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Theta and Alpha Neurofeedback for Age-Related Cognitive Deficits

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As pessoas têm medo das mudanças.

Eu tenho medo que as coisas nunca mudem.

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ABSTRACT

With the growing life expectancy, the number of elderly people is increasing tremendously worldwide. The progressive decrease of synaptic plasticity and neuronal inter-connectivity, concomitant with neurophysiological and behavioral alterations in the ageing brain, may be delayed by neurorehabilitation. Current approaches used to modify cognitive capabilities are often divided into behavioral training procedures and techniques for direct modulation of neural mechanisms, as neurostimulation or neurofeedback. Neurofeedback, which most of the times is based on electroencephalogram signals, is used to train individuals on learning how to influence their own brain functions based on the online-analysis of the brain activity. However, the potential greater effects of rehabilition through a combined methodology of these two trends are poorly investigated.

In the present study, we wanted to examine the effects of a protocol with neurofeedback training interleaved with neurocognitive tasks. It was hypothesized that the combined approach might have a superior impact on cognitive performance, in comparison with a neurofeedback training alone approach. A protocol for neurorehabilitation covering the two proposed methodologies was developed. It supports Alpha and Theta neurofeedback up-training, and can be interleaved with neurocognitive tasks, namely the n-Back Task (the 1-back and the 2-back versions) and the Corsi Block-Tapping Task (either in forward or in backward order). Then, 10 participants from a Health Care Centre from Braga, aged above 55 years-old, were intervened in a twelve-day protocol with either a neurofeedback-combined cognitive protocol or a neurofeedback-single protocol.

In general, the protocol established appear to induce an enhancement of Alpha and Theta activity as an enhancement in working-memory overall state. However, no clear conclusions could be drawn about the real effects of the intervention due to the small sample size and inter-individual differences.

With a forthcoming increase in the number of participants (with more participants already being recruited and intervened) we hope to better address the potential enhancement effects of the combined approach of behavioral training and neurofeedback, as well as understand the possible explanations in the origin of these effects.

RESUMO

Com o aumento da esperança média de vida, a população idosa tem vindo a aumentar exponencialmente no mundo inteiro, e Portugal não é excepção. O decréscimo progressivo da plasticidade sináptica e da inter-conectividade neuronal, em simultâneo com as alterações neurofisiológicas e comportamentais, decorrentes do envelhecimento do cérebro, podem ser atenuados pela neuro-reabilitação. As actuais abordagens utilizadas para estimular as capacidades cognitivas podem ser divididas entre treino comportamental e técnicas de modulação directa de mecanismos neuronais, como a neuroestimulação e o neurofeedback. O neurofeedback, que na maioria dos casos é baseado em sinais de electroencefalograma, é usado para treinar sujeitos no sentido de uma aprendizagem de como influenciar as suas próprias funções cerebrais com base numa análise em tempo real da actividade cerebral. Ainda assim, um possível benefício acrescido da reabilitação através de uma combinação metodológica destas abordagens não tem sido explorado com afinco.

No presente estudo, procurámos examinar os efeitos de um protocolo de treino de neurofeedback intercalado com tarefas neurocognitivas. Partindo da hipótese de que uma abordagem combinada poderia ter um impacto positivo mais significativo no comportamento cognitivo, quando comparada com um treino de neurofeedback em exclusivo, desenvolveu-se um protocolo para neuroreabilitação abrangendo as duas metodologias propostas. O referido protocolo promove um treino de aumento da potência de Alfa e Teta e pode ser intercalado com tarefas neurocognitivas, nomeadamente a tarefa n-Back (as versões 1-back e 2-back) e a tarefa Corsi Block-Tapping (quer directa quer invertida). De seguida, foram recrutados 10 participantes do Centro de Saúde de Braga, com idade superior a 55 anos, que participaram num protocolo de treino de 12 dias, ou com um protocolo neurocognitivo e de neurofeedback combinado, ou com um protocolo simples de neurofeedback.

Em termos gerais, o protocolo estabelecido poderá induzir um aumento da potência de Alfa e Teta, bem como uma melhoria da memória de trabalho. Salienta-se, contudo, que devido à pequena amostra de indivíduos neste trabalho, nenhuma conclusão exacta acerca dos efeitos da intervenção pode ser retirada.

De todo o modo, com o aumento do número de participantes, esperamos estar aptos a responder e a providenciar um entendimento mais conclusivo acerca dos efeitos de uma abordagem combinada de treino comportamental e de neurofeedback, sem descurar as possíveis explicações na base destes efeitos.

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ABBREVIATIONS LIST

ADHD	Attention Deficit and Hyperactivity Disorder
BCI	Brain-Computer Interface
EEG	Electroencephalogram
fm	Frontal-midline
Fp	Fontral-polar
GDS	Geriatric Depression Scale
Hz	Hertz
ICA	Indepedent Component Anlysis
MMSE	Mini-Mental State Examination
ms	Milliseconds
NC	Neurocognitive
NF	Neurofeedback
NFB	Neurofeedback training blocks
PEBL	Psychology Experiment Building Language
PFC	Pre-Frontal Cortex
s	Seconds
SEM	Standard Error of the Mean
TMT	Trail-Making Test
WCST	Wisconsin Card Sorting Test
WM	Working-Memory

1. INTRODUCTION

1.1. STATE OF THE ART

Continued increases in life expectancy implies fundamental changes in population structure representing an exponential increase in the number of elderly people (Lutz, Sanderson, & Scherbov, 2008; Oeppen & Vaupel, 2002). One out of every ten persons is now 60 years old or above, and this number is expected to increase to one out of five in 2050 (DESA United Nations, 2001). In Portugal, in the past 10 years elderly population (> 65 years old) increased from 16% to 19% (Instituto Nacional de Estatística, 2012).

As a consequence of this population ageing, the burden of age-associated disorders, such as Alzheimer and other kinds of dementia, is also exponentially growing, affecting about 50% of all elderly patients, with a high cost to society and a major impact on family and caregivers (Vicioso, 2002). In 2005, Ferri et al. globally estimated that 24 million individuals were living with dementia, and that this number would double in 20 years (Ferri et al., 2005). Impaired cognitive status is perhaps the single most disabling health condition in seniors and a hallmark of dementia. Poor cognitive abilities have been associated with a number of risk factors including decreased physical activity (Laurin, Verreault, Lindsay, MacPherson, & Rockwood, 2001; Yaffe, Barnes, Nevitt, Lui, & Covinsky, 2001), low levels of education (Callahan et al., 1996; Paulo et al., 2011), the lack of social engagement (Paulo et al., 2011), health conditions such as diabetes and hypertension (Kuo et al., 2005), and the presence of certain pathological and genetic traits (McKeith et al., 1996). Also at academic research, these concerns are reflected in the increasing number of articles on cognitive ageing in the last years (R Cabeza, Nyberg, & Park, 2004). In sum, these population changes are likely to have a profound influence on individuals' lives and society at large.

Therefore, it is of enormous relevance for the actual society to understand how the brain ages and develop strategies to preserve and promote elderly cognitive abilities. The age at which cognitive decline begins still remains subject of much debate (Finch, 2009; Nilsson, Sternäng, Rönnlund, & Nyberg, 2009; Salthouse, 2009). In 2004, there was not any reports on evidence of cognitive losses before the age of 60 (Hedden & Gabrieli, 2004) but in 2012 cognitive decline has been described to be apparent in middle-age (age 45-49) (Singh-Manoux & Kivimaki, 2012). This threshold appears to be very important to behavioral, pharmacological or even neurophysiological interventions designed to delay cognitive ageing trajectories since they seem to have better results if applied when individuals first begin to experience decline. These cognitive intervention approaches are nowadays ever more relevant and attractive and have boosted a market of products aimed at preventing or even reversing the effects of age on cognitive and mental abilities (Rabipour & Raz, 2012).

1.1.1. AGE-RELATED COGNITIVE DECLINE

Ageing is associated with brain structural and physiologic transformations that impair functional abilities. As we age, our physical, physiological, and psychological functions begin to deteriorate, which ends in a progressive loss of capabilities.

Healthy ageing (i.e. elders who are free of overt diseases) has been frequently associated with decreased cognitive performance and alterations in neural features and brain activity. Both cross-sectional and longitudinal studies on cognitive functions in healthy ageing reported a decreased performance on perceptual processing speed (Salthouse, 1996), a reduced capacity of encoding new memories of episodes or facts (Balota, Dolan, & Duchek, 2000), and a deficit in inhibitory processing (Kramer, Humphrey, Larish, & Logan, 1994). Moreover, there are also considerable age-related differences in tasks involving working-memory (WM) (Grady & Craik, 2000; Salthouse, 1994), attention (Connelly, Hasher, & Zacks, 1991; Madden, 1990) and cognitive flexibility (Cepeda, Kramer, & Gonzalez de Sather, 2001; Kramer, Hahn, & Gopher, 1999), all of which categorized in the high level 'executive' functions. Working-memory (WM), i.e. the ability of short-term retention of information, while allowing it to be prioritized, modified, utilized and protected from interference, is an essential feature in human cognition and it is typically reduced in older adults.

Alongside with these behavioral alterations, there are some neurophysiological characteristics altered in the ageing brain, as the proportion of neuron and glial cells, the cortical volume, the blood flow and the synaptic density and neuronal inter-connectivity (Rossini, Rossi, Babiloni, & Polich, 2007). Typically, during healthy ageing, all brain regions experience some loss in white matter integrity (O'Sullivan et al., 2001; Raz et al., 2005) and in white and gray matter volume, with the largest changes happening in the frontal cortex (Fjell & Walhovd, 2010; Salat et al., 2004). These ageing brain changes appear to be concomitant with decreased synaptic density (Terry, 2000) and can be accompanied by changes associated with various neurotransmitters' concentrations, transporters availability and receptors density and with modifications in connections between regions (Hedden & Gabrieli, 2004).

Additionally and consistent with behavioral data, reduced brain activity in older adults has been described during a variety of memory tasks (R Cabeza et al., 2004; Grady & Craik, 2000). However, increased brain activity in older adults, compared with that in young adults, have also been reported associated with a better cognitive performance (Roberto Cabeza, Anderson, Locantore, & McIntosh, 2002). It is thought that this process may be explained by an over recruitment of brain activity to compensate for age-related changes in brain structure and function (Grady, 2008).

Indeed, not only behavior and morphology changes with age, but also brain activity patterns, which can be measured by means of electroencephalography (EEG). EEG can reflect different cognitive, sensory or motor processes and may help to explain what happens in the ageing brain.

1.1.2. ELECTROPHYSIOLOGY OF THE AGEING BRAIN

Concerning the alterations in the ageing brain, studies in patients of Alzheimer Disease (Babiloni et al., 2000) and mild cognitive impairment (Rossini et al., 2007) suggested that patients' temporal and spectral EEG features significantly differ from healthy subjects'.

EEG has emerged as the most important methodology for acquiring brain signals in humans. EEG signals measure bioelectric potentials, recorded from electrodes placed on the scalp, which may echo the spatial-temporally collective activity of large populations of cortical neurons located underneath the sensor position. The signals typically reveal oscillatory activities in specific frequency bands and may reflect neural mechanisms enabling brain communication and cognition (Christoph S Herrmann, Munk, & Engel, 2004). Additionally, EEG is low-cost, robust and potentially mobile, and is its high temporal resolution (usually around few milliseconds) that makes it ideal for cognitive neuroscience of ageing research and for real-time Brain-Computer Interface applications (details in section 1.1.3).

A. EEG and cognition

Several studies have demonstrated a close relationship between increases in theta oscillation (4–8 Hz) and alpha oscillation (8–12 Hz) power and cognitive task performance (Wolfgang Klimesch, 1999; Mitchell, McNaughton, Flanagan, & Kirk, 2008).

Concerning working memory, some reports have shown an increase of theta activity during the encoding and retrieval of information (Karrasch, Laine, Rapinoja, & Krause, 2004; Wolfgang

Klimesch, 1996, 1999). Moreover, specifically frontal-midline theta has been associated with focused attention, sustained concentration (Pennekamp, Bösel, Mecklinger, & Ott, 1994), and higher cognitive functions such as increasing working-memory load or cognitive demands (Grunwald et al., 2001; Jensen & Tesche, 2002).

Regarding alpha band, it has been characterized as being reflective of cognitive functioning in general. Alpha has been associated to attention and binding processes (C S Herrmann & Knight, 2001; Wolfgang Klimesch, 1999) and its power increase is closely related to the successful inhibition of irrelevant information (Werkle-Bergner, Freunberger, Sander, Lindenberger, & Klimesch, 2012). Also, alpha power has been shown to increase with working memory load and to be a good predictor of good working memory performance.

Moreover, Beta waves (12–20 Hz) commonly associated with motor functions are also assumed to be involved in the activation of attentional processes (Fan et al., 2007), memory (Hanslmayr, Staudigl, & Fellner, 2012), and language processing (Weiss & Mueller, 2012). Finally, gamma activity (>30 Hz) may play a 'universal' role in sensory and cognitive processing (Başar, Başar-Eroğlu, Karakaş, & Schürmann, 2000) and has been linked with fluid intelligence (Jaušovec & Jaušovec, 2005; Stankov et al., 2006) and memory functions (Fell, Fernández, Klaver, Elger, & Fries, 2003; Jensen, Kaiser, & Lachaux, 2007; Sederberg, Kahana, Howard, Donner, & Madsen, 2003).

B. Abnormal EEG and ageing

A challenge in cognition-related EEG field has been to understand the brain mechanisms that might underlie a better or a worse performance in the elders.

It has been clearly established that ageing involves a general decrease of EEG activity (Obrist, 1954), although some evidence have been reported too of an abnormally enhanced theta associated with a greater cognitive impairment in patients with either mild cognitive impairment or dementia (Rossini et al., 2007). Furthermore, alpha-peak frequency, which is the individual's dominant frequency in the alpha range, has been negatively associated with ageing and Alzheimer's disease (W Klimesch, Vogt, & Doppelmayr, 1999). The individual's dominant frequency in a given band seems to vary considerably as a function of age, neurological diseases, and brain volume (Wolfgang Klimesch, 1999). Indeed the considerable individual differences in the alpha-peak have led to an individual adjusted frequency procedure with alpha and sometimes adjacent frequencies (Wolfgang Klimesch, Schimke, & Pfurtscheller, 1993).

Recently, our group has studied the associations between ageing and cognitive performance and its markers on EEG – Figure 1. It appears that theta power is sensible to age and performance and that alpha power seems to be more related with age than with performance, decreasing in the elders. The study tried to investigate cognitive decline EEG phenotypes by applying to young and elderly subjects the Wisconsin card sorting test (WCST). In this test, cognitive processes like working memory and rule shifting are evaluated. Both theta and alpha activity seem to be lower in elders and poorer performers (Ferreira & Dias, 2012) .

