



UNIVERSIDADE FEDERAL DE UBERLÂNDIA
FACULDADE DE ENGENHARIA ELÉTRICA

EVALUATING THE EFFECT OF GAMMA BINAURAL BEAT ON
WORKING MEMORY PERFORMANCE AND BRAIN ACTIVITY

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Uberlândia, MG

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Area: Biomedical Engineering

Advisor: Prof. Dr. Eduardo Lázaro Martins Naves

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LUDYMILA RIBEIRO BORGES

EVALUATING THE EFFECT OF GAMMA BINAURAL BEAT ON
WORKING MEMORY PERFORMANCE AND BRAIN ACTIVITY

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"If we knew what we were doing, it would not be called research."

Albert Einstein

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Abstract

The cognitive function of memory undergoes development from childhood to aging and can be stimulated and improved by various techniques. Binaural beats (BB) have been proposed as a potential enhancement method for memory performance and treatment of psychological disorders. BB stimulation in the gamma frequency band (30-100Hz) has been associated with increased transmission of information across functional neural circuits, leading to enhanced memory performance. The current literature presents controversial approaches, including variability in stimulation parameters and participant numbers. Few studies analyzed the correlation between BB stimulation in the gamma frequency band and memory tasks. This makes it challenging to determine the optimal therapeutic stimulation parameters and underlying neural dynamics involved in gamma frequency stimulation. To address these limitations, this study proposes a detailed methodology to better understand the relationship between gamma-BB stimulation and memory performance. We collected electroencephalographic data (EEG) from 30 subjects in 3 phases - a baseline, with gamma-BB stim, and control stim - in a resting state, with eyes closed - and while performing memory tasks. Both behavioral and neurophysiology data were analyzed. The study found no significant changes in memory task performance or EEG power spectral density between experimental and control conditions. However, significant differences in brain connectivity were observed using phase-locked value (PLV) analysis in frontal and parietal areas, which are essential for memory processes. This study highlights the importance of exploring the underlying neural dynamics involved in BB stimulation and memory performance. The findings provide insight into using PLV as a metric for measuring brain connectivity and its potential application in future research. This research contributes to the development of optimal therapeutic stimulation parameters for improving memory performance.

Keywords: working memory, brain stimulation, binaural beats, EEG, power spectral density, functional connectivity, memory performance.

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List of Abbreviations

A-:	Negatively charged proteins
AD:	Alzheimer's Disease
ADHD:	Attention Deficit Hyperactivity Disorder
ANT:	Advanced Neuro Technology
ASMR:	Autonomous Sensory Meridian Response
BB:	Binaural beat
CN:	Cochlear nucleus
COWAT:	Controlled Oral Word Association Test
CRS-R:	Coma Recovery Scale-Revised, a scale used to assess individuals with disorders of consciousness
CTC:	Communication Through Coherence
CSV:	Comma-Separated Values
dB:	decibel
EDA:	Electrodermal activity, a measure of skin conductance
EEG:	Electroencephalography
FFR:	Following Frequency Response
FFT:	Fast Fourier Transform
Freq.:	Frequency
GENUS:	Gamma Entrainment Using Sensory Stimuli
Hz:	Hertz
IC:	Inferior Colliculus
ICH:	imaginary coherence
ITMS:	interactive template matching and suppression
k Ω :	kiloohm
LFPs:	Local field potentials
MEG:	Magnetoencephalography
MMSE:	Mini-Mental State Examination
MOCA:	Montreal Cognitive Assessment test
MRI:	Magnetic resonance imaging
Na+:	Sodium
N.R.:	Not reported

PCA: Principal Component Analysis
PFC: Prefrontal Cortex
PICOS: Population, Intervention, Comparison, Outcome, and Study design
PLV: Phase Locked Value
POMS: Profile of Mood States
PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analysis
PSD: Power spectral density
RAVLT: Rey Auditory Verbal List
RCT: Randomized Clinical Trials
SD: Standard Deviation
SOC: Superior Olive Complex
STAI: State-Trait Anxiety Inventory
tACS: Transcranial Alternating Current Stimulation
TBI: Traumatic Brain Injury
WMC: Working memory capacity
WM: Working memory
.wav: Waveform Audio File Format

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1 INTRODUCTION

Brain activity results from neurons' actions embedded in networks communicating through excitatory and inhibitory synaptic connections. This electrochemistry phenomenon causes rhythmic oscillations in extracellular field potential, which may influence near networks.

Memory functions are associated with oscillations of electrical activity in the brain. Literature suggests that cortical theta, alpha, and gamma bands play a mechanistic role in multiple aspects of memory. This role includes the representation and offline maintenance of events and sequences of events, the assessment of novelty, the induction of plasticity during encoding, and the consolidation and retrieval of stored memories [1].

Rhythmic brain activity and its entrainment in cognitive functioning have been a rapidly growing area of research interest, focusing on the role of time-frequency oscillation patterns in memory mechanisms [2]. Several techniques have been employed to modulate human memory through brainwave entrainment by focusing on non-invasive modalities, such as auditory stimulation with binaural beats (BB) [3].

Periodic auditory stimulation induces rate-independent increases in brain waves and changes in phase synchronization that may influence changes in cognitive functioning [4], [5].

During neural communication, phase synchronization assists working and long-term memory and promotes neural plasticity [6]. Some studies suggest that neuronal synchronization modulates memory performance and the potential cellular mechanism of memory storage [7].

Although specific studies support BB as a promising method for cognitive enhancement, major issues, such as incompatible methodological approaches, remain to be addressed. The protocol of the stimuli application has not been well established in the literature. The effect of brief BB stimulation on scalp EEG has not been conclusively demonstrated, especially with gamma stimulation frequency. Given the technique's growing popularity among neuroscientists, the current study adds further substance to the literature approaching memory modulation with BB.

We hypothesize that the noninvasive stimulation with BB in gamma frequency masked with classical music can change brain activity and improve working memory performance. Providing detailed documentation about the method, we aim to obtain significant results that can serve as parameters for other studies, as well as the development of new rehabilitation techniques.

1.1 Objective

1.1.1 General objective

To elucidate the effect of gamma auditory binaural beat stimulation in working memory by investigating behavior and neurophysiology aspects.

1.1.2 Specific objectives

- Analyze the influence of BB in memory behavior through memory tasks;
- Study the cortical brain alteration related to the presented stimulus by analyzing the power spectral density and connectivity;
- Analyze the correlation between cortical activity and memory task performance under BB stim.

2 LITERATURE REVIEW

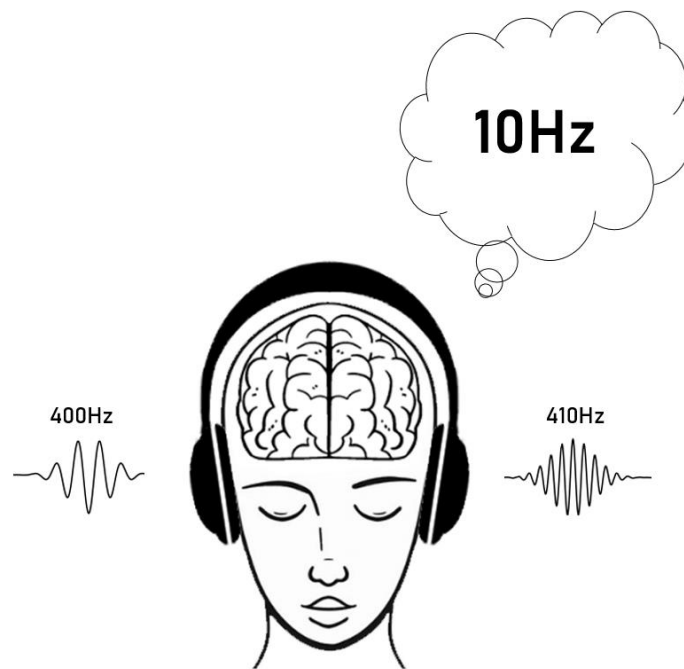
2.1 Binaural Beats

Over 150 years ago, Heinrich Wilhelm Dove discovered the Binaural Beats (BB) technique [8]. He used two tuning forks with different auditory frequencies that ran into two tubes — one frequency/tube for each ear. The participant receiving the auditory stimulation perceived only one beat, i.e., a third frequency that was the difference between the tuning forks' frequencies. Two decades later, binaural hearing studies were revolutionized with Alison's invention of the binaural stethophone [9], in which independent ear tubes enabled listening to two different sounds simultaneously. Using headphones directly the sounds towards the ears, guaranteeing that one frequency does not interfere with another.

Since then, investigators such as Licklider et al. [10] and Perrot & Nelson [11] have studied the frequency limit for BB perception. In 1973, Gerald Oster [8] demonstrated the process of binaural perception and its potential application in medical conditions. Oster found correlations between the ability to hear binaural beats and an early prediction of Parkinson's disease; during his experiments, some volunteers who could not hear the beats suffered from the disease. He also found differences in women's auditory perception and assigned it to the hormonal cycle. Besides, due to the Frequency Following Response (FFR), he suggested binaural beats could help in focus, memory, pain, and other cognition functions [8].

BB are two beats with slightly different frequencies provided in each ear separately, so the perception is the third sound from the difference between the two beats. For example, 410 Hz was provided to the left ear and 400 Hz to the right ear, and the brain perceived 10 Hz ($\Delta f = f_1 - f_2$) [8]. Figure 2-1. Representation of Binaural Beats perception illustrates BB perception.

Figure 2-1. Representation of Binaural Beats perception



Source: author

A sound wave is a vibration of molecules; the contact with each other transmits the energy characterized by:

- Frequency: the number of times that the pattern repeats in a second;
- Wavelength: the distance covered by the sound wave during one period;
- Acoustic power: the energy provided by a sound source in a given time interval;
- Sound intensity: the energy which, in each second, travels along a surface perpendicular to a sound wave, also known as amplitude, and it is measured in decibels (dB);
- Sound pressure: it corresponds to a force per surface unit;
- Duration: it is measured in seconds (s);
- Velocity: depends on the medium in which it is propagated; physical means of greater elasticity tend to propagate the sound more quickly due to the proximity between its molecules.

BB are characterized by dichotic auditory input [12]. The resulting perception— called "central beat" [13] — is due to the central processing of the third frequency in the superior olivary nuclei [14]. In contrast, monaural beats (MBs) use two different frequencies that

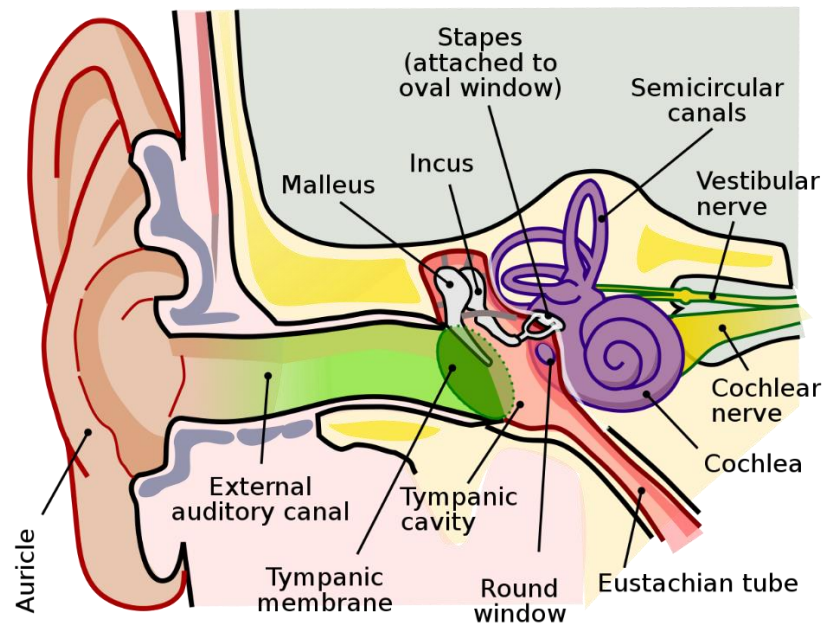
are merged and presented to both ears at the same time [15] (i.e., diotic stimulation), whereby the resultant "peripheral beat" perception is processed in the cochlea [13]. Both methods of auditory stimuli presentation have been used as neuromodulation techniques which involve only beats to affect the brain's entrainment. These methods do not require language, sound source localization, or selective attention processes commonly involved in dichotic listening tests. Brain activity recorded with electroencephalography (EEG) during BBs and MB stimulation shows differences in amplitude and phase, mainly due to differences in auditory processing mechanisms [13]. While EEG signals during MBs stimulation are high in amplitude, BBs mainly influence the signal phase because the neurons sensitive to time delays are primarily located in the brainstem, where their activation power is weaker [13], [15].

Binaural beats do not contain the third beat frequency – perceived by the subject – in their spectrum or envelope. However, this percept is presumably created in the brainstem. The auditory pathway is explained in the next section.

2.1 Auditory pathway

The organ responsible for the audition, the ear, receives the sounds, represented in Figure 2-2. The mechanical structure of the external and inner ear The mechanical vibration of air crosses the external acoustic meatus, an air-filled tubular space that conducts sound waves to the tympanic membrane. There are three little bones (malleus, incus, stapes) in the middle ear, which transfer the vibrations from the tympanic membrane into the waves in the fluid and, consequently, vibrate the membranes of the inner ear [16].

Figure 2-2. The mechanical structure of the external and inner ear



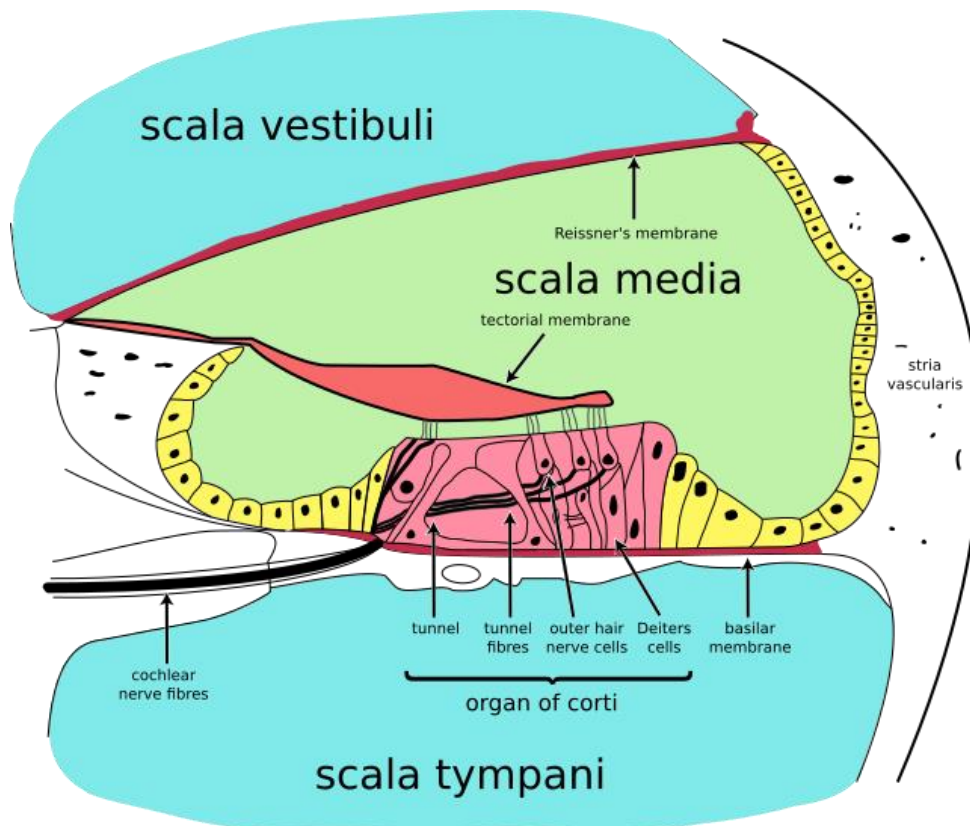
Source: [17]

The stapes impacts the oval window, causing movements in and out, like a piston, with sounds. The round window, located below the oval window, moves accordingly with fluid movement inside the cochlea [18], called perilymph. The two windows (oval and round) make possible the perilymph's vibration.

