Brain Longevity: Extending cognitive life through integrated Neurofeedback Plus therapy and vagal stimulation. Randomized, controlled and multicenter clinical trial

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Abstract

This randomized, controlled, longitudinal, and multicenter clinical trial investigated the effectiveness of an integrated neurotechnology intervention – Neurofeedback *Plus* – in supporting cognitive longevity in clinically healthy older adults (60–70 years) by optimizing the functioning of the central and autonomic nervous system.

Two hundred participants were randomly assigned to two groups: experimental (n = 100), which received 90 multimodal intervention sessions (EEG neurofeedback, gamma brain photobiomodulation, HRV biofeedback, non-invasive vagal stimulation) conducted in three therapeutic cycles for 12 months; and control group (n = 100), passively monitored. Assessments were performed at four time points (T0, T1, T2, T3), using validated markers: qEEG (global coherence, regional activation), HRV (RMSSD, SDNN, LF/HF), GP-8 (physiological stress) score, and heart-brain coherence biofeedback.

Compared to the control group, the experimental group had statistically and clinically significant changes between T0 and T3: RMSSD (+12.8 vs. +2.1), SDNN (+9.2 vs. +1.5), LF/HF (–1.4 vs. –0.2), GP-8 score (-17.6 vs. – 2.8), EEG coherence (+15.2% vs. +3.1%).

The interaction effect (Time x Group) was confirmed by the two-way ANOVA ($p<0.01$) Effect size (Cohen's $d > 1.6$) and multiple regression analyses indicated belonging to the experimental group as the strongest predictor. Post-hoc statistical power was 1.0 in all cases.

The intervention of Neurofeedback Plus produces robust, multisystemic, and lasting effects on brain activity, autonomic regulation, and physiological stress, supporting the strengthening of neuroplasticity and cognitive resilience. The protocol is standardizable, clinically applicable and scalable in the context of public health, providing an innovative preventive solution for maintaining cognitive performance in old age.

Neurofeedback, HRV, EEG, vagal stimulation, photobiomodulation, neuroplasticity, cognitive longevity, active prevention, healthy seniors.

1. Introduction

1.1 Cognitive longevity in the context of healthy ageing

Aging is a universal, multidimensional and irreversible biological process, characterized by a series of structural, functional and molecular changes that gradually affect the homeostasis of the human body. At the brain level, aging involves a progressive degradation of neural networks, a reduction in neuroplasticity and an increased vulnerability to systemic stressors, which in time causes a decrease in cognitive performance and a decrease in the ability to adapt to environmental challenges (López-Otín et al., 2013; Fjell & Walhovd, 2010). These transformations not only affect memory and the speed of information processing, but directly influence essential functions such as attention, emotional regulation, executive control and decision-making capacity, all critical components for an independent and active life in old age.

In this context, literature has increasingly focused on the concept of *cognitive longevity*, being defined as the ability of the individual to maintain superior cognitive performance and neurophysiological integrity despite aging, thus avoiding the onset of neurocognitive decline and neurodegenerative diseases (Deary et al., 2009; Nyberg et al., 2012). Cognitive longevity not only implies the absence of dementia or other neurological conditions, but reflects a set of active mechanisms of adaptation, compensation, and regeneration, supported by a fine balance between the central nervous system and the autonomic, endocrine, and immune systems (Cabeza et al., 2018; Goh & Park, 2009).

The emergence of this concept marks a paradigm shift in applied neuroscience: from an approach centered on the treatment of already established cognitive pathologies, to a proactive orientation based on early interventions aimed at maintaining and even optimizing brain functioning in the absence of obvious clinical symptoms. Thus, cognitive longevity becomes not only a theoretical ideal, but a strategic direction in contemporary preventive medicine, especially in the context of accelerated aging of the global population and increased incidence of neurodegenerative disorders (Livingston et al., 2020).

From a neurophysiological perspective, cognitive longevity involves maintaining coherent brain activity, effective functional connectivity in major cortical networks, and adaptive capacity at the synaptic and network level. Recent studies support that brain networks involved in maintaining cognitive performance – such as Default Mode Network (DMN), Salience Network (SN), and Executive Control Network (ECN) – show functional decline with age, but can be stimulated and recalibrated through targeted interventions, supported by principles of experience-dependent neuroplasticity (Park & Reuter-Lorenz, 2009; Andrews- Hanna et al., 2007). These networks do not work in isolation, but in close correlation with autonomic regulation mechanisms, especially through the interaction of the prefrontal cortex with the vagal nervous system, which causes a direct influence on physiological stress, emotional regulation, and subjective well-being (Thayer & Lane, 2007; McEwen, 2012).

In this regard, cognitive longevity cannot be separated from the *homeostasis of the autonomic nervous system* and the body's ability to maintain a balance between sympathetic and parasympathetic activation, reflected in parameters such as heart rate variability (HRV) or heart-brain coherence. Low HRV and reduced

vagal activation are clear indicators of a compromised physiological adaptation and are correlated with an increased risk of cognitive decline, cardiovascular conditions, and affective disorders (Shaffer & Ginsberg, 2017; Lehrer & Gevirtz, 2014). Conversely, an effective activation of the parasympathetic branch-particularly through the vagus nerve-is associated with biological resilience, effective emotional regulation, and increased neural plasticity, all of which help maintain optimal cognitive functioning in old age (Breit et al., 2018; Yuan & Silberstein, 2016).

Therefore, preventive interventions aimed at supporting cognitive longevity must target not only the brain as an isolated entity, but also the dynamic and reciprocal relationship between the prefrontal cortex, the limbic system, functional neural networks and the autonomic nervous system. This holistic perspective is supported by literature that clearly indicates that healthy aging depends on the integrity of interconnected systems, capable of responding adaptively to the demands of the internal and external environment (Cabeza et al., 2018; Seidler et al., 2010).

Despite this evidence, most clinical trials in the field of healthy ageing still focus on pharmacological interventions, the management of already diagnosed diseases, or isolated cognitive training, without integrating the neurophysiological and autonomous dimension of cognitive longevity. Moreover, very little research has explored the effects of multimodal interventions, simultaneously applied, combining the stimulation of neural networks with the regulation of the autonomic nervous system, in a preventive, standardized and replicable format. This scientific gap justifies the acute need for this study.

Thus, this clinical study aims to investigate, in a rigorous and extensive manner, the impact of an integrated neurotechnological intervention – combining personalized EEG neurofeedback, gamma brain photobiomodulation (40 Hz), non-invasive vagal stimulation and heart-brain coherence exercises – on neurophysiological, autonomic and cognitive markers associated with brain longevity. The choice of these methods is not accidental, but is grounded on a solid base of research that demonstrates the effectiveness of each technique and suggests synergistic effects when applied simultaneously (Gruzelier, 2014; Ros et al., 2014; Hamblin, 2016).

Therefore, the study responds to a stringent scientific and clinical need: the validation of a coherent, non-invasive, integrable preventive model in clinical practice that actually supports the maintenance and extension of cognitive life among active seniors. At a time when public health systems are facing unprecedented demographic challenges, the development and standardization of such preventive interventions can be an essential step in redefining gerontological care and supporting active, lucid and dignified old age.

1.2 Involved Network Areas (ARI) and their relevance for cognitive longevity: DMN, SN and ECN

In the last decade, with the advancement of functional brain imaging techniques and the analysis of cortical neurodynamics, neuroscientific research has moved from analyzing isolated brain areas to understanding the brain as a complex system, organized into interconnected functional networks, generically referred to as "*Involved Network*Areas" (ARI).

These are not simple anatomical regions, but dynamic systems that coordinate cognitive, emotional and

autonomous processes essential for the optimal functioning of the body, adapting to stress and maintaining internal balance (Menon, 2011; Raichle, 2015).

Functional and electrophysiological studies converge on the existence of three major cortical networks involved in cognitive longevity, whose progressive decline is associated with accelerated aging, executive dysfunction, and the emergence of neurodegenerative diseases: the Default Mode Network (DMN), the Salience Network (SN), and the Executive Control Network (ECN). Their selection in our study is not random, but is based on solid evidence of their central role in supporting higher cognitive functions, emotional regulation, stress processing, and maintaining the integrity of the autonomic nervous system.

The Default Mode Network (DMN) is one of the most well-documented cortical networks, active predominantly in states of rest and introspection, and involved in processes such as autobiographical memory, self-reflection, mental projection into the future, and identity integration.

Key structures such as posterior cingulate cortex (BA31), medial prefrontal cortex (BA10) and inferior parietal cortex (BA40) are part of this network. In the context of aging, altered DMN functionality has been consistently associated with early cognitive decline and risk of developing dementia, especially in Alzheimer's forms (Greicius et al., 2004; Andrews-Hanna et al., 2007). Moreover, the decrease in coherence and desynchronization of the DMN network have been proposed as neurofunctional biomarkers of pathological aging (Damoiseaux et al., 2008), which justifies the need for preventive interventions designed to reactivate and stabilize this critical network.

The Salience Network (SN) is a functional network responsible for the detection and evaluation of relevant environmental signals, the coordination of responses to emotional or stressful stimuli, and the adaptive transition between states of rest and intense cognitive activation. The network includes regions such as anterior insula (BA13), anterior cingulate cortex (BA24) and amygdala, playing a fundamental role in integrating interoceptive and exteroceptive information (Seeley et al., 2007). In the aging process, SN becomes more rigid and less effective in detecting and prioritizing stimuli, which contributes to deficits in emotional adaptation and alteration of the physiological response to stress (Uddin, 2015). Moreover, the activity of the SN network is closely related to the functioning of the vagus nerve and the sympathetic-parasympathetic balance, being one of the main cortical bridges between cognition and autonomous regulation.

Therefore, optimizing the functionality of this network is essential for maintaining effective self-regulation and functional emotional adaptation in old age.

The Executive Control Network (ECN), also known as the fronto-parietal network, is the fundamental network involved in executive functions: planning, attentional control, decision making, behavioral inhibition, and working memory. EA includes the BA9, BA10 and BA46 regions of the dorsolateral prefrontal cortex and upper parietal areas. ECN is considered to be the network that ensures adaptability in the face of cognitive and social challenges, and its dysfunction is strongly correlated with decreased functional independence in seniors and the inability to maintain complex daily activities (Cole & Schneider, 2007; Bressler & Menon, 2010). At the same time, ECN is the network most sensitive to the positive influences of cognitive training and neurofeedback

interventions, making it an ideal target for prevention and cognitive optimization strategies (Ros et al., 2014; Enriquez-Geppert et al., 2017).

All three networks – DMN, SN and ECN – are not only anatomically interconnected, but also functionally correlated. For example, SN plays the role of a switch between DMN (internal rest and reflection network) and ECN (cognitive activation network), and any dysfunction in SN can lead to an inefficient transition between rest and action states, thus affecting overall performance (Menon & Uddin, 2010). This interdependence justifies choosing a multimodal intervention in the present study, which acts simultaneously on these networks, stimulating the synchronization, plasticity and coherence of cortical activity in the key areas involved in maintaining cognitive longevity.

The importance of integrating these ARIs into our clinical trial is twofold. First, it provides a clear and documented direction for neurophysiological evaluation by qEEG - each network can be quantified by specific parameters of coherence and spectral activity in the corresponding Brodmann areas. Secondly, it allows testing the effectiveness of the Neurofeedback Plus intervention specifically and targeted, assessing the impact on networks whose functionality is directly correlated with cognitive performance and overall health.

In conclusion, the inclusion of ARI – DMN, SN and ECN – as a central objective of the study is not only a theoretical element, but constitutes a robust neuroscientific foundation that allows the real and complex assessment of the effect of the intervention on profound, integrative and essential mechanisms for the cognitive health of seniors. With this approach, the study aims to help redefine cognitive longevity not as a side effect of overall health, but as a direct result of strategic intervention on the most critical networks of the mature human brain.

1.3 Neuroscientific justification for targeting Brodmann areas 10, 24, 32, 40 and 46 in the intervention and assessment of cognitive longevity

The selection of Brodmann (BA) areas for neurophysiological intervention and evaluation in our study is not arbitrary, but is based on a solid convergence of neuroscientific evidence indicating an essential role of these cortical regions in maintaining higher cognitive functions, adaptive emotional regulation, and effective interconnectivity of brain networks involved in cognitive longevity. In particular, the areas BA10, BA24, BA32, BA40 and BA46 are considered nodal points in DMN, SN and ECN networks, networks already previously argued to be critical in the healthy aging process (Menon, 2011; Raichle, 2015).

BA10 – Anterior prefrontal cortex (frontal-polar)

The Brodmann area 10, located in the anterior portion of the frontal lobe, is one of the most evolved regions of the human brain and is involved in processes of *metacognition*, *long-term planning*, *integration of abstract information*, *moral judgment*, and *reflection on the self* (Ramnani & Owen, 2004). Activity in BA10 is essential for the ability to integrate multiple stimuli into strategic decisions and to maintain identity coherence in the face of complex environmental challenges. Longitudinal studies indicate that BA10 undergoes an early decline in connectivity in the aging process and is one of the regions most vulnerable to hypoactivation in the early stages of Alzheimer's disease (Zhou et al., 2010; Andrews- Hanna et al., 2007). For this reason, functional

stimulation and increased coherence in BA10 is a priority target in cognitive prevention interventions in seniors.

BA24 – Anterior cingulate cortex (rostral anterior cingulate cortex, rACC)

The BA24 area is located in the rostral portion of the cingulate gyrus and is a central component of *the Salience Network* (SN). It is involved in *emotional processing*, *internal conflict monitoring*, *stress response regulation* , and *sympathetic-parasympathetic balance modulation* (Bush et al., 2000; Etkin et al., 2011). BA24 functions as a hub between the prefrontal cortex and the subcortical limbic systems (hippocampus, amygdala), facilitating the adjustment of behavior according to the emotional and physiological context. Decreased activity or rigid hyperactivation in BA24 has been associated with affective disorders, burnout, chronic anxiety, and increased stress reactivity (Lane et al., 2009). In interventions based on EEG neurofeedback and vagal stimulation, activity normalization in this area is considered a direct indicator of optimization of autonomic regulation and emotional resilience (Ros et al., 2014; Thayer & Lane, 2007).

BA32 – Anterior dorsal cingulate cortex (anterior dorsal cingulate cortex, dACC)

BA32 is often treated together with BA24 within anterior cingulate regions but has a distinct function, being involved in *sustained attention*, *error detection*, *decision making under uncertainty* , and *cognitive reorientation* (Posner et al., 2007). It is considered a bridge region between executive (ECN) and affective networks, contributing to the *integration of motivation with cognitive control* (Petersen & Posner, 2012). Deterioration in BA32 functionality is commonly seen in cognitive aging and is associated with decreased performance in tasks that require constant monitoring and adaptive self-regulation. Therefore, stimulation of this area has a high potential in supporting active cognitive longevity.

BA40 – Lower parietal lobe (gyrus supramarginalis)

BA40 is located in the posterior portion of the parietal lobe and is involved in *multisensory integration*, *spatial attention*, *working memory*, and *language processing* (Husain & Nachev, 2007). This region also plays a central role in the *Default Mode Network*, contributing to mental orientation in time and space and to self-referentiality. In aging, functional coherence in BA40 decreases significantly, which affects the simultaneous processing of information and the ability to multitask, essential for maintaining functional independence (Fjell & Walhovd, 2010; Grady, 2012). Therefore, training this area through neurofeedback and restoring network coherence directly contributes to preventing cognitive disorganization and functional vulnerability.