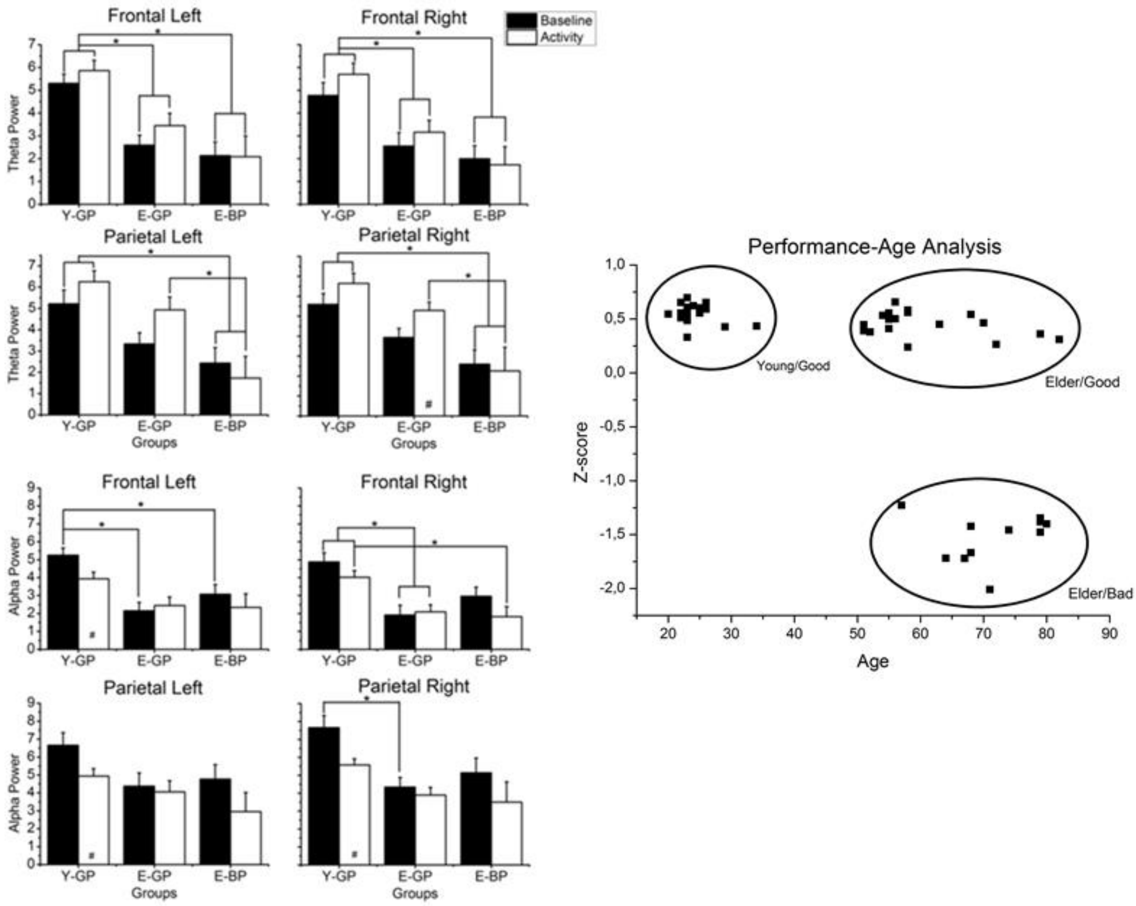

Figure 1. *Performance-Age Power Analysis.* The Wisconsin Card Sorting Test was applied to young and elder participants, to identify EEG phenotypes of deficits on cognitive performance. Three groups were established: young-good performance (Group 1), elder-good performance (Group 2) and elder-bad performance (Group 3). Differences in signal power on theta and alpha rhythms are presented. Adapted from Ferreira & Dias, 2012.

The correlations observed between certain EEG frequency bands and various aspects of cognition led to the conceptualization of neurofeedback (NFB) as an agent of cognitive change. Neurofeedback may be producing effects by enhancing synaptic strength through repeated firing, stimulating neuroplasticity.

Neuroplasticity is the capacity of the human brain, even an elder brain, to reorganize neuronal circuits and to produce new synapses throughout life (Eriksson et al., 1998). Plastic changes in the brain are characterized by neural redundancy and plastic remodeling of brain networking and can occur associated with training, practice or learning (Kolb & Whishaw, 1998) whenever task demands diverge from available capacities (Lövdén, Bäckman, Lindenberger, Schaefer, & Schmiedek, 2010).

1.1.3. BRAIN-COMPUTER INTERFACE AND NEUROFEEDBACK

Traditionally BCI applications are based on recordings of brain activity and aim at produce relevant data that assist human functioning. Applications of BCIs are among others ambulatory monitoring, control and communication devices, gaming and entertainment and even safety and security. However noticeable attention has been paid to BCI applications that address the functional recovery of the central nervous system to repair or improve either physical or mental abilities. Although these applications' context significantly differ from the classic BCIs (Nicolelis, 2001), the NFB-oriented BCI perspective has been suggested as a promising tool to enhance plasticity and able to provide new outcomes for cognitive functional recovery (Daly & Wolpaw, 2008).

Neurofeedback is a biofeedback technique used to train individuals to control or modify their cortical activity through learned self-regulation (Lecomte, 2011). Within a neurofeedback protocol, individuals receive continuous, real-time visual or auditory feedback over their brain activity patterns so they learn to modulate these signals in the desired direction (Heinrich, Gevensleben, & Strehl, 2007), typically up- or down-regulation of one's own brain activity.

An EEG neurofeedback protocol involves brain signal acquisition and recording, data pre-processing, feature extraction and generation and presentation of EEG signals to participants, in a way that they can be capable of modulating or altering their brain activity. Linking this feedback signal to a paradigm, for example, a computer game, it is possible for the subject to learn to control his neuronal rhythms. The success during the game dictates the success on rhythms control (Cohen & Evans, 2011).

The mechanism of neurofeedback is considered as an operant conditioning paradigm (Vernon et al., 2003) that might be able to guide neuroplasticity to an induced change in brain activity which may subsequently promote recovery of brain functions.

A. Neurofeedback clinical efficacy

Neurofeedback has been used for treating epileptic patients (Sterman & Egner, 2006) and for attention deficit and hyperactivity disorders (ADHD) symptoms relief (Butnik, 2005), but also in the context of addictions (Scott, Kaiser, Othmer, & Sideroff, 2005), depression and anxiety (Hammond, 2005), and chronic pain (Middaugh & Pawlick, 2002).

Without doubt ADHD has been the major focus of relevant clinical investigations being considered nowadays as "efficacious and specific" (Arns, de Ridder, Strehl, Breteler, & Coenen, 2009; Lofthouse, Arnold, & Hurt, 2012). Favoring neurofeedback are its long lasting changes in EEG activity (Gani & Birbaumer, 2008) its effects at the level of network connectivity (Ros et al., 2013); and also its comparability to pharmacologic approaches (Meisel, Servera, Garcia-Banda, Cardo, & Moreno, 2013).

Neurofeedback has been employed more recently to improve the physical or cognitive performance of human beings.

B. Neurofeedback in cognition

Based on the associations between alpha and memory, Bauer tried for the first time to study the effect of 4 NFB training sessions of alpha up-regulation on short-term memory in young adults. The results showed an increase of alpha activity but failed to observe a conclusively effect of NFB on memory performance (Bauer, 1976). Nevertheless, more recent neurofeedback procedures have successfully been used to alter participants' alpha activity and thereby increase cognitive capabilities in mental rotation and memory.

In a study of 18 young adults, Hanslmayr and colleagues investigated raising individual upper-alpha power versus reducing theta power in a single-session NFB training. They observed that 5 participants had learned to increase the power of the upper alpha waves, 6 participants had learned to decrease the power of the theta waves and 4 participants had learned to do both. 3 participants were unsuccessful in controlling either. They showed that upper-alpha stimulation improved spatial rotation accuracy while learned down-regulation of theta was not related to neither the behavioral nor the EEG outcome (Hanslmayr, Sauseng, Doppelmayr, Schabus, & Klimesch, 2005).

After that, other studies have been reporting similar results on alpha power NFB protocols. Zoefel and colleagues explored up-training of individual parietal upper-alpha in five daily sessions versus a non-training control group. Successful training was observed in 11of 14 participants, seen by a linear increase across days both in training and baseline EEG amplitude. They reported a rotation ability and upper-alpha amplitude higher due to neurofeedback training effects (Zoefel, Huster, & Herrmann, 2011). Similarly, in another study five consecutive days of parietal and occipital individual upper-alpha band training were compared to a no-training control, showing a better performance on working-memory in 6 out of 9 participants who were capable of NFB modulation (Escolano, Aguilar, & Minguez, 2011).

Working-memory has also been examined as an outcome measure comparing 16 participants who up-regulate central-midline individual upper-alpha with non-training controls (Nan et al., 2012). A memory enhancement correlated positively with an increase in relative alpha power during training but there was no effects in the post-training resting EEG, which they explained by a much less training density and duration compared to that in Zoefel et al., 2011.

Concerning theta, and based on assumptions that frontal theta has potential as a marker of executive functions and cognitive control, up-training of theta power in frontal-midline regions have been investigated and reported as an effective protocol for cognitive enhancement, specifically in attention and working memory.

Vernon et al. (2003) studied the effects of 8 sessions of NFB training on semantic working-memory and visual attention performance, in a protocol of stimulation of theta waves and inhibition of delta and alpha. The observations did not indicate any changes in EEG activity or in cognitive performance. On the other hand, more recently some studies had explored the trainability of frontal-midline (fm) theta in thirty one participants, who were either up-training theta or in a pseudo feedback protocol. The results showed a significantly enhanced fm-theta power only in the proper neurofeedback training group at the end of the training, as well as during the whole course of sessions (Enriquez-Geppert, Huster, Scharfenort, Mokom, Vosskuhl, et al., 2013; Enriquez-Geppert, Huster, Scharfenort, Mokom, Zimmermann, et al., 2013).

C. Neurofeedback in ageing

Despite several studies showing the effectiveness of neurofeedback on cognitive enhancement in young population, few previous studies have provided evidence showing that NFB can improve cognitive function in the elderly (Angelakis et al., 2007; Becerra et al., 2012; Lecomte, 2011; Wang & Hsieh, 2013).

Similarly to Hanslmayr in 2005, in 2007, Angelakis and colleagues studied six elderly individuals (aged 70–78 years) in contrasting neurofeedback protocols targeting peak alpha frequency (PAF) and alpha amplitude. With thirty one to thirty six sessions they concluded that peak alpha frequency training improved the speed of information processing as well as the resistance to interference; and that training the amplitude of alpha improved memory performance. Additionally, some pronounced effects on the EEG of frontal areas, following peak-alpha training, were described.

The implications of this pilot study were followed by Lecomte that studied the up-regulation of upper-alpha while down-regulating theta in 30 participants aged between 65 and 85 years who were assigned to one of three groups: 4 sessions NFB training protocol, a relaxation control protocol, or a no-intervention protocol. Cognitive improvements occurred in all groups but none of the improvements were associated with neurofeedback learning, although it was achieved by over half of the participants. The limited number of sessions of the study were surely too few with elderly participants (Gruzelier, 2013).

Regarding theta, Becerra and colleagues tried to reduce theta in fourteen participants ageing 60–85 years, who had evidence of abnormally high theta. Thirty 30-min sessions were given over 10–12 weeks, with theta power being successfully reduced, resulting in an improvement in EEG and behavioral measures. However, the control group also showed improved EEG values and memory performance.

In contrast, Wang and Hsieh investigated frontal-midline theta up-training in elders (61–72 years) compared with young students. 8 to 12 sessions over four weeks of theta training resulted in improved attention and working memory performance in ageing adults, accompanied by an increase in theta activity in the resting state. In addition, they reported that younger participants also benefited from the protocol in terms of improving their executive function.

Protocols on gamma and beta neurofeedback have also been reporting improved visual processing (Salari, Büchel, & Rose, 2012), enhanced managing of episodic retrieval (Keizer, Verment, & Hommel, 2010) and improved memory and intelligence in elders (Staufenbiel, Brouwer, Keizer, & van Wouwe, 2013), however in this case without a clear relation with improvements.

To complete, a number of exploratory attempts have been made for preserving cognitive functions in the healthy elderly, with the clear conclusion that age does not exclude neurofeedback ability to regulate brain activity.

1.1.4. NEUROREHABILITATION

Neurorehabilitation and mental activity foster the putative promise of enhancing or rehabilitating behavior and brain function (Rabipour & Raz, 2012). Brain training programs are used nowadays to decrease age effects on cognition, by increasing an individual's baseline level so that age-related declines begin to affect daily-life activities later in life ((Hultsch, 1998; Wilson et al., 2002). Such training can produce changes measured at the behavioral as well as at the neuroanatomical and functional levels. There is evidence on brain training effectiveness and durability (Lustig, Shah, Seidler, & Reuter-Lorenz, 2009) and it can be used to improve cognitive function when exercising attention (Rueda, Rothbart, McCandliss, Saccomanno, & Posner, 2005), working-memory (Klingberg, 2010; Klingberg et al., 2005), or executive and associative functions. In addition, extensive training may modify neural structures and functions by increasing gray matter volumes in specific brain regions (Draganski et al., 2004; Scholz, Klein, Behrens, & Johansen-Berg, 2009).

In healthy elderly populations, brain training delay the natural progression of cognitive decline (Buiza et al., 2009; Park, Kwon, Seo, Lim, & Song, 2009). Indeed, reasoning, memory and speed of process can be trained and a 10 1-h sessions seem to be effective in improving performance on elders in the specific trained abilities (Ball et al., 2002; Jobe et al., 2001). More specifically, training appears to improve memory in healthy ageing individuals (Dunlosky, Kubat-Silman, & Hertzog, 2003; Rebok, Carlson, & Langbaum, 2007) and even in individuals with mild cognitive impairment (Belleville et al., 2011) or mild-to-moderate Alzheimer's (Zanetti et al., 1997).

Even if brain training may improve specific cognitive abilities in health ageing, this type of intervention does not appear to improve overall cognitive function and also showed poor results on transferability of tasks for real-life activities (Lustig et al., 2009). Therefore, combination with other approaches are suggested in order to reach higher levels of rehabilitation (Lustig et al., 2009). As we have seen there are now a set of approaches used to alter cognitive capacities, summarized in Figure 2 – one is based on behavioral training procedures and the others on the up- or down-modulation of neural mechanisms by neurofeedback or neurostimulation (directly stimulation of specific brain regions via electrodes that are mounted on the scalp or via a magnetic field delivered by a coil) (Enriquez-Geppert, Huster, & Herrmann, 2013). It has been shown that both neurostimulation and neurofeedback affect the amplitude of cognitive related EEG oscillations (Demos, 2005; Egner, Strawson, & Gruzelier, 2002; Enriquez-Geppert, Huster, Scharfenort, Mokom, Zimmermann, et al., 2013; Hanslmayr et al., 2005; Zaehle, Rach, & Herrmann, 2010;

Zoefel et al., 2011). The combination of approaches may result in the cumulative effects of both, leading to higher benefits. The simultaneous cognitive training and neurostimulation protocol has already started to be investigated in improving numerical and response inhibition abilities (Cohen Kadosh, Soskic, Iuculano, Kanai, & Walsh, 2010; Ditye, Jacobson, Walsh, & Lavidor, 2012) with potential greater effects.