The cochlea organ is divided into three scalas (vestibular, media, and tympanic), shown in Figure 2-3. The scala vestibular and scala media are separated by Reissner's membrane. The basilar membrane separates Scala media and scala tympani [14]. The vibration of the fluid inside the cochlea goes forward the scala vestibule and returns by scala tympanic until it reaches the round window.

The vibrations of the fluid move the basilar membrane, and consequently, the organ of Corti lies on the basilar membrane. When vibrations occur, the stereocilia, sensory cells of the Corti organ, rub on the tectorial membrane. The movement of the hair cells bends them, and potassium channels open, causing the depolarization of hair cells [14].

Figure 2-3. The structure of the cochlea highlighting the Organ of Corti, responsible for transducing mechanical signals to electrical signals

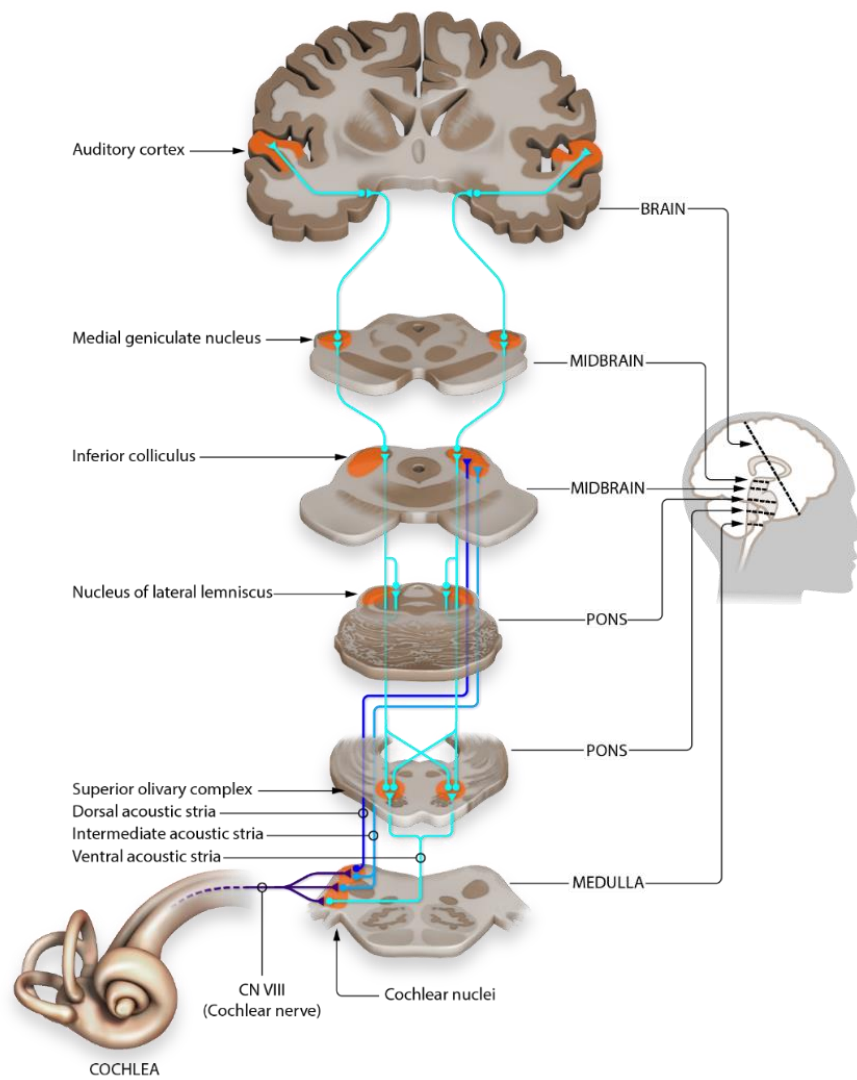


Source: [19].

The hair cells transfer the potential of action to the dendrites of sensitive (or afferent) neurons. The cellular corpora are in the spiral ganglion, and the axons reach the brainstem in cochlear nuclei.

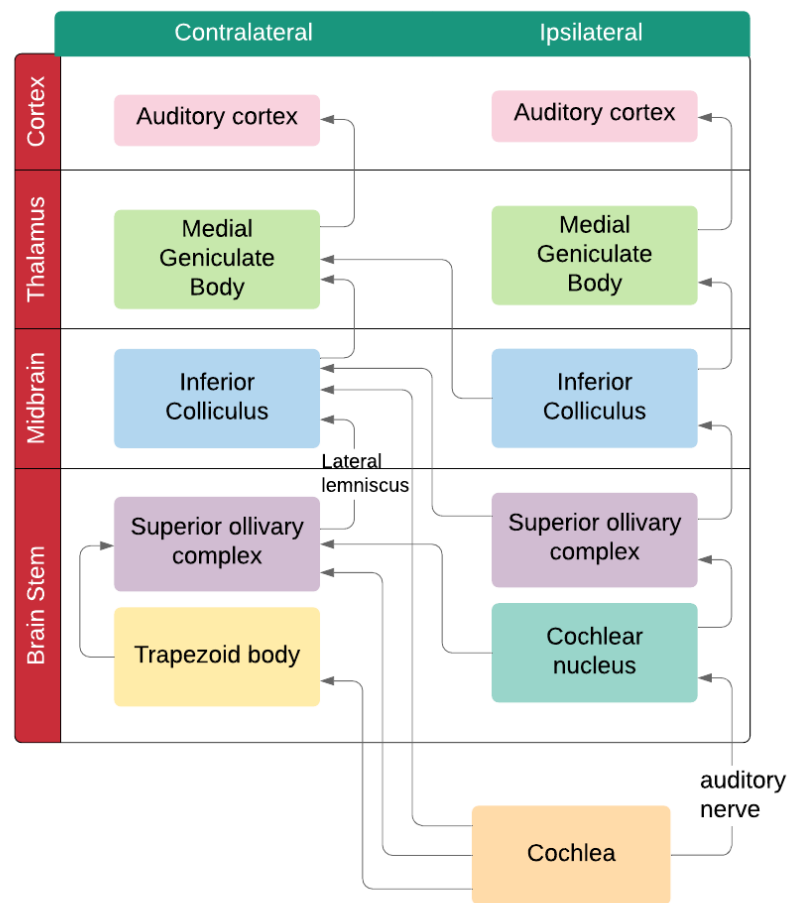
Most auditory fibers of the vestibulocochlear nerve end in the ventral and dorsal cochlear nuclei. Another connexion is made with the contralateral superior olivary complex [14]. The BB is perceived in the superior olive; these structures are responsible for sound localization because of detecting the time difference between the sound reaching each ear. Through the lateral lemniscus, the signal is sent from the superior olive to the inferior colliculus in the mesencephalon. Thus, the auditory signal passes to the thalamus structure and reaches the medial geniculate body, where occurred the preparation of motor response; and then passes to the primary auditory cortex on lateral fissure, the transverse gyrus of Heschl on the temporal area [20]. Figure 2-4 illustrates the afferent impulses in the neural mechanism of hearing. Figure 2-5 presents a schematic of the ipsilateral and contralateral auditory pathways.

Figure 2-4. The Neural Mechanism for Hearing



Source: [21]

Figure 2-5. Schematic of the pathway of auditory electrical stimuli in the ipsilateral and contralateral side



Source: Author

2.2 Non-primary auditory pathway

The system described above selects the sensory message that will receive attention first. The stimulus from the cochlear nuclei achieves the reticular formation in the mesencephalon (also called midbrain) through small fibers; this part is also responsible for receiving other sensory messages. Then, the auditory stimulus passes to non-specific thalamus nuclei and ends in the polysensory (associative) cortex. The thalamus nuclei also connect with the hypothalamus, and limbic cortex, where vegetative reactions originate, hormonal secretions, emotion motivation, voluntary motricity response, and conscious perception [12].

The non-primary pathway also called the reticular sensory pathway, is important in the context of this study. Regarding the attention concomitant between the auditory

stimulation BB and other tasks, the auditory information is expected to be processed unconsciously while the user performs a memory task.

2.3 Neural oscillations

Neural oscillations refer to the rhythmic patterns of electrical activity that are generated by groups of neurons in the brain. These oscillations can be measured using techniques such as electroencephalography (EEG), magnetoencephalography (MEG), and local field potentials (LFPs).

The neurons communicate among them through action potential. The origin of action potentials can be traced to the electrical properties of the cell membrane of neurons [22]. Neurons have a resting membrane potential, a negative electrical charge across the membrane when the cell rests. This resting potential is maintained by the unequal distribution of ions (charged particles) inside and outside the cell, with sodium (Na^+) and chloride (Cl^-) ions more concentrated outside the cell and potassium (K^+) and negatively charged proteins (A^-) more concentrated inside [22].

When a neuron receives a signal from other neurons, this can cause a temporary change in the distribution of ions across the membrane, leading to a depolarization of the membrane potential. If the depolarization reaches a certain threshold, it triggers the opening of voltage-gated ion channels in the membrane, allowing positively charged sodium ions to enter the cell rapidly, further depolarizing the membrane and causing an action potential to be generated [22].

Once an action potential is generated, it propagates down the neuron's axon, triggering neurotransmitters' release at the synapses between neurons, ultimately activating downstream neurons in the network.

Neural oscillations typically arise from the coordinated activity of large groups of neurons, and the timing and synchronization of action potentials within those groups can shape the oscillatory patterns [23].

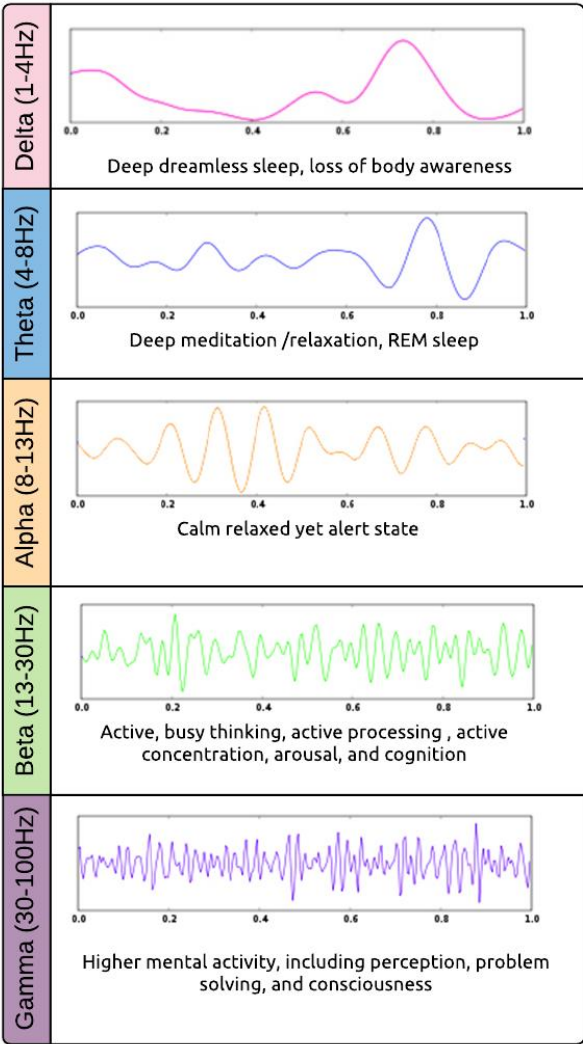
2.4 Frequency Following Response (FFR) and Gamma Entrainment

Brain entrainment, or Frequency Following Response (FFR), is not just some non-neural mechanism such as an acoustic vibration perceived in the records by the interface brain-electrode (cross-talk); it reflects a neural activity's response for exterior rhythmic

stimulation [24]. Although the neurological origin of FFR remains unclear, Smith et al. [25] suggest that the origin is in the inferior colliculus (IC). Others suggest that FFR origins are not only in the IC but also in the cochlear nucleus (CN), superior olive complex (SOC), and lateral lemniscus [26]. However, while some authors suggest that the FFR has only subcortical sources, Coffey et al. [27] suggest cortical contributions among FFR sources.

The FFR causes brainwave entrainment, and the oscillation pattern of cortical activity has been associated with different cognitive functions [28]. Figure 2-6 shows the five main brainwave state categories and their alertness state.

Figure 2-6. Overview of primary cognitive functions associated with brainwaves frequency



Source: Author

Modulations in rhythmic synchronization of the gamma-frequency are related to the binding process within sensory processing, attentional enhancement of sensory input, and both working and long-term memory [29].

Neural synchronization in the gamma frequency is often linked to short-range communication within brain areas. While long-range communication relates to neuronal phase locking in slower frequency bands [30] [31]. In line with this characteristic, increased gamma-band power improves the top-down control of feature bindings [32].

The frequency of gamma in synaptic transmission is related to the inhibitory (GABAergic) and excitatory (glutamatergic) activity [29]. The mechanism for the selective transmission of sensory information across different brain areas seems to be based on gamma frequency [28]. Gamma also works as neuroprotective, influences nonneuronal cell types, and affects synaptic plasticity, learning, and memory [33]. In patients with Alzheimer's disease (AD), the interareal gamma coherence and gamma power are disturbed [34]. The same occurred with mice models of AD, which presented reduced abnormal gamma activity [35].

Two theories are proposed for how gamma might contribute to functional neural circuits and enhance communication among brain areas: the communication through coherence (CTC) and the 'oscillations facilitating stimulus selection' proposals. In the CTC, the flow of information between two areas is optimized when gamma oscillations are coherent in both [23]. In the 'oscillations facilitating stimulus selection' proposal, the sender area induces gamma synchronization in the downstream areas [36]. Indeed, a recent study showed that 40 Hz stimulation enhanced coherence between the visual cortex, CA1 (hippocampus region), somatosensory and prefrontal cortices [35]. These findings reinforce that gamma sensory stimulation improves brain functions. However, further studies are required to understand how gamma induces synchronization in brain function.

Studies applying auditory stimuli in the gamma frequency in humans produced gamma entrainment in the frontal area [37] [38]. Another study [34] shows firing periodicity during auditory evoked gamma in the auditory cortex, a hippocampal region essential for memory (CA1), and the prefrontal cortex (PFC).

Adaikkan and Tsai [28] reported the use of GENUS (gamma entrainment using sensory stimuli) for 1h daily for 10 days in mice. They suggested that gamma entrainment could improve spatial learning and/or memory. On the 9th day, the short-term novel object

recognition and memory improved; on the 10th day, the object location memory improved. Nevertheless, on the 7th day, the stimulation did not affect these functions.

Applied in epileptic patients, the deep brain stimulation at 40Hz implanted in the rhinal cortex and hippocampus enhanced memory [39]. Using tACS, the authors in [40] entrainment the dorsolateral PFC at 80Hz coupled to the theta peak and boosted working memory performance, whereas 40 Hz entrainment in temporal-parietal areas improved working memory [41].

The neuroprotective gamma effect has been evaluated in mouse models, and fortunately, the sensory evoked in gamma frequency also seems feasible in humans [37], [38]. The authors in [42] found cross-frequency connectivity patterns using gamma and theta binaural beats but did not evaluate cognitive performance. In comparison, the authors in [43], with sessions of 20 minutes of gamma binaural beats, found that reaction time decreased in cognitive tasks. In this context, the binaural beats are a noninvasive approach that can potentially induce gamma frequency in area memory-related through sensory stimulation.