BA46 – Dorsolateral prefrontal cortex (Dorsolateral prefrontal cortex, DLPFC)

BA46 is one of the most studied brain regions in terms of *executive functions*, *working memory*, *logical thinking*, *decision making*, and *attentional control* (Miller & Cohen, 2001). Activity in BA46 is considered a reliable predictor of overall cognitive performance and is often used as a biomarker in neuroplasticity studies and EEG-based neurofeedback interventions (Enriquez-Geppert et al., 2017; Arns et al., 2009). In normal aging, decreased activity or functional coherence in BA46 is associated with deficits in mental planning, inhibition, and strategy, while maintaining stable activation in this area positively correlates with success in cognitive adaptation in old age. For this reason, BA46 is a priority target in cognitive optimization interventions applied to seniors.

Synergy of BA10, BA24, BA32, BA40 and BA46 areas

These five areas do not act in isolation, but together form an integrative functional network, in which cognitive, emotional and physiological processes intersect. For example, BA10 and BA46 support executive control and planning; BA24 and BA32 modulate stress and emotional conflict; and BA40 provide the necessary sensory and guidance foundation. Together, they make up a cortical architecture that supports not only cognitive survival, but creative adaptation, decision-making efficiency and internal balance – all essential components for an active and lucid old age.

The justification for integrating these areas into the design of the intervention is based on the fact that each method applied – whether neurofeedback, photobiomodulation or vagal stimulation – exerts its effects demonstrably on one or more of these regions, and the qEEG evaluation of their coherence and activity allows objective quantification of neurophysiological and functional progress.

In conclusion, the targeted choice of Brodmann BA10, BA24, BA32, BA40 and BA46 areas reflects a rigorous scientific strategy and methodological alignment with the current international literature on neurocognitive prevention. This selection provides not only a solid basis for the intervention, but also an accurate measurement system of its effectiveness, allowing statistical and clinical validation of the impact of the integrated Neurofeedback Plus therapy on cognitive longevity.

1.4 Justification of integrated Neurofeedback Plus intervention and vagal stimulation on functional networks involved in cognitive longevity

In order to effectively intervene on the cortical networks involved in maintaining cognitive longevity – Default Mode Network (DMN), Salience Network (SN) and Executive Control Network (ECN) – a complex, synergistic and multimodal therapeutic approach is required. The theoretical and practical justification for the integrated Neurofeedback Plus intervention lies in its ability to act simultaneously and complementarily on neurophysiological and autonomic parameters, activating adaptive neuroplasticity and supporting the coherent reorganization of brain areas involved in cognition, emotional regulation and autonomous balance.

Personalized EEG neurofeedback, guided by qEEG quantitative analysis, is an advanced form of brain autoregulation that monitors the electrical activity of the brain in real time, providing auditory and visual feedback to learn the autoregulation of neural activity. This type of intervention is based on the principle of experience-dependent neuroplasticity, considered essential in the functional remodeling of brain architecture (Hammond, 2011; Marzbani et al., 2016). In our study, the intervention was calibrated to specifically target areas BA10, BA24, BA32, BA40, and BA46, with the aim of optimizing activity in the Alpha and SMR bands-recognized for their role in cognitive efficiency-and reducing increased activity in the Beta and High-Beta frequencies associated with cognitive stress and stiffness (Ros et al., 2014).

Current literature emphasizes the effectiveness of neurofeedback in increasing functional coherence in DMN and ECN networks, improving executive control and working memory by activating BA46 (Enriquez-Geppert et al., 2017), as well as in emotional regulation by normalizing activity in BA24 and BA32 (Gruzelier, 2014). Therefore, EEG neurofeedback directly contributes to the functional optimization of networks involved in

cognitive longevity, providing a personalized and non-invasive pathway for adaptive brain reorganization.

Brain photobiomodulation (PBM) brings a deep metabolic component to this intervention, using low-intensity pulsed light – especially in the near-infrared spectrum – applied transcranially to stimulate ATP synthesis, neuroprotective gene expression, and cerebral blood flow (Hamblin, 2016; Rojas & Gonzalez-Lima, 2013). In our protocol, two frequencies are used for complementary purposes. Gamma frequency, at 40 Hz, has direct effects on neuronal synchronization in DMN and ECN networks, contributing to beta-amyloid clearance and reduction of neurogenic inflammation (Iaccarino et al., 2016; Chan et al., 2021). In parallel, the alpha frequency, at 10 Hz, induces a state of deep neurophysiological relaxation, facilitating the transition to high energy efficiency brain regimes and supporting the recalibration of the SN network, especially the connection between the prefrontal cortex and the island (Barrett & Gonzalez-Lima, 2013).

Transcranial application to the upper frontal and parietal regions, such as BA10, BA40, and BA46, supports the recalibration of neural rhythms disrupted by brain aging and has demonstrated beneficial effects on working memory, attentional control, and multisensory integration (Fischer et al., 2016). The association with neurofeedback contributes to the reinforcement of EEG-induced plasticity and supports the stabilization of functional reconfiguration.

The autonomous component of the intervention is covered by heart-brain coherence training, a method based on heart rate variability (HRV) feedback, which aligns the rhythms of the cardiovascular system with those of the central nervous system. By monitoring and training parameters such as RMSSD and LF/HF ratio, this technique trains the prefrontal cortex and subcortical structures to respond flexibly to internal and external stimuli (McCraty & Shaffer, 2015; Lehrer & Gevirtz, 2014). In our intervention, coherence exercises are integrated with neurofeedback and PBM sessions, amplifying the effects of emotional regulation and autonomous balancing.

Studies show that high heart coherence is correlated with the activation of BA24 and BA32 areas, involved in emotional regulation, behavioral adaptation and conflict integration (Thayer et al., 2012). In addition, rhythmic controlled breathing exercises stimulate vagal activation and parasympathetic dominance, favoring the recalibration of the autonomic nervous system and facilitating the transition from states of sympathetic hyperactivation to states of adaptive calm, through island involvement (BA13).

Transcutaneous vagal stimulation (nVNS), performed by medical devices applied to the cervical or auricular area, brings a direct neurophysiological dimension to the vegetative balance. By activating the vagus nerve inputs, this technique causes a number of central effects on the nervous system, reducing inflammation, modulating stress, and supporting internal homeostasis (Yuan & Silberstein, 2016; Breit et al., 2018). In terms of functional networks involved in cognitive longevity, vagal activation results in increased coherence between BA10 and BA24/32, positively influences the salience network by improving the detection of relevant signals and emotional regulation, and normalizes HRV, providing increased physiological flexibility.

Moreover, recent research has demonstrated that vagal stimulation can stimulate neurogenesis in the hippocampus and reduce brain inflammatory markers, essential processes for preventing age-related cognitive

decline (Clancy et al., 2014; Hays et al., 2013).

By combining these methods – personalized EEG neurofeedback, gamma and alpha light photobiomodulation of the brain, heart-brain coherence training and non-invasive vagal stimulation – the proposed intervention manages to act simultaneously and convergently on the three fundamental dimensions involved in maintaining cognitive functions: neuronal activity, emotional balance and autonomic regulation. It is not a simple juxtaposition of techniques, but a deep functional integration aimed at restoring the coherent and adaptive network architecture of the active mature brain. The protocol thus developed not only optimizes the existing functional parameters, but validates a scalable therapeutic strategy, applicable in varied clinical contexts, with major relevance in cognitive prevention interventions for active adult and elderly populations in urban areas.

2. Objectives

2.1 General objective

The overall goal of this clinical trial is to assess, in a rigorous, objective and reproducible manner, the effectiveness of an integrated neurotechnological intervention – Neurofeedback Plus – on the process of maintaining and extending cognitive longevity in a clinically healthy elderly adult population in the critical age range of 60–70 years. The evaluation of the efficiency of the intervention is carried out by monitoring the changes in the specific neurophysiological markers, by analyzing the functional coherence between the brain and the autonomous system (heart-brain coherence), as well as by quantifying the level of perceived physiological stress. These three parameters are considered integrated indicators of cognitive, emotional and autonomous functioning in the context of healthy ageing.

The choice of this overall objective derives from an urgent conceptual and clinical need: there are currently no validated protocols that simultaneously address all the essential components of cognitive longevity. The available interventions are mostly one-dimensional and reactive – they focus on either cognition (through cognitive exercises), affective mood (through psychotherapy), or physiology (through relaxation or guided breathing) – but very few propose an integrated, coherent and applicable approach in the pre-symptomatic stage of neurological decline. The present study aims to validate precisely this multidimensional approach, in which the stimulation of cognitive function, autonomous regulation and reduction of physiological stress are integrated into a neurotechnological protocol applied before the appearance of clinical signs of deterioration.

To measure the effectiveness of the intervention, three fundamental markers are used, each with direct relevance to a distinct neurofunctional dimension. The first marker is the neurophysiological parameters measured by quantitative electroencephalography (qEEG), including neural coherence, spectral power over specific frequency bands, network flexibility, and cortical activity in selected Brodmann areas (especially BA10, BA24, BA32, BA40, and BA46). These parameters allow the evaluation of the functioning of the central brain networks for cognitive longevity – Default Mode Network (DMN), Salience Network (SN) and Executive

Control Network (ECN) – recognized in the literature as predictors of healthy aging and the risk of neurodegenerative decline (Raichle, 2015; Cabeza et al., 2018).

The second marker, heart-brain coherence, is assessed by heart rate variability (HRV) and psychophysiological resonance scores. This coherence reflects the synchrony between the central and autonomic nervous systems and is considered an essential indicator of effective emotional regulation, physiological resilience, and behavioral adaptability. Recent studies have confirmed that high heart-brain coherence is associated with stable cognitive functioning and a reduced incidence of affective disorders in the elderly (Lehrer & Gevirtz, 2014; McCraty & Shaffer, 2015).

The third marker, perceived physiological stress, is assessed by validated biofeedback scales and clinical psycho-physiological scores, such as those provided by systems such as GP8. This indicator reflects the degree of chronic activation of the HPA (hypothalamic-pituitary-adrenal) axis and the sympathetic nervous system. High and persistent levels of stress activate neurotoxic mechanisms, increase systemic inflammation, and contribute to autonomic and cognitive dysfunction. Chronic stress is therefore an accelerating factor of neural aging and degeneration of networks involved in self-regulation and adaptation (McEwen, 2012; Sapolsky, 2004).

By combining these three dimensions – neurophysiological, autonomous and affective – the overall objective of the study is to comprehensively assess the impact of the intervention on cognitive longevity, in the sense of an active, quantifiable and sustainable maintenance of higher mental functions in a population on the threshold of neurological vulnerability, but still outside the pathological spectrum.

This approach is aligned with the principles of P4 medicine – predictive, preventive, personalized and participatory – formulated by Hood & Friend (2011), and responds to a real need to build proactive, reproducible and broadly applicable clinical protocols. The goal is not just to extend life span in general, but to extend the duration of cognitively active and functional life.

The proposed intervention does not aim to repair a damaged function, but to optimize and strengthen a fragile neurophysiological balance, on the verge of decompensation. From this perspective, the overall objective of the study also acquires a conceptual dimension: to transform the way prevention is understood in applied neurosciences – from a reactive intervention to disease, to an active support of neural, functional and emotional health in the preclinical phases of aging.

2.2 Improved activation and functional connectivity in ARI (DMN, SN, ECN)

The first specific objective of this clinical study is to evaluate and improve activation and functional connectivity within the three major neural networks involved in maintaining cognitive longevity: Default Mode Network (DMN), Salience Network (SN), and Executive Control Network (ECN). These functional networks make up the fundamental neurodynamic architecture that supports critical functions such as memory, emotional self-regulation, cognitive planning, attentive processing, and the ability to adapt to stress-all of which are essential for active, coherent, and autonomous old age (Raichle, 2015; Menon, 2011).

Default Mode Network is involved in processes of autobiographical memory, future-oriented thinking, self-reflection and identity coherence. The regions involved include the medial prefrontal cortex (BA10), the posterior cingulate (BA31), and the inferior parietal cortex (BA40) (Buckner et al., 2008). With age, the activation of this network decreases naturally, a phenomenon associated with loss of mental coherence, impaired temporal orientation, and reduced ability to integrate life experience (Damoiseaux et al., 2008). Reduction of connectivity in DMN is considered an early marker of Alzheimer's disease and neurocognitive decline (Greicius et al., 2004).

Salience Network plays an essential role in the detection of relevant stimuli, in emotional regulation and in facilitating the transition between resting states (supported by DMN) and cognitive activation states (supported by ECN). It consists of structures such as anterior insula (BA13), anterior cingulate (BA24 and BA32) and amygdala. This network functions as a dynamic switch between introspection and externally oriented action, allowing rapid adaptation to environmental changes (Seeley et al., 2007; Uddin, 2015). Dysfunctions within SN are correlated with stress hyperreactivity, affective disorders, and behavioral inhibition difficulties, traits that become common in the emotional aging process (Menon & Uddin, 2010).

The Executive Control Network is responsible for functions such as planning, decision making, focused attention, working memory and cognitive inhibition – fundamental skills for maintaining personal and social autonomy in old age. The network is anchored in the dorsolateral prefrontal cortex (BA46) and in the upper parietal regions, being particularly vulnerable to decreased activation and circuit desynchronization with aging, especially in the absence of constant cognitive stimulation or under chronic stress conditions (Spreng et al., 2010; Cole & Schneider, 2007).

The multimodal intervention proposed in this study – Neurofeedback Plus – aims to restore the functional balance between these networks through cortical regulation training, biophoton stimulation and vagal activation. This intervention aims at increasing coherent activation in the nodal regions of each network (BA10 for DMN, BA24 and BA32 for SN, BA46 for ECN), recalibrating functional connectivity between networks, with a particular focus on reactivating the SN network that regulates the adaptive transition between DMN and ECN (Menon, 2011), and restoring the balance between rest and cognitive activation networks, balance often damaged in physiological aging and accelerated by chronic stress.

The evaluation of activation and connectivity in DMN, SN and ECN is performed through a combination of objective methods. Quantitative electroencephalography (qEEG) allows the analysis of relevant brain frequencies – in particular Alpha frequencies for DMN and SMR/Low Beta for ECN – as well as the measurement of intra- and interhemispheric coherence in the targeted regions. Cortical maps generated by qEEG analysis also allow detailed functional assessment of Brodmann areas of interest (BA10, BA24, BA32, BA40, BA46). Monitoring of changes is done through repeated measurements, before and after the intervention, but also through intermediate assessments performed after each therapeutic cycle, in order to capture the dynamic evolution and cumulative effects of the applied program.

The obtained data will be statistically analyzed by significance tests (such as paired t-test and ANOVA

with repeated measurements), and the correlations between cortical activation and autonomic regulation parameters (such as HRV) will be used to highlight the functional link between neural networks and neurovegetative balance. This approach allows testing the central hypothesis of the study: that an integrated neurotechnological intervention can restore and even amplify the functionality of brain networks involved in cognitive longevity, at a critical time in life when prevention has the greatest impact (Fratiglioni et al., 2004; Goh & Park, 2009).

Validating this goal would mean demonstrating that functional reorganization of brain networks is possible even after the age of 60, in the absence of a manifest clinical decline, thus supporting a paradigm shift – from a reactive medical approach, to a preventive, optimizing approach, oriented towards the active extension of cognitive life.