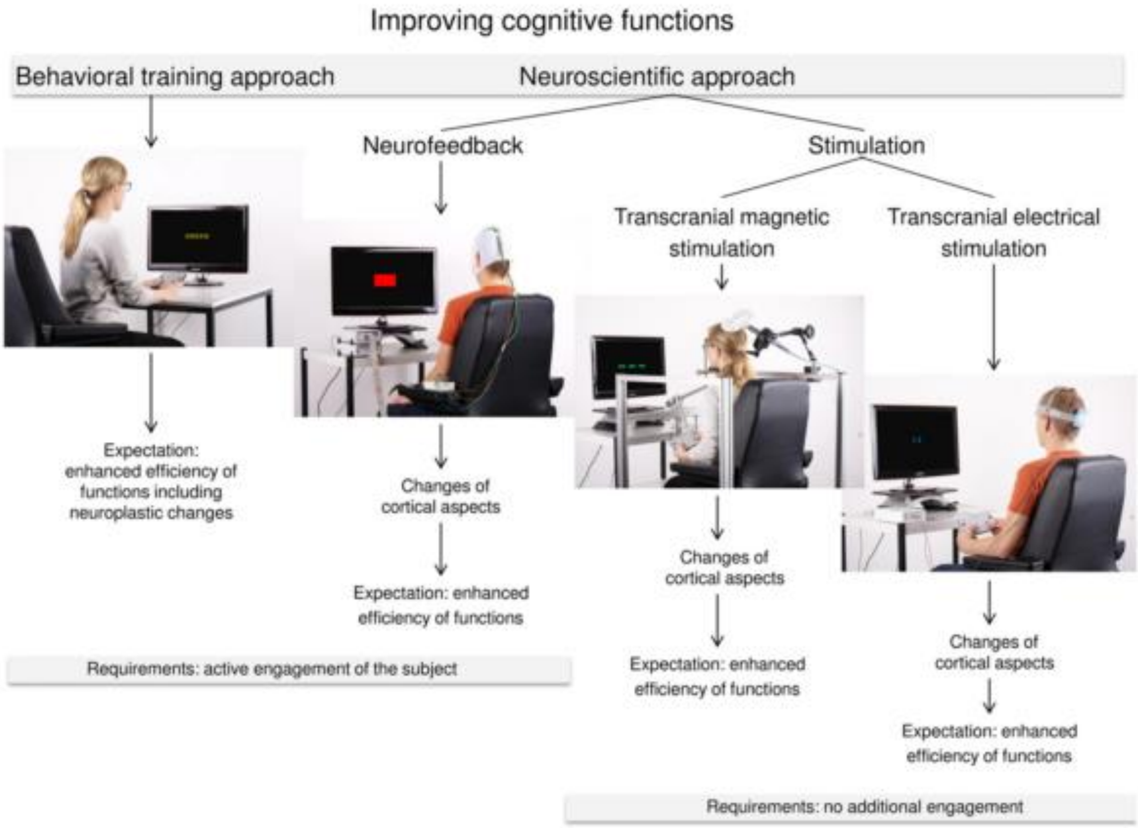

Figure 2. *Training approaches for cognitive rehabilitation.* Behavioral/neurocognitive training, neurofeedback and neurostimulation can be used nowadays to enhance cognitive capacities (Enriquez-Geppert, Huster, & Herrmann, 2013).

1.2. RESEARCH OBJECTIVES

Considering there are few studies in the literature investigating the combined effects on cognitive enhancement in the elderly, and none of them involve neurofeedback technique, the aim of the present study was to examine the effects of a protocol with NFB training interleaved with classic neurocognitive tasks. The central hypothesis is that the combined approach might have a greater impact on cognitive performance, in comparison with a neurofeedback training alone approach.

First, based on evidences that both frontal-midline theta and alpha activity are potential effective parameters for cognitive enhancing in elderly population, and based on proven effects of working-memory training in delaying cognitive ageing decline, a combined protocol that comprised both approaches was developed (Portugal, Ferreira, Reis, Pinho, & Dias, 2013) – Annexes 1.

Then, a twelve-days cognitive intervention protocol for memory training was design, combining neurofeedback training (Alpha and Theta power enhancement) with common neurocognitive tasks (working-memory), and validated on 10 Portuguese participants (> 55 years-old), whom were submitted to one of the two cognitive intervention approaches:

- Experimental Group 1: Neurofeedback (NF) training single-methodology;
- Experimental Group 2: Neurofeedback training and Neurocognitive training (NF+NC) combined-methodology.

Considering that this work is included in a broader project which will also recruit subjects for other experimental groups aiming at controlling both neurofeedback and neurocognitive interventions, the scientific goals of this report are to:

- Identify NF+NC intervention effects on EEG power spectrum, working-memory and cognitive flexibility performances, as a potentiation technique for NF.
- Assess dynamic changes on EEG power spectrum and behavioral measures across training sessions for both experimental groups.

Ultimately we wanted to evaluate, and compare, the alterations induced by both rehabilitation intervention protocols on subjects' cognitive performances. In addition, this study may support investigations concerning theta and alpha enhancement as a promising parameter in neurofeedback for cognitive enhancement.

2. MATERIAL AND METHODS

2.1. PARTICIPANTS CHARACTERIZATION

For this project 10 participants (4 males and 6 females) were recruited from a Health Care Centre from Braga and are community-dwelling individuals living in the Minho Region of Portugal. Only participants without any diagnosed dementia, cerebrovascular or neurological pathology were invited to the study. The participants did not present a high academic level (mean years of schooling: 5,4 years ± 1,68 years) and were unemployed or retired. At the beginning of the experiment they answered some questions about their educational qualifications, current or previous occupation and prescribed medication.

The cohort was established in accordance with the principles expressed in the Declaration of Helsinki and the work approved by the national ethical committee (Comissão Nacional de Protecção de Dados) and by local ethics review boards.

All participants were right-handed and presented normal or corrected vision. The Edinburgh Handedness Test was used to determine if the subjects were either right-handed or left-handed.

All the participants joined voluntarily the study, after they had the experimental nature and protocol procedure explained to them. At the time, all the participants sign a voluntarily informed consent for the use of the collected data.

The participants' cognitive and mood profile was assessed previously to the training protocol by a team of trained psychologists and MD students. Briefly, cognitive state was evaluated using a battery of neurocognitive tests to measure general cognition, attention, learning, short-term memory, verbal memory, cognitive flexibility, verbal fluency and processing speed. Additionally, psychological tests were applied for assessing mood, anxiety and stress profile, personality, functional ability, quality of life and memory perception. Most notably, Geriatric Depression Scale (GDS) and the Mini-Mental State Examination (MMSE) were comprised in this battery. Regarding GDS, participants mean score was 14,8 ± 7,4, which characterize this sample as "mildly depressed" (Yesavage et al., 1983). In the MMSE the participants mean was 19,4 ± 2,32.

Participants within each gender were randomly assigned to either the Neurofeedback (NF) training-group (N = 5, mean age of 61,2 ± 4 years, range: 55– 66 years) or the Neurofeedback and Neurocognitive (NF+NC) training-group (N = 5, mean age of 61,8 ± 5,8 years, range: 55– 67 years).

At the end of the study, all participants completed a questionnaire about their general opinion of the study.

2.2. ELECTROENCEPHALOGRAM ACQUISITION

Two systems were used for the electroencephalogram signal acquisition. They are both based on the international 10-20 system (32 channels standard electrode layout – Figure 3, with ground and reference electrode). EEG signals were acquired with either the QuickAmp®, Brain Products, GmbH or the ActiCHamp®, Brain Products, GmbH.

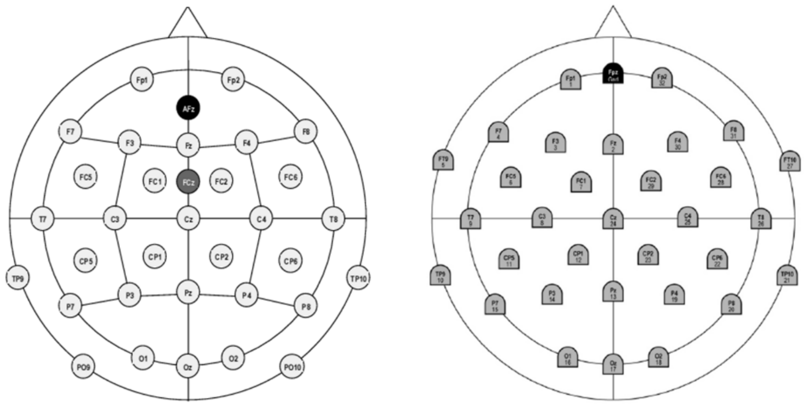

Figure 3: *Electrodes layout for the EEG acquisition.* Left: electrodes layout of the QuickAmp equipment; Right: electrodes layout of the ActiChamp equipment.

The whole system was constituted by: Ag/AgCl active electrodes, a cap for the placement of the electrodes – actiCAP or EASYCAP (Brain Products, GmbH) – electrolyte gel (to decrease the contact impedance between electrodes and the scalp) and straps to keep the cap in place. Ground was located at forehead and reference was FCz channel when using QuickAmp equipment and Cz when using the ActiCHamp equipment.

For each participant, the same equipment was used for all EEG data acquisitions. During recordings, all participants were instructed to not make any movements beyond the required ones and, when necessary, to answer always with the same hand.

2.3. REHABILITATIVE PROTOCOL

All the participants followed a protocol of 12 days accordingly to the diagram in Figure 4.

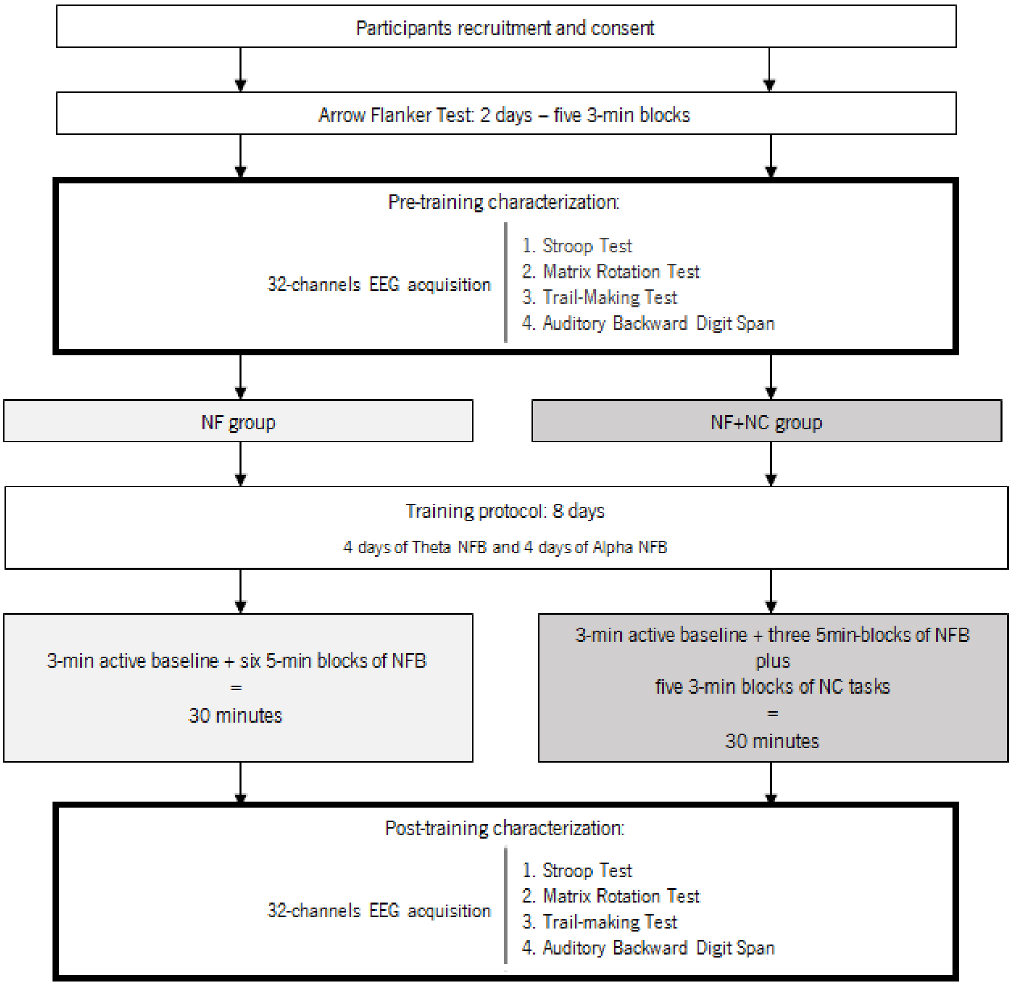

Figure 4. *Schematic diagram of the design and procedure in the present study protocol.*

During the intervention, participants were sited in an illuminated and acclimatized room, distancing 50-80 cm from a 17 inch computer screen, with touch technology. All the moments of the study were conducted in 'Centro Clínico Académico' in Hospital de Braga.

2.3.1. ARROW FLANKER TEST

In the first 2 days of the protocol all participants were submitted to an attention test: the Arrow Flanker Test during approximately 15 minutes (5 blocks of 85 trials). This step aimed at guarantee that all participants presented minimum attention level in order to be able to perform the subsequent requested tasks of the intervention.

The Arrow Flanker Test was implemented in the framework used during the training protocol, the BCI++ (details below). It was adapted from the Flanker Task in the PEBL (Psychology Experiment Building Language) Test Battery and it was used for attention domain assessment. In each trial of the test, the participant is presented with a set of arrows. He has to pay attention to the central target arrow and give a directional response – left or right – within a timeout of 1500 milliseconds. The target arrow can be flanked by stimuli in the same direction (congruent stimuli), in the opposite direction (incongruent stimuli), or not be flanked (neutral stimuli) (Figure 5). A fixation cross is presented before every trial. Measures of mean accuracy, mean response time (RT) and conflict (difference in response time between incongruent and congruent stimuli) are reported at the end of the task. Additionally, measures of accuracy and response time for each stimuli condition can be assessed.

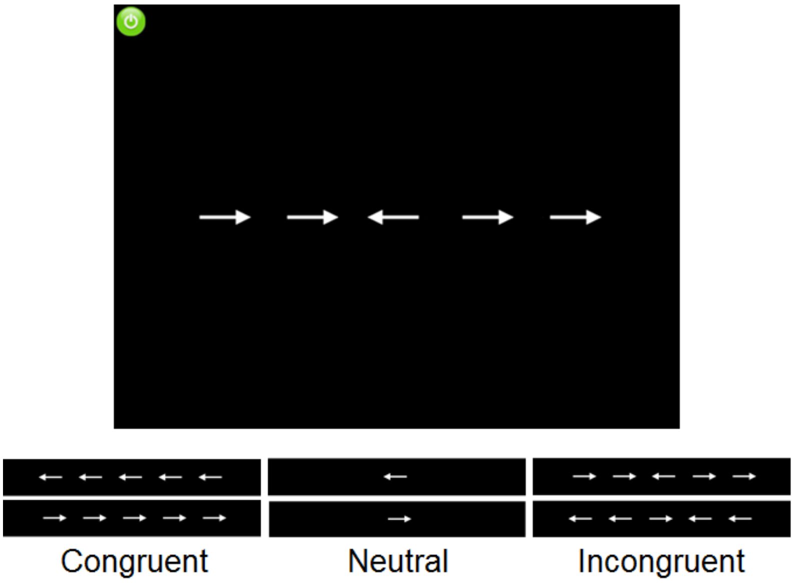

Figure 5. *Representative image of the Arrow Flanker Test implemented in the protocol.* A set of arrows is presented and the participant has to give a directional response to the central arrow (target), in a determined set of time (1500 ms). The type of stiimulus that can flank the target arrow are also represented at the bottom of figure.

Direction responses were given on a modified keyboard, either by pressing a yellow key on the left of the keyboard or a blue one on the right, whether the answer was *left* or *right* direction, respectively.