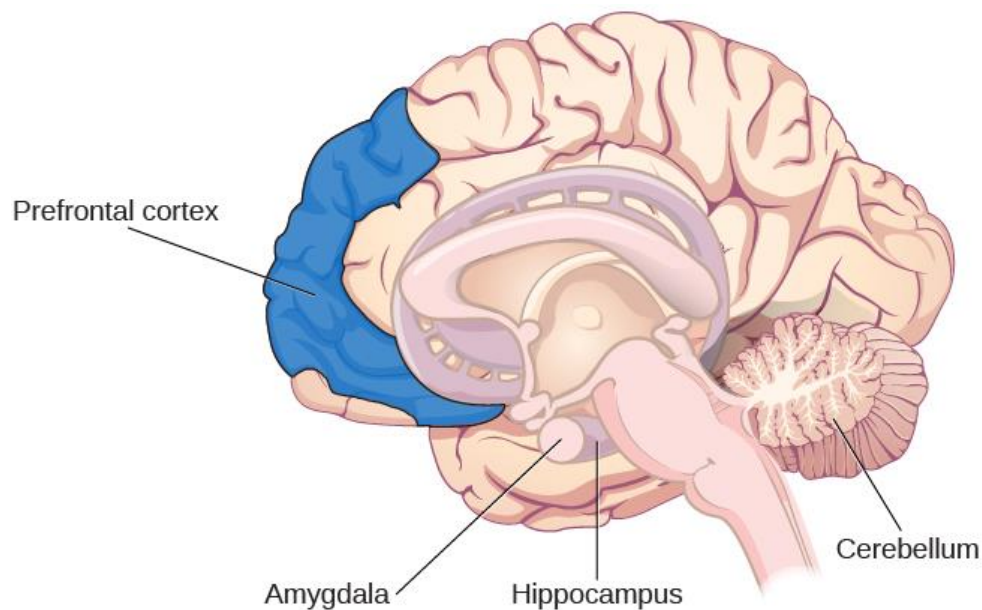
2.5 Memory

Memory is the capability of encoding, storing, retaining, and recalling information when needed [44]. It is one of the cognitive functions among attention, language, perception, and executive functions. The name "memory" comes from the Latin *memoria* and *memor*, meaning "mindful" or "remembering".

Memory is thought to involve changes in the strength and connectivity of synapses, the junctions between neurons, and the activation of specific neural circuits in response to sensory input and other cues.

Memory functions involve many different brain regions and neural circuits working together. Some key brain regions and structures involved in memory are the amygdala, the hippocampus, the basal ganglia, the cerebellum, the medial temporal lobe, the neocortex, and the prefrontal cortex [44], illustrated in Figure 2-7.

Figure 2-7. Some parts of the Brain Involved in Memory



Source: [44]

Amygdala - is an almond-shaped structure in the brain's temporal lobe, responsible for regulating emotions, such as fear and aggression, and the memory stored, influenced by stress hormones [44]. The amygdala also consolidates memory, transferring new learning into long-term memory. It facilitates encoding memories at a deeper level when the event is emotionally arousing.

Hippocampus - located in the brain's temporal lobe, related to recognition and spatial memory. It is the part where new memories are encoded. It also makes part of memory consolidation, transferring stored memories to the neocortex, and connecting them with other connected memories, giving meaning to them [44]. It is typically divided into four distinct regions: the dentate gyrus, CA1, CA2, and CA3.

- The dentate gyrus is primarily involved in pattern separation, which is the process of distinguishing between similar but distinct experiences or memories;
- The CA1 region plays a role in the consolidation of memories, helping to transform short-term memories into long-term memories;
- The CA2 region is less well understood but is thought to play a role in social memory or the ability to remember social interactions with others;

- The CA3 region is involved in pattern completion, which is the ability to retrieve complete memories based on partial or incomplete cues.

Basal ganglia - is located deep within the brain and is involved in the formation and retrieval of procedural memories, which are memories related to skills and habits.

The Cerebellum - a separate structure located at the rear base of the brain, is associated with the basal ganglia, which lies deep within the brain. It coordinates implicit memories, such as procedural, motor learning activity, movement, and habit formation [44].

The medial temporal lobe - including the hippocampus and other structures- is involved in forming and retrieving declarative memories, which are memories related to facts and events.

Neocortex - the brain's outer layer- stores and retrieves long-term memories.

The prefrontal cortex (PFC) - is part of the neocortex and sits at the front of the brain. Its functions correspond to encoding, retaining, and retrieving information related to short-term working memory [44].

Some subareas of the prefrontal cortex are involved in different aspects of memory processing. These include:

- The dorsolateral prefrontal cortex (DLPFC): This area is involved in working memory. It is also important for executive functions like planning, organization, and problem-solving.
- The ventrolateral prefrontal cortex (VLPFC): This area is involved in semantic memory. It is also important for language processing and comprehension.
- The orbitofrontal cortex (OFC): This area is involved in episodic memory. It is important for emotional processing and decision-making.
- The medial prefrontal cortex (mPFC): This area is involved in autobiographical memory. It is important for social cognition and theory of mind, which is the ability to understand and interpret the mental states of others.

2.5.1 Memory models

Many authors have proposed theories about memory models [45]–[51]. Here are some of the most well-known:

- **Richard Atkinson and Richard Shiffrin** proposed the multi-store model of memory, which suggests that information is processed through three different memory stores: sensory memory, short-term memory, and long-term memory [45].
- **Endel Tulving** proposed the dual-process model of memory, which suggests two types of memory: semantic memory (memory for general knowledge and facts) and episodic memory (memory for personal experiences) [46].
- **Fergus Craik and Robert Lockhart** proposed the levels-of-processing model, which suggests that information is processed more deeply and retained better when processed meaningfully [47].
- **Daniel Schacter** proposed the seven sins of the memory model, which suggests seven common types of memory failures: transience, absent-mindedness, blocking, misattribution, suggestibility, bias, and persistence [48].
- **Martin Conway** proposed the autobiographical memory model, which suggests that personal experiences are stored in a way that reflects their personal significance and the individual's knowledge and beliefs [49].
- **Eric Kandel**, a Nobel Prize-winning neuroscientist, proposed a memory model based on the physiological changes that occur in the brain during the formation and storage of memories. Kandel's model is known as the synaptic plasticity model of memory [50]. His model proposes that memories are formed by changes in the strength of connections between neurons in the brain. These changes are called synaptic plasticity and are believed to be the basis of learning and memory.
- **Allan Baddeley's** memory model is called the "Working Memory Model". It was proposed in the 1970s as a new way of understanding short-term memory. The model suggests that short-term memory is not a single, unitary system but consists of multiple components working together [52]. His working memory model consists of four main components: phonological loop, visuospatial sketchpad, central executive, and episodic buffer [53].

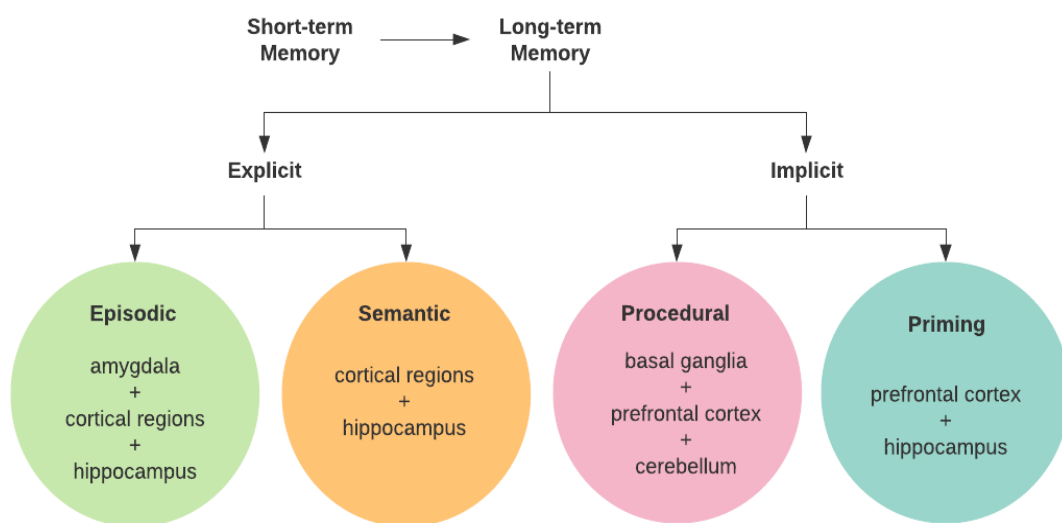
For our purposes, we adopted the following types of memory definitions:

Short-term memory is the ability to hold small amounts of information over a brief period. Also known as working memory could last a few minutes and provide data accessibility while being processed and manipulated [47]. The working memory is most processed in

PFC and parietal cortex, as well as interactions between these regions and other brain parts [54].

Unlike short-term memory, long-term memory presents unlimited capacity and is divided into explicit and implicit memory. Explicit memory is one in that we can consciously recall and describe information. Episodic memory, constituted by events and personal experiences, and semantic memory, based on facts or general knowledge about the world, are included in explicit or conscious memory [54]. The explicit memory is processed in the amygdala, cortical regions, and hippocampus [44] and is affected by neurodegenerative diseases such as Alzheimer's. Implicit memory is stored and retrieved without conscious effort. Procedural memory involves acquiring motor skills, such as bike riding and is processed in the basal ganglia, PFC, and cerebellum [44]. Priming memory is involved in the recall process by association, which occurs when exposure to one stimulus influences our brain's response to another. For example, the word "bread" is associated with the word "butter" faster than the word "tire" [55]. Both procedural and priming memory is related to implicit memory. Studies of dementia indicate different reactions of patients with Alzheimer's disease regarding implicit memory; while they show impaired priming, motor skill learning remains intact [56]. Figure 2-8 illustrates the types of memory and the brain regions involved.

Figure 2-8. The types of memory



Source: Author adapted from [57]

Overall, the memory process in the brain is complex and involves multiple neural systems and procedures, including attention, perception, encoding, consolidation, and retrieval. Advances in neuroscience research continue to shed light on the underlying mechanisms of memory and may lead to new strategies for improving memory function in various contexts.

3 SYSTEMATIC REVIEW

A systematic review was performed on experimental studies investigating the effects of BBs on healthy participants' working memory and short & long-term memory. This study has followed the Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) guidelines. The eligibility criteria were framed using the PICOS approach, which identifies the population (P), intervention (I), comparison (C), outcome (O), and study design (S). Thus, studies were selected if they met the following inclusion criteria:

- Population — sample populations were identified as cognitively and physically healthy participants with no age or gender limits.
- Intervention — auditory binaural stimulation.
- Comparator — studies comparing pre-/post-BBs stimulation effects; experimental vs. control condition (with no beat, pink or white noise).
- Outcome — memory performance assessment tools such as neuropsychological tests/tasks.
- Study design — experimental research, including prospective and pre- vs. post-intervention studies, were reviewed regarding BB stimulation effects on cognitive enhancement as per the above outcome measures.

Database searches used the English language terms "binaural beats", "binaural beating", "auditory beat stimulation", and "binaural auditory beats" for the intervention — while for the outcome, they were "memory", "cognition", "brain connectivity", "auditory cognitive neuroscience" and "cognitive function". These keywords were combined two by two — intervention and outcome — with Boolean AND in each search string to ensure consistency across the different databases. Each generated a total of 20 searches effectively. The databases accessed in March 2022 (without publication date limits) were Scopus, PubMed, Google Scholar, BIREME, Science Direct Elsevier, IEEE Xplore, and Web of Science. In Google Scholar, the keywords terms must be in the publication title. All the other databases did not have such restrictions. When the database could expand the results, we did so, including conference full articles and theses. Exclusion criteria were only abstracts, posters, and papers without results, proposals, and studies that did not use BB as a memory improvement method.

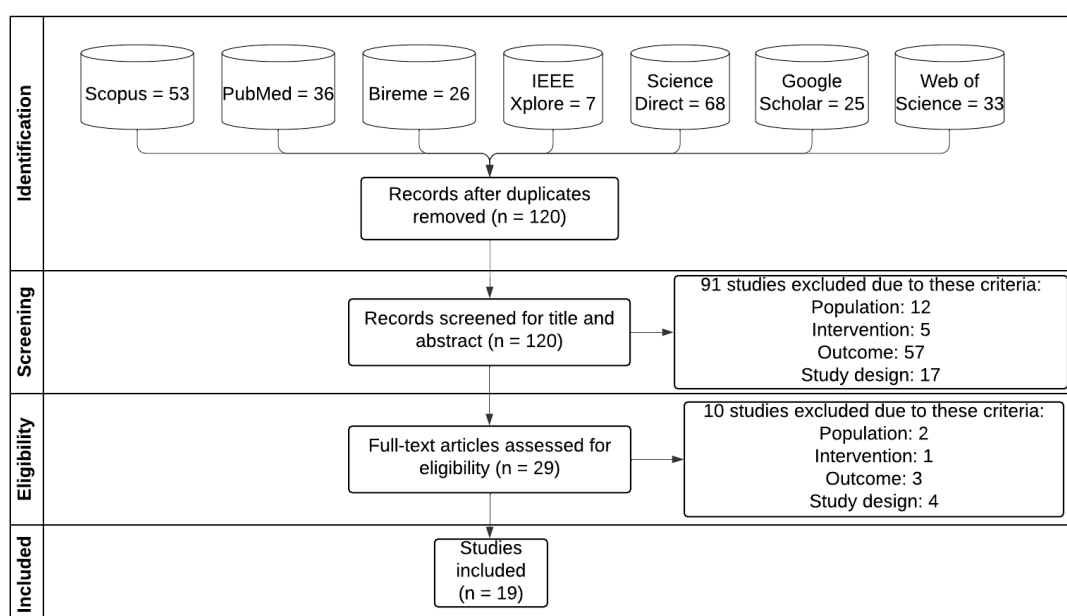
All studies matching the inclusion criteria were considered. As there was not a large volume of published work in this area, the study parameters employed were heterogeneous such as volunteer groups, age, gender, assessment tool, and data processing methods. The data extracted from the articles were title, authors, year, sample size, participants' characteristics, stimulation frequency, carrier tone, beat frequency in the right and left ear, exposure duration, number of sessions, assessment tool, and main results.

The results are presented in Figure 3-1. After eliminating the duplicates, we found 120 studies from 7 databases. These 120 studies were screened by title and abstract, from which 12 were excluded according to the population criterion: ADHD (1), depressive disorder (1), schizophrenia (1), epilepsy patients (2), animal experiments (3), Parkinson's disease (1), autism (1), borderline personality disorder (1), and in vivo animal cell (1).

As BB were the primary intervention of interest, another five studies were excluded due to the method used: monaural beats (1), techno music (1), autonomous sensory meridian response (ASMR) (1), Steady-state visual evoked potential (1) and the big data approach with COVID data (1). Fifty-seven (57) studies examined different outcomes other than memory: brain alterations that did not evaluate memory functioning (12), auditory detection (11), attention (10), alteration of emotional states (4), stress (4), anxiety (2), tinnitus (2), mental fatigue (2), induction of dreams and sleep (2), sedation pre-surgery (1), lucid dreams (1), drug addiction (1), premenstrual syndrome (1), dental anxiety (1), motor response (1), creativity (1), mood and depression (1). Regarding study design, 17 articles were excluded: descriptive or theoretical studies (8), review studies (3), system development (3), abstracts or posters (2), and simulation in virtual brain model (1).

The full texts of 29 studies were screened and evaluated in the next step, leaving 19 studies for further analysis. The exclusions of 10 studies were due to: population criteria — presurgical epilepsy patients (2); intervention criteria — pre-study for BBs stimulation without any subsequent stimulation (1); outcome — cognitive flexibility and decision-making (1), attention (1), EEG signal analysis that did not evaluate memory functioning (1); and study design — abstract or poster (3) and descriptive or theoretical studies (1).

Figure 3-1 PRISMA flow diagram illustrating the study selection process



Study characteristics

The characteristics of each study are summarized in Table 3-1, which reports the participant sample size (including males and females), participants' age, and the assessment tool/methods used.