2.3 Increasing overall EEG coherence and neural coherence in selected Brodmann areas

One of the fundamental objectives of the clinical trial is to increase overall EEG coherence as well as regional neural coherence in Brodmann strategic areas BA10, BA24, BA32, BA40 and BA46, using quantitative electroencephalography (qEEG) as an assessment tool. This objective is aimed at highlighting positive changes in the functional organization of the brain, as a result of multimodal neurotechnological intervention Neurofeedback Plus, being designed as a direct indicator of adaptive neuroplasticity and functional synchronization in the brain networks of the mature brain.

EEG coherence is defined as a mathematical index reflecting the temporal synchronization of electrical activity between two cortical regions, thus representing the degree of functional connectivity between neural networks in various frequency bands (Thatcher et al., 1986). Increased coherence denotes more effective information integration, stable neural communication, and coherent functional brain organization – all of which are essential conditions for maintaining higher cognitive functions and neurofunctional adaptation in older age (Thatcher, 2010; Barry et al., 2020). Conversely, the decrease in EEG coherence – especially in fronto-parietal and interhemispheric networks – is correlated with physiological aging (Grandy et al., 2013), with executive and affective dysfunction (Harmony, 2013), as well as with the onset of neurodegenerative disorders, including early forms of dementia (Prichep et al., 2006).

From this perspective, EEG coherence can be considered a first-rate electrophysiological biomarker for cognitive longevity – not just in terms of sustained brain activity, but above all as an expression of coherent, dynamic and adaptive networking in the adult brain.

In the study, the analysis of neural coherence is centered on several strategic regions with scientifically documented functions in cortical networks involved in cognition and emotional regulation. BA10, located in the anterior prefrontal cortex, has a central role in the Default Mode Network and is involved in integrating autobiographical experiences and mentally anticipating future events (Ramnani & Owen, 2004). The BA24 and BA32 areas, components of the anterior cingulate, are essential in regulating stress responses, emotional awareness, and behavior adjustment (Bush et al., 2000; Lane et al., 2009). BA40, located in the lower parietal lobe, participates in spatial attention, sensory integration, and mental orientation (Husain & Nachev, 2007).

BA46, part of the dorsolateral prefrontal cortex, is integrated into the executive core network and supports working memory, planning and control of cognitive inhibition (Miller & Cohen, 2001).

Increasing inter- and intrahemispheric coherence in these areas reflects superior neural integration and effective network communication, both of which are indispensable for maintaining cognitive functions in advanced ages (Thatcher, 2010; Rossini et al., 2007).

Neurofeedback Plus intervention is structured to stimulate increased EEG coherence through four synergistic mechanisms. First, personalized EEG neurofeedback guides the individual to voluntarily alter his or her cortical activity, orienting it toward a balanced distribution of frequency bands. Through repetitive training, this process causes an increase in interhemispheric coherence and more effective communication between the frontal and parietal regions (Gruzelier, 2014; Ros et al., 2014), especially by strengthening the Alpha and SMR frequencies – associated with effective cognitive states and mental clarity (Hammond, 2011).

Second, gamma and alpha pulsed light photobiomodulation of the brain stimulates network synchronization through transcranial application to the frontal and parietal areas.

Gamma oscillations, in particular, are fundamental to higher-order cognitive coherence (Iaccarino et al., 2016; Chan et al., 2021), while alpha frequencies facilitate neural energy efficiency and coherence of background rhythms. This method additionally contributes to reducing brain inflammation and optimizing neuronal metabolism (Hamblin, 2016).

The third mechanism is heart-brain coherence training, achieved through heart rate variability biofeedback (HRV). This training facilitates functional connectivity between the prefrontal cortex and the brain stem vegetative centers (Lehrer & Gevirtz, 2014), supporting the synchronization between BA24, BA32 and BA10 within the salience and executive network (Thayer & Lane, 2007). Transcutaneous vagal stimulation, as the fourth therapeutic pillar, activates the parasympathetic nervous system and modulates cortical excitability, favoring network synchronization in SN and ECN networks (Breit et al., 2018; Clancy et al., 2014). By reducing background cortical noise and decreasing dysfunctional activity in fast frequencies (High-Beta), a neurophysiological environment conducive to sustained and stable EEG coherence is created.

EEG coherence assessment is performed by using high-resolution cortical maps, generated by medically certified software, recording activity from 19 EEG channels in the 10–20 system. Both interhemispheric and intrahemispheric coherence scores will be calculated in the targeted areas, before and after the intervention, as well as in intermediate stages. Differences in coherence will be correlated with changes in HRV and physiological stress scores to assess the overall and zonal effects of the intervention.

Statistical analyses will include t-tests for paired samples, ANOVA models with repeated measurements and the calculation of the effect size (Cohen's d), with a planned statistical power of at least 0.80, in order to validate the robustness of the observed effects.

Increased EEG coherence is not only a technical indicator, but a validated clinical marker of healthy, synchronized and effective brain function that supports mental adaptation, cognitive flexibility and functional resilience in the face of biological and psychological challenges of age. Validating this goal would mean

demonstrating that network reorganization of the mature brain is possible by non-invasive methods without medication,

and would support the expansion of proactive interventions in preventive medicine. Within this framework, Neurofeedback Plus would become a strategic reference for interventions aimed not only at preventing cognitive degradation but also at stimulating greater adaptation in key stages of neurological maturity.

2.4 Vagal tone stimulation and HRV improvement (RMSSD, LF/HF, SDNN)

A central objective of this clinical study is to improve the functioning of the autonomic nervous system by increasing vagal tone and heart rate variability (HRV), as measured by standardized indicators such as RMSSD (Root Mean Square of Successive Differences), LF/HF (Sympathetic to Parasympathetic Activity Ratio), and SDNN (Standard Deviation of NN intervals). These parameters directly reflect the body's ability to respond flexibly and adaptively to stress, to regulate internal psychophysiological tensions, and to maintain autonomous homeostasis – essential components for supporting cognitive longevity.

HRV is one of the most sensitive and validated measures of the balance between the sympathetic and parasympathetic branches of the autonomic nervous system, being correlated with a wide spectrum of cognitive, affective and physiological functions (Thayer et al., 2012). High levels of HRV are associated with increased vagal tone, a high emotional and physiological adaptive capacity, a reduced risk of cardiovascular disease, anxiety or dementia, as well as increased overall biological flexibility (Shaffer & Ginsberg, 2017; Lehrer & Gevirtz, 2014). In contrast, low HRV is considered an early marker of accelerated aging, reduced brain plasticity, the emergence of cognitive dysfunction, and a diminished capacity for self-regulation in the face of external stressors (McEwen, 2012; Malik et al., 1996).

In this study, three main indicators of HRV are analyzed, each with distinct value in assessing neurovegetative functioning. RMSSD expresses the square mean of the differences between successive RR intervals and is recognized as the most accurate indicator of parasympathetic activity and vagal tone, respectively. An increase in RMSSD indicates an increased capacity for physiological relaxation, better emotional resilience, and an effective adaptive response to internal and external changes (Shaffer et al., 2014). The LF/HF ratio reflects the balance between the sympathetic and parasympathetic components of HRV. An optimal ratio, ideally located between 0.8 and 1.5 values, signals an autonomous functional homeostasis. Imbalances in favor of low frequency (LF) are interpreted as signs of persistent sympathetic hyperactivation, correlated with chronic stress, while an excessive ratio in favor of high frequency (HF) may indicate phenomena of hypotonia or ineffective parasympathetic inhibition (Shaffer & McCraty, 2017).

SDNN, as a standard deviation of NN intervals, provides a global picture of heart rate variability, being influenced by both branches of the autonomic system and recognized as a predictor of overall mortality and cardio-neurovegetative integrity (Malik et al., 1996).

Neurofeedback Plus intervention acts on HRV through several synergistic pathways, built to simultaneously stimulate both autonomous regulation and its integration into cognitive and emotional

functioning. Transcutaneous vagal stimulation activates the related fibers of the vagus nerve, especially in the cervical area, and causes a significant increase in parasympathetic tone. Research shows that consistent use of this technique leads to increases of up to

to 20–30% in RMSSD after only 6–10 weeks of application (Clancy et al., 2014; Yuan & Silberstein, 2016). Heart-brain coherence biofeedback complements this mechanism through rhythmic breathing training and visual feedback, which allows participants to optimize their HRV and stabilize the LF/HF ratio. This process, repetitive and teachable, has cumulative effects in emotional regulation and strengthening vegetative flexibility (Lehrer et al., 2013; McCraty & Zayas, 2014).

Cortically, interventions through EEG neurofeedback and gamma-frequency photobiomodulations support the induction of neurocognitive states of active calm, reduce cortical hyperactivation, and contribute to the normalization of autonomic rhythms. The activation of the prefrontal (BA10) and anterior cingulate (BA24) areas, involved in executive control and top-down autonomous regulation, contributes to the stabilization of the cortico-subcortical connections involved in HRV (Thayer & Lane, 2007).

The HRV evaluation is performed with certified medical devices, with millisecond accuracy, in accordance with standardized protocols – the recording taking place at rest, in a sitting position, for 10 minutes, before and after each 10-week cycle of intervention. Individual scores are calculated for each parameter analyzed, and the data obtained will be subjected to detailed statistical analyses, including paired t-tests, ANOVA models with repeated measurements and effect size analysis (Cohen's d), for robust evaluation of intra- and intergroup differences.

Increasing HRV, especially RMSSD and balancing the LF/HF ratio, are correlated with improving concentration and sustained attention (Hansen et al., 2003), reducing the risk of cognitive decline and dementia (Zeki Al Hazzouri et al., 2014), optimally regulating emotional response, effectively adapting to stress, and delaying the onset of inflammatory and degenerative processes associated with aging (Thayer et al., 2012; McEwen, 2007).

These indicators not only allow objective monitoring of the effectiveness of the applied intervention, but also provide the ability to predict cognitive longevity in an integrative, neurophysiological and autonomous perspective. In addition, HRV provides an interdisciplinary communication framework, being a recognized parameter both in cardiovascular medicine and in neuroscience and psychophysiology, which favors the translation of the results of this study to international protocols and preventive health-oriented public policies.

2.5 Reduction of physiological and emotional stress.

Another key specific objective of this clinical study is to reduce the level of physiological stress and optimize the capacity for emotional regulation, both of which are recognized as central pillars of neurophysiological health and major predictors of cognitive longevity. Within the current paradigm of applied neuroscience, chronic stress is not only interpreted as a subjective psychological experience, but as a profound physiological process with direct effects on brain architecture, functioning of the autonomic nervous system,

cardiovascular rhythms, and efficiency of neural networks involved in self-regulation, memory, attention, and adaptation (McEwen, 2007; Sapolsky, 2004).

Persistent physiological stress, triggered by hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, causes chronic increases in cortisol levels, systemic inflammation, neuronal excitotoxicity, and inhibition of neurogenesis, especially in essential brain structures such as the hippocampus and prefrontal cortex (Lupien et al., 2009; Joëls et al., 2007). In the long run, these cumulative effects lead to deterioration of executive and emotional regulation functions, cognitive rigidity, functional connectivity disorders in DMN, SN and ECN networks and, implicitly, to acceleration of age-related neurocognitive decline.

Physiological stress is objectively quantifiable through a number of standardized psycho-physiological parameters, including heart-brain coherence scores, psycho-physiological stress scores (e.g. GP8), reactivity indices, and assessment of heart and respiratory rhythms in resting and activation states. These indicators directly reflect the degree of functional dissociation between the prefrontal cortex and the limbic system, as well as the level of respiratory stiffness and the absence of voluntary control over physiological stress responses (Lehrer & Gevirtz, 2014; Shaffer & Ginsberg, 2017).

The capacity for emotional self-regulation is defined as the ability to recognize, understand, and modulate one's own emotional reactions in an adaptive and contextualized way. This function is neurally supported by several critical structures: the anterior cingulate (BA24 and BA32), involved in detecting internal conflicts and adjusting behavioral response; the anterior insula (BA13), responsible for interoceptive awareness and integration of bodily signals into emotional experience; and the dorsolateral prefrontal cortex (BA46), which provides voluntary cognitive control over affective reactions. The dysfunction of these regions is commonly encountered in disorders such as anxiety, depression, burnout and adjustment disorders, all with increased incidence in old age and with negative impact on cognitive functioning (Etkin et al., 2011; Lane et al., 2009).

The study proposes an integrated neurotechnological intervention, combining scientifically validated methods in order to restore psycho-physiological balance. Through heart-brain coherence biofeedback, participants learn to regulate the respiratory rhythm in real time in relation to cardiac activity, increasing their voluntary control over stress reactions and strengthening the functional connection between the prefrontal cortex and the brain stem, involved in autonomous integration (McCraty & Shaffer, 2015). Transcutaneous vagal stimulation, applied to the cervix or ear, reduces sympathetic reactivity, decreases overall hyperactivation tone, and supports a rapid physiological recovery after exposure to emotional or cognitive stimuli (Clancy et al., 2014; Breit et al., 2018).

The personalized EEG neurofeedback, oriented towards the training of BA24, BA32 and BA13 areas, has the effect of reducing hyperactivation in High Beta fast bands – associated with rumination and hyperalertation states – and stimulating the alpha and SMR bands in the limbic regions, favoring states of calmness, concentration and integrative processing. This type of training contributes to the regulation of activity in the Salience Network, responsible for the detection and filtering of relevant environmental signals (Ros et al., 2014).

In parallel, brain photobiomodulation applied in the 10 Hz and 40 Hz frequencies reduces cortical inflammation, decreases oxidative stress, has anxiolytic effects, and favors brain rhythm synchronization, essential for mental clarity and emotional balance (Barrett & Gonzalez-Lima, 2013; Iaccarino et al., 2016).

Physiological stress will be assessed through standardized biofeedback tools, including HRV scan, coherence score, tension level and clinical scores (e.g. GP8). Assessments will be performed at the beginning and end of each 10-week cycle under controlled resting conditions. Data will be analyzed statistically by t-tests, ANOVA, effect size analysis (Cohen's d), multiple regressions, and Pearson correlations to identify relationships between physiological stress, emotional self-regulation, and overall neurofunctional improvement.

Reducing physiological stress and improving emotional regulation prevent chronic activation of the HPA axis, reduce neuroinflammation, and limit the dysfunctional impact of negative emotions on executive networks. At the same time, they strengthen the connection between cognitive and autonomous networks, substantiating effective adaptation to stress, mental clarity and affective stability in advanced stages of life. These effects result in increased concentration, psychological tolerance, speed of emotional recovery and social adaptability – all key elements in maintaining and extending the duration of cognitively active life.

2.6 Assessment of cumulative and longitudinal effects of intervention on neurophysiological, autonomic and emotional markers

The last specific objective of this study is to evaluate the cumulative and longitudinal effects of multimodal neurotechnological intervention, in order to determine the durability and stability over time of the improvements obtained on neurophysiological, autonomic and emotional markers. This approach is not limited to measuring the immediate effect of the intervention, but aims to monitor the persistence and possible amplification of beneficial effects during the therapeutic rest periods and after the completion of all training cycles.

In modern neuroscience, a clear distinction is made between immediate effects – those observable changes in proximity to the intervention –, cumulative effects – results that accumulate gradually following regular repetition of an intervention – and longitudinal effects – those transformations that persist and stabilize over time, signaling a strengthening of neuroplasticity (Draganski et al., 2006; Ros et al., 2014). This distinction is fundamental to the validation of any protocol of preventive value, as an effect that disappears with discontinuation of treatment cannot be considered relevant to the maintenance of long-term health.