2.3.2. PRE AND POST-TRAINING CHARACTERIZATION

At the third and at the last day, EEG signals from 32-channel were acquired from all the participants while performing a battery of cognitive tests. This battery was applied to evaluate mainly working-memory and cognitive flexibility. It comprises the Stroop Test, the Matrix Rotation Test, the Trail-Making Test and the Auditory Backward Digit Span Test. The EEG data was used for further power analysis.

A. EEG recordings

During the characterization moments, *OpenVibe* was used for acquiring, plotting and recording the EEG signal (Renard et al., 2010). Also, it was used to implement the synchronization between the EEG data and the software of visual stimulation (PEBL) with markers for offline analysis. The four cognitive tests were preceded by a one-minute length eyes-open baseline, where the participants were instructed to minimize blinking and to stare at the center of the computer screen in grey, the more relaxed as possible.

B. Cognitive battery

PEBL (Psychology Experiment Building Language) was used for stimulus presentation. PEBL is a psychology software for designing and running computer-based tests and experiments, which has ready-made paradigms for assessing cognitive and psychological domains (Mueller, 2010). The four tests used in the battery were already implemented on PEBL Test Battery, and were adapted for the cognitive characterization.

1) *Stroop Test (Stroop):* it is a popular neuropsychological test with different test variants (Lezak, 2004) considered to measure selective attention, cognitive flexibility and processing speed (Lezak, 2004; Strauss, Sherman, & Spreen, 2006). The version applied here consisted of two blocks. On the first one, the participant read random color names (red, green, blue, yellow) printed in colored ink (red, green, blue, yellow) or black ink, ignoring the color of the print – in equal proportions the print color could correspond to the color name (Condition 1), the print color could not match the color name (Condition 2), or the print color could be black (Condition 3). In the second block, the participant had to name the ink color in which the color names were printed

and to ignore their verbal content – similarly to the previous block, stimuli color and reading name could match, stimuli color could not match the reading name or stimuli could consist of groups of letters "xxxx" in a given color. 60 stimuli were presented in each block of the test. Every stimulus must be paired "correctly" for the test to proceed, which means participants may make multiple errors before complete a trial. Four labels with the four color names (red, green, blue, yellow) printed in the matching color ink were visible on the bottom of the screen for responses.

2) *Matrix Rotation Test:* it is a test that evaluates spatial working-memory and mental rotation skills, described in the Unified Tri-Service Cognitive Performance Assessment Battery (UTC-PAB) (Englund et al., 1987). A set of 4 to 4 cell matrices were presented to the participant, each with 4 highlighted yellow cells. The participant was required to compare successive matrices and determine if they had the same pattern, but turned 90 degrees to the left or right, or if they were different matrices, regarding the immediately preceding matrix. Participants decided on the time they needed to memorize the study matrix. There were 10 matrices per condition (same and different). Two labels of response (designated as "same" or "different") were visible on the bottom of the screen.

3) *Trail-making Test (TMT):* it is used to measure attention, speed, and cognitive flexibility. There were two parts of the test, both consisting of 26 circles spread over the screen. In part 1, the circles were numbered 1 to 26 consecutively, and the participant had to connect the numbers in ascending order. In part 2, the circles included both numbers (1 – 13) and letters (A – N), and the participant had to connect the circles in an ascending pattern, but alternating between numbers and letters (i.e., 1-A-2-B-3-C, etc.). The trial finished when all the circles had been successfully clicked in the correct order. Two trials were performed for each part of the test.

4) *Auditory Backward Digit Span (Backward Digit Span):* it is a test for assessing working-memory and consists of a series of trials presenting random digits at the rate of one digit per second. In each trial, the participant had to listen the digits sequence. Then, he was instructed to enter the digits, in the exact inverse order, in a digital keyboard on the screen. The test started with sequence length 3 and had 2 trials at each length. The length increased until the participant failed twice to recollect every digit.

All the responses were given using the computer touch screen. The tests were preceded by practicing. For all the participants, tests were applied in the same order.

2.3.3. TRAINING SESSIONS

Depending on the cognitive intervention group, participants were randomly allocated to the neurofeedback training protocol or to the combined neurofeedback and neurocognitive training protocol.

During the 8 sessions of training, participants were submitted to a 30-minute intervention protocol each day, since this training intensity seems to produce effects on behavior and electroencephalogram measures (Ball et al., 2002; Keizer et al., 2010; Ros et al., 2013), according to their experimental group:

- NF training-group – participants performed only neurofeedback training (NFB) – modulation of theta and alpha rhythm. The 30-minute training period consisted of six 5-minutes blocks of a neurofeedback task, preceded by a 3-min baseline measurement.

- NF+NC training-group – participants performed training of neurofeedback (NFB) – modulation of theta and alpha rhythm – and a set of neurocognitive tasks: *Corsi Block-Tapping Test* and *n-Back Test* (see details below). The 30-minutes training comprised three 5-minutes blocks of a neurofeedback task, preceded by a 3-min baseline measurement, plus five 3-minutes blocks of neurocognitive tasks.

The participants were asked about motivation and interest for attending the sessions prior to the beginning of each session; and were asked about general concentration and train difficulty at the end of it. Exceptional stress or tiredness was documented for posterior analysis.

The 8-days protocol of NFB enhancement was conducted separately for alpha and theta rhythms, each with a duration of 4 days. The initial frequency to be trained was randomly selected for each participant.

For this part of the intervention protocol it was used a software dedicated to the development and fast prototyping of Brain-Computer Interface systems and pc-driven protocols, the BCI++ platform (Maggi, Parini, Perego, Andreoni, & Milano, 2008). BCI++ is divided in two main interconnected modules: one module oriented for the acquisition and real-time processing of the EEG signals, and a second module responsible for providing visual and auditory stimuli to the participant. Both modules have customized blocks for the development and implementation of C++ or Matlab based algorithms and paradigms.

During the training protocol, EEG signals were acquired continuously, sampled at 500 Hz, from the Fp1, Fp2, Fz and Pz channels. Alpha or theta feedback was calculated from the Fz channel and Fp1 and Fp2 channels were used for detection of ocular movements.

A. Neurofeedback training procedure

For NFB training, EEG data was processed in real-time by a custom algorithm developed in C++ and Matlab® (Matlab® Engine).

Every 200 ms, data was filtered and baseline corrected, and Fast Fourier-Transforms (FFT) were computed, using a spectrum estimation function of the Chronux matlab toolbox (Andrews et al., 2008), for calculation of theta or alpha power. The spectrum measurement was based on 1 s data windows (containing the last 200 ms of data and 800 ms of outdated data), which provides the participants with a smooth appearance of the visual feedback and avoids large shifts in feedback.

At first, a baseline measurement of 3 min was recorded, during which the measurement of power spectrum was recorded. At the end, the amplitude of theta and alpha rhythms was calculated in all artifact-free data windows. An average of all power spectrum estimations was used for detection of the individual alpha peak frequency (IAPF). Then, theta and alpha frequency bands were defined from 4 Hz to IAPF− 3 Hz and from IAPF− 2 Hz to IAPF + 2 Hz, respectively. The baseline power, calculated as the mean power of all segments, was then used as a participant-specific reference for the feedback training.

During the training blocks, feedback was given as the ratio of the power amplitude measured at Fz channel to the baseline power amplitudes, updated for each training session. Feedback was updated 5 times per second.

The neurofeedback training task, where visual feedback was provided to the participant about his own brain rhythms, was design with a therapeutic approach in order to keep participants motivated and stimulate them to improve. So, a virtual scenario is presented to the participant, as seen in Figure 6, with a human head, three neuronal cells and a fire. During NFB blocks, the feedback was given by means of a blue bar (symbolizing water) that comes out of the first neuronal cell and must reach the fire. The length of the bar indicated the power amplitude in relation to the baseline measurements.

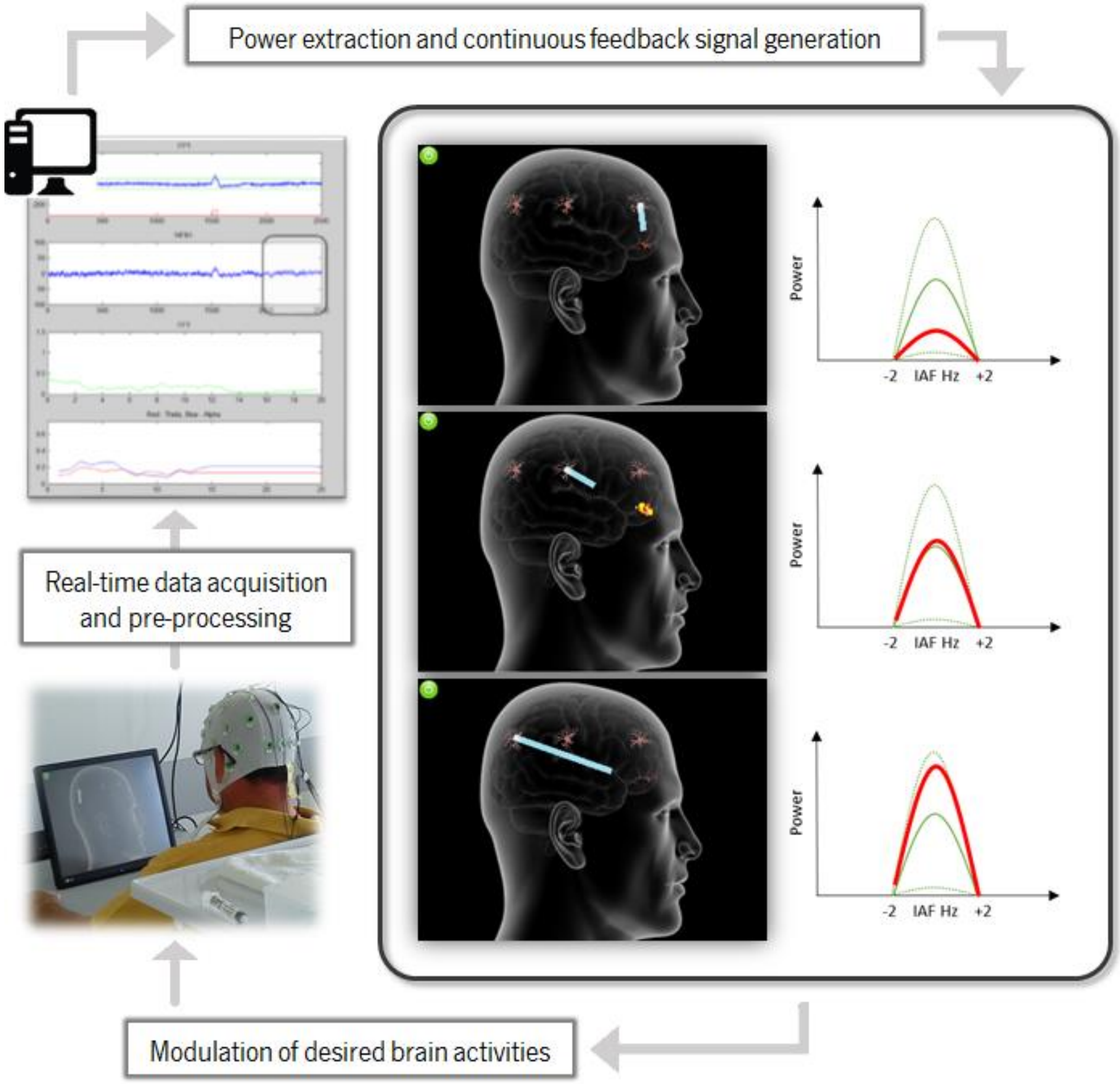

Figure 6. *Representative images of the Neurofeedback task implemented in the protocol.* An online power analysis of the EEG is computed for Fz, updated every 200 ms. Results of this analysis are visually presented as a blue bar (symbolizing water) whose length, depending on the current power amplitude, was displayed as longer, closer to the fire, whenever the power amplitude was increased and the bar turned shorter, further from the fire when the power amplitude was attenuated, in comparison to the individual baseline power measurement. The three levels of the game are displayed. Red lines represent current power amplitude and green lines represent the minimum, medium and maximum reference values – 2,5 percentil, 55 percentil and 97,5 percentil.

Depending on the current power amplitude, the bar length was displayed as longer, closer to the fire, whenever the power amplitude was increased and the bar turned shorter, further from the fire when the power amplitude was attenuated, in comparison to the baseline power measurement.

In the game paradigm, the three neuronal cells indicated the three levels of the game. The length scale covered 95% of the amplitude range measured during the baseline. At the first level, values below the 2,5 percentile were displayed with no bar and full-bright fire, while values above the 55 percentile were displayed with a full length bar and total extinction of the fire. A level update happened when the mean value for the last 10 seconds was above the 40 percentile. At the second level, values below the 2,5 percentile were again displayed with no bar and full-bright fire, while values above the 75 percentile were displayed with a full length bar and total extinction of the fire. At this moment, a level update could happen when the mean value for the last 10 seconds was above the 60 percentile or below the 40 percentile. If the mean value was below 40 percentil, the participant went back to the first level scenario. If the mean value was above the 60 percentil, the participant went to the third level. At this level, values below the 2,5 percentile were displayed with no bar and full-bright fire, while values above the 97,5 percentile were displayed with a full length bar and total extinction of the fire.. The mean value for level adaptation was monitored after 12 seconds in the same scenario level, and afterwards was updated with the same frequency as the feedback.

To assure the comparability of the baseline measurements and the NFB blocks, the virtual scenario of the first level was presented during recording of baseline, with the blue bar randomly changing its length. In order to maintain the subject cognitively active, they were asked to count the number of times the bar approached the fire (Enriquez-Geppert, Huster, Scharfenort, Mokom, Zimmermann, et al., 2013; Zoefel et al., 2011).

Because artifact contaminated feedback signals may influence the neurofeedback learning outcome and since their power unfolds in frequency bands often used for neurofeedback training (Huster, Mokom, Enriquez-Geppert, & Herrmann, 2013), as with Theta, we discard from feedback data windows showing contamination of ocular artifacts, like eye blinks, detected whenever the signal amplitude of the Fp1 or Fp2–Fp1 exceeded an adjustable threshold. In this case, the feedback was suppressed and the length of the bar did not change.

A set of strategies which earlier studies reported as successful were given to the participants. In the first sessions they could test these strategies and find the most effective strategy. The strategies listed were separated in positive (like love, family or friendship), negative (death, diseases or conflicts) or neutral (like visual attention to the bar, mental operations and calculus or breathing). Participants were informed that they could use these strategies favoring an increase of the bar and thus the fire extinction. Also, they were instructed to be concentrated and keep pursuing this goal as much as possible. At the end of each block of training, the strategy used and its effects were reported. Additionally, average measures of theta and alpha power in each block were kept for posterior analyses.

B. Neurocognitive tasks

For the neurocognitive practice, two working-memory neurocognitive tests were implemented: the Corsi Block-Tapping Test, adapted from PEBL Test Battery, and the n-Back Test. Responses to the tasks were given using the touch screen.

1) *Corsi Block-Tapping Test (forward and backward):* is a classic test to assess visual-spatial short-term working memory (Corsi, 1973). The player has to track a sequence of up to nine identical spatially separated blocks being highlighted and reproduce it, either in forward or backward order - Figure 7. The sequence starts with length two (two blocks highlighted), but when the player repeats correctly the sequence, the length is increased in the next trial. On the other hand when the participant repeats wrongly, the length decreases. The task finishes when the participant performs 15 trials. At the end, measures of block memory Span, number of corrected trials, mean length of the sequence and a combined score of memory block span and corrected trials were reported.