Table 3-1. Characteristics of 19 studies in the systematic review

Study	N (male, female)	Age (range, mean, and SD)	Assessment tools/methods
Ahmadi et al., 2021 [58]	60 (N.R.)	19–25 years	Corsi block tapping test
Khattak, 2021 [59]	60 (30, 30)	M = 21.97 (SD = 2.59)	Word-free recall test
Mujib et al. 2021 [60]	60 (16, 44)	M = 25.73 (SD = 2.02)	Digit span task, EEG
Sharpe et al., 2020 [61]	9 (N.R.)	N.R.	Mathematical problems, recall tasks
Dy et al., 2020 [62]	89 (74, 15)	18–23 years M = 20	Word list recall
Cortez et al., 2019 [63]	74 (38, 38)	M = 16	Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was converted to

			index scores ranging from 40–160
Engelbregt & Deijen, 2019 [15]	24 (16, 8)	18–35 years M = 22.3 (SD=3.42)	Flanker task; Klingberg test
Cristóbal, 2019 [64]	28 (14, 14)	19–34 years M = 23.36 (SD = 3.92)	Visual-spatial task
Shekar et al., 2018 [65]	40 (20, 20)	22–30 years M = 23	Auditory reaction time, visual reaction time, short-term memory test
Lim et al., 2018 [66]	25 (<i>N.R.</i>)	20–50 years	D2-test, digit span test, Corsi block-tapping test, picture recognition test, EEG
Garcia-Argibay, et al., 2017 [65]	32 (23, 9)	14–51 years M=22.77 (SD=10.56)	Word list recall
Jirakittayakorn & Wongsawat, 2017 [65]	47 (16, 31)	M = 20.6 (SD = 1.4)	Brunel Mood Scale (BRUMS), EEG, word list recall
Calomeni et al., 2017 [67]*	Elderly without dementia = 15 Children with normal cognitive development = 10	Elderly M = 76 (SD = 8) Children M = 11 (SD = 4)	Digit span test, pictures of children (e.g., crying, smiling), EEG
Colzato et al., 2017 [68]	40 (30, 10)	18–28 years M = 22.10 (SD = 2.82)	The task measured binding-related effects by combining stimulus features (e.g., shape and color) and responses
Beauchene et al., 2017 [67]	34 (15, 19)	18–46 years M = 27.1	The n-back task, cortical network topology via EEG
Beauchene et al., 2017 [67]	28 (12, 16)	19–46 years M = 27.6	Visuospatial working memory task, network topology measured by EEG
Kraus & Michaela, 2015 [67]	40 (29, 11)	M = 21.63	Automated Operation Span Task
Wahbeh et al., 2007 [69]	4 (1, 3)	43–57 years M = 51	Rey Auditory Verbal List (RAVLT), Profile of Mood States (POMS), State-Trait Anxiety Inventory (STAI), Controlled Oral Word

			Association Test (COWAT), blood pressure, spectral and coherence analysis on EEG
McMurray, 2006 [70]	20 (<i>N.R.</i>)	67–80 years M = 65	Mini-Mental State Examination (MMSE), digit span task, continuous performance task, EEG power

N.R. = not reported

*As the population inclusion criteria were cognitively and physically healthy participants, this study [67] will be included in the systematic review but only for the elderly without dementia and children with normal cognitive development.

The sample sizes varied from 4 to 89. The range of 1–74 and 3–44 was, respectively, for females and males.

Several assessment tools were used. The most common working memory instruments were the digit span test, visual-spatial task, and word list recall. Several studies [60], [66], [67], [69]–[73] also analyzed EEG signals pre-post and/or during BB stimulation.

Table 3-2 summarizes the BB intervention characteristics, including stimulation frequency (in the left and right ear), the number of sessions, exposure time, carrier tone, masking stimulus, and control condition.

Table 3-2. BBs intervention protocols across the 19 studies

Study	BBs stimulation (Left ear and Right ear) (Hz)	Sessions (exposure time)	BBs before or during the task	Carrier tone (Hz)	Masking stimulus	Control condition
Ahmadi et al., 2021 [58]	9.5 (230 and 220.5), 15 (255 and 240), 6 (250 and 256)	1 session (12min)	before	230 and 220.5 255 and 240 250 and 256	<i>N.R.</i>	None

Khattak, 2021 [59]	40 (<i>N.R.</i>)	1 session (5min)	before and during	450 and 410	<i>N.R.</i>	white noise
Mujib et al. 2021 [60]	10, 14, 30 (400 and 410, 414, 430)	1 session (15min divided into 3 sub-sessions with 5 min)	before	400 and 410, 400 and 414, 400 and 430	<i>N.R.</i>	None
Sharpe et al., 2020 [61]	25, 40, 100 (<i>N.R.</i>)	8 sessions (5min)	before	<i>N.R.</i>	<i>N.R.</i>	None
Dy et al., 2020 [62]	20 (<i>N.R.</i>)	1 session (15min)	before	<i>N.R.</i>	<i>N.R.</i>	Delta binaural beat
Cortez et al., 2019 [63]	5 (125 and 130)	5 days (15min)	before	125 and 130	<i>N.R.</i>	Vivaldi's Four Seasons "Spring Movement"
Engelbregt & Deijen, 2019 [15]	40 (<i>N.R.</i>)	1 session (<i>N.R.</i>)	during	440 and 480,	white noise	white noise
Cristóbal, 2019 [64]	4 and 7 (220 and 227, 224)	2 blocks (8min) for each condition (4hz, 7hz and control)	before and during	220 and 227, 220 and 224	10% of pink background noise	non-binaural beat (220 Hz)
Shekar et al., 2018 [65]	10 and 40 (<i>N.R.</i>)	1 session for each condition (<i>N.R.</i>)	<i>N.R.</i>	<i>N.R.</i>	<i>N.R.</i>	constant tone (340Hz)
Lim et al., 2018 [66]	10 and 7–4 (<i>N.R.</i>)	1 session (20 min) for each condition	Before	<i>N.R.</i>	piano music and natural sounds	Mechanical massage and

		with a 1-week interval				relaxation (none)
Garcia-Argibay, et al., 2017 [65]	20 and 5 (390, 395 and 410, 400)	1 session (17 min)	before and during memorization	390 and 410, 395 and 400	<i>N.R.</i>	White noise generated in a range of 1–22,038 Hz
Jirakittayakorn & Wongsawat, 2017 [65]	40 (<i>N.R.</i>)	1 session (30min and 20min)	before	250 and 290	<i>N.R.</i>	None
Calomeni et al., 2017 [67]	8, 10, 12, 4, 15 (<i>N.R.</i>)	10 sessions (15min BBs and light - 3min each condition) carried out on alternate days for a total of 20 days	before	<i>N.R.</i>	<i>N.R.</i>	None
Colzato et al., 2017 [68]	40 (320 and 360)	1 session (10min)	before and during	320 and 360	All tones were embedded in white noise	340Hz
Beauchene et al., 2017 [67]	5, 10, 15 (245, 250, 255, and 240)	1 session (<i>N.R.</i>)	during	240 and 245, 240 and 250, 240 and 255	<i>N.R.</i>	None, pure tone (240 Hz) and classical music
Beauchene et al., 2017 [67]	5, 10, 15 (245, 250,	1 session (30min)	during	240 and	<i>N.R.</i>	None, pure tone (240

	255, and 240)			245, 240 and 250, 240 and 255	Hz) and classical music	
Kraus & Michaela, 2015 [67]	9.55 (<i>N.R.</i>)	1 session (12min)	before	230 and 220.45	Music with a BB	music without BBs
Wahbeh et al., 2007 [69]	7 (133 and 140)	1 session (30min)	before	133 and 140	Pink noise resembled the sound of rain	rain sounds
McMurray, 2006 [70]	7-11 (<i>N.R.</i>)	1 session (<i>N.R.</i>)	during	<i>N.R.</i>	<i>N.R.</i>	soft music

N.R. = not reported

Across all reviewed studies, the minimum binaural frequency was 4Hz and the maximum 100Hz. BBs stimulation within the gamma frequency band was presented in seven studies, while the other ranges were found in eight studies each.

Most studies (68.4%) did not report whether the BB stimulation was embedded with a masking stimulus. Six studies masked the stimulation with either white noise, pink noise, piano music, or natural sounds.

Six studies did not report the carrier tone frequency that was used. Across all reviewed studies, the minimum tone was 125Hz, and the maximum 480Hz. Ten studies (52.6%) did not report the frequency used in each ear. Six studies used a minor frequency in the left ear than in the right ear.

The BB stimulation time varied from 3 to 30 minutes. Four studies did not report this exposure time [15], [65], [70], [72]. Most studies (14 of 19) performed only 1 session of BB stimulation.

Ten studies delivered BBs only before the assessment task. Four studies [15], [70], [72], [73] stimulated only during the tasks, while another four [59], [64], [68], [74] applied the BBs stimulation both before and during the tasks. One study [65] did not report if the BBs occurred before, during, or both.

3.1 Risk of bias assessment

Across all the included studies, we used The Cochrane Collaboration's tool – RevMan, to assess the risk of bias in randomized trials [75]. Seven sources of bias in clinical trials were evaluated. Table 3-3 summarizes the studies' risk of bias across each attribute.

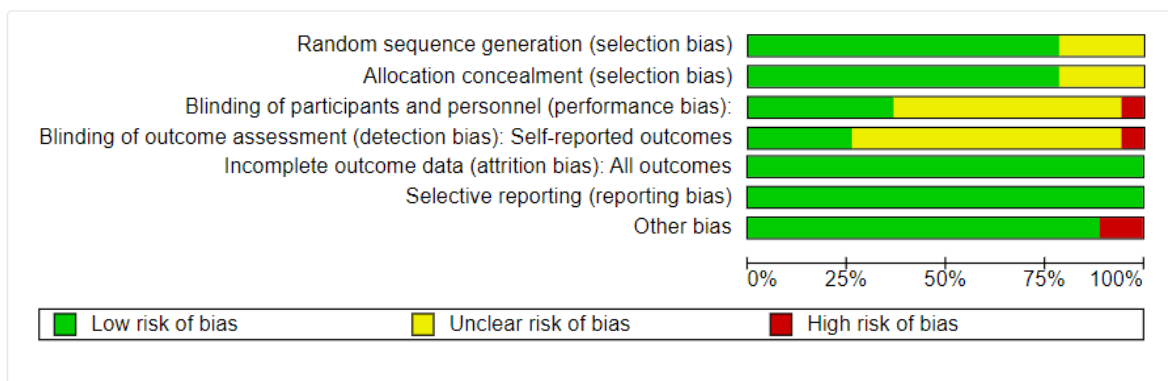
Table 3-3. Risk of bias: review authors' judgments about each risk of bias item for each included study

Study	Risk of bias						
	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias (carry-over effect)
Ahmadi et al., 2021 [58]	Low	Low	Unclear	Unclear	Low	Low	Low
Khattak, 2021 [59]	Low	Low	Unclear	Unclear	Low	Low	Low
Mujib et al. 2021 [60]	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Sharpe et al., 2020 [61]	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Dy et al., 2020 [62]	Low	Low	Low	Low	Low	Low	Low
Cortez et al., 2019 [63]	Low	Low	Low	Low	Low	Low	Low
Engelbregt & Deijen, 2019 [15]	Low	Low	Low	Unclear	Low	Low	Low
Cristóbal, 2019 [64]	Low	Low	Unclear	Unclear	Low	Low	Low
Shekar et al., 2018 [65]	Low	Low	Unclear	Unclear	Low	Low	Low
Lim et al., 2018 [66]	Low	Low	High	High	Low	Low	Low
Garcia-Argibay, et al., 2017 [65]	Low	Low	Low	Unclear	Low	Low	Low
Jirakittayakorn & Wongsawat, 2017 [65]	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Calomeni et al., 2017 [67]	Low	Low	Unclear	Unclear	Low	Low	High
Colzato et al., 2017 [68]	Low	Low	Low	Low	Low	Low	Low
Beauchene et al., 2017 [67]	Low	Low	Unclear	Unclear	Low	Low	Low

Beauchene et al., 2017 [67]	Low	Low	Unclear	Unclear	Low	Low	High
Kraus & Michaela, 2015 [67]	Low	Low	Low	Unclear	Low	Low	Low
Wahbeh et al., 2007 [69]	Low	Low	Low	Low	Low	Low	Low
McMurray, 2006 [70]	Unclear	Unclear	Unclear	Unclear	Low	Low	Low

All 19 studies were assessed via the risk of bias summary chart (see Figure 3-2).

Figure 3-2. Risk of bias summary



Overall, most studies characterized a low risk of selection, attrition, and reported bias. Only one study, Lim et al., 2018 [66], was marked with a high risk for performance and detection bias concerning blinding attributes. In this study, the blind test could not be performed because the subject could know the applied treatment, which involved sitting on a massage chair, receiving a massage, and listening to BBs.

Conversely, the "other bias" category (carry-over effect) was the most common bias to be of high risk in two studies [65] and [71]. Due to the lack of information in the studies regarding knowledge of the allocated interventions by outcome assessors, the blinding of outcome assessment (detection bias) was the most unclear risk.

This systematic review included 19 studies investigating BBs intervention on working memory and short and long-term memory among healthy participants. The information extracted and depicted in the preview tables is discussed below:

3.2 Sample characteristics

Although some studies reported differences between males and females concerning changes in auditory perception [8], especially in speech-evoked auditory brainstem response peaks [76] and differences during the menstrual cycle [77], none, however, evaluated gender differences. In one study [59], the numbers of males and females were equal to avoid gender influence on the results, and the difference between genders was not assessed.

The literature reports age-related differences concerning the auditory system's perception. While young adults have great magnitude responses and hemispheric asymmetry in cortical activity, older adults have small magnitude responses and reduced hemispheric asymmetry [78]. Moreover, Grose & Mamo [79] suggested deficits in age-related temporal processing, while Bellis & Wilber [80] indicated that auditory temporal patterning decreases between 40 and 55 years. Nevertheless, none of the studies in the systematic review evaluated age differences.

3.3 Assessment tools

Interpreting the effects induced by BB stimulation is a complex task because the BB stimulation has been assessed using different variables (neurophysiological, cognitive behavioral) with heterogeneous methodologies,

Variance exists among assessment tools for measuring working, short-term, and long-term memory [81]. As such, the convergence to a standard experimental approach among the studies hinders the comparison of the findings. According to Baddeley's theory [51], human memory has at least three subsystems: visuospatial sketchpad, phonological loop, and central executive, which can be further fractionated. The central executive component is assumed responsible for the attentional control of working memory, reasoning, decision-making, strategy planning, and behavior control. Cognitive tasks involving the central executive include mental arithmetic, recalling long lists of digits, logical reasoning, semantic verification, and recalling long-term memory events.

The collaborators in Jaeggi et al. [82] affirm that N-back, used in Beauchene [72], is a convenient tool for experimental investigations concerning working memory (WM), as it allows for the manipulation of the load. According to Clair-Thompson [83], the same task could be a WM measurement for children and a short-time memory measurement for adults. They highlighted that storage, processing, strategy use, and domain-specific

abilities influence the performance of such tasks. In light of the aforementioned, adults do not seem to need executive-attentional resources as a child does. The authors suggest considering individual abilities for choosing the memory task.

The study by Wilhelm et al. [84] affirms that only one cognitive test could not assess all aspects of WMC, so they recommend measuring memory through a heterogeneous set of paradigms to avoid mono-operation bias. Nevertheless, only one or two tests are usually feasible due to limited time and resources. In this context, four task classes (complex span, recall N-back, updating, and binding) are good indicators of working memory capacity (WMC). These match the general WMC mechanism of building, maintaining, and updating arbitrary bindings. These tasks require participants to remember a small number of items in their correct order or location. Each step needs to update the bindings between the information and its location. Then, these bindings must be continuously updated. The most common WM instruments used in the studies were the digit span test and word list recall [59]–[62], [66], [67], [70]–[72], [74]. These are particularly well suited for measuring the ability of individuals to build and maintain temporary bindings in WM.

3.4 Choice of frequency

The studies encountered used different carrier frequencies. Perrot & Nelson [11] stated that the probability of beat detection was maximal at 500Hz and decreased as frequency increased. Based on synchronous hearing in the two auditory nerves, Licklider et al. [10] found that the third sound perceived from the difference between the two beats was maximal at approximately 35Hz and that 400Hz was a most distinguishable carrier tone. Following these results, only the studies [15], [59], [60], [74] would be near these recommendations due to their using 440, 390, 410, and 400Hz, respectively, as carrier tones. Engelbregt & Deijen [15] evaluated gamma binaural beat (40Hz) but found no influence on working memory performance. The study of Garcia-Argibay et al. [74], which investigated beta (20Hz) and theta (5Hz), found better performance in the beta condition, which also decreased the vigilance and regulation system. Research by Khattak [59] evaluated gamma and found better scores in the experimental condition than in the control condition. Furthermore, Mujib et al. [60] found a decrease in reaction time for alpha and gamma groups after evaluating alpha, beta, and low gamma groups.

