the ON condition and highlight heterogeneity across participants.

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