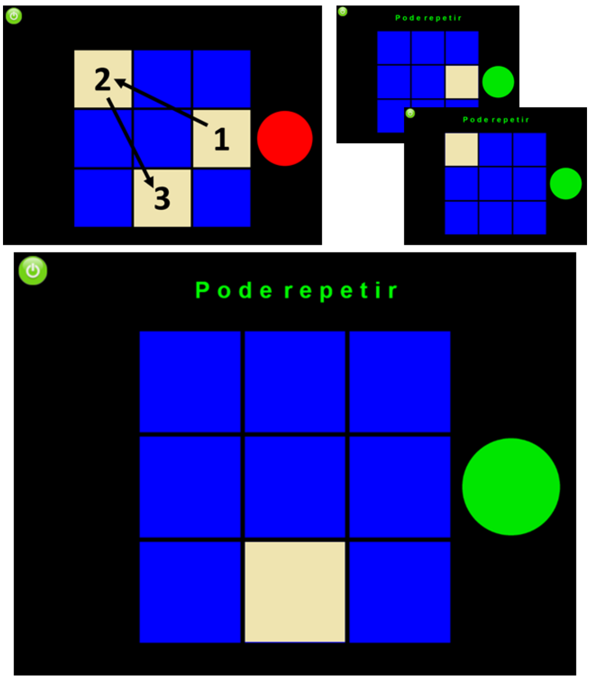

Figure 7. *Representative image of the Corsi Block-Tapping Task implemented in the protocol.* There are 9 blue blocks and a sequence of them is presented to the player, who has to reproduce it. When the player reproduces correctly, the number of the next sequence is increased (up to nine), and when it reproduces it incorrectly the number is decreased.

2) *n-Back Test (1-back and 2-back):* is a commonly accepted task to measure cognitive performance in working memory domain. The participant is presented successively with digits and has to indicate whether or not the current digit matches the one *n* instances before (1-back – the previous digit; 2-back – the digit that appeared before the previous digit) – Figure 8. In the 1-back task, which was originally introduced by Kirchner (1958), the participant only has to evaluate if the current digit is the same as the previous one. In the 2-back task, a variation proposed by Jaeggi et al. (2003), the participant has to remember and compare the current digit to the digit that appeared prior to the previous one (Jaeggi et al., 2003; Kirchner, 1958). Measures of accuracy and reaction time, for every type of stimuli (one-back, two-back, or random) were reported at the end. Each block had 65 stimuli.

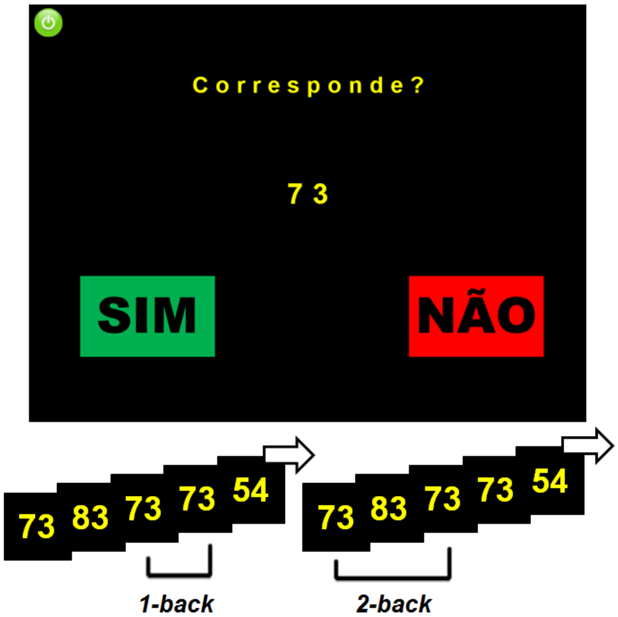

Figure 8*.* *Representative image of the 2-Back Task implemented in the protocol.* A digit is consecutively appearing in the screen, and the player has to warn if it matches the digit presented two times earlier in the game.

2.4. OFFLINE SIGNAL PROCESSING

Analyzer 2®, Brain Products, GmbH and Matlab, The MathWorks, Natrick, USA were used for offline EEG processing and power analysis.

At first, all raw signals were filtered with a notch filter to reject the 50 Hz band, and a *bandpass* filter from 0,3 to 100 Hz.

2.4.1. 32-CHANNELS EEG CHARACTERIZATION

An algorithm for correction of ocular artifacts based on independent component analysis (ICA) (Hyvärinen & Oja, 2000) was applied, and segments with motor artifacts were removed by rejecting segments of the signal based on threshold criteria of signal amplitude , difference between the maximum and minimum in a time envelope and signal gradient. The adequate thresholds were adjusted for every session and every task.

In order to increase the independence of the signals on neighbouring electrode locations, the current source density (CSD) method was applied on the data. In this way, the channels generated did not have any specifications with respect to the channel reference (Makeig, Jung, Bell, Ghahremani, & Sejnowski, 1997).

From the 32 electrodes recorded, only Fz, F4, F3, FC2, FC1, FC6 and FC5 were used to power spectrum estimation. The last 6 channels were selected to observe hemispheric lateralization.

At the end, EEG signals were segmented. The signal of every cognitive test was separated in Baseline (EEG acquired during resting state) and Activity (EEG acquired during task performance), and these were divided in segments of 1 s length with 0,8 s overlap. A baseline correction was applied in each segment.

2.4.2. EEG RECORDINGS OF TRAINING SESSIONS

Segments with ocular and motor artifacts were removed by rejecting segments of the signal based on threshold criteria of signal amplitude , difference between the maximum and minimum in a time envelope and signal gradient. The adequate thresholds were adjusted for every session and block. Only the Fz channel were used to calculate the power spectrum.

For the neurocognitive blocks, the EEG data was separated in Baseline and Activity.

Then, all EEG signals were divided in segments of 1 s length with 0,8 s overlap. A baseline correction was applied in each segment.

2.4.3. POWER ANALYSIS

The signal power was calculated through the Fourier transform (Sanei & Chambers, 2007) in the Analyzer software. The average of the calculus of the Fourier transform in all the segments was exported to Matlab.

In Matlab, the signal power was analysed for each frequency band: theta (4-8 Hz) and alpha (8-13 Hz). The limits for each frequency bands were adjusted accordingly to the individual alpha peak frequency (IAPF). The IAPF was calculated as the frequency with the highest peak in the alpha band range (8-13 Hz). For each of the characterization moments, the IAPF measurement was based on the average of the four baselines performed. In the case of the training sessions, IAPF detection was based on the start baseline of each day. Theta and alpha were defined as the frequency bands from 4 Hz to IAPF – 3 Hz and IAPF – 2 Hz to IAPF + 2 Hz, respectively. Then, power for each individual frequency band was extracted.

2.5. STATISTICAL ANALYSES

Considering the low number of available subjects and not assuming the normal distribution of the population, non-parametric tests were used for statistical analyses. For comparisons between groups and conditions the Kruskal-Wallis ANOVA was used in order to assess if there were any significant differences (considered for p-values below 0.05). For testing positive or negative effects of the intervention (testing if the median is greater or lesser than 0) the one-sample Wilcoxon signed-rank test (significance was considered for p-values below 0.05) was used. Spearman´s Rank Correlation Coefficient Test was performed to observe statistical dependence between EEG and behavioural measures. The statistical analyses were performed using OriginLab®.

2.5.1. EVOLUTION OF ALPHA AND THETA POWER ACROSS SESSIONS

Dynamical changes in power due to NFB training were identified analyzing variations in power measurements throughout the 8 days. Thus, for each participant, the power amplitudes in the theta and alpha band were extracted and averaged across blocks, for each training session. Power was extracted for baseline EEG as well.

In order to analyze the training effects on the EEG amplitudes, it was examined separately the four days corresponding to theta NFB and the four days corresponding to alpha NFB. To investigate the relationship between the amplitudes in the above mentioned EEG bands and session number a regression line was fitted for each subject.

As previous studies have reported, a subset of subjects does not respond to NFB training (Enriquez-Geppert, Huster, Scharfenort, Mokom, Zimmermann, et al., 2013; Hanslmayr et al., 2005; Zoefel et al., 2011). Thus, statistics was performed only considering responders. Training results were inspected and a participant was classified as responder if the gradient of training was positive, and as non-responders if this gradient was null or negative.

Gradients were used to test differences between groups (NF vs. NF+NC). Also, differences between starting the training with alpha NFB and starting with theta NFB were examined.

With regard to the mental strategy analysis, strategies employed in each block were collected and the most frequently used were reported for each participant.

In the same way as with the power in NFB, performance in the neurocognitive tasks were assessed. Measures of performance along the 8 days were extracted and a regression line was fitted for each participant of the NF+NC group. Gradients were used to investigate if there was an improvement over the days in the cognitive performance measures.

2.5.2. TRAINING EFFECTS ON BEHAVIOR AND ALPHA THETA POWER

The cognitive battery of four tests and the EEG recordings while performing them were used for assessment of training effects.

For each participant, Fz-power of theta and alpha frequencies was extracted for the first and final characterization moments. Baseline and activity was discriminated for each of the four tests.

For the cognitive evaluation, different measures were collected from the first and last tests applications. In the case of Stroop, the measures adopted were the error rate and the mean response time for condition 2 of the second block. A measure of interference between conditions 1 and 2 was also taken into account. For the Matrix Rotation test, the mean accuracy, study time and response time were extracted. Regarding the TMT, measures of accuracy and mean response time concerning only the part 2 of the test were registered. In the Digit Span, a combined measure was calculated based on the memory span – the last digit that was successfully recollected – and the number of correct trials.

At the end, differences between the pre-training moment and the post-training moment were calculated in order to evaluate and compare the alterations induced by both intervention protocols on participants' behavioral performance and neurophysiologic state.

Outlier observations were detected if a value was two standard deviations away from the mean.

2.5.3. BRAIN LATERALIZATION

For each participant, power of theta and alpha bands was extracted in F4, F3, FC2, FC1, FC6 and FC5 channels. To observe hemispheric lateralization power ratios of F4 to F3, FC2 to FC1, and FC6 to FC5 were calculated both in the pre- and post-characterization, discriminating baseline and activity for each of the four tests. Differences between the pre-training moment and the post-training moment were also calculated.

3. RESULTS

This section presents the results obtained after data analysis and their statistical testing. We begin by verifying that participants in both experimental groups (NF and NF+NC) had no significant differences in age, education and score obtained in both the Geriatric Depression Scale and the Mini Mental State Examination.

3.1. PERFORMANCE IN THE ARROW FLANKER TEST

During the first two days of the intervention protocol participants had to perform 5 3-min blocks of the Arrow Flanker Test, which is an attentional test. Based on researcher observations, it was clear that all participants understood the instructions that were given to them, they answered appropriately and kept engaged in the task. Most importantly, individual reports of the task were analyzed and all participant had very few number of errors in each block (maximum 6 errors in 85 stimulus).

Accuracy, response time (RT), and conflict (difference in time response between incongruent and congruent stimulus) are presented for both groups in Table 1. There were no significant differences between the groups for any of the measures, which suggests there were no performance differences between the NF and the NF+NC group in the Arrow Flanker Test.

Table 1. Performance measures in the Arrow Flanker Test. Mean and standard error of the mean (SEM) are discriminated for accuracy, response time and conflict (difference between incongruent and congruent stimulus).

		MEAN	**SEM**
ACCURACY (%)	NF	98,6	0,6
	NF+NC	98,1	0,6
RT (ms)	NF	844	73
	NF+NC	807	72
CONFLICT (ms)	NF	20	12
	NF+NC	38	23

Additionally, all participants presented a mean accuracy in the Arrow Flanker Test greater than 97,5 % (Z = 2,344 and p-value = 0,0068). Additionally, mean response time was lower than 1000 milliseconds (Z = -2,548 and p-value = 0,0029).

3.2. INTERVENTION EFFECTS ON BEHAVIOR, THETA AND ALPHA POWER

The main objective of this study was to address the effects of the intervention protocol on cognitive state and brain activity, hence behavioral measures and EEG power were obtained during the four cognitive tests applied before (initial battery) and after (final battery) the training procedure. Differences between the two moments were calculated for each participant and averaged for experimental groups. Since the four cognitive tests in the battery evaluate different cognitive domains, the analysis was done separately for each test.

3.2.1. STROOP TEST

Performance results in the Stroop Test are presented for each training group in Figure 9. Figure 9.A show differences between pre- and post-assessment moments for NF group and NF+NC group concerning error rate, which should be lower for an enhanced cognitive performance in Stroop Test. As the error rate, the response time is expected to be lower for an improved performance. Although the results seem to indicate that group NF had greater improvement in the two measures while group NF+NC only appears to improve in error rate, it was not found any significant difference between the groups.

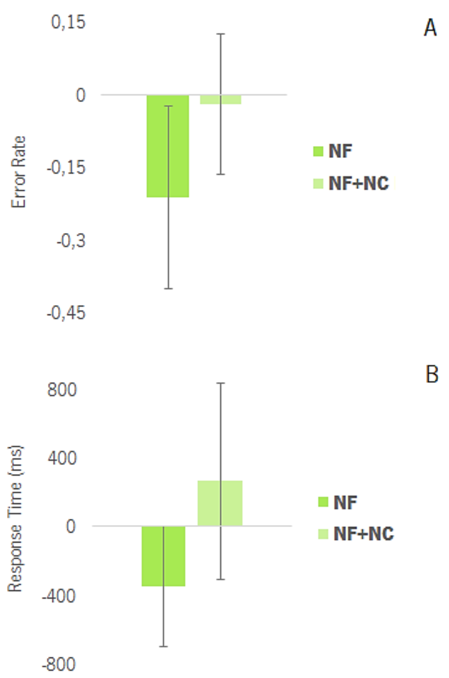

Figure 9. *Behavioral effects of the intervention protocol in the Stroop Test.* Data is presented as mean and SEM in terms of differences between initial and final Stroop assessment in A) Error Rate, and B) Response Time, for both experimental groups.

Regarding EEG power values, Figure 10 presents the results of the average of Alpha and Theta power differences in channel Fz, for Baseline and Activity during Stroop performance. No significant differences were found between the two experimental groups. However, all the participants seem to had an increased training-associated power from the pre-training to the post-training characterization, as indicated by a significant positive difference in both frequencies for baseline and activity (EEG acquired during performance of the test) (Alpha/baseline: N=9, Z = 2,488 and p-value = 0,0039; Alpha/activity: N=10, Z = 2,752 and p-value = 0,0009; Theta/baseline: N=10, Z = 2,548 and p-value = 0,0029; Theta/activity: N=10, Z = 2,752 and p-value = 0,0009). When analyzing within groups, NF-participants (N=5) showed positive results of training in Alpha and Theta power, both in baseline and activity (Alpha/baseline: Z = 1,888 and p-value = 0,0313; Alpha/activity: Z = 1,888 and p-value = 0,0313; Theta/baseline: Z = 1,888 and p-value = 0,0313; Theta/activity: : Z = 1,888 and p-value = 0,0313). Slightly different, the NF+NC (Alpha/baseline: N=4; Alpha/activity: N=5; Theta/baseline: N=5; Theta/activity: N=5) participants showed enhancement of Alpha and Theta power only for the activity periods (Alpha/activity: Z = 1,888 and p-value = 0,0313; Theta/activity: : Z = 1,888 and p-value = 0,0313).