In addition, Licklider et al. [10] reported that at 250Hz, most beats were too low a value for the third frequency, so studies by Ahmadi et al. [58], Jirakittayakorn & Wongsawat [71], Beauchene et al. [72], Beauchene et al. [73], Kraus et al. [85], and Cristóbal [64] that used carrier tones near to 250Hz would better evaluate minor cortical frequencies such as theta. In fact, Ahmadi et al. [58], Beauchene et al. [72], Beauchene et al. [73], and Cristóbal [64] assess the BBs in the theta band. However, the outcomes of Ahmadi et al. [58], Beauchene et al. [72], and Beauchene et al. [73] showed better memory performance on the beta-BB, and Cristóbal [64] did not find any difference in WMC.

Other studies provided carrier frequency information, including Colzato et al. [68] that used 320Hz, which belongs to the best-perceived range (250-500Hz), and have found enhancement selectivity in updating episodic memory traces after stimulation in gamma frequency; two studies [69] and [63] used 133Hz and 125Hz, respectively, which are much lower than the tone indicated in previous studies. Their results did not show significant differences in stimulation conditions, and the authors reported that the carrier frequency might be too low to achieve entrainment.

There is no evidence that the choice of the beat frequency applied to each ear influences the outcomes (i. e., if the highest frequency is played on the right or left ear) since part of the auditory electrical impulse is crossed to the contralateral side of the head [14].

One study [64] evaluated the WMC listening BBs of 4 and 7, and the authors applied the carrier tone at 220Hz and an exposure time of 8 minutes. In another study [63], the stimulation frequency was 5Hz, and classical music was the control condition. Both [64] and [63] found BBs increased memory to the same degree as the control condition but did not find statistical differences. Given that the theta frequency is predictive of how well the item was transferred to long-term memory [86] and could also be responsible for transferring the information into and out of long-term memory [87], it was not expected that theta could improve WM as evaluated by [64] and [63]. Following these literature reports, studies [58], [72]–[74] compared theta stimulation with other frequencies. They found that the highest frequency showed better working memory enhancement than the theta frequency.

Eleven studies affirmed some improvements after BBs intervention [58]–[61], [67], [68], [70], [71], [73], [74], [85]. In [65], the time spent on the task was reduced in the

experimental stimulation compared to the control condition; however, there was no statistically significant difference between the frequencies (alpha and gamma).

3.5 Exposure time

In the studies for this systematic review, the time of exposure to stimulation varies between 3 and 30 minutes. The authors of [88] found that higher exposure times were associated with larger effect sizes. This observation could explain the results in Cristobal [64], which used the third shortest stimulation duration among the remaining studies and did not improve memory capacity. Four studies by Engelbregt & Deijen, Beauchene et al., 2017, Beauchene et al., 2016, and McMurray [15], [70], [72], [73] opted to stimulate with BBs only during the tasks. However, [15] did not demonstrate significant differences between the experimental and the control condition. Additionally, Garcia-Argibay et al. [88] suggest that binaural-beat exposure before and before & during the task produces superior results than exposure just during the task.

3.6 Bias risk

To reduce or eliminate some experimental bias sources, the trial must be blinded (i.e., the information that may influence the participants and researchers is hidden until the experiment is complete) to ensure that the results are not affected by the power of suggestion [89]. In this context, two blinded biases were evaluated: performance bias due to knowledge of the allocated interventions by participants and personnel during the study; detection bias due to knowledge of the allocated interventions by outcome assessors. The studies poorly reported the second of the two and, due to this fact, the most inadequately assessed attribute.

The study by Lim et al. [66] could not prevent participants from knowing which treatment they received. In the study, three treatments were compared: having rest while sitting on a massage chair (Treatment A), receiving a mechanical massage with a massage chair (Treatment B), and mechanical massage while listening to the BBs using the massage chair (Treatment C). Despite the absence of blinding, the data were objectively quantified using the frequency power spectrum analysis of EEG instead of a subjective method such as a survey. Thus, they minimized the risk of bias regarding blinding. The study [66] found that the treatments in B and C improve sustained attention, nonverbal long-term memory, and verbal short and long-term memory via mental fatigue recovery.

One risk identified as "other bias" was the carry-over effect, mainly due to the absence of a "washout" period, which is a time break built into the study design to reduce the carry-over effects of treatments [90]. We reviewed whether carry-over effects were mentioned anywhere in the studies classified as high and how these were addressed in the data analysis of treatment effects. But no mention was made of it.

3.7 General

In addition to the preview studies [87] and [90], we proceeded with an accurate qualitative and descriptive reporting of experimental protocols. We summarized all related studies, including gray literature and those not providing sufficient information to infer statistical analysis. We also included and analyzed technical details of the key methodological parameters mentioned as crucial by Chaieb et al. [91].

There is still a long way for science to elucidate the relationship between the synchronization of brainwaves with binaural stimuli and memory. While experimental research investigates this approach, companies and startups combine their expertise to advance in this promising field.

The Bio-Acoustical Utilization Device (BAUD) [92], a battery-powered handheld device, provides the binaural sound effect. The results effectively reduced emotional issues, from severe phobias and post-traumatic stress disorder (PTSD) to everyday anxieties, decreasing cravings or urges, substance abuse, and chronic pain of all kinds [92].

Several apps were developed for BBs entrainment practice, such as Brainaural [93]: a parametric binaural beat generator that gives the user complete control over frequencies; Zen Wellness [94]: audios and videos for Relaxation and meditation, BB therapy, mantras, Ho'oponopono, among other features. In the context of the COVID-19 crisis, an Israeli–Estonian startup developed an app, Synctuition, that offers relaxation experiences based on BB technology [95].

Some examples of BB stimulation are available for free on the Internet. Video sites have extensive playlists with different tones used in treatments. Another option is the streaming apps that have playlists with varied options. Mobile delivery and free availability on the Internet of BB stimulation have several advantages over traditional clinic-based therapies. Besides being cheaper to implement, they also can reach more people. As such, barriers such as access, distance, or engagement can be overcome.

However, despite popularizing such a feasible technique, the apps are not guaranteed to be implemented correctly. Such solutions have not been scientifically validated or tested for efficacy, where the risk of offering the population nothing but a placebo exists. Similarly, app users may misuse BB stimulation without expert guidance or in a manner not intended by its creators; also, this stimulation may not be appropriate for everyone. Additionally, the protocol of the stimuli application has not been well established, as analyzed in this systematic review section, and there are no existing studies concerning possible side effects.

The design of our experiment was based on the literature findings and it is described in the next section.

4 MATERIALS AND METHODS

4.1 Experimental design

We recruited 30 healthy adults (18 to 33 years old, 9 males) for a placebo-controlled, cross-over, double-blind trial. The Ethics Committee of the Universidade de Uberaba – UNIUBE approved the experimental protocol with the number CAAE 79586917.8.0000.5145.

All volunteers were assessed using the Montreal Cognitive Assessment test (MOCA) – in annex 1 – as inclusion criteria ($M=25$, $SD=2$). The volunteers had no neurological or psychiatric history, such as epilepsy, seizures, attention deficit hyperactivity disorder, or hearing or vision deficiency. They were not taking any medication at the time of the experiment. They also abstained from alcohol and caffeinated beverages 12 hours before the testing sessions.

The audio files were created using the software Audacity (version 1.2.3). For the experimental stim condition, two sine tones of 400 and 440 Hz were played separately and simultaneously in the right and left ear, respectively, to produce 40 Hz BB. The BB were masked with the classical music track *La Stravaganza* by Antonio Vivaldi. The control stim condition consisted of the same classical music without the BB. The audio files were saved in uncompressed .wav format to avoid frequency distortion during the encoding process. An uninvolved person named the recordings to support a double-blind experiment. The auditory stimuli were played through a stereo headset (Sony EH-1000XM3) with noise canceling.

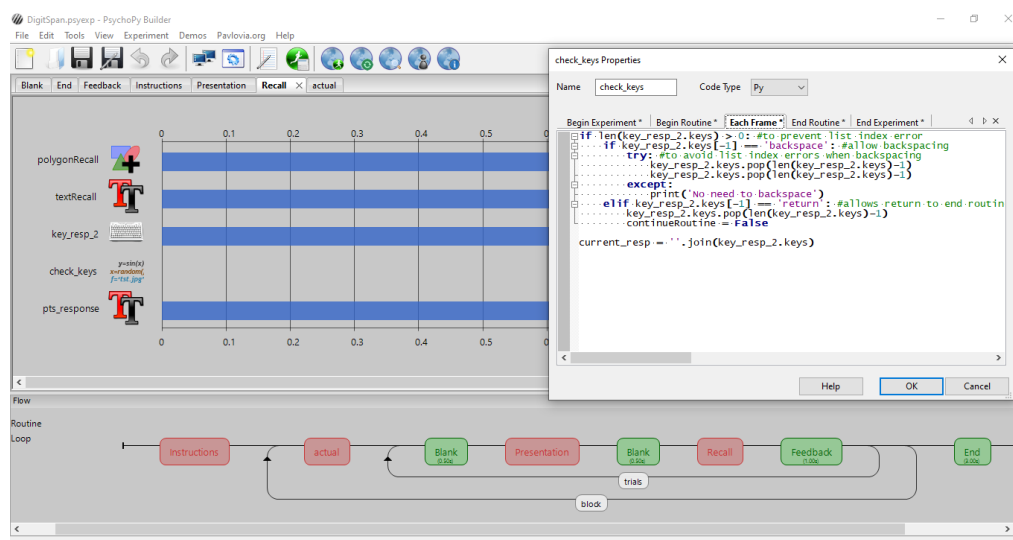
The experiment was divided into 3 phases: without stim, with the experimental stim, and with the control stim. All participants performed the three phases. In each phase, we collected EEG data both in a rest state (with eyes closed) and while performing memory tasks. The volunteers sat facing a computer while we collected their EEG signals throughout the experiment. The experiment took approximately 2 hours, including the setup process.

Without any auditory stim, the first phase began with the EEG baseline collection in a resting state, with eyes closed, for 2 minutes. Subsequently, the participants performed two memory assessment tasks in alternated sequences to avoid any order effect. The two tasks were:

- Digit span task: A commonly used measure of short-term memory [53]. First, a random sequence of numbers appears on the screen. Second, users press the numbers on the keyboard in the same order the numbers were presented. The first tentative starts with one digit (0-9), incrementing by one digit after a correct answer in each trial. The performance is indicated by the highest number of digits correctly remembered. The test ends after two consecutive errors.
- N-back task: Continuous recognition measures present visual stimuli in sequence. The volunteer judges whether the current target matches the one that appeared N item back for each item. This protocol has 3 phases: 1, 2, and 3-back. Each phase consists of 60 trials. We saved the scores for the target (when they should press a keyboard button, confirming the target matches) and non-target (when they should not press a button because the target does not match) across the three phases and total.

The memory tasks were developed in PsychoPy [96], an application for the creation of experiments in behavioral science. It has a Python graphic interface and code environment, illustrated in Figure 4-1.

Figure 4-1. Digit Span task memory screen developed in the PsychoPy environment

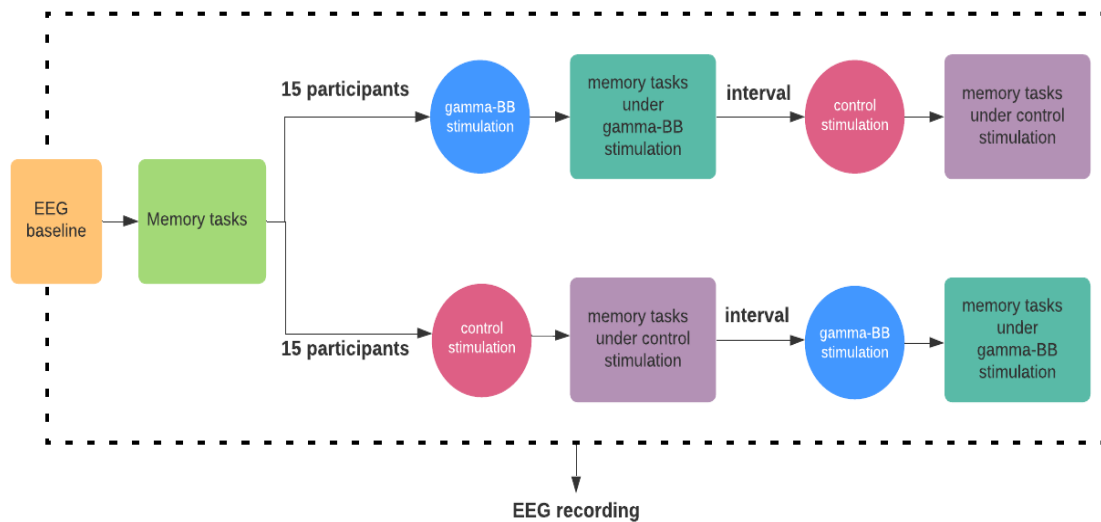


Source: Author

The scripts are available at <https://gitlab.pavlovvia.org/Ludymila/digitspan-randomnumbers> under Memory Task - Digit Span and Word list recall. Project ID: 44706.

In the stim phase (second and third phases), the volunteers were equally and randomly allocated to a group. The first group started with gamma-BB stimuli. They listened to the prepared audio recording for 10 min with their eyes closed, then performed the memory tasks (Digit Span and N-back). After a 5-minute interval, the subjects were presented to the control stim for 10 minutes, with eyes closed, and then performed the same memory tasks. The second group had this sequence inverted. The auditory stimuli continued until the tasks were completed in each phase. The order of the two memory tasks was presented on a rotating basis known as "Latin Squares" to prevent any carryover effects from one task to another. Figure 4-2 illustrates the experimental procedure.

Figure 4-2. Diagram of the experimental protocol



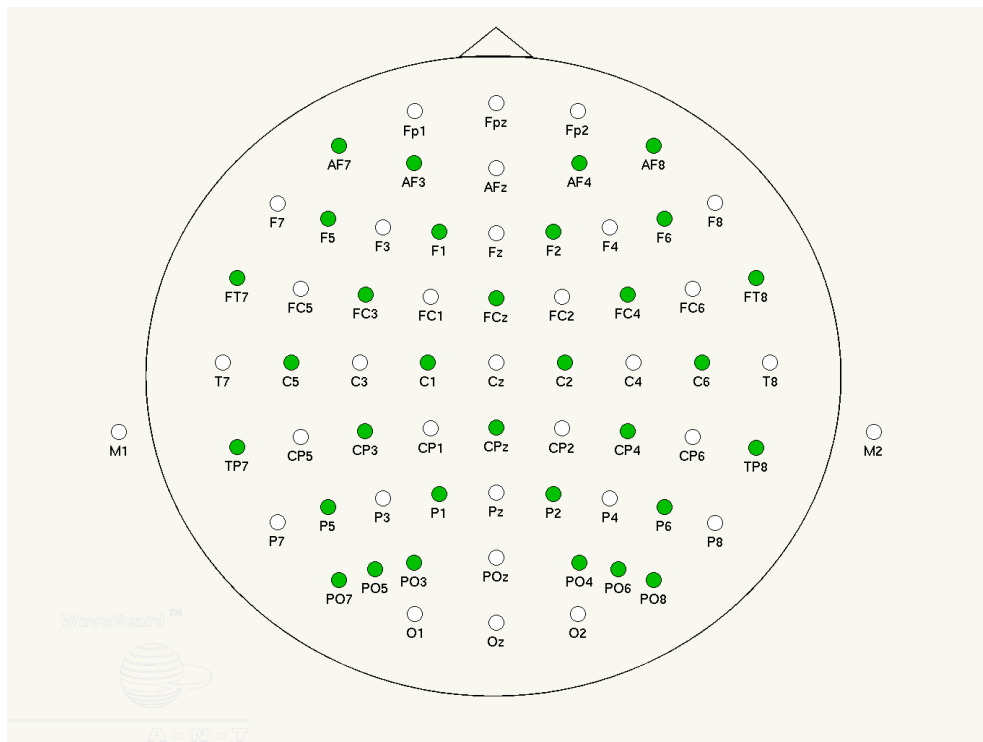
The amplifier device used to record the EEG signals was the EEGO™ rt, Figure 4-3, with a waveguard™ original cap of 64 electrodes in 10/20 configuration, Figure 4-4, from ANT Neuro corporation. We connected the ANT-Neuro device through the OpenViBE platform to acquire the signal at a sampling rate of 1024 Hz. A notch filter of 60 Hz was applied to mitigate external interference. Then, the EEG signal was saved in two extensions: .ov (OpenViBE extension) and .csv (Comma-Separated Values extension) for posterior analysis in Matlab. All 64-electrode-skin impedances were maintained below 10 kΩ during the experiments.