The objective of this study is to demonstrate not only the ability of the Neurofeedback Plus intervention to induce neurophysiological changes, but especially to facilitate the stabilization of sustainable functional reconfigurations capable of supporting the cognitive longevity and biological resilience of the mature brain. The applied protocol is structured in three therapeutic cycles, each of ten weeks of intensive intervention, alternated with ten-week rest periods, over an entire year. This structure allows a rigorous analysis of how physiological, autonomic and emotional parameters evolve not only during exposure to therapy, but also in its absence, in contexts of spontaneous integration of neurophysiological learning.

This therapeutic architecture can precisely follow the continuous evolution of the measured markers, identify trends to maintain or amplify the results between cycles, and observe functional strengthening phenomena comparable to those encountered in sustained behavioral or cognitive interventions (Lubar et al., 1995; Enriquez-Geppert et al., 2017). The repeated but time-spaced application of an active neurotechnological intervention allows the consolidation of the newly formed functional routes and the strengthening of the synapses involved in cognitive and emotional regulation, resulting in a sustainable brain reorganization, measurable by qEEG and HRV.

To test this hypothesis, the study will include four assessment times: T0 – baseline, T1 - after the first cycle of intervention, T2 – at six months, after the second cycle and a rest period, and T3 – at the end of the three cycles, respectively at the end of the year. The parameters evaluated will be: quantitative EEG activity (overall EEG coherence, spectral power in frequency bands, activation of BA10, BA24, BA32, BA40 and BA46 areas); autonomous parameters (RMSSD, LF/HF, SDNN); physiological stress (biofeedback scores, physiological stress and reactivity indices); and heart-brain coherence (psycho-physiological resonance scores).

The differences between the evaluation moments will be analyzed statistically, in order to confirm not only the immediate effectiveness of each intervention cycle, but also the durability of the induced changes.

The analysis will focus on identifying stabilization or regression trends between cycles in order to determine whether the results can be maintained or even amplified in the absence of direct intervention. The working hypothesis, supported by current literature, is that spacing effect interventions facilitate gradual synaptic consolidation and an increase in global functional self-regulation (Pascual-Leone et al., 2005), particularly in cortical networks involved in memory, attention, and emotional regulation (Goh & Park, 2009).

Moreover, controlled rest periods are not passive interruptions of the therapeutic process, but contexts in which the nervous system has the opportunity to integrate and automate the learned regulatory mechanisms. This transition from “active training” to “optimized functioning” in everyday life is one of the most important evidences of genuine neurophysiological strengthening.

The validation of this objective has direct implications on the real applicability of the proposed intervention. Demonstrates that Neurofeedback Plus is not a temporary solution, but an intervention with sustained and cumulative neurophysiological impact. It also supports the integration of this method into annual or semi-annual therapeutic regimens as part of a cognitive maintenance and neurophysiological prevention program in seniors. Through its non-invasive character and ability to support biological and functional self-regulation, this intervention provides a real alternative to pharmacological treatments and can form the basis for the development of national prevention programs dedicated to elderly people at increased cognitive risk.

3. Methodology and study design

3.1 Overall study design

The present study was designed as a randomized, controlled, longitudinal, multicenter, double-arm (intervention vs. control) clinical trial, conducted over a period of twelve months within the national network of BrainMap Neuroscience clinics in Romania. The choice of this methodological design aimed to maximize internal validity, strengthen causal inference, and ensure robust external generalizability of results, in line with international standards of good clinical practice and methodology specific to randomized trials in applied neuroscience (Friedman et al., 2015; Schulz et al., 2010).

The participants included in the study were randomly assigned into two numerically equal groups: an experimental group that benefited from multimodal Neurofeedback Plus intervention, applied in three distinct therapeutic cycles, and a control group that was monitored in parallel, without receiving the active intervention, but subjected to the same evaluation procedures, with the same frequency and temporal structure. Randomization was performed by a layered computerized method, which took into account variables such as age (60–70 years), gender, educational level and city of origin, thus ensuring the balancing of groups and reducing the risk of selection bias (Suresh, 2011).

The two-arm model – intervention versus control – is currently the reference methodological standard in clinical research, as it allows direct comparison between the changes arising from the application of the intervention and the natural evolution of the population without active treatment (Hariton & Locascio, 2018). In addition, an essential element of the design is the longitudinal structure, which involves the repeated evaluation of each participant at four distinct moments: T0 (baseline), T1 (after 10 weeks), T2 (at 6 months) and T3 (after 1 year), in order to capture the dynamics of the effects, the stability over time, the accumulation of the results and the clear differentiation between immediate, intermediate and sustainable effects.

The literature firmly argues that neurotechnological interventions aspiring to the status of preventive solutions must demonstrate not only punctual efficiency, but longitudinal impact, functional stability and adaptive integration in everyday functioning (Kazdin, 2017; Pascual-Leone et al., 2005).

Conducting the study in all BrainMap Neuroscience clinics in Romania – Bucharest, Brasov, Timisoara, Cluj, Iasi, Bacau, Constanta, Craiova, Arad, Baia Mare and Targu Mures – gives the design a multicenter structure with national relevance. This methodological choice ensures increased geographical and sociocultural representativeness, provides a real framework for testing the feasibility of the intervention in varied clinical contexts, and contributes significantly to the external validation of the results, reducing the risk that they reflect the peculiarities of a single location (Grobbee et al., 2007).

Each unit involved followed the standardized protocol of intervention and evaluation, using identical equipment, uniformly trained therapists and documented procedures. The quality monitoring of the implementation was carried out through centralized supervision and periodic internal audits, thus ensuring the

consistency of the methodological application and the integrity of the collected data.

The total duration of twelve months was strategically chosen to allow the application of three sequential ten-week treatment cycles alternating with equal duration rest periods. This temporal structure provides the necessary framework for assessing the cumulative effects and neurophysiological consolidation in the medium and long term, as well as for observing how therapeutic benefits are transferred or maintained in everyday life in the absence of continuous intervention.

Shorter intervention durations, of 3–4 months, have often proven insufficient to produce and stabilize significant changes in the brain and autonomy among elderly populations (Draganski et al., 2006). Longitudinal studies dedicated to neuroplasticity have shown that the processes of synaptic consolidation and functional reorganization in networks involved in memory, executive control, and emotional regulation require repeated, sustained, and structured exposure over time (Enriquez-Geppert et al., 2017; Arns et al., 2009).

Thus, the overall study design was built to meet all international scientific validation criteria applicable to clinical trials in the field of neuroscience and preventive medicine: controlled randomization, inter-group comparability and intra-group evolution, longitudinal structure with multiple assessments, national multicentricity and clinical translatability.

Through this rigorous approach, the study not only provides a point validation of an intervention, but proposes a replicable, scalable clinical model with the potential for systemic integration into public health practices and neurocognitive prevention at national and international level.

3.2 Sample characteristics

The total study sample consisted of 200 participants, equally distributed between the two comparison groups: the experimental group (n = 100), which benefited from the integrated Neurofeedback Plus intervention carried out in three consecutive therapeutic cycles over one year, and the control group (n = 100), which was monitored in parallel, without receiving the active intervention, but subjected to the same standardized evaluation procedures (qEEG, HRV, biofeedback), under the same time and environment conditions. The structure thus designed ensures maximum comparability between groups and allows the isolation of the specific effect of the intervention, through rigorous control of demographic, cognitive and physiological variables.

The sample size was determined by a statistical power calculation, starting from an estimated average level effect size (Cohen's $d \approx 0.60$), a significance level $\alpha = 0.05$ and a target statistical power of over 0.80, parameters considered standard in rigorous clinical research (Cohen, 1988). Similar neurophysiological intervention models, documented in literature, reported consistent effects on HRV parameters, EEG activity, and emotional regulation with comparable samples (Enriquez-Geppert et al., 2017; Ros et al., 2014).

The selection of participants was carried out on the basis of a strict set of inclusion and exclusion criteria, with the aim of ensuring the homogeneity of the sample and reducing the potential impact of confusion variables. Only participants aged 60 to 70 years were included, with no significant differences between the two groups. This interval was chosen strategically, being considered a critical period in the transition to senescence,

in which cognitive decline is still reversible, and neuroplasticity can be supported by targeted interventions (Nyberg et al., 2012; Goh & Park, 2009).

In order to ensure the accessibility to the intervention and the coherence of the participation conditions, only people from the urban environment were selected. At the same time, participants were required to have completed secondary or higher education, as an indicator of a level of pre-existing cognitive reserve and higher capacity for active engagement in complex therapeutic interventions (Scarmeas et al., 2006). Previous professional activity was also a selection criterion – only individuals with predominantly intellectual occupational history (areas such as education, administration, IT, health, culture, economics or engineering) were included to control non-neurocognitive influences, such as severe physical wear and tear, on autonomous operation.

General health was another central criterion: only clinically healthy participants without active major chronic diseases and with independent functional capacity (ADL/IADL intact) were included. Initial neuropsychological assessment was applied to all participants and included only those with normal scores, without signs of dementia, mild cognitive impairment or acute psychiatric pathologies, criteria confirmed by standardized clinical interviews and validated scales.

Participants with major neurological disorders (Alzheimer's, Parkinson's, epilepsy, stroke, multiple sclerosis), severe psychiatric disorders (schizophrenia, active major depression, bipolar disorder, severe PTSD), chronic consumption of alcohol or neurotoxic substances, unstable cardiovascular conditions or pacemaker carriers for which vagal stimulation would have been contraindicated, as well as those involved in other clinical trials or similar therapies, were excluded from the study.

Participants with severe cognitive or sensory deficits (aphasia, blindness, deafness, advanced motor disorders) that would have interfered with the proper conduct of the assessment and intervention protocol were also excluded.

The distribution in the two groups was achieved with the maintenance of a balanced distribution in terms of gender, age and educational level. Thus, the final structure of the sample is characterized by approximately 50% women and 50% men, 65% participants with higher education and 35% with secondary education, distributed proportionally by geographical regions and clinical centers, covering the entire BrainMap network.

This rigorous sample structuring allows direct comparability between the experimental group and the control group, ensuring high internal validity and significantly reducing the influence of external variables on the results. In addition, the strategic selection of the targeted population –

clinically healthy seniors, but in a neurocognitively vulnerable phase – provide the ideal conditions for the validation of a preventive intervention, designed not to treat installed deficits, but to support and expand active cognitive functions.

Unlike studies focused on patients with dementia or advanced mental disorders, this study aims to evaluate the effectiveness of a proactive intervention, applied before the manifest decline. The approach is aligned with current directions in personalized and preventive medicine, aimed at early interventions in people

with latent cognitive risk but without obvious clinical symptomatology (Sperling et al., 2011; Livingston et al., 2020). Through this approach, the study contributes to the redefinition of neurocognitive prevention as an active intervention, oriented towards functional optimization and extending the duration of active cognitive life.

4. Structure of multimodal neurotechnological intervention

The therapeutic protocol applied in this study was intended exclusively for the experimental group and aimed at stimulating neuroplasticity, optimizing brain networks involved in higher cognitive functions, regulating autonomic balance and reducing physiological stress, through an integrated, multimodal structured intervention, called Neurofeedback Plus.

The intervention lasted for twelve months and was organized into three distinct therapeutic cycles, each lasting ten weeks, followed by periods of ten weeks of active therapeutic break, designed to allow neurophysiological consolidation and adaptive integration of effects in the absence of direct stimulation.

This cyclic structure was inspired from validated sequential neurofunctional training models, in which rest periods not only have a recovery role, but become windows of cortical integration, grid stabilization, and autonomous recalibration (Enriquez-Geppert et al., 2017; Gruzelier, 2014). Each cycle included ten weeks of active intervention, with a frequency of three sessions per week (non-consecutive), totaling thirty sessions per cycle and ninety sessions for the entire annual schedule. This frequency was chosen to comply with the principle of optimal intensity in neuroplasticity induction: frequent enough to generate durable adaptive changes, but also spaced enough to prevent cortical saturation and psycho-physiological fatigue (Ros et al., 2014; Arns et al., 2009).

The duration of one session was sixty minutes, divided into four sequentially applied therapeutic components, each aimed at a distinct level of brain and autonomous functioning. The first component, personalized EEG neurofeedback (20 minutes), was the foundation of cognitive intervention. The protocol was individually calibrated based on an initial qEEG assessment, with registration in the 10–20 system, focusing on the Brodmann areas BA10, BA24, BA32, BA40 and BA46. The objectives of this component were to increase activity in the Alpha and SMR bands, reduce activation in High Beta and High Beta, and regulate neural coherence and interregional connectivity. Scientific justification derives from studies attesting to the EEG neurofeedback's ability to induce adaptive functional reorganization, boost connectivity in networks involved in cognitive longevity, and normalize activity in the Default Mode Network and Executive Control Network (Ros et al., 2014; Hammond, 2011; Enriquez-Geppert et al., 2017).

The second component of the intervention was gamma-frequency brain photobiomodulation (10 minutes), achieved by transcranial application of pulsed LED light in the near-infrared range (810-850 nm), with a frequency of 40 Hz. The devices were positioned bilaterally, frontally and parietally. This frequency was chosen because it stimulates gamma oscillations, correlated with cognitive coherence, multisensory integration, and grid synchronization (Iaccarino et al., 2016; Chan et al., 2021). In addition, photobiomodulation supports

neuronal metabolism by increasing ATP synthesis, reduces neurovascular inflammation, and promotes clarification of neurotoxic proteins (Hamblin, 2016). In the context of the elderly population, PBM is a safe, non-invasive intervention with documented effects in preventing subclinical degeneration of the cortex.

The third stage of each session was the heart-brain coherence biofeedback (15 minutes), performed using a clinical system validated by HRV biofeedback, which

record heart rate and breathing in real time. Participants were guided to practice controlled breathing (6 cycles/minute), synchronized with cardiac activity, aiming to achieve a state of physiological coherence between the central nervous system and the autonomic nervous system. This component is justified by the close link between high HRV and effective emotional regulation, vagal control, and cognitive adaptation (Lehrer & Gevirtz, 2014; McCraty & Shaffer, 2015). At the neuroanatomical level, this exercise indirectly activates the anterior cingulate area (BA24/32) and insula (BA13), involved in body awareness and affective integration.

The last component of the session, transcutaneous vagal stimulation (15 minutes), was performed with a CE/FDA certified medical device, applied at the cervical or auricular level (zona tragus), with a frequency of 25 Hz and subjectively adapted intensity, under therapeutic monitoring.

This intervention aims to directly stimulate the vagus nerve, increasing parasympathetic tone and reducing background sympathetic activation. Studies have shown positive effects of this method on regulating the HPA axis, reducing cortisol, controlling systemic inflammation, and increasing RMSSD values (Breit et al., 2018; Yuan & Silberstein, 2016; Clancy et al., 2014). In addition, vagal stimulation helps improve connectivity in the Salience Network and supports emotional adaptation by reducing chronic physiological reactivity.

Through this coherent combination, the four components of the intervention work synergistically. EEG neurofeedback provides cortical autoregulation, gamma photobiomodulation supports metabolic and neuroprotective reorganization, HRV biofeedback allows psycho-physiological recalibration, and vagal stimulation completes the intervention by autonomously stabilizing and reducing systemic stress. The unitary structure of the session allows simultaneous action on the brain networks critical for cognitive functioning – DMN, SN and ECN – as well as on the parameters that support the stability of these networks: HRV, physiological stress and emotional coherence.

By rigorously standardizing each component and integrating them into a scalable clinical protocol, Neurofeedback Plus intervention becomes not only replicable, but also translatable into practices of cognitive prevention, health education or neurofunctional rehabilitation, thus contributing to the redefinition of the active seniors care paradigm.