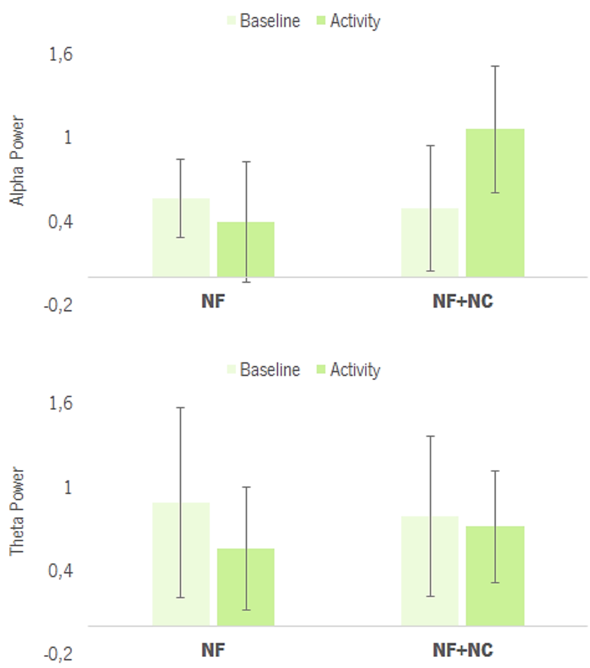

Figure 10. *Effects of the intervention protocols in the power of Alpha and Theta rhythms measured during the performance of Stroop Test, in Fz channel.* Data is presented as mean and SEM in terms of differences in Alpha and Theta power between initial and final Stroop assessment, during baseline and during activity, in Fz, for both experimental groups.

3.2.2. Matrix Rotation Test

Behavioral performance on the Matrix Rotation Test was assessed by calculating the mean accuracy for all the stimulus presented in the test. Furthermore, mean response time and mean study time (time participants took to memorize each stimuli) were also calculated for additional support. The differences between pre- and post-training are shown in Figure 11, for both experimental groups. In respect to performance accuracy in Matrix Rotation test (Figure 11.A), participants (N=8) had greater performance after the intervention (Z = 2,303 and p-value = 0,0156). Although the performance measures of NF and NF+NC subjects do not differ statistically, in respect to time measures, the NF group seemed faster than the NF+NC group (Figure 11.B).

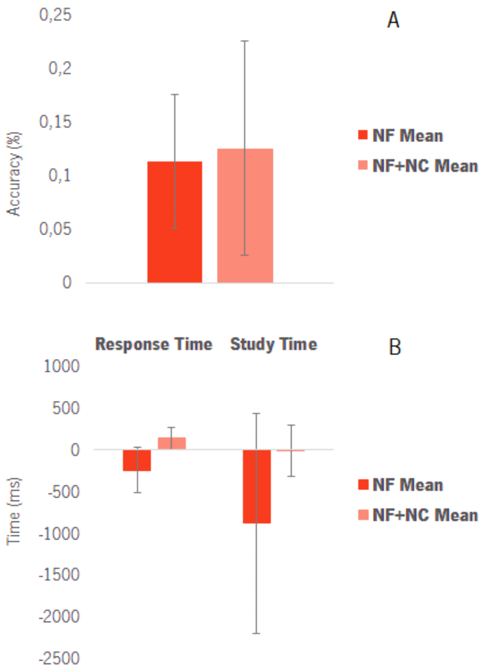

Figure 11. *Behavioral effects of the Matrix Rotation Test.* Data is presented as mean and SEM in terms of differences between initial and final Matrix Rotation Test assessment in A) Accuracy, and B) Study and Response Time, for both experimental groups.

In Figure 12, the variation of Alpha and Theta power in Fz channel are shown,, either measured in baseline or activity, while performing the Matrix Rotation Test. Together, participants (N=10) had an increase in Alpha and Theta power from the initial to the final testing with the Matrix Rotation test, indicated by a positive difference in Alpha and Theta power only during activity (Alpha/activity: Z = 1,937 and p-value = 0,0244; Theta/activity: Z = 1,835 and p-value = 0,0322). No significant differences were found between the two experimental groups.

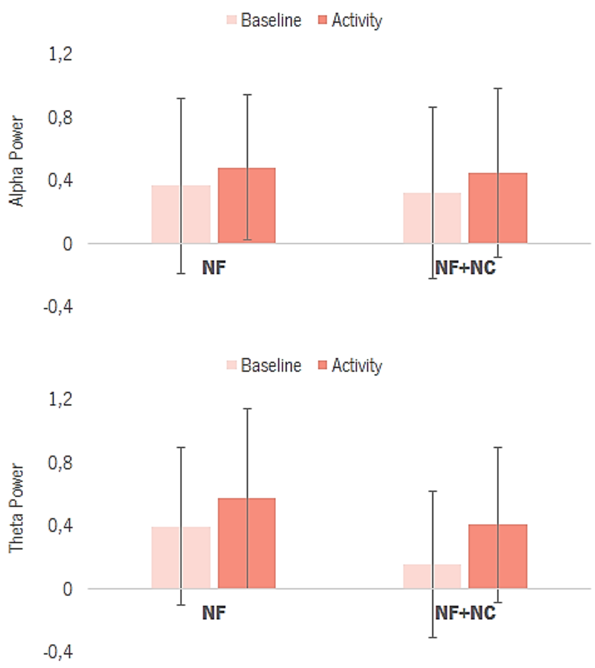

Figure 12. *Effects of the intervention protocol in Alpha and Theta power measured in the Matrix Rotation Test, in Fz channel.* Data is presented as mean and SEM in terms of differences in Alpha and Theta power between initial and final Matrix Rotation Test assessment, during baseline and during activity, in Fz, for both experimental groups.

3.2.3. Trail-Making Test

In order to assess behavioral results concerning the Trail-making Test only the Part 2 of this test was considered. Mean accuracy and mean response time are presented in Figure 13. Both groups appear to have decreased their response time post-intervention (Figure 13.B); whereas in accuracy (Figure 13.A), only the NF+NC group seems to have an enhancement. However, statistical assessment suggests that NF and NF+NC group do not differ concerning accuracy or response time differences (NF: N=4; NF+NC: N=5).

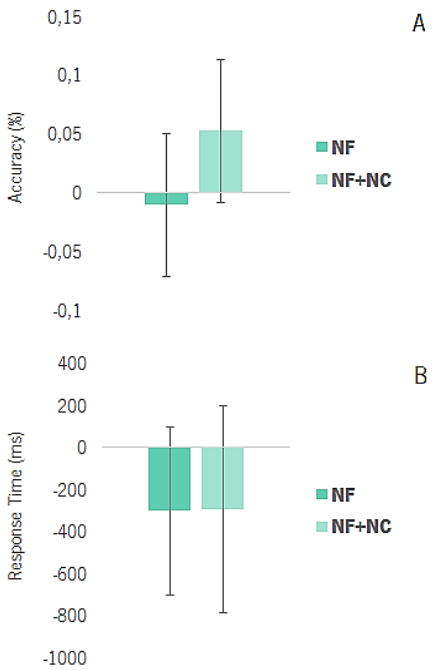

Figure 13. *Behavioral effects of the intervention protocol in the Trail-Making Test.* Data is presented as mean and SEM in terms of differences between initial and final TMT assessment in A) Accuracy, and B) Response Time, for both experimental groups.

As shown in Figure 14, Alpha and Theta power seem to increase in Fz channel, with respect to final – initial testing with TMT. An increase in power may have occurred after training, revealed by a positive effect in Alpha power during baseline and activity, and in Theta power during activity (Alpha/baseline: N=9, Z = 1,777 and p-value = 0,0371; Alpha/activity: N=10, Z = 2,2425 and p-value = 0,0098; Theta/activity: N=10, Z = 2,141 and p-value = 0,0137). Despite no group differences have been found, NF+NC experimental group presents a significant increase in Theta power from pre- to post-intervention assessments, concerning activity measurements (N=5, Z = 2,2425 and p-value = 0,0098; Theta/activity: N=10, Z = 1,888 p-value = 0,0316).

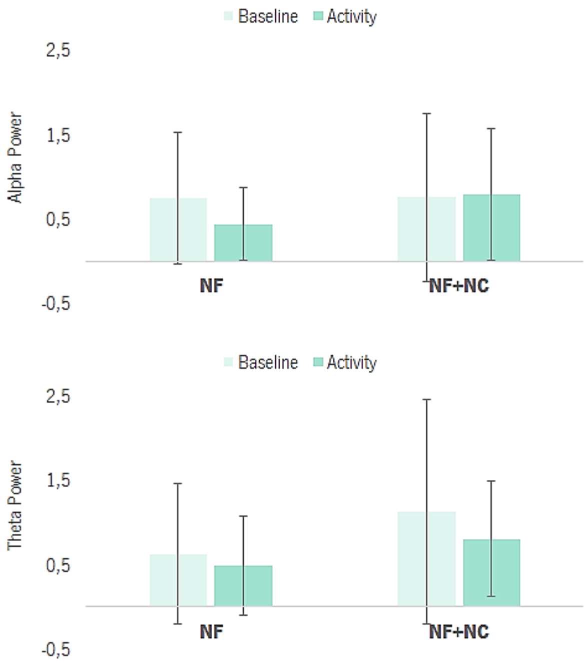

Figure 14. *Effects of the intervention protocol in Alpha and Theta power measured during the Trail-Making Test, in Fz channel.* Data is presented as mean and SEM in terms of differences in Alpha and Theta power between initial and final TMT assessment, during baseline and during activity, in Fz, for both experimental groups.

3.2.4. Auditory Backward Digit Span

In order to assess the training effects on behavior concerning the Auditory Backward Digit Span, a combined score was calculated and extracted for each participant. The score is calculated based on number of trials correct and memory span achieved in the task (Number of trials correct × Block Memory Span). The difference between post- and pre-training scores is presented for both intervention groups in Figure 15. Statistical assessment suggests that the NF (N=5) and the NF+NC (N=4) group did not differ concerning the Digit Span performance, though it seems the NF+NC group had a greater increase in score. As a whole, the increase in the combined score was greater than 0 (Z = 2,047 and p-value = 0,0468), suggesting a positive outcome of the intervention in this specific test.

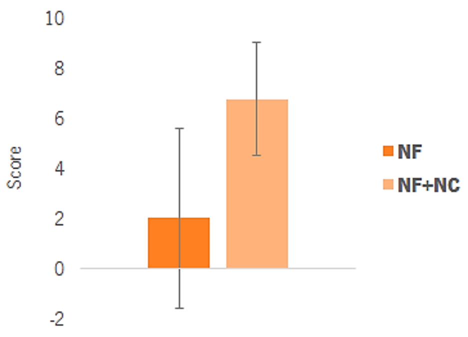

Figure 15*. Behavioral effects of the intervention protocol in the Auditory Backward Digit Span.* Data is presented as mean and SEM in terms of differences between final and initial Digit Span application in Score (Number of trials correct × Block Memory Span) for both experimental groups.

In order to observe the neurofeedback training effects in EEG activity, the difference between post- and pre-training Alpha and Theta power in Fz channel are presented in Figure 16, for baseline and activity periods. The Alpha and Theta power increased following the neurofeedback training, in both groups, suggesting positive effects of the intervention in EEG measures. This is indicated by a generally greater difference in Alpha and Theta power (Alpha/baseline: N=9, Z = 2,488 and p-value = 0,0039; Alpha/activity: N=9, Z = 2,752 and p-value = 0,0009; Theta/baseline: N=10, Z = 2,548 and p-value = 0,0029; Theta/activity: N=10, Z = 2,752 and p-value = 0,0009). Additionally, the Alpha power during baseline showed a stronger gain only within the NF training group (N=5, Z = 1,887 and p-value = 0,0313).

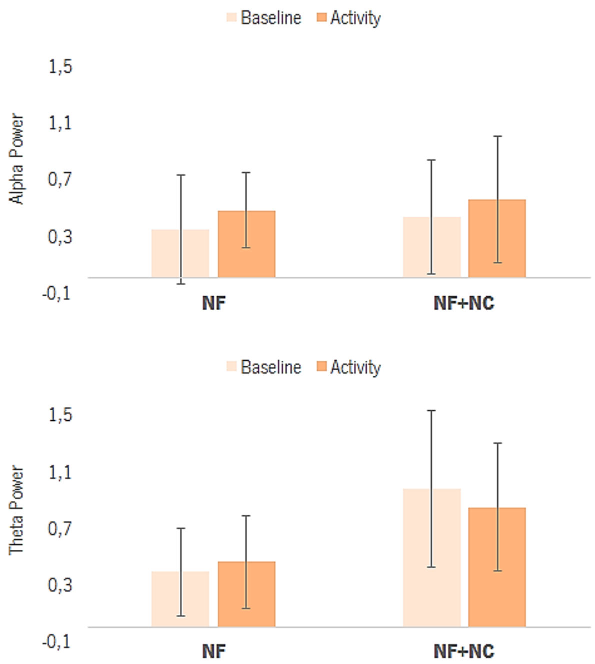

Figure 16. *Effects of the intervention protocol in Alpha and Theta power measured in the Auditory Backward Digit Span, in Fz channel.* Data is presented as mean and SEM in terms of differences in Alpha and Theta power between initial and final Digit Span assessment, during baseline and during activity, in Fz, for both experimental groups.

3.2.5. Brain Lateralization

In order to investigate possible differences in brain lateralization patterns presented previously and following the intervention protocol, a ratio of the power in FC2 channel to the power in FC1 channel was calculated and averaged for both groups. A ratio close to 1 shows a bilateral pattern of brain activation (symmetrical activation), whereas a ratio lower than 1 indicates a left-sided frontal activation (left asymmetry) and a ratio higher than 1 means a right-sided activation (right asymmetry). Results concerning the four tests were examined and the patterns obtained during the performance of Digit Span and TMT are presented in Figure 17. Although no significant effects were found, a difference on the lateralization pattern in the two experimental groups following the intervention seems to be present. Even though both groups appear to have a ratio close to 1 before the intervention, in the post-characterization moment it seems the NF group has a ratio above 1, while the NF+NC group seem to have a ratio below 1.

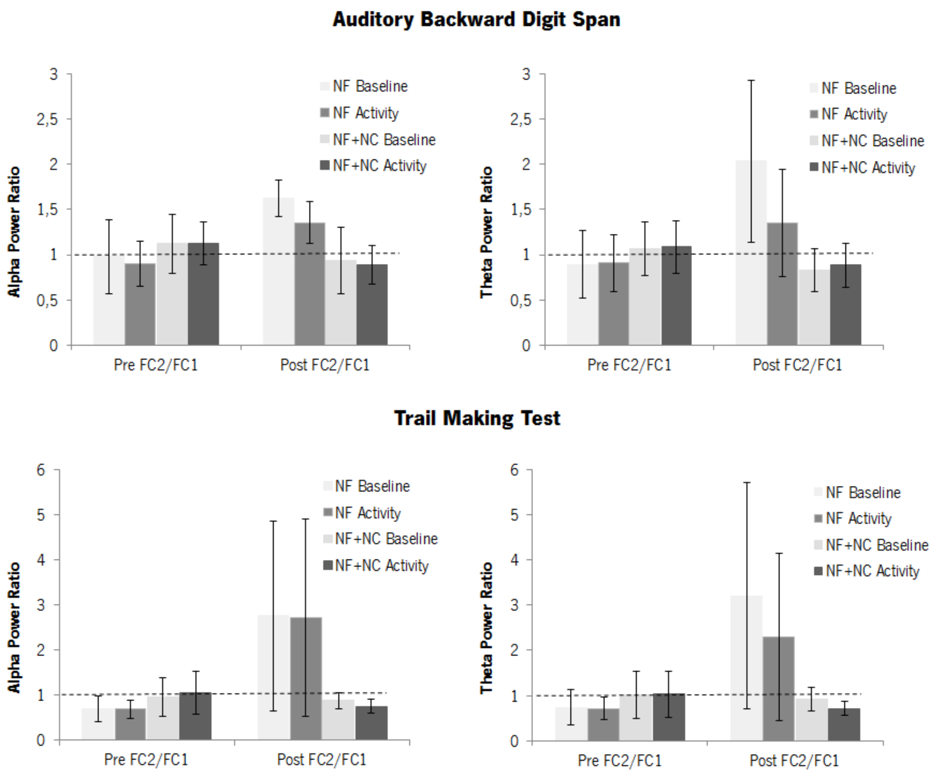

Figure 17. *Patterns of lateralization in the pre- and post-intervention evaluation.* Data is presented as mean and SEM in terms of ratio of the power of Alpha and Theta in FC2 channel to the power in FC1 in initial (Pre) and final (Post) Digit Span and TMT assessment, during baseline and during activity, in Fz, for both experimental groups. A ratio = 1 shows a bilateral pattern of brain activation, a ratio < 1 indicates a left-sided frontal activation and a ratio > 1 means a right-sided activation.