Figure 4-3. ANT-Neuro EEG amplifier device and the waveguard cap



Source: [97].

Figure 4-4. The electrode layout 64 channels cap



Source: [97].

4.2 Pre-processing stage

In the pre-processing stage, we applied an interactive template matching and suppression (ITMS) procedure to detect and suppress blink artifacts [98]. The ITMS approach estimated a waveform of the blink-artifact after ten interactions from the original blink-artifact template resampled at the sampling rate of the EEG device (1024 Hz). Then, the blink-artifact model was suppressed from the raw EEG data. The ITMS algorithm is single-channel interaction-based. We applied the algorithm to the electrodes from the frontal lobe [99], channels: FP1, FPz, FP2, AF7, AF3, AF4, and AF8. Then, the data were filtered using a designed FIR filter, order 400, with cut-off frequencies of 0.1 to 100Hz. Additionally, we proceeded with a visual inspection to confirm that no movement artifacts remained.

4.3 Features extraction - PSD

Power Spectral Density (PSD) measures the power distribution across different frequencies in a signal. PSD is commonly used in signal processing and time series analysis to extract useful information about a signal's frequency content.

We extract the PSD using the sine-multitaper approach described by Barbour and Parker [100]. This recursive method is suited to long time series, in which parameter tuning such as time bandwidth or segment length is not required. We extracted the relative PSD for the delta (0.1 to 4 Hz), theta (4 to 8 Hz), alpha (8 to 12 Hz), beta (12 to 30 Hz), and gamma (30 to 80 Hz) range.

To compare the experimental to the control stim, without and during tasks, the features – PSD and task scores – of the phase without stimulation were subtracted from the stimulation phase (experimental and control). The learning effects that can be caused by the performance of memory tasks repeatedly were decreased by Latin squares rotation and the standardization by the phase without stimulus.

We performed the Principal Component Analysis (PCA) to reduce dimensionality using the algorithm of Singular Value Decomposition (SVD), the default mode of the Matlab function *pca()*. This function extracts data in the directions with the highest variances. We have several matrices 30 x 64 (one matrix for each condition and each frequency band), composed by the PSD values, where 30 was the number of volunteers, and 64 was the number of channels. We reduced to matrices 30 x 1 (the first component representing all channels). Then, we stored the first component of each phase (10 min stimulation and

memory tasks under gamma-BB stim and control stim). We also extracted five channels that most contributed to the first component.

4.4 Features extraction - connectivity

To assess the connectivity of brain activity, we utilized the Phase Locking Value (PLV) as a measure of phase synchronization between pairs of signals. It quantifies the consistency of the phase relationship between two signals over time, with values ranging from 0 (no phase locking) to 1 (perfect phase locking). A high PLV indicates a consistent and synchronized phase relationship between two signals, while a low PLV suggests an inconsistent and unsynchronized phase relationship.

Bandpass filters were designed to extract the frequency bands of interest (delta, theta, alpha, beta, and gamma). The data was obtained from a .mat file containing EEG data. Each EEG channel was filtered separately using the designed filters.

To compute the PLV, we first applied a Hilbert transform to obtain the instantaneous phase of each signal. Resting-state data (without task), including baseline, gamma-BB, and control stim, were windowed into 1-second epochs. We then computed the circular mean of the phase differences between the two signals at each time point, resulting in a single PLV value for each pair of electrodes. Each participant resulted in a functional connectivity matrix for each frequency band. Heatmaps were generated to visualize the connectivity matrices.

4.5 Statistical analysis

We compared the memory task scores under experimental to control stimuli using the non-parametric paired test Wilcoxon, correcting for multiple comparisons and controlling for false discovery rate (FDR). We used the Matlab function *fdr_bh()*.

We performed the non-parametric permutation test (50000 permutations) using the first component of PCA and PSD of all channels. Then, we compared these features for gamma-BB and control stim in a resting state, with eyes closed, and while performing memory tasks; across the delta, theta, alpha, beta, and gamma frequencies range. All the groups had the same size (30 samples).

Subsequently, we detected the outliers using a boxplot with outliers labeled in Jasp software (version 0.16.4.0). We compared the groups again after excluding the outliers from both groups to keep the same size.

For the connectivity analysis, as the matrices did not adhere to a normal distribution, we employed the Friedman test while controlling for multiple comparisons and adjusting for a false discovery rate. Specifically, we performed comparisons between gamma-BB stim and baseline, gamma-BB stim and control stim, as well as control stim and baseline.

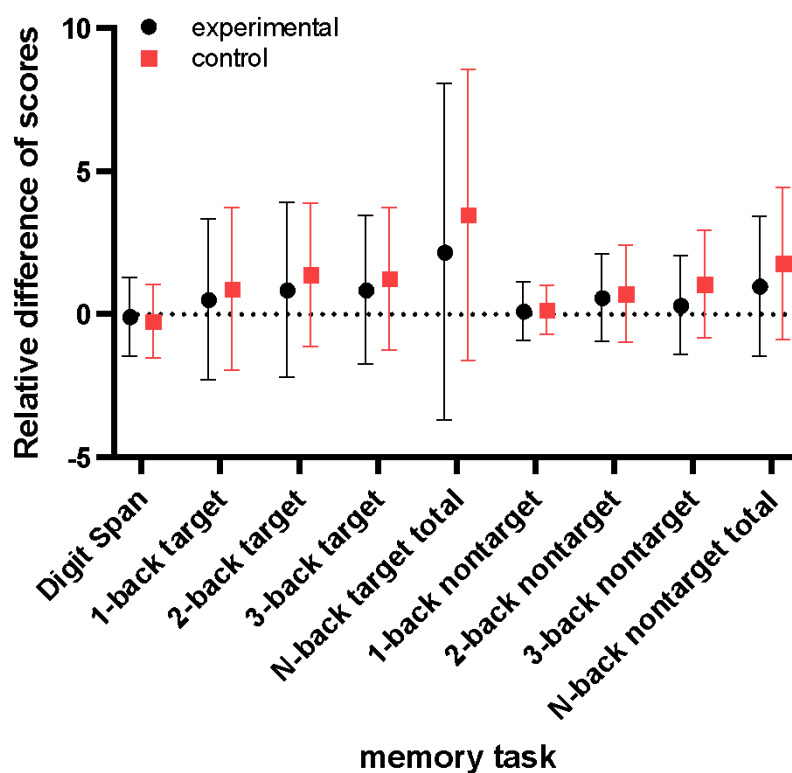
For all analyses, the threshold for significance was $p < 0.05$.

5 RESULTS

5.1 Behavior data

Figure 5-1 shows the results for the memory task scores. The positive values of the y-axis indicates a higher score for the stim phase compared to the without stim, while negative values indicate the opposite. The results were not significant after correction for multiple comparisons and FDR.

Figure 5-1. Relative difference of task score for experimental and control stimulation condition



5.2 Neurophysiology - PSD

Figure 5-2 shows the raw EEG signal and the results after applying the ITMS algorithm and filtering in the pre-processing stage.

Figure 5-2. Results of the pre-processing stage in the signal and its respective PSD

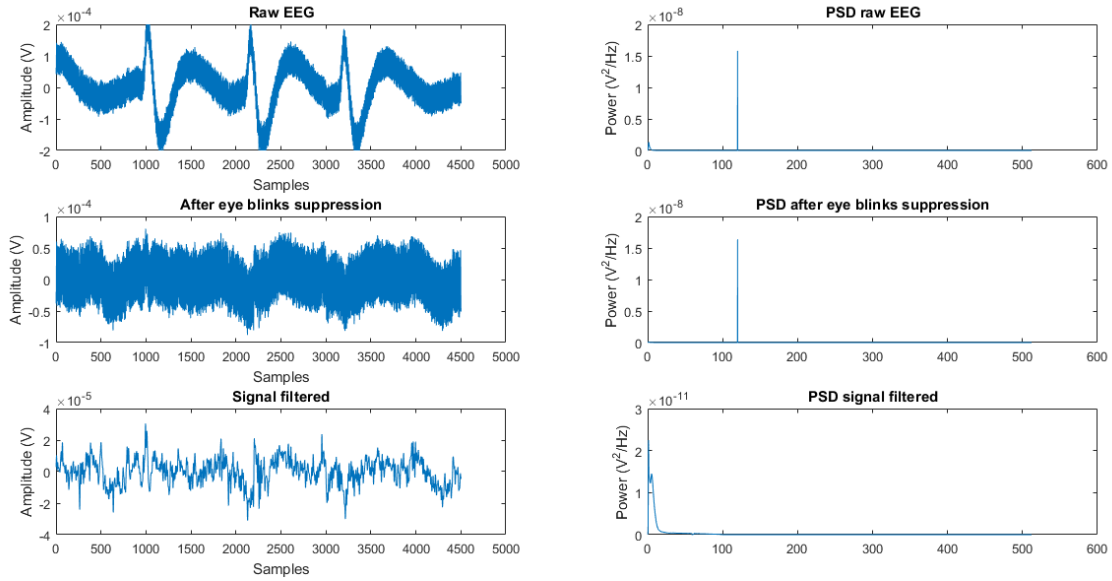


Figure 5-3 shows each frequency band's first component scores per volunteer. Table 5-1, Table 5-2, and Table 5-3 show the five channels that most contributed to the first component, how much this component explains the data, and the p -values for the permutation test using the first component. Channel F2 was the most highlighted across all conditions.

Figure 5-3. Plot of the scores of the first component per volunteer for the stimulation phase in a rest state across the bands (delta, theta, alpha, beta, and gamma)

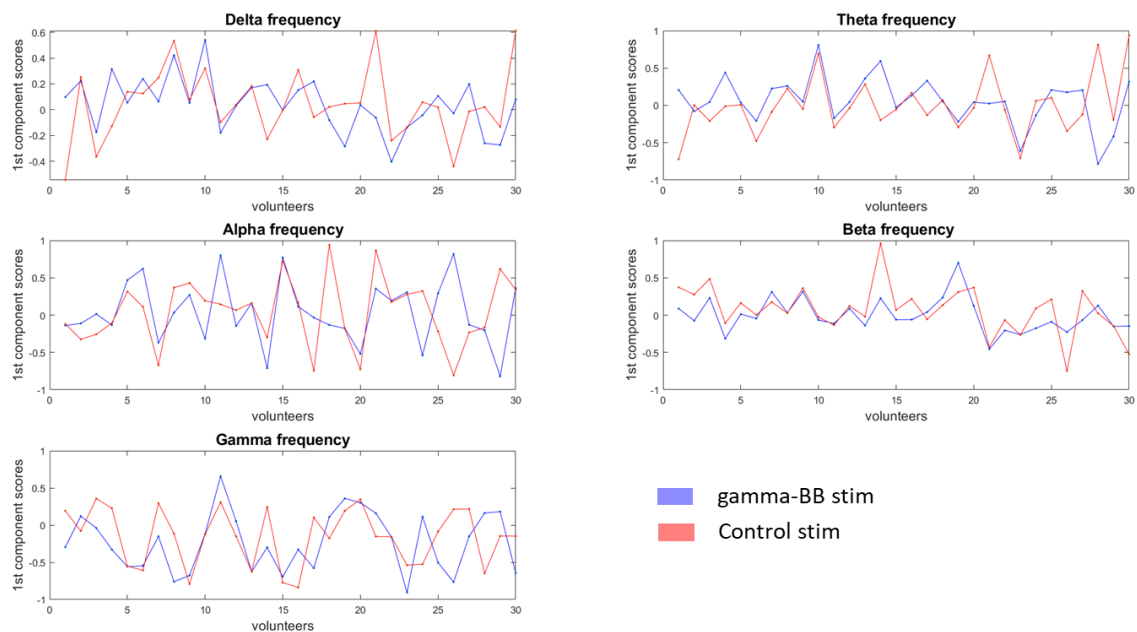


Table 5-1 The five channels contributed most to the PCA first component, total variance explained by the first component, and p-values for permutation test results for experimental and control stimulation in a rest state

Freq.	Gamma-BB stimulation without task	Explained (%)	Control stimulation without task	Explained (%)	p-value
Delta	F2, F3, F1, Fz, F4	61	F2, Fz, F4, F6, F8	58	0.5560
Theta	FC6, FC5, C5, FT7, C6	84	F2, AF7, F7, FCz, Fz	72	0.6317
Alpha	TP8, P2, PO8, Pz, C1	79	P2, Pz, PO8, PO6, PO4	79	0.9798
Beta	Pz, P1, P2, C1, Cz	72	C5, C3, C1, PO7, P1	76	0.2670
Gamma	C5, C6, FC5, C3, FT7	79	C1, CP4, CP3, TP8, Pz	76	0.7234

Table 5-2. The five channels contributed most to the PCA first component, total variance explained by the first component, and p-values for permutation test results for experimental and control stimulation during the digit span task

Freq.	Digit span with Gamma-BB stimulation	Explained (%)	Digit span with Control stimulation	Explained (%)	p-value
Delta	F7, FC5, F1, F3, FC3	52	F2, Fz, F1, F3, F7	70	0.1737
Theta	FC3, F1, FC1, F2, C3	61	AF3, AF8, F2, Fz, AF4	69	0.4415
Alpha	C5, C4, C3, CP3, FT7	56	PO6, PO8, P8, O1, P6	72	0.6782
Beta	AF4, AF3, FCz, F2, FC3	71	F2, FP1, Fz, F1, AF4	74	0.2295
Gamma	C3, FC3, F1, CP2, P2	64	Fz, F2, AF3, F1, FCz	68	0.6192

Table 5-3. The five channels contributed most to the PCA first component, total variance explained by the first component, and p-values for permutation test results for experimental and control stimulation during the n-back task

Freq.	N-back with Gamma-BB stimulation	Explained (%)	N-back with Control stimulation	Explained (%)	p-value
Delta	F2, F7, F8, FCz, F1	83	F2, FCz, F7, Fz, F1	75	0.8736
Theta	FPz, FP1, FCz, AF4, AF3	79	AF4, FCz, AF3, F2, FPz	63	0.9725
Alpha	CP3, P5, P3, CP5, FC6	67	P4, CP3, CP1, P2, CP4	55	0.3467
Beta	F7, F2, FCz, AF8, FC2	73	F2, Fz, FCz, F1, C2	79	0.8759
Gamma	FC2, FCz, F2, C2, Cz	80	FCz, C2, P2, F2, C1	71	0.9506

Figure 5-4, Figure 5-5, and Figure 5-6 show the violin graph of the relative PSD of channel F2, in experimental and control conditions across the delta, theta, alpha, beta, and gamma frequencies and their respective p-value. We plotted only channel F2 for visualization purposes but tested all 64 channels. The positive values of the y-axis mean

higher energy for the stim phase compared to the without stim, while the negative values indicate the opposite.