5. Measurements and evaluation tools

To objectively assess the impact of multimodal intervention on cognitive longevity and overall neurophysiological functioning, the study integrated a complex battery of scientifically validated assessments, conducted at four distinct times: T0 – baseline assessment, prior to the start of the intervention; T1 –

immediately after the first therapeutic cycle (10 weeks); T2 – interim assessment, at six months (after the second cycle and a rest period); and T3 – final assessment, at twelve months, after the end of the three therapeutic cycles.

This longitudinal structure was chosen to allow not only the capture of immediate effects (T1), but also the analysis of cumulative trends (T2) and the durability of changes (T3), in line with international methodological recommendations on applied neuroplasticity studies (Pascual-Leone et al., 2005; Draganski et al., 2006).

The neurophysiological evaluation was performed by quantitative electroencephalography (qEEG – quantitative EEG), using a standard medical system with 19 channels, in 10–20 configuration, recorded both at rest with eyes closed and with eyes open. The EEG analysis followed global and regional coherence – a parameter that expresses the degree of functional synchronization between different brain regions, directly correlated with the efficiency of information processing (Thatcher, 2010; Barry et al., 2020) – and spectral power in the frequency bands Delta, Theta, Alpha, SMR, Beta, High Beta and Gamma. Each of these bands is associated with distinct states of cognitive, emotional, and autonomic activation, and changes in their relative strength allow fine evaluation of the obtained functional adjustments. For example, increased power in Alpha and SMR is associated with states of active calm, focus, and self-regulation, while hyperactivation in High Beta is an indicator of cortical stress and rumination (Hammond, 2011).

The assessment focused on the Brodmann areas BA10, BA24, BA32, BA40 and BA46 – strategic areas involved in major brain networks associated with cognitive longevity: Default Mode Network, Salience Network and Executive Control Network (Raichle, 2015; Menon, 2011). In addition to the analysis of local activation and intrahemispheric coherence, functional mapping of connectivity between the main nodes of these networks was also carried out to document the network reorganization induced by the intervention (Greicius et al., 2004; Cole & Schneider, 2007).

All records were processed with medically certified software, capable of generating comparative cortical maps for each moment of the assessment (T0-T3), allowing individual and group evolution to be tracked.

The evaluation of the autonomic balance was carried out by analyzing the heart rate variability (HRV), using CE/FDA certified devices, under controlled conditions (quiet environment, sitting position, 10 minutes of recording). The extracted parameters included RMSSD – considered the most accurate marker of vagal tone and ability to rapidly return to balance after a stressor (Shaffer & Ginsberg, 2017); SDNN – expression of total heart rate variability and general neurophysiological resilience indicator (Malik et al., 1996); and LF/HF ratio – reflecting the balance between sympathetic and parasympathetic components of the autonomic system, a balanced ratio indicating effective autonomic adaptability (Thayer et al., 2012). These markers are critical to understanding how the intervention

influences the recalibration of the vegetative nervous system – a key aspect in maintaining brain function in old age.

Physiological stress was assessed by the GP-8 score, a clinically validated psycho-physiological scale that integrates general neurophysiological tension, stress reactivity, and perception of somatic self-regulation (Barrios-Choplin et al., 2001). In addition, physiological indicators such as muscle tension, respiratory rate and pulse variation were recorded during biofeedback exercises, which were integrated into a composite physiological stress score.

These parameters provide an objective picture of the internal alert state of the nervous system, directly correlated with the capacity for emotional regulation, cognitive clarity and autonomous stability (McEwen, 2012).

Heart-brain coherence was evaluated through a specialized clinical HRV biofeedback system, which provided real-time scores on alignment between heart rate and respiratory rate, HRV resonance index (a parameter of psycho-physiological efficiency), and HRV variation amplitude synchronized with respiration, a marker of functional connectivity between the prefrontal cortex and the autonomic nervous system. The increase in these scores is recognized for its positive impact on emotional adaptability, decreased sympathetic activation, and support for optimized autonomous functioning (Lehrer & Gevirtz, 2014; McCraty & Shaffer, 2015).

All assessment tools used in this study are medically certified (CE, FDA), validated in international clinical trials and applied uniformly in all participating centers by therapists trained according to a standardized protocol. This complex and integrated evaluation system allows objective measurement of functional neuroplasticity (through qEEG), quantification of autonomic balance (through HRV) and documentation of the capacity for emotional regulation and management of physiological stress (through biofeedback and clinical scores). By integrating these valid measurements into a rigorous longitudinal design, the study provides a clear, reproducible and scientifically supported picture of the effectiveness of Neurofeedback Plus intervention in supporting active cognitive longevity.

6. Data analysis

6.1 Descriptive results and statistical interpretation

The analysis of the results obtained from the *integrated Neurofeedback Plus* intervention revealed significant and consistent changes in the evaluated neurophysiological, autonomic and emotional parameters. Comparisons made both **intra-group** (T3 vs. T0) and **intergroup** (experimental group vs. control group) demonstrate a clear, cumulative and statistically significant effect of the applied intervention on key markers of cognitive longevity.

During the 12 months of intervention, the experimental group showed significant improvements in the autonomic, neurophysiological and psycho-physiological parameters. The observed mean changes between T0 and T3 are shown in Table 1, followed by the specific interpretation for each parameter.

Table 1- Evolution of physiological and neurophysiological parameters in the experimental group between T0 and T3

	Mean T0	*SD T0*	*Mean T3*	*SD T3*	*Δ Mean (T3-T0)*	*Clinical interpretation*
RMSSD (ms)	28.7	10.4	41.5	12.1	12.8	Increased vagal activation, effective autonomous self-regulation (Shaffer & Ginsberg, 2017)
SDNN (ms)	38.2	9.7	47.4	10.2	9.2	Extended autonomic adaptability, enhanced cardiovascular health (Malik et al., 1996)
LF/HF Ratio	2.18	0.73	0.83	0.41	-1.35	Sympathetic-parasympathetic balance restored (Thayer et al., 2012)
GP-8 score (0–100)	64.1	11.8	46.5	10.3	-17.6	Marked reduction of perceived physiological stress (Barrios-Choplin et al., 2001)
Overall EEG Coherence (%)	61.4	8.3	76.6	7.9	15.2	Improved network synchronization brain (Thatcher, 2010; Barry et al., 2020)

At the end of the intervention, the experimental group showed a significant increase in vagal activity, expressed by a positive change in the mean values of RMSSD, from 28.7 ms (SD = 10.4) to 41.5 ms (SD = 12.1), which corresponds to an increase of 12.8 units and indicates an effective parasympathetic self-regulation (Shaffer & Ginsberg, 2017).

There was also an increase in SDNN, from an initial average of 38.2 ms (SD = 9.7) to 47.4 ms (SD = 10.2), with an average gain of 9.2 ms, signaling a generalized increase in physiological and cardiovascular resilience (Malik et al., 1996).

The LF/HF ratio decreased from a mean value of 2.18 (SD = 0.73) to 0.83 (SD = 0.41), indicating an effective rebalancing between sympathetic and parasympathetic systems, reflecting a strengthened vagal tone and a decrease in chronic sympathetic hyperactivation (Thayer et al., 2012).

In terms of physiological stress, the GP-8 score fell on average by 17.6 points, from 64.1 (SD = 11.8) to 46.5 (SD = 10.3), suggesting a significant reduction in internal psychophysiological tension (Barrios-Choplin et al., 2001).

In addition, overall EEG coherence – expressed in percentages – increased from an average value of 61.4% (SD = 8.3) to 76.6% (SD = 7.9), with a difference of 15.2 percentage points, indicating a substantial improvement in functional connectivity between cortical networks involved in self-regulation, attention, and cognition (Thatcher, 2010; Barry et al., 2020).

During the 12 months of monitoring without active intervention, the control group recorded minor changes in physiological and neurophysiological parameters. These variations fall within the expected limits of natural fluctuation and do not indicate a significant self-regulatory effect or spontaneous neuroplasticity.

Table 2- Evolution of physiological and neurophysiological parameters in the control group between T0 and T3

	Mean T0	*SD T0*	*Mean T3*	*SD T3*	*Δ Mean (T3-T0)*	*Clinical interpretation*
RMSSD (ms)	28.4	9.6	30.5	10.1	2.1	Slight physiological variation without functional significance (Shaffer & Ginsberg, 2017)
SDNN (ms)	38.5	8.9	40	9.2	1.5	Minor change, compatible with natural autonomous fluctuation
LF/HF Ratio	2.15	0.71	1.95	0.68	-0.2	Marginal decrease, irrelevant clinic (Thayer et al., 2012)
GP-8 score (0–100)	63.9	11.6	61.1	10.9	-2.8	Slight reduction in perceived stress, possible placebo effect Minimal
Overall EEG Coherence (%)	60.9	8.1	64	7.8	3.1	Marginal increase in cortical timing, no functional relevance

At the end of the 12-month period, the control group – which did not benefit from the Neurofeedback Plus intervention – showed only minor variations in the analyzed parameters. The mean value of RMSSD increased slightly from 28.4 ms (SD = 9.6) to 30.5 ms (SD = 10.1), corresponding to a difference of +2.1 units, not associated with a clinically significant improvement in parasympathetic activity (Shaffer & Ginsberg, 2017).

SDNN increased by 1.5 units (from 38.5 ms to 40.0 ms) and the LF/HF ratio decreased from 2.15 to 1.95, but these changes are within the margin of spontaneous variability and do not reflect a significant autonomous recalibration (Thayer et al., 2012).

The GP-8 score declined on average by 2.8 points (from 63.9 to 61.1), a change that can be interpreted as a marginal placebo effect or a result of contextual adaptation with no objective physiological correlations.

The overall EEG coherence increased slightly, from an average of 60.9% (SD = 8.1) to 64.0% (SD = 7.8), which represents a variation of +3.1 percentage points – a level considered insignificant in terms of network reorganization or functional neuroplasticity (Thatcher, 2010).

Intra-group comparison (T3 − T0 differences)

For **the experimental group**, all variables analyzed showed statistically significant changes between baseline (T0) and final assessment (T3), confirming the effectiveness of the intervention:

- **RMSSD**: t (99) = 26.80, $p < .001$
- **SDNN**: t (99) equals 112.70, $p < .001$
- **LF/HF Ratio**: t (99) = –18.70, $p < .001$
- **GP-8 score**: t (99) = –43.80, $p < .001$
- **Overall EEG coherence**: t (99) = 33.10, $p < .001$

These results demonstrate a significant improvement in vagal activity, sympathetic-parasympathetic balance, physiological stress, and brain connectivity following Neurofeedback Plus intervention.

For **the control group**, the paired t-tests applied between T0 and T3 showed no statistically significant

difference ($p > .05$), which supports the absence of a spontaneous or placebo physiological effect.

Table 3- T-test results for paired samples – intra-group comparison T0 versus T3

Parameter	*Group*	*t(99)*	*p*
RMSSD	Experimental	26.8	0.001
	Control	1.41	0.162
SDNN	Experimental	112.7	0.001
	Control	1.02	0.311
LF/HF Ratio	Experimental	-18.70	0.001
	Control	-1.67	0.098
GP-8 Score	Experimental	-43.8	0.001
	Control	-1.81	0.073
Overall EEG Coherence (%)	Experimental	33.1	0.001
	Control	1.92	0.058

Intergroup comparison (T3 − T0 differences)

To assess the effectiveness of the intervention against natural progression, differences between endpoint (T3) and baseline (T0) were compared in the two groups by the t-test for independent samples. The results indicate **statistically significant differences for all variables analyzed**, supporting a clear effect of the Neurofeedback Plus intervention. Significant results:

- **RMSSD**: t (198) = 13.24, $p < .001$
- **SDNN**: t (198) = 14.61, $p < .001$
- **LF/HF Ratio**: t (198) = –12.47, $p < .001$
- **GP-8 score**: t (198) = –17.33, $p < .001$
- **Overall EEG coherence**: t (198) = 18.21, $p < .001$

All *p-values* are below the materiality threshold ($\alpha = 0.05$), confirming that the improvements observed in the experimental group cannot be attributed to natural fluctuation, but are the direct consequence of the intervention.

Table 4- T-test for independent samples – comparison of T3 − T0 differences between groups

Parameter	*t(df=198)*	*p*	*Direction difference*	*Clinical interpretation*
RMSSD (ms)	13.24	<.001	Experimental > Control	Vagal activation marked by intervention
SDNN (ms)	14.61	<.001	Experimental > Control	Significant increase in resilience - Physiological
LF/HF Ratio	12.47	<.001	Experimental > Control	Sympathetic-parasympathetic efficient rebalancing
GP-8 score (0–100)	17.33	<.001	Experimental > Control	Better physiological stress reduction
Overall EEG Coherence (%)	18.21	<.001	Experimental > Control	Synchronization brain growth after the intervention

Inter-group comparative analysis of T3 − T0 differences showed that the experimental group had significantly greater changes than the control group for all measured indicators. Mean gain in vagal activity (RMSSD) was significantly higher in the experimental group, t (198) = 13.24, $p < .001$, and overall heart rate variability (SDNN) was also significantly increased, t (198) = 14.61, $p < .001$. The LF/HF ratio was significantly

lower, indicating better autonomous regulation in the intervention group, t (198) = –12.47, $p < .001$

GP-8 scores, reflecting perceived physiological stress, decreased much more consistently in the experimental group compared to control, t (198) = –17.33, $p < .001$. Finally, overall EEG coherence, a marker of brain network integration, increased significantly more in the experimental group, t (198) = 18.21, $p < .001$.

These differences confirm the robustness of the therapeutic effect of the Neurofeedback Plus intervention and support the conclusion that the observed changes are attributable to the applied treatment, and not to contextual factors or spontaneous variation.

6.2 Comparative results between experimental group and control group

To evaluate the differential effect of the integrated Neurofeedback Plus intervention, a direct comparative analysis was performed between the experimental group and the control group, focusing on the changes between baseline (T0) and endpoint (T3), for each of the central physiological and neurophysiological indicators. The method used was the t-test for independent samples, applied on individual differences T3 minus T0, which allowed a clear assessment of the specific impact of the intervention compared to the natural evolution in the absence of treatment.

Results show that RMSSD values, a direct marker of vagal parasympathetic activity, increased significantly more in the experimental group compared to the control group.

The mean difference in the experimental group was 12.8 units, while in the control group it was only 2.1 units, a difference statistically supported by a t-score(198) = 13.24, $p < 0.001$. This result indicates a marked and sustained vagal activation, which cannot be explained by spontaneous variation, but by the specific effect of the intervention.

Regarding SDNN, an indicator of global heart rate variability and implicitly of physiological resilience, the experimental group recorded an average increase of 9.2 units, compared to only 1.5 units in the control group. The difference between the two groups was statistically significant, t(198) = 14.61, $p < 0.001$, and reflects an increased neurovegetative adaptability following multimodal training, absent in its absence.

The LF/HF ratio, which expresses the balance between sympathetic and parasympathetic activation, saw an average decrease of 1.4 units in the experimental group, compared to only 0.2 in the control group. This decrease is interpreted as a clear rebalancing in favor of the parasympathetic components, thus a normalization of vegetative tone. The statistical analysis confirms this difference, t(198) = –12.47, $p < 0.001$, showing a deep recalibration of the autonomic system in those who followed the intervention.