3.3. TRAINING LONGITUDINAL ANALYSIS

3.3.1. ASSESSMENT OF MOTIVATION, INTEREST, CONCENTRATION AND TASK DIFFICULTY

Subjects' self-reports over all training sessions were collected and motivation, interest, concentration and difficulty were averaged for each participant. Then, mean and standard error of the mean was discriminated for both groups (Table 2). Visual inspection of the results reveals similarly high motivation and high interest to participate in the study; as similar good levels of concentration during the sessions and similar small experienced training difficulty.

Table 2. Mean levels of motivation, interest, concentration and difficulty experienced during the training for both experimental groups.

	Motivation	**Interest**	**Concentration**	**Difficulty**
NF	3,58 (0,46)	3,7 (0,42)	3,36 (0,29)	2,21 (0,20)
NF+NC	3,68 (0,24)	3,6 (0,28)	3,31 (0,29)	2,22 (0,10)

3.3.2. ALPHA AND THETA POWER OVER THE TRAINING SESSIONS IN RESPONDERS

In order to assess the evolution of the training-associated rhythm power, we obtained the slope gradients of a first order regression function that best fits power time-series of all 4 training sessions (fitted line between Alpha or Theta power along the four trainings sessions) – Figure 18. It was examined separately the four days corresponding to Alpha up-training and the four days corresponding to Theta up-training. The analysis excluded non-responders (i.e. participants who did not learn to modulate their EEG power over the course of the training) for each of the frequencies. The capacity of success in neurofeedback training was defined as a positive gradient, either if it was on the baseline measurement or in the NFB blocks. A null or negative gradient was considered as indicative of a resistance to training, so its participant was considered a non-responder for that rhythm. In the NF group, 3 participants were classified as responders to Alpha modulation and 3 participants were classified as responders to Theta modulation. Only one participant in this group was considered non-responder for both frequencies trained. In the NF+NC training group, 3 participants were considered responders to Alpha modulation and 4 were considered responders to Theta modulation. In this group no participant was considered non-responder for both rhythms trained.

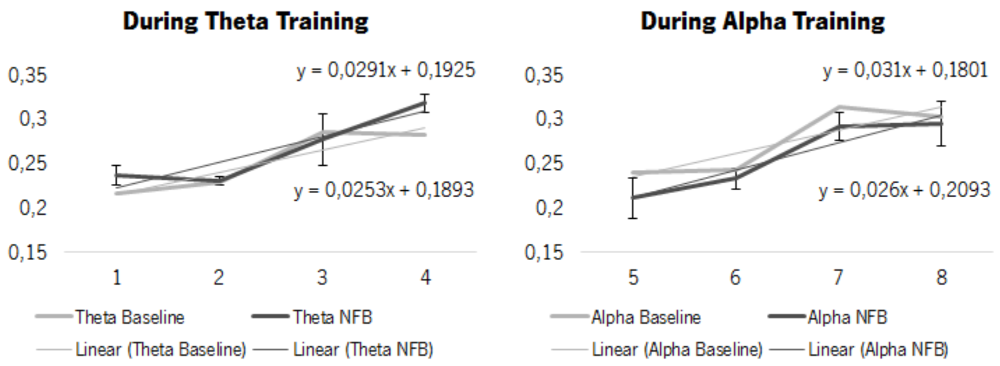

Figure 18. *Representative Power time-series of the 4 training sessions corresponding to Theta-uptraining (left) and to Alpha up-training (right).* Data is presented in terms of power in baseline measurements and as mean and SEM in terms of power in the NFB training blocks, for one participant.

A. During Alpha up-training

Gradients of the responder participants of each group were averaged and the mean and standard error of the mean are presented in Figure 19, both for active baseline measurements and NFB average blocks. It is expected that the Alpha power increase throughout the training sessions, which is indicated by a significant positive gradient. This is the case when testing for the gradients of all participants (N=6; NFB: Z = 2,097 and p-value = 0,0156), during training. Remarkably an increase of power in the baseline measurement can be observed as well (Alpha/active baseline: Z = 2,097 and p-value = 0,0156). Although no significant differences were observed between the experimental groups, it is observed a slightly higher gradient for baseline measurement in the NF+NC group.

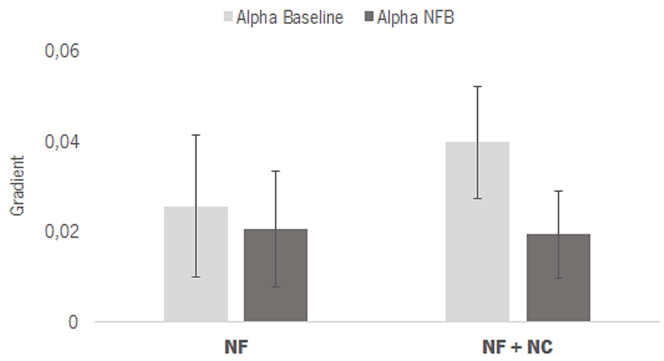

Figure 19. *Gradient of Alpha power increases throughout the four training days.* Data is presented as mean and SEM in terms of slope gradient of Alpha up-training for responders participants in both experimental groups.

B. During Theta up-training

Following the same procedure as for the Alpha up-training analysis, gradients of the participants who were not resistant to Theta training were averaged for the experimental groups. Mean and standard error of the mean can be observed in Figure 20. In this case, it appears that the NF group has a higher increase in Theta power across the four sessions compared to NF+NC group, in particular in the baseline period. Nonetheless, significant differences were not found between the groups. When testing all participants, a strong enhancement of Theta power throughout the days is exhibited for during NFB blocks and active baseline measurements (N=7; Theta/NFB: Z = 1,775 and p-value = 0,0391; Theta/active baseline: Z = 2,113 and p-value = 0,0156).

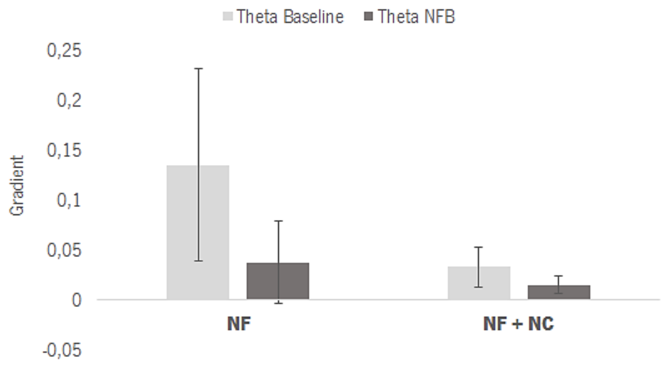

Figure 20. *Gradient of Theta power increases throughout the four training days.* Data is presented as mean and SEM in terms of slope gradient of Theta up-training for responders participants in both experimental groups.

3.3.3. DIFFERENCES IN ALPHA AND THETA TRAINABILITY

During the training procedure, participants had experienced two different neurofeedback protocols – one where they had to modulate their Alpha rhythm and other where they had to modulate their Theta rhythm. In the subsequent analyzes we tried to investigate differences between the Alpha neurofeedback protocol and the Theta neurofeedback protocol. All participants' results were considered independently of the behavior.

A. Assessing prospective differences in beginning intervention protocol with Alpha or with Theta up-training

For the 10 participants that undertook the proposed intervention protocols, 5 started the intervention with Alpha power modulation and 5 started with Theta power modulation. After 4 days, they interrupted the training of the initial rhythm and continued the intervention with modulation of the other rhythm. Based on these two different conditions, two groups were established – one that began the intervention with Alpha modulation and another that began the intervention with Theta modulation. The slopes of power evolution throughout the sessions were averaged for the two groups and the results are presented in Figure 21. The two groups statistically differ in capacity of enhancing Alpha power throughout the baseline measurements (Chi-Square = 3,938 and p-value = 0,0472), suggesting a possible better effect of starting the training with Alpha modulation. No further effects were found.

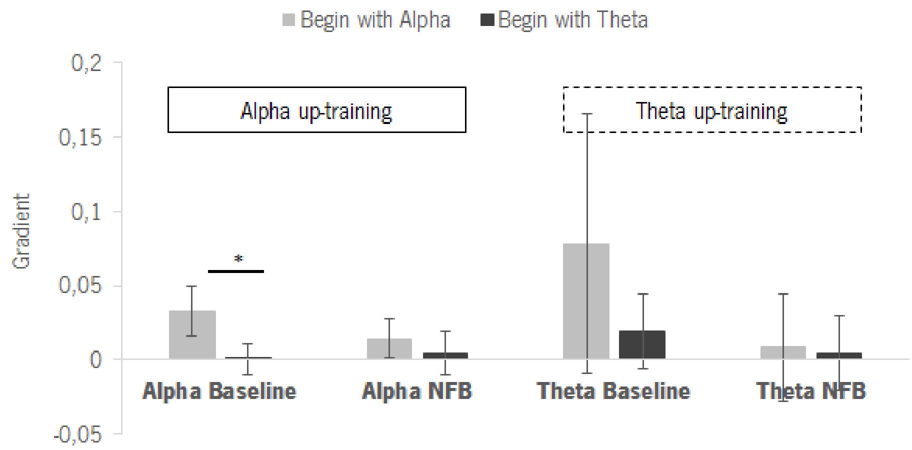

Figure 21. *Differences in Alpha and Theta power increases throughout sessions according to the starting modulation of Alpha or Theta.* Data is presented as mean and SEM in terms of slope gradient during Alpha up-training or Theta-up-training, for two established groups – participants who begin the protocol training the Alpha rhythm or participants who begin the protocol training the Theta rhythm (*p<0.05).

B. *Mental strategies applied for Alpha and Theta Neurofeedback*

Participants were asked to report the strategy used in the neurofeedback task after every block of NF training. The strategies were collected for all participants, independently of the success they had on power enhancement. Participants could use any strategies they like, however if the strategy applied was successful they were instructed to keep using the same strategy. Some strategies were unspecific, since the participants were not focused in a particular one, and they comprise 11 % of the total strategies used. Moreover, 9 % were memories, which included childhood memories, family memories, as well as memories of negative events. All the other strategies were grouped into three types: positive, neutral and negative. Among them, positive strategies represent 27 % of the total, which included faith/religion, love, family (grandchildren, sons, family elements far-away), friendship (friends, friends support, and friends' reunions) and positive thinking (optimism, imagination of future successful events). Neutral strategies represent 46 % of the total, which contained mental calculation, leisure/hobbies, landscapes, traveling, fire (nature), breathing and visual attention to the task. Negative strategies represent 6 % of the total, which consisted of thinking about personal or family problems and feeling sad. Then, strategies were discriminated for Alpha and Theta training sessions, and are presented in Figure 22. Although almost all strategies were used in either of the rhythms trained, it appears that during Theta up-training more strategies were employed, while during the Alfa up-training there was a small group of strategies which have been mostly used (e.g. visual attention, breathing and family).

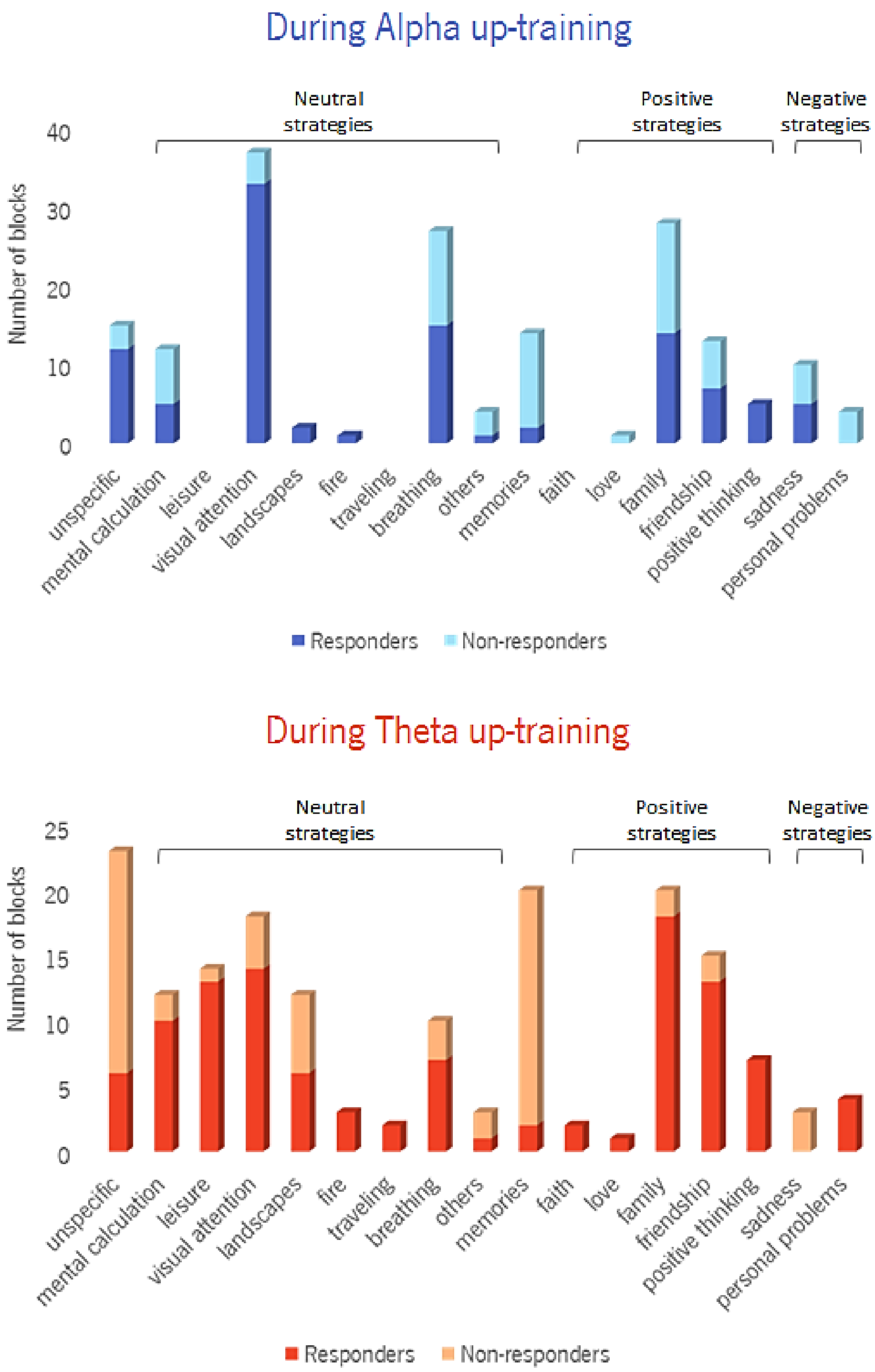

Figure 22. *Different mental strategies applied for Alpha and Theta up-training.* Data is presented in terms of total number of NFB blocks in which each strategy was used, considering all participants in the study.