Figure 5-4. Relative difference of PSD for F2 channel during experimental and control stimulation without a task in a rest state

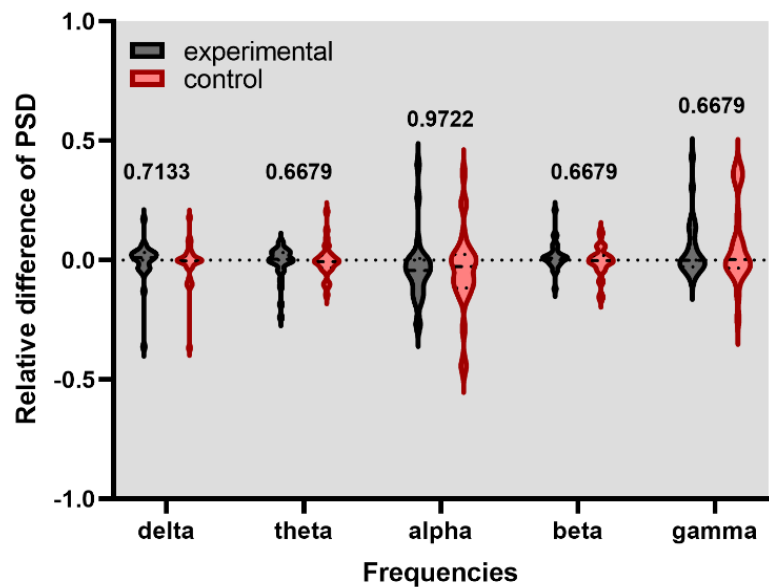


Figure 5-5. Relative difference of PSD for F2 channel during the digit span task

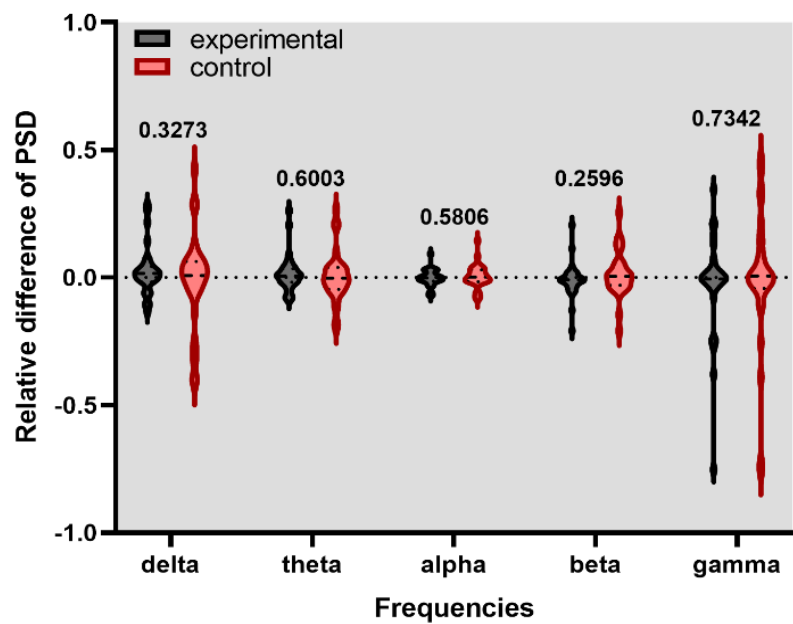
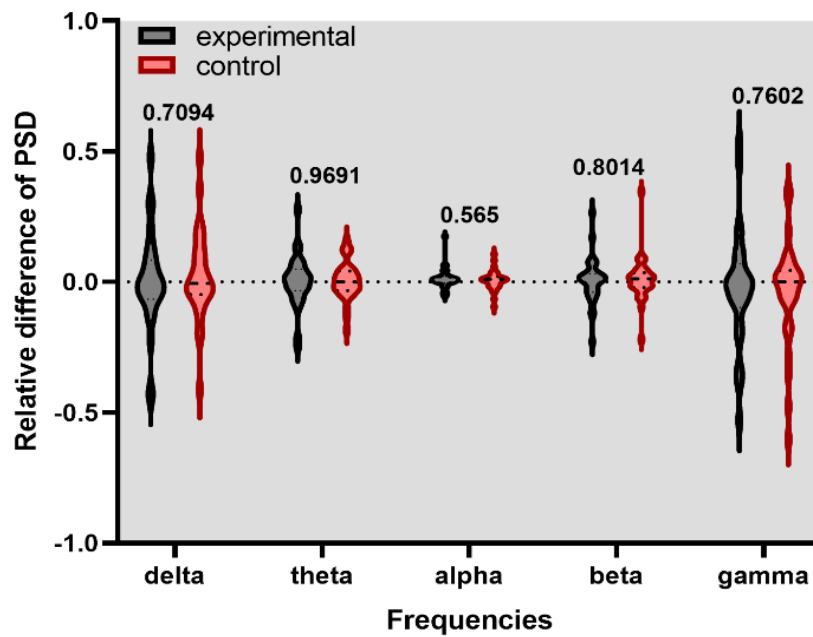
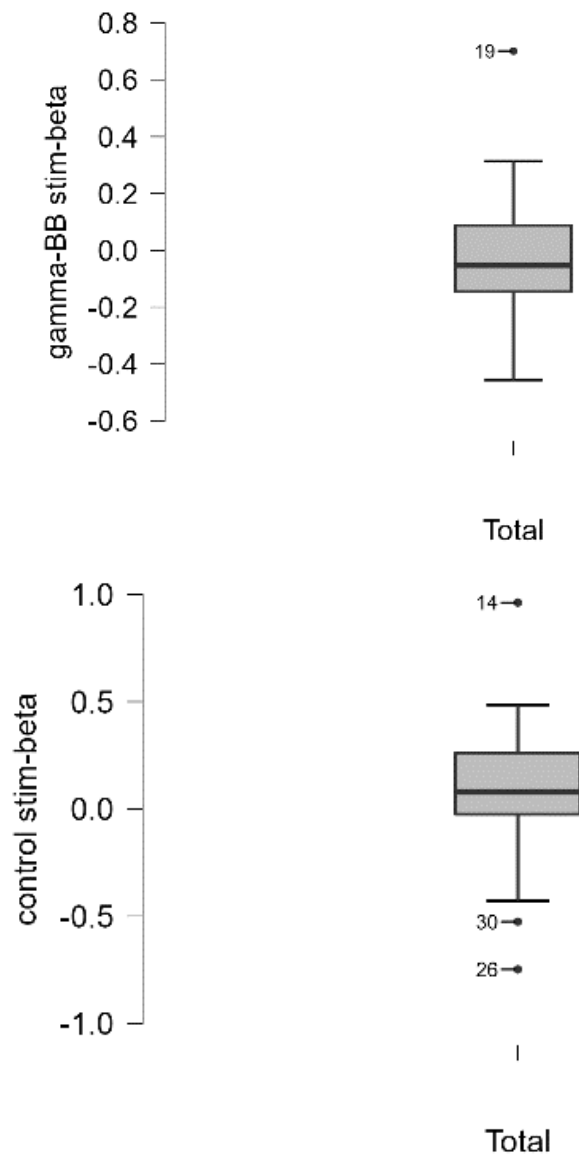


Figure 5-6. Relative difference of PSD for F2 channel during the N-back task



The groups did not present differences considering the first component of PCA and channel by channel, nor after outlier detection and exclusion for any condition. Figure 5-7 exemplifies how we identified the outliers using a boxplot in Jasp software.

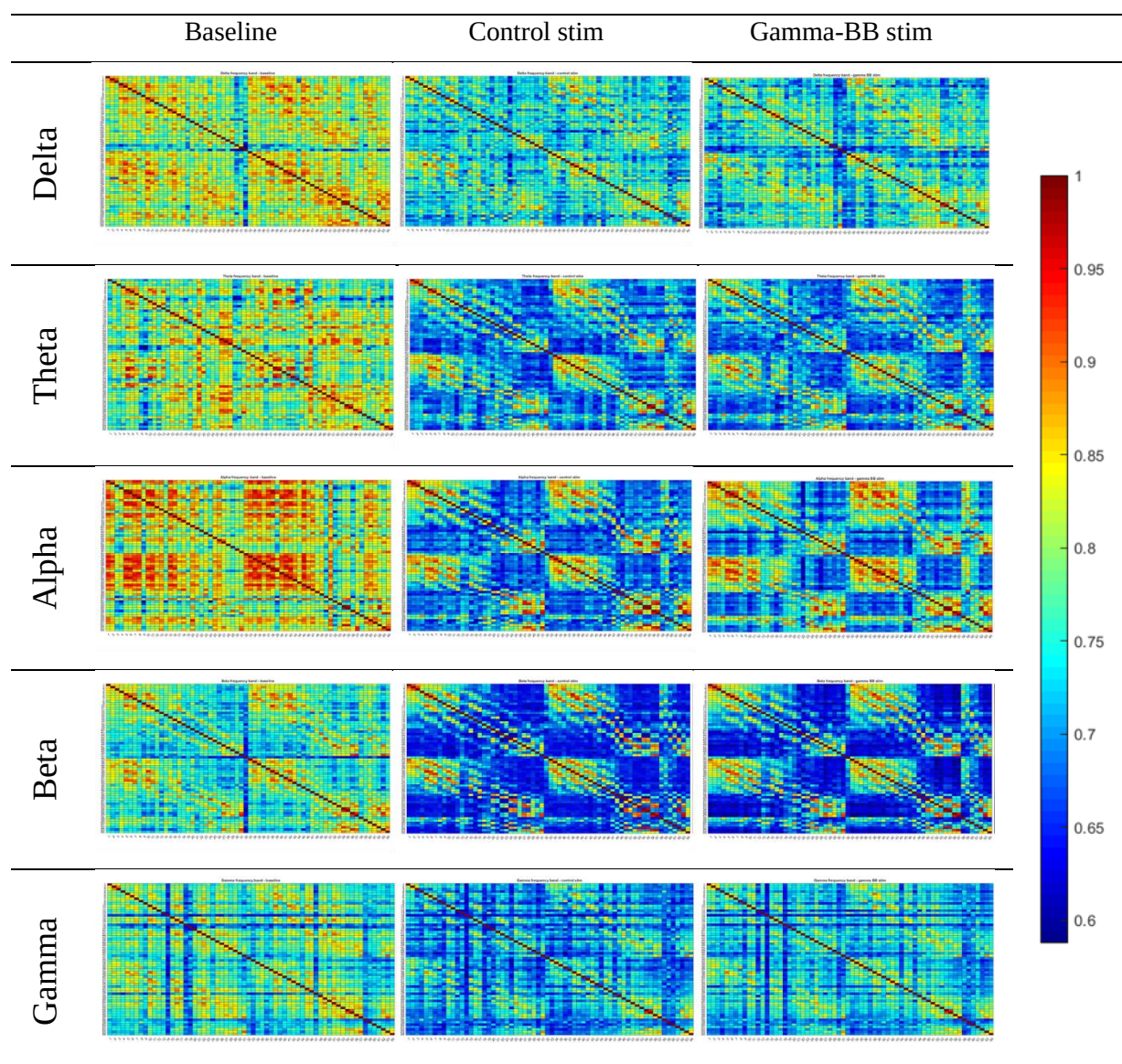
Figure 5-7. Boxplot of 1st component in resting state in beta frequency with outliers labeled for gamma-BB and control stim



5.3 Neurophysiology – Connectivity

Figure 5-8 presents the average connectivity matrices for each frequency band and moment, calculated from the data of 30 volunteers. The matrices are represented by values ranging from 0 (denoting no phase locking) to 1 (indicating perfect phase locking).

Figure 5-8. Average connectivity matrices



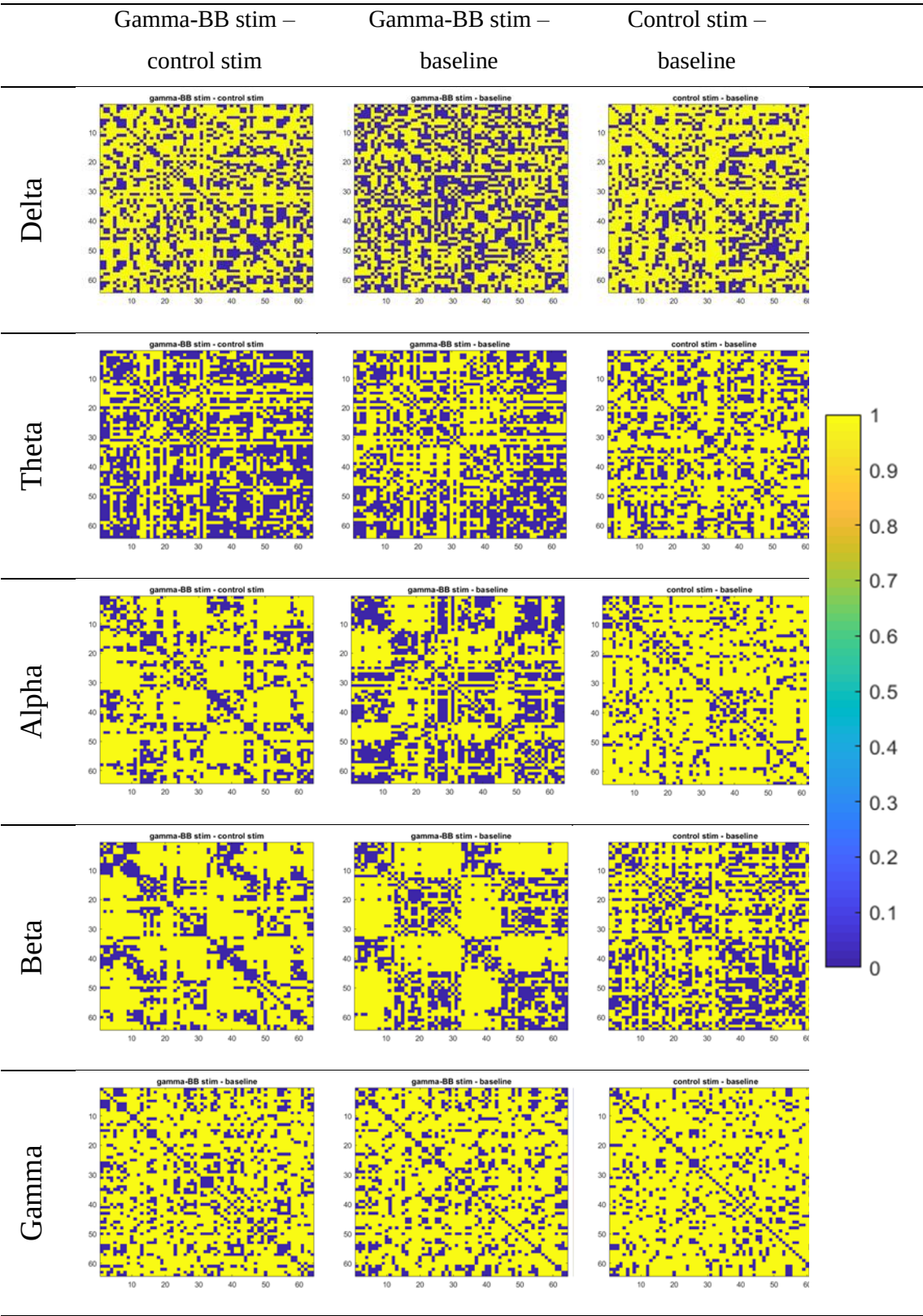
The auditory stimulus highlights differences in connectivity between brain regions, as both gamma-BB stim and control stim exhibit a stronger blue color in the correlation of paired channels compared to the baseline. In other words, silence can enhance connectivity in the whole brain, particularly in the alpha frequency band. These findings suggest that auditory stimuli can optimize connectivity between brain regions, selectively activating specific areas related to memory and/or attention processes.

It is possible to observe a more pronounced correlation in alpha, beta, and gamma frequency bands when comparing gamma-BB stimulation with control stimulation.

The outcomes of the matrices comparisons performed with the Friedman test and FDR correction for each frequency band are presented in Figure 5-9. The resulting values of 1

indicate the presence of statistically significant differences, whereas 0 shows no statistically significant differences.