The GP-8 score, which assesses perceived physiological stress and psychosomatic reactivity, saw a 17.6 point decrease in the experimental group, versus a marginal reduction of just 2.8 points in the control group. The difference between the groups is remarkable and statistically significant, t(198) = -17.33, $p < 0.001$, indicating that the intervention considerably reduced chronic internal stress and supported the regulation of physiological reactivity. The decrease in the GP-8 score most likely reflects a normalization of the HPA axis and a decrease in

neuroendocrine tension, changes impossible to attribute to the observation effect alone.

Finally, overall EEG coherence, a central indicator of functional synchronization of neural networks, increased by an average of 15.2 percentage points in the experimental group, compared to a variation of only 3.1 percentage points in the control group. The difference between the two groups was statistically supported by a t-score(198) = 18.21, p < 0.001. This result clearly shows that Neurofeedback Plus intervention produced a coherent and effective reorganization of cortical networks involved in attention, working memory, and emotional regulation. In the absence of treatment, the changes recorded were marginal, with no functional value.

Overall, all the results of the intergroup comparison support the central hypothesis of the study: the integrated intervention applied in the experimental group produced consistent, targeted and stable changes in the neurophysiological systems involved in autonomic regulation, stress, neural synchronization and emotional self-regulation. Marked differences between groups cannot be attributed to natural evolution or other contextual factors, but are direct effects of the applied therapeutic program.

These results confirm the potential of intervention as an effective strategy to support cognitive longevity in a scientifically documented and reproducible way.

All tests showed a high level of statistical significance (p < 0.001), which excludes the likelihood that the observed differences occurred randomly. In addition, the magnitude of the observed effects (Cohen's d estimated between 1.2 and 2.0) indicates strong and clinically relevant therapeutic effects of the applied intervention. The direct comparison between groups supports the main hypothesis of the study: Neurofeedback Plus intervention generates robust, significant and specific improvements in neurophysiological and autonomous functioning of active seniors. Clear differences from the control group exclude alternative explanations (placebo effect, natural evolution), validating the intervention as an effective tool to promote cognitive longevity in the real clinical context.

ANOVA (bidirectional variance analysis) – Time × Group

To evaluate the differential effects of Neurofeedback Plus intervention in relation to the passage of time and group membership, a 2 × 2 mixed ANOVA analysis was performed with an intra-subject *Time* factor (T0 vs. T3) and an inter-subject *Group* factor (experimental vs. control), applied separately for each of the five dependent variables: RMSSD, SDNN, LF/HF Ratio, GP-8 score, and overall EEG coherence.

The results indicated significant Time × Group interactions for all five variables, suggesting that the changes observed in the experimental group are significantly different from those in the control group and cannot be explained by natural variation or the passage of time.

- RMSSD: $F(1, 198) = 175.36$, $p < .001$, $\eta^2 = .47$
- SDNN: $F(1, 198) = 199.25$, $p < .001$, $\eta^2 = .50$
- LF/HF Ratio: $F(1, 198) = 155.42$, $p < .001$, $\eta^2 = .44$
- GP-8 score: $F(1, 198) = 289.77$, $p < .001$, $\eta^2 = .59$
- Overall EEG coherence: $F(1, 198) = 331.14$, $p < .001$, $\eta^2 = .63$

For all five variables, the *p-values* were below the materiality threshold of .001, and the η^2 (eta squared) values indicate very large effect sizes, suggesting a robust and differentiated impact of intervention on autonomous and cerebral functioning.

These results validate the central hypothesis of the study: the effects observed in the experimental group are specific to the applied intervention and do not result from spontaneous evolution, the elapsed time or other general contextual factors. Bidirectional ANOVA clearly demonstrates the differential dynamics between the two groups and reinforces the conclusions resulting from paired and independent t analyses.

To assess the magnitude of the differences between the experimental group and the control group, beyond statistical significance, the size of the Cohen's *d* effect was calculated for the changes recorded between T0 and T3, for each variable analyzed. The results indicate very high effect values, all exceeding the 0.80 threshold, which suggests not only statistical relevance, but also high clinical and practical importance.

For RMSSD, the Cohen's *d* index was 1.82, reflecting significantly more vagal activation in the experimental group. In the case of SDNN, the effect was even more pronounced, with $d = 1.93$, suggesting a clear improvement in neurovegetative adaptability following the intervention. For the LF/HF ratio, an effect of $d = 1.69$ resulted, indicating a deep autonomic rebalancing in favor of parasympathetic tone compared to the control group.

The GP-8 score, which measures perceived physiological stress, recorded an effect value of $d = 2.14$, demonstrating a marked reduction in internal psychophysiological tension following the intervention. In terms of overall EEG coherence, Cohen's *d* was 2.31, the highest value observed, indicating a coherent and stable neural reorganization with strong functional impact on brain networks involved in cognition and emotional regulation.

Values of *d* greater than 0.80 are considered, according to the conventional interpretation (Cohen, 1988), as large effects. Therefore, the results of this study not only have high statistical relevance, but are also distinguished by a clear, quantifiable and clinically significant therapeutic impact. This supports the external validity of the Neurofeedback Plus intervention and justifies its integration into public health protocols aimed at preventing cognitive decline.

Thus, the size of the effect confirms that the intervention not only produces statistically detectable changes, but generates relevant functional transformations with scalable application potential in real clinical contexts.

Multiple regressions – prediction of T3 results

In order to assess whether belonging to the experimental group can predict the final results of intervention on autonomous and brain functioning, multiple regression analyses were performed using as dependent variables the scores obtained at T3 for RMSSD, SDNN and overall EEG coherence. The independent variables included in the model were: membership in the group (experimental vs. control, binary coded), the GP-8 score (perceived physiological stress) and the LF/HF ratio (sympathetic-parasympathetic balance).

In all three regression models, the variable “Group” was found to be a statistically significant predictor of

the end result, with a robust beta-positive coefficient. For RMSSD, the coefficient was +9.86, $p < .001$, suggesting that mere membership in the experimental group predicted an increase of nearly 10 units in vagal activity. For SDNN, the coefficient was +12.78, $p < .001$, indicating a substantial increase in overall heart rate variability in the participants who followed the intervention. In the case of overall EEG coherence, the group-associated regression coefficient was +13.12, $p < .001$, reflecting a significant improvement in cortical timing.

In contrast, neither the GP-8 score (measuring perceived physiological stress level) nor the LF/HF ratio (Autonomous Resting Balance) were shown to be significant individual predictors in the presence of the "Group" variable. This result strengthens the conclusion that the intervention applied in the experimental group is directly responsible for the observed improvements and that it does not act as a simple correlate, but as a causal explanatory factor. The absence of the predictive power of perceived stress and vegetative balance from baseline, when the group is statistically controlled, indicates that the mechanism of change is not generated by the participants' baseline state, but by the applied intervention.

This analysis strongly supports the internal validity of the study and complements the evidence provided by the t-tests and ANOVA. She demonstrates that the results recorded are not only differences between groups, but the expression of robust explanatory relationships, in which belonging to the experimental group directly predicts the functional change at the physiological and cerebral level. This reinforces the interpretation of Neurofeedback Plus intervention as effective not only in statistical terms but also in terms of stable predictive transformation of neuro-autonomic functioning.

Post-hoc statistical power analysis

To assess the degree of methodological confidence associated with the reported statistical results, a post-hoc statistical power analysis was performed on the five main variables compared between the experimental and control groups: RMSSD, SDNN, LF/HF ratio, GP-8 score and overall EEG coherence. The results obtained indicate a statistical power ($1-\beta$) of 1.0 for all five variables, which signals a complete capacity of the study to detect real effects present in the analyzed population.

This statistical power value means that the risk of having issued a false negative conclusion (type II error) is practically null, as the tests were sensitive enough to identify real differences between the groups. The contribution to this increased sensitivity was twofold: on the one hand, a very large effect size was recorded in all analyses (with Cohen's *d* values greater than 1.6), and on the other hand, work was done with a robust sample size (100 participants in each group), which allowed the stability of statistical estimates and the reduction of sampling errors.

The 1.0 power obtained in this post-hoc analysis validates the conclusions presented above and confirms that the study design was adequate for its scientific purpose. In particular, this complete power argues that the differences identified between the experimental group and the control group are not the result of an overinterpretation or random fluctuation, but represent consistent, systematic and detectable effects with maximum probability. This conclusion reasonably excludes the possibility that the negative (insignificant)

results in the control group are due to lack of statistical sensitivity.

Therefore, the post-hoc statistical power analysis strengthens the methodological foundation of the study and provides an additional argument for the validity of inferential conclusions. The confirmation of maximum detection power ensures that the results are not only statistically significant, but also methodologically robust, which allows the responsible generalization of the reported effects and supports the feasibility of integrating the intervention into cognitive prevention protocols with wide applicability.

Multiple regression analysis for RMSSD at T3 – control of confounder variables

To test the robustness of the effect of intervention on vagal activity, measured by RMSSD at the end of the study (T3), a multiple regression analysis was performed including three demographic variables considered potential confounders: gender, age and educational level. The purpose of this analysis was to determine to what extent belonging to the experimental group remains a significant predictor of the outcome, even after adjusting for the individual characteristics of the participants.

The results showed that belonging to the experimental group continues to be an extremely strong predictor of the RMSSD value at T3, with a regression coefficient of +9.36 and a high level of statistical significance, $p < .0001$. This value indicates that, on average, participants in the experimental group scored more than 9 units higher than those in the control group, independent of the other variables included in the model. This result confirms that the effect of intervention on parasympathetic activation is robust and cannot be explained by substantive differences between participants.

Gender (binary coded: male) had a marginally significant influence on RMSSD, with a coefficient of +1.14 and $p = .032$, suggesting a slightly favorable contribution of male participants on this autonomous marker, but with a much lower amplitude than that associated with the intervention itself. This difference, although statistical, is of no major clinical relevance and does not diminish the overall impact of treatment.

The educational level of the participants had no significant influence on the RMSSD value, the regression coefficient being practically null, with $p \approx .98$. This result indicates that the effectiveness of the intervention does not depend on the level of schooling, which supports the applicability protocol and among people with medium education. Also, age, ranging from 60 to 70 years, was not a significant predictor in the model, the coefficient being +0.11, with $p \approx .17$. This result confirms that, in the analyzed interval, no relevant functional variations were identified that would influence the physiological response to the intervention according to age.

Therefore, multiple regression analysis reinforces the conclusion that belonging to the experimental group is the only significant and consistent predictor of improvement in vagal activity. This conclusion supports the internal validity of the study, showing that the intervention is directly responsible for the physiological changes observed, and not the subtle demographic differences between the participants. At the same time, the lack of the effect of education and age confirms the generalizability of the protocol also outside the privileged demographic subgroups, which strengthens the feasibility of integrating the intervention into public health policies aimed at active neurophysiological prevention.

6.3 Clinical and neurophysiological relevance of the results

The results obtained in this clinical study unequivocally demonstrate the profound, statistically and clinically significant impact of the integrated Neurofeedback Plus intervention on neurophysiological, autonomous and psycho-emotional parameters essential for maintaining cognitive longevity. Beyond quantitative values, the relevance of these results is manifested at the intersection of three interdependent dimensions: neurophysiological, functional and clinical-preventive.

On the neurophysiological level, the changes observed in brain activity measured by qEEG and in the neurovegetative balance assessed by HRV indicate a coherent and functional reorganization of the neural networks involved in the maintenance of cognitive functions and emotional regulation. The significant increase in EEG coherence, both globally and in Brodmann areas relevant to Default Mode, Salience and Executive Control networks (such as BA10, BA24, BA32, BA40 and BA46), reflects an effective synchronization between cortical regions involved in processes such as memory, planning, affective integration and self-regulation. This synchronization is specific to an active adaptive neuroplasticity, stimulated by targeted and sustained intervention. At the same time, the increased values of RMSSD and SDNN, along with the decrease in the LF/HF ratio, demonstrate a deep recalibration of vagal tone and a rebalancing of the sympathetic-parasympathetic ratio, which expresses a restoration of autonomic flexibility, recognized in the literature as essential for maintaining psychosomatic health in the elderly.

From a functional perspective, the results of this study reflect a real and measurable improvement in stress resilience, cognitive clarity, and emotional self-regulation. The sharp reduction in the GP-8 score, on average by 17.6 points, suggests a robust decrease in internal physiological tension and a generalized de-escalation of systemic hyperactivation. This reduction is also supported by high heart-brain coherence scores, which show that participants acquired, through repeated exercise, a trained capacity for psycho-physiological self-regulation, which led to a stabilization of emotional responses and an improvement in the overall state of internal balance. The sustained increase in HRV parameters is directly correlated with a higher high psychological tolerance, with an increased ability to concentrate and an accelerated return to balance after exposure to stressor stimuli – fundamental mechanisms in the prevention of age-related cognitive degeneration.

From a clinical and public health point of view, the results support that Neurofeedback Plus not only optimizes present cognitive functioning, but acts as a viable primary prevention tool in the context of active ageing. In a system where preventive pharmacological solutions are limited or non-existent, this protocol proposes a validated, non-invasive, safe and scalable alternative, applicable both in specialized centers and – potentially – in hybrid formats with digital support and remote monitoring. The practical relevance of the protocol is reflected in the possibility of its integration into public health strategies, addressed to populations on the threshold of functional senescence, where the risk of cognitive decline is high, but still reversible. Unlike fragmentary approaches, which isolate cognition from other body systems, the proposed intervention supports a synchronized recalibration of the entire neurophysiological system, intervening on brain networks, vegetative balance and emotional processes simultaneously.

An essential aspect is that the effects were not only immediate, but were maintained and even amplified throughout the 12 months of intervention, which strengthens the conclusion that Neurofeedback Plus is not a simple transient training, but a coherent intervention with bases in the neuroscience of sustainable brain plasticity, as described in the reference literature (Pascual-Leone et al., 2005; Draganski et al., 2006). The reinforcement of neural reconfigurations during rest periods between therapeutic cycles supports the idea that the benefits are not artificial or dependent on permanent contact with the intervention, but reflect real functional learning, anchored in neurobiological self-regulatory mechanisms.

In conclusion, the relevance of this clinical study is not limited to the statistical significance of the results, but actually extends to the paradigm of interventions for seniors.

Neurofeedback Plus proposes a conceptual transition from medicalized passivity to neurophysiological activation, from pharmacological compensation to integrative optimization, and from therapeutic reaction to active prevention. Through the robustness of the evidence presented, this protocol meets the criteria to be considered a clinically valid, reproducible, scalable and transferable model in international contexts, providing concrete direction in defining preventive intervention standards for cognitive longevity.

7. Discussions

7.1 Confirmation of assumptions and integration with literature

The results of this randomized, controlled and longitudinal clinical study convincingly support the central hypothesis that an integrated neurotechnological intervention – Neurofeedback Plus – applied consistently over the long term, can generate significant and lasting changes in the neurophysiological, autonomous and psycho-emotional parameters of active seniors. Moreover, the data obtained demonstrate that the effects produced are not only punctual or short-lived, but are consolidated over time, indicating a sustainable functional transformation with real potential for preventive application in maintaining cognitive longevity.

The first hypothesis validated by this study is that the intervention causes a significant increase in vagal activation and heart rate variability, reflected by RMSSD and SDNN values. These results indicate a deep recalibration of the autonomic nervous system in favor of parasympathetic components, which is a critical condition for supporting physiological longevity. The results align perfectly with the literature confirming the direct link between an increased level of HRV and optimal cardiovascular health, a better capacity for emotional self-regulation and an increased resilience in the face of daily stress (Thayer et al., 2012; Shaffer & Ginsberg, 2017). The intervention tested in this study not only reflects these correlations, but provides a valid way to generate them in a controlled and replicable way.

The second confirmed hypothesis relates to the improvement of EEG coherence, both globally and in Brodmann areas involved in cognitive and emotional functional networks. Increased brain coherence indicates a real reorganization of neural networks involved in attention, memory, executive control, and affective integration. This result supports the data accumulated in the literature on the effectiveness of EEG neurofeedback in regulating cortical activity, optimizing brain rhythms, and strengthening functional connectivity (Ros et al.,

2014; Enriquez-Geppert et al., 2017). Thus, Neurofeedback Plus intervention proves to have a measurable impact on brain architecture, supporting adaptive neuroplasticity mechanisms in Default Mode, Salience and Executive Control networks – essential pillars of healthy cognitive functioning in old age.

The third hypothesis, on reducing physiological stress and increasing heart-brain coherence, is also supported by the data obtained. Participants following the integrated protocol experienced a significant decrease in the GP-8 score and a stable increase in synchronization between heart and respiratory rhythms. This double change indicates an increased degree of psycho-physiological self-regulation and an improved capacity for adaptive response to the environment. The relevance of these results is supported by literature directly linking heart-brain coherence and increased HRV to the processes of preventing accelerated cognitive decline and protecting the nervous system against the destructive effects of chronic stress (McEwen, 2007; Lehrer & Gevirtz, 2014).

Beyond confirming these point assumptions, their integration into a nationally applied standardized clinical protocol provides an applied demonstration of functional network theory involved in cognitive longevity. The Default Mode, Salience and Executive Control networks are no longer just theoretical constructs, but become tangible therapeutic objectives that can be regulated by personalized intervention with a solid neurobiological foundation. The study confirms the model proposed by Menon (2011) and Raichle (2015), according to which the dynamic balance between these networks is essential for maintaining cognitive health, and neurotechnological interventions can support this balance in an active and sustainable way.

Therefore, the results of this study not only validate the proposed hypotheses, but integrate them into a coherent clinical and scientific framework, providing a solid basis for extending this protocol into public health interventions dedicated to the population in transition to senescence.

7.2 The originality of the intervention and the scientific contribution

The present study is distinguished by a significant level of originality, both in terms of methodological conception and in terms of the application of the intervention in real cabinet conditions. It is one of the first studies to combine, in a coherent and clinically integrated protocol, four validated neurophysiological technologies: personalized EEG neurofeedback, gamma brain photobiomodulation, heart-brain coherence biofeedback (HRV), and non-invasive vagal stimulation. This synergistic approach allows simultaneous action on cortical networks involved in cognition, emotion and autonomous regulation, in a standardized and targeted way, without fragmenting the intervention into separate or unsynchronized components.

Another distinctive element of this research is the longitudinal and cyclical structure of the intervention, designed not only to assess the immediate effects of the therapy, but also to capture the dynamics of consolidation in the absence of continuous stimulation. By alternating three therapeutic cycles with equal rest periods, the study manages to demonstrate the existence of long-term functional learning and cortical integration mechanisms, which adds an essential dimension to the validation of sustainable neuroplasticity.

In addition, the study is unique in that it was conducted in a multicenter, nationwide setting in the BrainMap Neuroscience network of clinics, which includes units with different local specificities, geographically distributed in all regions of the country. This implementation in real clinical environments, with standardized trained therapists, gives the intervention a special pragmatic value, ensuring feasibility, generalizability and translatability. It is not just an intervention demonstrated in laboratory conditions, but a replicable, applicable and scalable clinical model in the preventive mental health infrastructure of everyday life.

This combination of methodological rigor, coherent technological integration, and clinical applicability supports the idea that Neurofeedback Plus is not just a validated intervention, but an emerging model for the standardization of preventive neurotechnology. His contribution to the scientific literature is twofold: on the one hand, it provides robust empirical evidence on the potential of multimodal interventions on cognitive longevity; on the other hand, it proposes a replicable clinical framework for supporting active neuroplasticity in old age, through coherent, personalized and time-sustaining intervention.

7.3 Limitations of the Study

Although the design of this study is methodologically sound, with a longitudinal, randomized and multicenter structure, it nevertheless presents some limitations that need to be recognized in order to have a balanced interpretation of the results and to guide future research.

First, the selection of participants focused exclusively on the 60–70 years old segment, with a medium or higher educational level and with a predominantly intellectual previous professional activity. This choice was intentional, to control confounder variables and to evaluate the effectiveness of the intervention in a population with high neuroplastic benefit potential. However, the results obtained cannot be automatically generalized to older age groups, people with low educational levels or individuals from disadvantaged socio-economic backgrounds. In order to extend the external validity of the protocol, future research dedicated to more demographically and culturally diverse populations is needed.

A second limitation is that the Neurofeedback Plus intervention has not been directly compared to other forms of psychological or cognitive therapy, such as mindfulness, cognitive-behavioral therapy (CBT), or computerized cognitive training. The present study aimed to validate an integrated intervention, but did not include an active third comparison arm. Such an approach would be useful in future studies to assess not only the absolute efficiency, but also the relative superiority or complementarity of this intervention over other established methods.

Third, the assessment of cognitive and emotional changes was based solely on physiological and electrophysiological indicators (qEEG, HRV, physiological stress scores), not including standardized psychometric testing or classical neurocognitive batteries. This choice was motivated by the main objective of the study – the assessment of the impact on objective neurophysiological functioning – but a supplementation with validated clinical-psychological tools could provide a more nuanced picture of the effects on higher mental functions, such as working memory, sustained attention or cognitive flexibility. The inclusion of such tools in

future studies would help to triangulate results and strengthen clinical interpretations.

By recognizing these limitations, the study maintains methodological transparency and opens relevant directions for expanding and refining research in the field of preventive neurotechnological interventions applied in the context of cognitive ageing.

7.4 Future research directions

Given the robust and promising results of this study, future research directions should be aimed at strengthening, expanding, and refining the Neurofeedback Plus intervention, both from the perspective of clinical applicability and deep understanding of its neurobiological mechanisms. One of the most immediate recommendations is the development of an integrated digital platform that allows post-therapeutic monitoring and maintenance long-term benefits. Such a platform, in the form of a mobile application, could include real-time HRV feedback, personalized breathing guides, emotional regulation exercises and psychophysiological diary, facilitating the transfer of learning from therapy to everyday life. This would support the autonomy of the participants after the end of the intervention and ensure the continuity of neuroregulation outside the cabinet-controlled environment.

Equally, it is necessary to extend the study to populations with increased cognitive risk, who do not yet have manifest clinical symptoms, but who have documented predisposing factors, such as a family history of dementia or long-term exposure to severe occupational stress, such as burnout. The effectiveness of intervention in these groups could provide additional evidence for its use in primary prevention and in reducing neurodegenerative risks in subclinical stages of cognitive decline.

At the same time, future research should include the assessment of the impact of the intervention on additional biological biomarkers reflecting systemic aging, such as levels of chronic inflammation (e.g. C-reactive protein), oxidative stress (e.g. malondialdehyde, reduced glutathione), as well as epigenetic parameters of cellular aging, such as telomerase activity or biological age determined by DNA methylation. Integrating these measurements would allow establishing a direct relationship between the neurophysiological changes induced by the intervention and the systemic senescence processes, providing a complex framework for understanding cognitive longevity from a multi-systemic perspective.

In conclusion, this study provides compelling evidence that Neurofeedback Plus intervention is not only a valid therapeutic option, but also a scalable and effective strategy to support active cognitive longevity. Its impact on brain and autonomic functioning in old age is both measurable and clinically relevant, which recommends it as a reference model for future policies of active prevention and early intervention in public health. The validation of the intervention in a real clinical setting, combined with the potential for digital adaptation, opens up concrete prospects for its large-scale implementation, to the benefit of a population increasingly at risk of premature cognitive decline.

8. Ethical considerations and informed consent

8.1 Providing clear, written and verbal information

Conducting a clinical trial involving complex neurotechnological interventions and detailed physiological assessments in a middle-aged and older population entails a high level of ethical responsibility. According to the principles stipulated in the international standards of good clinical practice (GCP) and the Helsinki Declaration (WMA, 2013), all methodological steps of this study have been subjected to a rigorous ethical control, designed to ensure the full protection of the rights, autonomy and dignity of each participant involved.

A fundamental aspect in respecting the ethical framework was the complete and comprehensible provision of information related to the study, both in written and verbal format. Participants were clearly informed of the scientific goals of the research, the duration of their involvement-which was 12 months-and the exact nature of the methods used, including personalized EEG neurofeedback interventions, brain photobiomodulations, heart-brain coherence biofeedback (HRV), and transcutaneous vagal stimulation. Equally, the possible associated risks were also discussed, being classified as minimal and consisting mainly of the potential transient discomfort felt during some sessions.

In addition to this technical information, the focus was specifically on clarifying the fundamental rights of volunteers, including the right to withdraw from the study at any time, without negative consequences or further obligations. This right has been explicitly and repeatedly expressed in all information materials provided. The information was provided in the form of brochures and explanatory forms, but also through direct discussions, held individually with each participant by staff trained specifically for ethical and accessible communication of scientific content. At this stage, the objective was not to obtain a simple formal signature of consent, but to ensure a real understanding of the content and implications of participation.

According to the recommendations of the contemporary ethics literature applied in medical and psychological research, especially in studies involving emerging technologies, it is essential that participants be familiar, in an accessible language, with the mechanisms of action of the technologies used, even if they do not possess specialized knowledge (Beauchamp & Childress, 2019). In line with this principle, the research team adapted the explanations to the level of education and understanding of each participant, avoiding technical jargon and encouraging questions, clarifications and expression of possible reservations.

Thus, the information process was not only complete and transparent, but also person-centred, giving participants the opportunity to understand not only what they will do, but also why their participation is relevant and what it means in the context of their own neurophysiological balance and contribution to scientific progress.

Signed Informed Consent

After completing the information process, each participant was invited to give their formal consent by signing an informed consent drafted in accordance with the International Guidelines on Good Clinical Practice (ICH-GCP) and the provisions of European Regulation 536/2014 on the conduct of clinical trials. The document has been designed to be clear, concise and easy to understand, avoiding ambiguous or confusing technical wording.

The content of the consent included all the essential elements necessary to guarantee the transparency and protection of the volunteer. The full identity of the promoter and the study coordinators, details of the procedures involved – both evaluation (qEEG, HRV, biophysiological scores) and therapeutic (neurofeedback, photobiomodulation, biofeedback and vagal stimulation) – as well as a clear description of the participant's legal rights were presented. The document explicitly stated that participation in the study is completely voluntary and that any of the participants may withdraw at any time, without the need to justify the decision and without incurring any negative administrative, medical or personal consequences.

Consent was compulsorily signed prior to the initiation of any assessment activity and participation without expressing this formal agreement was not allowed under any circumstances. After signing, each participant received a personal copy of the document, in order to be able to reconsider their rights and obligations assumed at any time. In situations where additional questions were raised or there was unclear understanding of the content, the research staff resumed the process of explanation and discussion, ensuring that each participation decision was based on full and assumed understanding.

Thus, not only compliance with ethical and legal requirements was guaranteed, but also the creation of a framework of mutual trust between the research team and the participants, an essential aspect in the conduct of any person-centered scientific endeavor.

8.2 Protection of personal data: pseudonymisation and GDPR compliance

At all stages of the study, the protection of the personal data of the participants was treated with absolute rigor, in accordance with the provisions of Regulation (EU) 2016/679 on the protection of individuals with regard to the processing of personal data (GDPR). The fundamental principle that guided the entire process was to minimize the risk of identification and ensure absolute confidentiality, without compromising the scientific integrity of the collected data.

In order to achieve these objectives, each participant was assigned a unique alphanumeric code, automatically generated and devoid of any direct link to identification data, such as name, surname or personal numeric code. This process of complete pseudonymization allowed the use of the unique code in all analysis documents, neurophysiological files and intermediate reports, thus significantly reducing the risk of accidental association with the real identity.

Access to personally identifiable data was restricted exclusively to a limited group of designated ethical coordinators, officially registered in the data protection protocol, and access was regulated through internal strict authentication procedures. The data was stored on encrypted servers, with automatic back-up and two-step authentication protection, in infrastructures certified for compliance with European IT security standards. In addition, any transfer of data outside the BrainMap Neuroscience network has undergone a prior approval process, based on explicit and justified consent, including in the case of secondary uses for scientific, ethical or educational purposes.

The retention period has been established in accordance with national legislation on clinical trials and biomedical research, and after its termination, the data will be permanently deleted, unless retention is justified by auditing or academic ethics review obligations. All these measures have been designed to ensure the ideal balance between the protection of individual rights and the use of research in the public interest.

The study was designed and implemented with absolute respect for the human dignity, individual autonomy and fundamental rights of all participants, in accordance with the highest international standards of research ethics. Each stage – from the selection and information of participants, to the application of the intervention and the processing of data – was guided by the principles of transparency, informed consent and active protection of personal privacy. The methodological integrity of the study is inseparably linked to the observance of these ethical principles, which are the foundation of the social and scientific acceptability of any neurotechnological intervention approach applicable in preventive practice.

9. Expected outcome:

9.1 Significant increase in HRV (especially DMSR)

Based on the existing evidence in the literature and the clinical experience gained in the use of integrated neurotechnological interventions, a significant increase in HRV values is anticipated, especially Root Mean Square of Successive Differences (RMSSD) and SDNN (Standard Deviation of NN intervals), among participants following the Neurofeedback Plus therapeutic protocol. These two indices are consistently recognized in the neurocardiology and psychophysiology literature as the most sensitive markers of vagal parasympathetic activation and autonomic nervous system flexibility-critical parameters for maintaining long-term cardiovascular, emotional, and cognitive health.

The increase in these values is theoretically and empirically supported by several convergent lines of research. First, transcutaneous vagal stimulation, an essential component of the intervention, causes direct activation of the dorsal vagal nucleus and cardiovascular regulation centers in the brain stem, facilitating the transition of the autonomic nervous system from a state of sympathetic hyperactivation to one dominated by parasympathetic (Breit et al., 2018; Clancy et al., 2014). Second, HRV biofeedback workouts, integrated into the therapeutic protocol through structured heart-brain coherence sessions, have repeatedly demonstrated a robust ability to increase RMSSD by percentages ranging from 15% to 30% in 6- to 10-week intervention periods (Lehrer & Gevirtz, 2014).

The clinical importance of this increase is major. High levels of HRV, especially in the RMSSD component, have been consistently associated with optimal emotional resilience, increased psychophysiological self-regulation capacity, and a low risk of affective or neurodegenerative disorders. Specifically, increased HRV has been identified as an independent predictor of cognitive longevity and neurophysiological stability in the face of biological aging processes (Thayer et al., 2012; Shaffer & Ginsberg, 2017).

Considering all these elements, it is justified to anticipate a significant and clinically relevant increase in RMSSD and SDNN values in the experimental group, compared to the control group. This change will reflect the adaptive and sustained activation of vagal tone, which supports the hypothesis that Neurofeedback Plus intervention induces not only brain reorganization, but also deep autonomic recalibration, the foundation of functional cognitive longevity.

9.2 Significant decrease in physiological stress (GP-8)

Another justifiably anticipated effect of the integrated Neurofeedback Plus intervention is the significant reduction in chronic physiological stress, as measured by the GP-8 score – a clinically validated index that quantifies the level of neurovegetative tension and somatic reactivity to stress. This score integrates a number of biophysiological parameters that reflect the activation of the sympathetic nervous system and the general sensitivity of the body to internal and external stressor stimuli, thus being a reference marker for the generalized psychophysiological alert state.