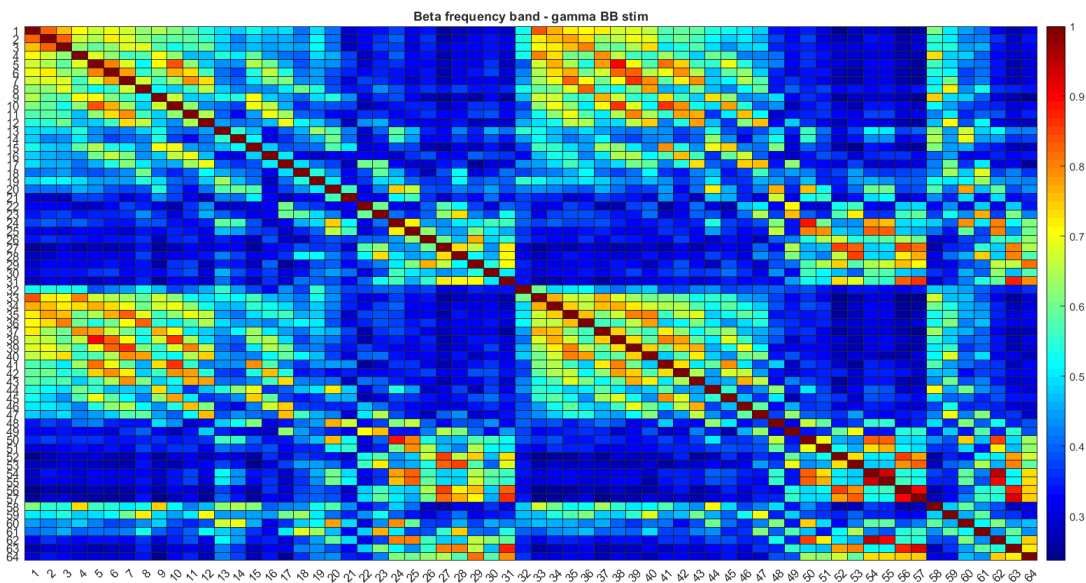
Figure 5-9. Comparison of connectivity matrices



The results reveal a noticeable predominance of yellow color in the alpha, beta, and gamma matrices when compared to the delta and theta matrices. Moreover, it is evident that gamma-binaural beats stimulation results in patterns of connectivity, which are particularly salient in the beta frequency when compared to baseline and distinguishable when compared to the control stimulation.

Upon a detailed examination of the connectivity matrix in the beta band and under gamma-BB stimulation, as presented in Figure 5-10, it is possible to discern which channels, as outlined in annex 2, exhibited perfect synchronicity of PVL and which did not show any phase locking. Specifically, the channels colored in red, yellow, and green (33-43) were identified as AF7, AF3, AF4, AF8, F5, F1, F2, F6, FC3, FCz, and FC4, respectively. The channels numbered 50-57 were P5, P1, P2, P6, PO5, PO3, PO4, and PO6. Conversely, the remaining channels exhibited no phase locking.

Figure 5-10. Connectivity matrix in the beta band and under gamma-BB stim



6 DISCUSSION

BB is a technique that is growing in popularity among neuroscientists. Among the sparse and controversial studies found in the literature, only Mujib et al. [60] investigated gamma-BB stimulation with EEG and working memory performance analysis, similar to our approach. However, our study presents some improvements, such as the presence of a control condition with a potential placebo effect, besides a detailed cross-over experiment with new approaches for signal processing.

6.1 Behavior data

Contrary to what we expected, we found no evidence of any influence of BB on working memory scores. Some studies, such as Khattak [59] and Sharpe et al. [61], found memory enhancement after gamma-BB exposure. The study of Khattak [59] considered a larger sample size ($N=60$). The participants who listened to the gamma frequency performed better on the word-free recall test than on the white noise condition. Sharpe et al. [61] did an exploratory pilot study with 9 participants. The participants performed eight sessions over four weeks of pre- and post-exposure evaluation to a 5-minute binaural beat. They considered 25, 40, and 100 Hz for BB stimulation, and the memory score improved at a greater significance for 40 Hz.

Analogous to the present study's findings, Shekar et al. [65] did not find a statistically significant improvement between listening to the BB frequency of alpha and gamma and a constant tone of 340 Hz on memory task scores. However, they found a significant decrease in auditory and visual reaction time after entrainment with alpha and gamma binaural beats. It suggests that BB can enhance attention, not specifically memory performance.

Engelbregt et al [15] compared white noise (WN), 40 Hz gamma BB and 40 Hz gamma monaural beat (MB). The participants ($N = 24$) performed two tasks under WN, BB, and MB stimulation conditions in a within-subject cross-over design. The authors masked the MB and BB conditions with white noise. They did not find significant results for working memory measured by Klingberg task scores. However, they found reduced reaction times for attention measured by the Flanker task in BB and MB conditions.

Moreover, Jirakittayakorn & Wongsawat [71] found that the average numbers of words recalled by each participant before and after listening to the stimulus were not

significantly different. In their protocol, the word list recall task was conducted before and after listening to the gamma-BB (40 Hz) for 20 minutes. However, after BB stimulation, better scores were found in remembered words at number 8.

Mujib et al. [60] evaluated participants' short-term memory performance through digit span tasks under alpha (10 Hz), beta (14 Hz), and gamma (30 Hz) BB stimulation. They found increases in the cognitive score for the alpha condition, while a significant decrease in reaction time was noted for alpha and gamma.

Even though we did not analyze the reaction time feature, the studies conducted by Engelbregt et al. [15], Shekar et al. [65], and Mujib et al. [60] support the notion that faster attention processing may be attributed to the influence of gamma-BB. However, these studies did not find evidence that gamma-BB significantly influences working memory. Therefore, they further substantiate our findings.

6.2 Neurophysiology PSD data

This study investigated if brain entrainment may occur via gamma-BB stimulation and whether that could improve working memory. The examined data demonstrated that neither the PSD of the 64 channels nor the first main component of PCA significantly changed after listening to either experimental or control stimulation conditions. It implies that gamma-BB stimulation did not result in brain entrainment.

Interpreting the effects induced by BB stimulation is complex since the BB stimuli has heterogeneous methodologies. It is still unclear whether BB stim causes a frequency following the response of the presented frequencies or whether it evokes different responses in the brain. Some studies report brain entrainment with BB stim. Jirakittayakorn and Wongsawat [71] showed that the FFT absolute power of gamma oscillation increased with time, especially in the frontal and central regions of the brain after the participants were exposed to the 40 Hz BB. The greatest induced changes were found within 15 minutes of listening. Similarly, Mujib et al. [60] found significant differences in the group with BB stimulus at 30 Hz. The authors observed an increase in the frontal gamma band power during the stim stage and both theta and gamma power increase in the bilateral frontal and left parietal cortex post-stim state.

Additionally, Corona-Gonzalez et al. [101] found no significant differences between two BB stimulation (theta and beta BB) sessions in brain entrainment. Similarly, in Wahbeh [69], no significant differences existed between the experimental (BB frequency in 7 Hz)

and control conditions in any of the EEG measures. Furthermore, Gao et al. [102] identified no apparent brainwave entrainment effect. However, connectivity changes were detected following relative power variation during BB stim. Their finding confirms the importance of further research into functional brain connectivity under BB stim.

Brain activity during BB stimulation mainly effects the signal phase, since neurons that are sensitive to time delays are mostly found in the brainstem, where their activation power is weaker [13], [15]. As Gao et al. [102] reported, these results imply that BB may influence functional brain connectivity, though perhaps not always via evoking a frequency-following reaction.

It is crucial to research the brain regions responsible for processing working memory and auditory stimulation in order to comprehend the mechanism of BB stimulation and memory enhancement. [103]. Although there were no statistically significant differences between the PSD of the F2 channel, this channel appears to be crucial for memory, especially in theta frequency [104]. Given that the frontal cortex is thought to be a source of top-down modulation of cortical sensory activity during goal-directed behavior, this channel may be crucial for memory. Numerous studies have shown that the medial prefrontal cortex's spectral power increases when the brain is working hard, such as when short-term memory encoding calls for increased focus. Studies applied auditory stimuli in the gamma frequency in humans, producing gamma entrainment in the frontal area [37], [38]. Also, topographical distributions in Scholz et al. [105] suggest the generation of memory-related low beta oscillations in medial and frontal neural structures.

According to the methodology used in this study, the first component of the PCA method, which lowers dimensionality by linearly combining the directions with the highest variance, takes into account a variety of channels and weights. Because of these disparities in the spatial source dependency, there cannot be any discernible findings when comparing the initial components. Therefore, we conducted a paired analysis for all 64 channels, with and without the outliers.

6.3 Neurophysiology connectivity data

The PLV is commonly used in neuroscience to study the synchronization of brain activity between different regions or networks. From our systematic review, the studies of Beauchene et al. [73] [72] extracted PLV through a graphical network analysis and connectivity ratio features, and Mujib et al. [60] extracted imaginary coherence (ICH).

While PLV measures the consistency of the phase relationship between signals over time and is used to identify phase synchronization between different brain regions at different frequencies, imaginary coherence is a measure that considers the nonlinear interactions between signals. It is calculated from the Hilbert transform of the signals and provides a measure of nonlinear coupling between two signals.

In line with our results, some studies have found binaural beats eliciting activity outside of the frequency range of the beat. The study of Perez [42] analyzed PLV and imaginary coherence during MB and BB stim. They found that gamma-BB stim elicits a widespread connectivity pattern at alpha frequency, while theta-BB stim elicits activity at higher frequencies (40 Hz). Also, Gao et al. [102] extracted PLV and Cross-Mutual Information and analyzed the changes in connectivity measures across time. They found differences only in the last minute of 5 minutes of stimulation. Under delta, alpha, and beta BB stim, they found increased connectivity in the theta band. Furthermore, Mujib et al. [60] found an increase of theta and gamma ICH in post-BB stim for alpha and gamma BB stim.

Besides our BB stimulation in gamma frequency, we found prominent differences in connectivity measures in beta frequency. Beta frequencies are often observed in the frontal and parietal lobes of the brain and have been linked to various cognitive processes, including working memory. Research has shown that beta oscillations are significant for working memory [106]. Studies have found that beta oscillations increase during the encoding and maintenance of information in working memory tasks [106]. Moreover, the strength of beta oscillations during these tasks has been shown to correlate with individual differences in working memory performance [73].

Additionally, studies have suggested that beta oscillations may play a role in the active maintenance of information in working memory. Specifically, beta oscillations may help to "refresh" or "reactivate" neural representations of the information held in working memory, allowing us to keep this information active and available for use in ongoing tasks [107].

Our results show high connectivity in frontal and parietal areas under gamma-bb stim in beta frequency. Similar to Beauchene et al. [72], the channels with the highest degree values in each hemisphere correspond to the electrodes over the frontal and parieto-occipital cortices. The frontal area of the brain, specifically the prefrontal cortex and anterior cingulate cortex, is important for memory and attention. The brain's parietal lobe

is also important for memory and attention but primarily plays a different role than the frontal lobes. While the frontal lobes are more involved in executive functions, such as planning and decision-making, the parietal lobes are more involved in sensory processing and spatial awareness.

The parietal cortices are responsible for the initial processing of visual signals [108] and providing feedback for visual attention [109]. In the Digit Span task, the numbers are processed as both visuospatial and verbal. While in N-back task is processed in visuospatial. By examining the strengths of the regional connections, we can gain insight into the brain's overall functional connectivity and performance in working memory tasks. A study by [73] showed that the impact of binaural beats on cortical connectivity was more significant in individuals with higher working memory capacity. This suggests that individual differences in cognitive ability may influence the effects of binaural beats.

6.4 General

Most studies found in the literature did not report whether the BB stim was embedded with a masking stimulus. In contrast, others masked the stimulation with white noise, pink noise, piano music, or natural sounds [15], [66], [68], [69], [85]. Inspired by Lim et al. [66] and Kraus et al. [85], we masked the BB stim with music to amuse the long protocol; it is worth highlighting that it is a common way to find BB stim on the internet. However, unmasked BB stim expressed larger effects than those masked with music [88]. It can be one of the reasons for not achieving brain entrainment through our approach in PSD analysis.

7 CONCLUSIONS

In conclusion, auditory binaural stimulation (BB stim) is a simple, non-invasive technique that can be used for rehabilitation. However, the role of BB stimulation in the brain and the frequency choice for memory enhancement is still unclear. Working memory performance and power spectral density (PSD) features did not significantly change between experimental and control conditions, according to our research. However, connectivity analysis revealed that gamma-BB influences the most important links within the working memory network, which may reflect attention and faster reaction.

While some studies have examined the relationship between auditory binaural stimulation and memory in healthy individuals, much is still unclear, such as the optimal frequency and carrier tones for enhancing memory performance, the duration of stimulation, and the effect of auditory beats on brain entrainment or connectivity. Furthermore, the time course of BB's effects on memory is still unknown.

Our study contributes to the growing literature by presenting a detailed and reproducible method for analyzing the effects of BB in the gamma frequency band on working memory task performance and neurophysiological data. We employed different approaches for signal processing and data analysis and recommend future studies to trigger tasks to analyze different phases of working memory, such as encoding and retrieval, and to design experiments without music masked with carrier tones to amplify the effect.

Our study highlights the need for further research to clarify the mechanisms underlying BB stimulation and its potential for memory enhancement.

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Annex 1 – MOCA (Montreal Cognitive Assessment)

Portuguese version used for this study

MONTREAL COGNITIVE ASSESSMENT (MOCA)		Nome: _____		Data de nascimento: ____/____/____				
Versão Experimental Brasileira		Escolaridade: _____		Data de avaliação: ____/____/____				
		Sexo: _____		Idade: _____				
VISUOESPACIAL / EXECUTIVA		 Copiar o cubo Desenhar um RELÓGIO (onze horas e dez minutos) (3 pontos)		Pontos				
[] []		[] Contorno	[] Números	[] Ponteiros	___/5			
NOMEAÇÃO		 [] [] []		___/3				
MEMÓRIA	Leia a lista de palavras, O sujeito de repeti-la, faça duas tentativas Evocar após 5 minutos		Rosto	Veludo	Igreja	Margarida	Vermelho	Sem Pontuação
	1ª tentativa							
	2ª tentativa							
ATENÇÃO	Leia a sequência de números (1 número por segundo)	O sujeito deve repetir a sequência em ordem direta	[]	2 1 8 5 4				___/2
		O sujeito deve repetir a sequência em ordem indireta	[]	7 4 2				
Leia a série de letras. O sujeito deve bater com a mão (na mesa) cada vez que ouvir a letra "A". Não se atribuem pontos se ≥ 2 erros.		[] FBACMNAAJKLBAFAKDEAAAAMOFAB						___/1
Subtração de 7 começando pelo 100		[] 93	[] 86	[] 79	[] 72	[] 65		___/3
		4 ou 5 subtrações corretas: 3 pontos; 2 ou 3 corretas 2 pontos; 1 correta 1 ponto; 0 correta 0 ponto						
LINGUAGEM	Repetir: Eu somente sei que é João quem será ajudado hoje.	[]	O gato sempre se esconde embaixo do Sofá quando o cachorro está na sala.	[]				___/2
Fluência verbal: dizer o maior número possível de palavras que comecem pela letra F (1 minuto).		[]	_____ (H ≥ 11 palavras)					___/1
ABSTRAÇÃO	Semelhança p. ex. entre banana e laranja = fruta	[]	trem - bicicleta	[]	relógio - régua			___/2
EVOCAÇÃO TARDIA	Deve recordar as palavras SEM PISTAS	Rosto []	Veludo []	Igreja []	Margarida []	Vermelho []	Pontuação apenas para evocação SEM PISTAS	___/5
OPCIONAL	Pista de categoria							
	Pista de múltipla escolha							
ORIENTAÇÃO	[] Dia do mês [] Mês [] Ano [] Dia da semana [] Lugar [] Cidade							___/6

© Z. Nasreddine MD www.mocatest.org
 Versão experimental Brasileira: Ana Luísa Rosas Sarmiento
 Paulo Henrique Ferreira Bertolucci - José Roberto Wajman

TOTAL Adicionar 1 pt se ≤ 12 anos de escolaridade ___/30

(UNIFESP-SP 2007)

Annex 2

Relation between numbers and EEG channels names

1. FP1	23. CP6	45. C1
2. FPz	24. P7	46. C2
3. FP2	25. P3	47. C6
4. F7	26. Pz	48. CP3
5. F3	27. P4	49. CP4
6. Fz	28. P8	50. P5
7. F4	29. Poz	51. P1
8. F8	30. O1	52. P2
9. FC5	31. O2	53. P6
10. FC1	32. EOG	54. PO5
11. FC2	33. AF7	55. PO3
12. FC6	34. AF3	56. PO4
13. M1	35. AF4	57. PO6
14. T7	36. AF8	58. FT7
15. C3	37. F5	59. FT8
16. Cz	38. F1	60. TP7
17. C4	39. F2	61. TP8
18. T8	40. F6	62. PO7
19. M2	41. FC3	63. PO8
20. CP5	42. FCz	64. Oz
21. CP1	43. FC4	
22. CP2	44. C5	