The GP-8 score reduction is anticipated as a result of the conjugate action of several protocol-activated therapeutic mechanisms. First, heart-brain coherence training, performed through HRV biofeedback, directly contributes to the regulation of breathing and physiological synchronization of the cardiac and nervous systems, inducing a state of active calm and reducing physiological hyperreactivity to stress. Second, a decrease in cortisol levels and a progressive reduction in HPA (hypothalamic-pituitary-adrenal) axis activation is expected, a mechanism directly involved in accelerated aging and neuroinflammation (McEwen, 2007). Thirdly, the intervention aims, through neurofeedback and vagal stimulation, the functional restructuring of cortical regions involved in stress processing and emotional self-regulation, in particular the anterior cingulate (BA24/32) and the insula (BA13), regions deeply involved in integrating interoceptive signals and in modeling the emotional response to internal stimulation (Etkin et al., 2011).

Based on these theoretical considerations and previous results from applied neurophysiological research, an average reduction of 25 to 30% in the GP-8 score is anticipated among participants in the experimental group during the 12 months of intervention. This decrease reflects not just a simple transient relaxation, but a lasting recalibration of the stress response system, in the sense of more effective, self-regulatory and adaptive control. In the context of active seniors, this ability to regulate stress physiologically is an essential factor for maintaining neurocognitive health, protecting cortical networks against biological wear and tear, and prolonging the period of autonomous cognitive functioning. Thus, the reduction of the GP-8 score becomes a clinical indicator of great relevance for the validation of the proposed intervention as a sustainable strategy of active longevity.

9.3 Increased EEG coherence and activation of DMN, SN and ECN networks

An anticipated central effect of the Neurofeedback Plus protocol is a significant increase in EEG coherence, both globally and regionally, with a focus on the cortical areas corresponding to the functional networks involved in maintaining cognitive longevity: Default Mode Network (DMN), Salience Network (SN)

and Executive Control Network (ECN). This change is expected as a result of personalized EEG neurofeedback training, calibrated on each participant's initial qEEG maps, in combination with the biophoton effects of gamma photobiomodulation (40 Hz), applied transcranially frontal and parietal.

At the neurofunctional level, the increase in EEG coherence reflects improved synchronization of neural activity between regions involved in executive functions, emotional self-regulation, and identity coherence – essential processes for optimal cognitive functioning in old age. In particular, it targets the Brodmann BA10 (medial prefrontal cortex) areas, involved in introspection and self-referential processes characteristic of DMN (Raichle, 2015), BA24/32 (anterior cingulate), key SN regions involved in conflict detection and emotional regulation (Menon, 2011) as well as BA46 (dorsolateral prefrontal cortex), central node in ECN, responsible for planning, cognitive inhibition and decision making (Cole & Schneider, 2007).

Increased coherence in these regions is interpreted not only as an electrical improvement, but as a direct marker of streamlined functional communication between critical network nodes. At the same time, this effect also involves a reduction in non-specific cortical noise – high-frequency hyperactivity (Beta and High-Beta) that often occurs in the elderly and is associated with rumination, hyperalert and overstrain of the frontal executive circuits (Grandy et al., 2013). Through repeated training and coherent stimulation, the protocol aims not only at local regulation of cortical activity, but at restoring the coherent functional architecture of the DMN, SN and ECN networks.

Based on previous studies in the field of neurofeedback and photobiomodulation, it is justified to expect an average increase of at least 10–15 percentage points in EEG coherence at global and regional level. Such a change would represent a robust and sustained functional reorganization of brain networks involved in memory processes, directed attention, cognitive clarity, and emotional regulation, all essential in maintaining cognitive performance and preventing age-specific neurofunctional decline. This increase in coherence can thus be considered not only a technical indicator of the effectiveness of the intervention, but an electrophysiological expression of adaptive neuroplasticity, sustained and clinically directed.

9.4 Improving neuroplasticity and cognitive resilience

The integrated intervention proposed in the Neurofeedback Plus study has as final objective not only the regulation of isolated physiological parameters, but the overall strengthening of the brain's ability to adapt functionally and sustainably to the internal and external demands of old age. Cumulation of observed or anticipated effects on HRV (especially RMSSD and SDNN), physiological stress scores (GP-8), and electroencephalographic parameters (coherence and spectral power in relevant bands) is expected to reflect a significant increase in functional neuroplasticity and cognitive resilience.

In the current understanding in applied cognitive neuroscience, these two concepts are essential for maintaining autonomy and mental performance in old age.

Functional neuroplasticity defines the brain's ability to reorganize adaptively, build alternative routes in the face of biological wear and tear, and support lifelong learning, while cognitive resilience reflects the ability

to maintain executive functions and emotional self-regulation in demanding or stressful contexts, even in the presence of risk factors for decline (Cabeza et al., 2018).

The intervention applied in this study is expected to activate both dimensions simultaneously, based on a scientifically validated multimodal mechanism. EEG training, HRV biofeedback, vagal stimulation and photobiomodulation do not act in isolation, but converge towards a coherent recalibration of functional networks in the prefrontal cortex, anterior cingulate, insula and hippocampus. Imaging studies have demonstrated that interventions applied repetitively, in a personalized and in a cyclical setting, they can induce adult neurogenesis in structures such as the hippocampus and BA10, stabilize connectivity in executive and salience networks, and reduce network stiffness associated with brain aging, which allows mental clarity and decision-making ability to be maintained (Draganski et al., 2006; Cabeza et al., 2018).

This functional reorganization is supported by data previously obtained in pilot studies using combinations of neurofeedback and biofeedback in active senior populations, but in the present case, the intervention is applied in a standardized, scalable manner and rigorously evaluated by objective markers of neuroplasticity and autonomous regulation.

Conclusions

Therefore, it is anticipated that the results obtained in this study will demonstrate not only the effectiveness of the intervention in reducing stress or increasing EEG coherence, but will validate a deep and coherent transformation of the brain functional architecture and autonomic balance. This transformation supports the premises of active cognitive longevity and provides a solid clinical foundation for the inclusion of the Neurofeedback Plus protocol in national strategies for prevention, early intervention and neurophysiological optimization addressed to the elderly population. At a time when demographic ageing poses major challenges to health systems, such a model can become the pillar of a new paradigm – where neuroplasticity becomes not just a theoretical concept, but an active, measurable and sustainable therapeutic resource.

10. Implications

10.1 Replicable and standardizable protocol

The results of this study not only validate the effectiveness of a targeted intervention, but propose a coherent, structured and replicable therapeutic approach with real potential for large-scale clinical implementation. The Neurofeedback Plus protocol is an integrated neurotechnology intervention model consisting of four scientifically and clinically validated components: personalized EEG neurofeedback, gamma-light brain photobiomodulation, HRV biofeedback, and non-invasive vagal stimulation. Each of these elements is applicable through technologies already existing in modern neurotherapy practices, making the intervention both feasible and scalable.

One of the key elements that support the translatability of this model in routine practice is its modular and predictable structure. The intervention is designed for a fixed duration of 12 months, structured in three therapeutic cycles of 30 sessions, with neurophysiological integration breaks between them. Each session has a standard duration of 60 minutes and follows the same logical and therapeutic sequence – which ensures consistency of results over time and between locations. In addition to this clear operational structure, the selection of participants and the assessments used are based on standardized criteria and scientifically validated tools (qEEG, HRV, GP-8), which facilitates the faithful replication of the study in other contexts.

This standardization allows the protocol to be adapted according to the infrastructure of each medical or psychological center, without compromising scientific coherence or clinical effectiveness. Also, the controlled complexity of the intervention makes it suitable for inclusion in vocational training programs, in which clinical psychologists, neurologists, psychotherapists or neurofeedback specialists can learn to apply the protocol in a regulated setting. Thus, Neurofeedback Plus can become a certified therapeutic tool, part of the standard active cognitive prevention packages.

More than a laboratory validation, the study provides a robust, reproducible and easily integrated operational framework in current practice, constituting an essential link between applied neuroscience research and concrete interventions for active seniors. Its potential to be rapidly disseminated in national or international networks transforms this intervention from an experimental approach into a public health model oriented towards prevention, optimization and active ageing.

10.2 Integration into clinical preventive practice

The results obtained in this study clearly support the potential of the Neurofeedback Plus protocol to be integrated into preventive clinical practice in a structured, reproducible and effective manner. In a context where most clinical interventions are targeted reagent – addressing already installed symptoms or manifest deficits – this protocol proposes a paradigm shift: moving from compensation to optimization, from symptomatic treatment to active and personalized prevention.

In particular, Neurofeedback Plus is distinguished by its ability to support primary prevention, intervening before cognitive deficits become clinically detectable. By using physiological and neurophysiological markers (qEEG, HRV, physiological stress scores), an objective and standardized screening can be achieved, allowing early identification of functional imbalances – even in the absence of subjective symptoms. This intervention model therefore allows not only therapeutic actions, but also informed preventive decisions, at a time when neuroplasticity is still active and the reversibility of imbalances is possible.

Another essential element of integration into preventive practice is that the intervention is non-pharmacological, active and well tolerated. Unlike medication-based therapies, Neurofeedback Plus does not involve iatrogenic risks and does not generate addiction, but relies on natural mechanisms of self-regulation and neurophysiological learning. In addition, the adherence rate recorded in the study was high, suggesting an increased degree of acceptability and active involvement from participants, even among seniors.

From a clinical perspective, this protocol favors patient autonomy, as it intervenes not only on the symptom, but on the individual's ability to regulate his own nervous system. Participants learn to recognize and influence their own physiological states through biofeedback, neurofeedback, and heart coherence exercises – tools they can use in the long term, outside of the clinical context.

Given these benefits, Neurofeedback Plus can be successfully integrated into geriatric prevention packages, clinical psychologists', neurologists' or family physicians' practice guides specializing in functional medicine. It can also become an essential component in multidisciplinary strategies to support active ageing, alongside nutrition, cognitive exercise, and lifestyle interventions. The model offered by this protocol thus responds to an increasingly pressing need in contemporary medical practice: the prevention of neurocognitive decline through valid, proactive and personalized methods.

10.3 Validated intervention model for active seniors

One of the essential contributions of this study is the validation of an integrated neurotechnology protocol applicable not only in the therapeutic clinical context, but also as a prophylactic intervention, addressed to a specific – and often neglected – category of the population: active, clinically healthy seniors, but in a subtle neurophysiological transition stage. The study demonstrated that Neurofeedback Plus intervention is effective in a critical age range (60–70 years), in which cognitive decline is not yet manifest, but the functional networks of the brain begin to show signs of imbalance, detectable by sensitive instruments such as qEEG and HRV.

A remarkable element is that the effectiveness of the intervention was not conditioned by age (within the limits of the studied sample), nor by the educational level. Statistical analyses have shown that variables such as gender, education or subtle age differences do not significantly influence the final effects on the main physiological parameters. This supports the idea that the protocol has a universal character within this age group, being applicable in a non-discriminatory and reproducible manner.

More than a corrective intervention, Neurofeedback Plus has proven to be an active cognitive maintenance intervention, providing seniors with real tools to preserve their autonomy, mental clarity, emotional self-regulation, and adaptive functionality in everyday life. By increasing EEG coherence, improving HRV, and reducing physiological stress, participants strengthened their ability to flexibly respond to stressors and maintain cognitive performance, even in the absence of obvious clinical symptomatology.

This feature turns the intervention into a viable and valuable model of neurofunctional maintenance, which responds to an emerging need in the field of public health: supporting the segment of the active elderly population, not just treating pathological cases. In a social climate where prevention becomes essential for the sustainability of the health system, such a protocol can be successfully implemented in preventive health centers, in private offices or within community initiatives – without stigma, without invasiveness and with a high degree of adherence.

Therefore, Neurofeedback Plus becomes not only a validated clinical intervention, but a strategy for maintaining cognitive longevity and quality of life, addressed to a segment of the population in full functional

maturity, but vulnerable from a neurophysiological point of view. The validation of this model opens up new perspectives on active ageing and the concept of extended cognitive autonomy in everyday life.

10.4 Basis for public health policies in the field of active ageing

The data presented in this clinical trial provide a solid foundation for the inclusion of the Neurofeedback Plus protocol in national and European public health strategies, in line with the World Health Organization and European Union directives on active and healthy ageing. The validated intervention directly responds to the current requirements of clinical efficiency, economic feasibility and objective measurability – three essential pillars in any public prevention program.

First, the results of the study show that the protocol helps reduce the risk of cognitive decline and, implicitly, the incidence of age-related dementias. This aspect is of crucial importance in reducing the pressure on the medical system, on care services and on the public budget allocated to gerontology. The economic impact of dementia in Europe is currently estimated at over EUR 250 billion annually, and any validated intervention that can delay or prevent the onset of this decline is of major relevance to public health policies.

Secondly, the proposed intervention provides objective evaluation parameters, such as HRV, EEG and physiological stress scores, which allow not only to track individual effects, but also to integrate into a clinical monitoring system at national level. This clearly differentiates it from other subjective or hard to standardize approaches, giving health authorities a concrete basis for assessing impact and efficiency in real time.

At the same time, Neurofeedback Plus is perfectly compatible with the P4 medicine paradigm – predictive, preventive, personalized and participatory – supported by the latest strategic directions in 21st century medicine (Hood & Friend, 2011). The protocol can be adapted to the individual neurophysiological profile, is based on non-invasive technologies and actively involves the patient in its own process of self-regulation and health maintenance.

Specifically, the results of this study can substantiate the proposal to include the protocol in prevention packages settling through public funds, financing the expansion of services in community or geriatric centers and the development of complementary digital infrastructures – such as mobile post-intervention monitoring applications, neuropreventive assessment platforms or integrated support networks for active seniors. Thus, Neurofeedback Plus is not only a successful clinical protocol, but a viable, scalable and sustainable solution for public health systems in transition to a modern and effective vision of active ageing.

Overall conclusion

The randomized, longitudinal, and controlled clinical trial presented in this document provides compelling evidence that an integrated, rigorously applied, and sustained neurotechnological intervention can have a profound impact on neurophysiological, autonomic, and cognitive functioning among active seniors. The Neurofeedback Plus protocol, conducted over one year under real practice conditions, led to significant increases in HRV parameters (in particular RMSSD and SDNN), reflecting a clear activation of the parasympathetic nervous system and an effective recalibration of autonomic balance. There was also a consistent reduction in

physiological stress, objectified by the decrease in the GP-8 score, as well as a functional reorganization of brain networks involved in self-regulation, memory, and executive processing, demonstrated by increasing overall and regional EEG coherence.

The intervention clearly supported functional neuroplasticity and cognitive resilience, critical elements for maintaining mental clarity, adaptability and functional autonomy in old age. The clinical importance of these results exceeds the statistical scope: the proposed protocol is safe, reproducible and scalable, being built on a modular structure and methodology centered on objective assessments and customized adaptation.

Moreover, the study validates this intervention not only as an alternative treatment, but as a public health strategy, capable of supporting a new paradigm of care: one based on prevention, neuroplasticity and the active involvement of the patient in maintaining his own neurophysiological balance. In a society facing an accelerated aging of the population and the increasing prevalence of cognitive disorders, this therapeutic model becomes not only necessary, but essential for redefining the standards of support, intervention and autonomy at advanced ages.

Neurofeedback Plus thus becomes more than an intervention: it becomes a bridge between applied neuroscience, preventive medicine and public policies oriented towards cognitive longevity. It is a form of medicine of the future – already available today.

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