3.3.4. PERFORMANCE IN NEUROCOGNITIVE TASKS OVER TRAINING SESSIONS IN NF+NC GROUP

During the 8 training days, participants in NF+NC group performed 5 daily blocks of cognitive training. Four tasks were selected for this purpose, which were the Forward Corsi-Blocks Tapping Test (Corsi Test), the Backward Corsi-Blocks Tapping Test (Corsi Test), the 1-back Test (n-Back) and the 2-back Test (n-Back). Participants practiced the neurocognitive tasks in this exact order every day, ending with one of the four that changed from day to day. Overall blocks, each participant performed each task 10 times. To assess the effects of training practice, daily measurements were collected for each task – in the case of Corsi tasks, a combined score of block span and number of correct trials were extracted for each block; in the case of n-Back, the mean accuracy for each block was used. A regression line was fitted for each task – Figure 23, and to investigate if there was an improvement in the behavioral training over the days, the gradients obtained for each participant were averaged and the mean and standard error of the mean are presented in Figure 24. As expected, concerning the Corsi Task, it seems there is an improvement over the days for the total score. In fact, regarding Corsi Forward, the gradient is significantly greater than 0 (N=5; Z = 1,888 and p-value = 0,0313). However, the same results are not observed in respect to n-back. Indeed, the 2-back performance appears to have decreased over the days, although it was not found to be statistically different than 0.

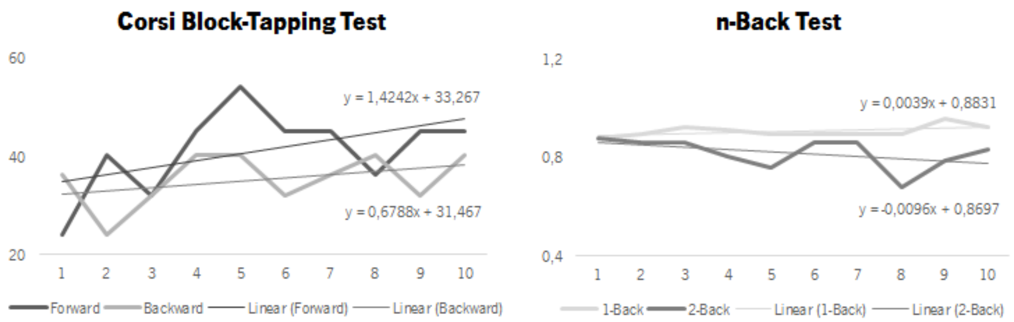

Figure 23. *Representative performance time-series of the 10 training blocks corresponding to Corsi Block-Tapping Test (left) and to n-Back Test (right).* Data is presented in terms of combined score (block span × number of correct trials) for Corsi Test and mean accuracy for n-Back, for one participant.

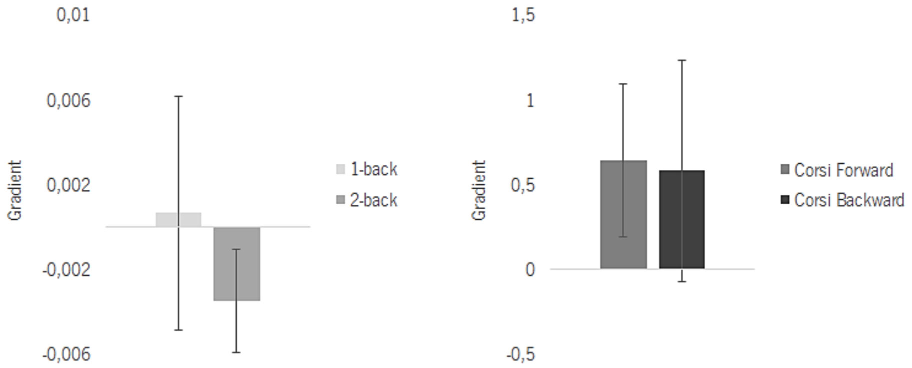

Figure 24. *Gradient of behavioral performance evolution throughout the eight training days, for the four neurocognitive tasks applied.* Data is presented as mean and SEM in terms of slope gradient of n-Back Test (left) and Corsi Test (right) performances.

3.4. CORRELATIONS OF MMSE SCORE AND TRAINING EFFECTS ON THE TRAIL-MAKING TEST

In order to explore correlations between the behavioral results and rhythm's power, several correlations in the data were tested for all the 10 participants. It was observed that the MMSE score, applied previously to the intervention protocol, presented a significant negative correlation with the accuracy difference in the TMT (Spearman Corr. = -0,848 and p-value = 0,0020) and with power differences in Fz channel during TMT (Alpha/baseline: Spearman Corr. = -0,841 and p-value = 0,0023; Alpha/activity: Spearman Corr. = -0,910 and p-value = 0,0003; Theta/baseline: -Spearman Corr. = -0,817 and p-value = 0,0040; Theta/activity: Spearman Corr. = -0,885 and p-value = 0,0007) – Figure 25. Additionally, the MMSE score also correlated negatively with other performance measures, as the accuracy in Matrix Rotation (Spearman Corr. = -0,785 and p-value = 0,0072) and the Error Rate in Stroop (Spearman Corr. = -0,645 and p-value = 0,0441). This suggests that the MMSE score could be predicting a lower effect of the intervention on behavioral and EEG measures, although the opposite effects are apparent for Stroop performance.

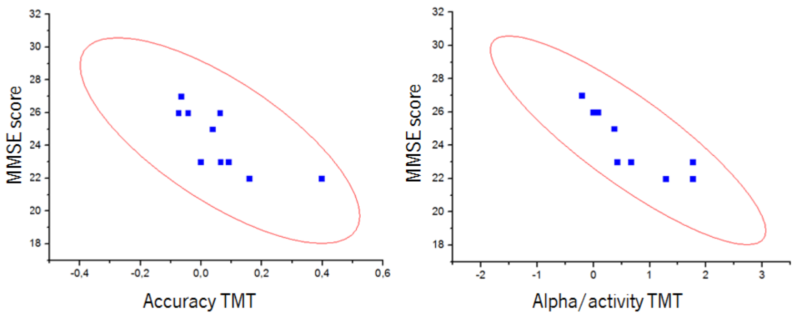

Figure 25. *MMSE score significant negative correlations with performance and Alpha and Theta activity diferences between initial and final TMT assessments.* Differences observed in accuracy in TMT and associated Alpha power after the intervention correlate negatively with MMSE total score. Each dot represents one participant.

4. DISCUSSION AND CONCLUSIONS

4.1. NEUROCOGNITIVE AND NEUROPSYCHOLOGICAL PROFILE OF PARTICIPANTS AND PERFORMANCE ON THE ARROW FLANKER TEST

Prior to the beginning of the intervention protocol, all participants were characterized regarding their cognitive abilities and their neuropsychological condition. Concerning the general cognitive state, based on the Mini Mental State Examination, and considering participants' age and level of education, these individuals seem to be have a medium to poor cognitive performance (Santos et al., 2012). Mood profile was assessed by the Geriatric Depression Scale, which characterized participants as "mildly depressed" individuals. In fact, most participants in the present study have prescribed medication for mood and anxiety disorders. In previous studies based on similar cohorts, mood show to be a key determinant of cognitive status in older individuals (Santos et al. 2012) and it may also have effects on EEG patterns. The results presented in this work, are not sufficient to predict this cognitive and mood profile effects on the outcome of neurofeedback training, but we think that further investigations should address this question.

In the first two days of the intervention, participants had do perform the Arrow Flanker Test, in which they all had a very good performance, in general with no more than 3 errors in 85 stimulus. The response time was also very lower compared to the time out (1500 milliseconds), suggesting that the pre-defined timeout could be reduced to a further better discrimination of participants' attentional abilities. Nevertheless, the two days dedicated to the attentional test seem to have unforeseen benefits, as the familiarization of participants with the computer and the hospital environment, which led to a lower state of anxiety and stress when participants got to the first assessment moment.

4.2. EFFECTS OF THE COGNITIVE INTERVENTION PROTOCOL

One of the main objectives during this study was the development of the intervention protocol. So, regardless of the type of methodology applied, we wanted to investigate possible effects of the protocol on improving cognitive behavioral measures and EEG activity associated with Alpha and Theta rhythms. Since both training methodologies, neurofeedback training and neurocognitive tests, have previously been showing positive effects on cognition, an enhanced performance would be expected. In order to assess intervention protocol effects on cognition, we examined whether the participants exhibited a gain in performance from the pre- to the post-training cognitive assessments. After 8 sessions of neurofeedback, all participants seem to be able to perform better on the Digit Span and Matrix Rotation, both evaluating working-memory abilities, reflecting a possible improvement in this cognitive domain. It is nonetheless important to consider that effects of practice may have occurred during the post-training assessment moment. The initial and final cognitive assessments were only ten to twelve days apart, hence test/re-test effects cannot be excluded and are an important factor concerning the behavioral outcomes of the intervention.

It is important to notice, however, that the same did not happen neither in Stroop Test nor in Trail-Making Test (TMT). Stroop and TMT, both assessing cognitive flexibility, were applied in order to investigate possible transfer effects of a working-memory training into another cognitive dimension. If supported by further studies, these observations may be explained by a lack of transfer effects of the protocol.

Although not supported by statistical evidence, it appears that the two intervention approaches could have a slightly different effect on cognitive performance. The combined NF and NC (NF+NC) training seem to have a greater gain in both the Digit Span and Matrix Rotation. If supported by a higher sample size and significant differences, it could indicate greater effects due to the combination of neurofeedback and neurocognitive training, which is believed to lead to higher levels of rehabilitation (Enriquez-Geppert, Huster, & Herrmann, 2013; Lustig et al., 2009). No effects were observable in the TMT, but in the Stroop Test an opposite effect seems to occur, with the NF group showing a higher gain compared to the NF+NC group – though once again no statistical evidence is clear.

The neurofeedback training effects on the enhancement of EEG power of Alpha and Theta rhythms following intervention were investigated, regardless of training intensity, which was different for the experimental groups. Both Alpha and Theta power measured in baseline (EEG acquired during resting state) and activity (EEG acquired during task performance) seem to be increased in the post-training assessment as compared to the pre-training assessment. There were no noticeable differences regarding the power gain between training protocols, suggesting that NF training intensity *per si* might not be a determinant feature in training effects when supplemented by other methodology.

Additionally, some distinctive brain activation patterns, noticed during data analysis, appeared to be altered after training. This asymmetries in brain activation, or lateralization patterns, are often associated with depressive mood and anxiety disorders, and not much is known about its involvement in cognition. However, these particular observations puzzled us and led us think in exploring this research topic better in the future.

Going back to the neurocognitive and neuropsychological characterization applied to all participants prior to the beginning of their training, some correlations were observed between the Mini Mental State Examination score with performance and EEG activity training outcomes. Although it is essential to increase sample size to fundament these associations, it is interesting to observe that a possible lower general cognitive state of participants resulted in a higher gain in cognitive and EEG power measures.

4.3. NEUROFEEDBACK TRAINING PROCEDURE

The results presented in this study are highly preliminary to clearly show if the neurofeedback training led to an enhancement in Alpha or Theta power throughout the eight days of intervention. The small sample size was even shortened when considering only the responder participants in the analysis, resulting in a lack of conclusions drawn for the statistical assessments. Even so, for the participants that were considered to respond to training, both baseline measurements for Alpha and Theta power seemed to respond to the training modulation, which is in line with previous reports of increasing baseline amplitudes across days (Cho et al., 2008; Zoefel et al., 2011).

Regarding the inter-individual differences, in response to training, important considerations should be addressed. In this work, some participants seemed able to learn how to increase the power of Alpha and Theta rhythms throughout the protocol, whereas some of them were only able to modulate one of the two rhythms. This 'resistance' to the neurofeedback training had been reported in a considerably amount of studies (Doehnert, Brandeis, Straub, Steinhausen, & Drechsler, 2008; Enriquez-Geppert, Huster, Scharfenort, Mokom, Zimmermann, et al., 2013; Fuchs, Birbaumer, Lutzenberger, Gruzelier, & Kaiser, 2003; Hanslmayr et al., 2005; Lubar, Swartwood, Swartwood, & O'Donnell, 1995; Zoefel et al., 2011), regardless of the efficacy and intensity of neurofeedback training, with a substantial proportion of the so-called non-responders – typically in the range of 15-30 % (Allison & Neuper, 2010).

The specific reasons why some participants could not achieve significant control over their own brain rhythms are still unknown yet in focus of the current neurofeedback research. Some studies are investigating predictors of neurofeedback responsiveness and success, reporting neurophysiological and neuroanatomical foundations (Enriquez-Geppert, Huster, Scharfenort, Mokom, Vosskuhl, et al., 2013; Hinterberger, Kübler, Kaiser, Neumann, & Birbaumer, 2003), psychological factors (concentration, mood, or motivation) (Kober, Witte, Ninaus, Neuper, & Wood, 2013; van Gerven et al., 2009) and also environmental effects, such as physical activities or engagement in cognitively stimulating events, that affect neuroplasticity. Another reason might be the excessive artifacts that some participants produce, which might disturb the feedback signal and hamper the learning effect in neurofeedback (Allison & Neuper, 2010). In fact, in our experimental study, there were some reported observations concerning the amount of artifact some participants seem to produce consistently, although we could not, at this moment, take conclusions regarding the outcomes in neurofeedback responsiveness and success.

Another focus of current neurofeedback research concerns the mental strategies used for controlling brain activity. In our study, it was seen that a lot of mental strategies were used by participants, and the same strategy did not trigger the same sense of effect on different participants. Other studies had reported that the same mental strategy, when applied by different participants, led to different outcomes in neurofeedback success (Linden et al., 2012). Addressing differences in individual strategies for EEG modulations, Nan et al. (2012) analyzed the association of mental strategies with training success in an upper alpha band neurofeedback training experiment, and reported strategies related to positive thinking to be the most successful ones.

However, more recent studies indicated that not giving a set of strategies to participants could have better results. Their results reported a higher neurofeedback success for individuals that do not had a specific strategy when modulating their feedback signals. They suggested that participants stating vivid reports on strategies to control probably overload cognitive resources, which could be counterproductive in terms of modulating the specific rhythm (Kober et al., 2013).

In the presented results, the effects of the mental strategies on neurofeedback success were not considered. However, further studies will address specifically the effects that each strategy provoked in Alpha and Theta power enhancement.

4.4. PROTOCOL CONSIDERATIONS

Increasing age is associated with more intra- and inter-individual variability both in cognitive performance and associated neuronal activity (Li, Brehmer, Shing, Werkle-Bergner, & Lindenberger, 2006; MacDonald, Nyberg, & Bäckman, 2006). Indeed, participants' results presented quite diverse values regarding the differences on behavioral performance in the cognitive battery and the power values measured in EEG. Due to low sample size, these variations affected considerably data analysis and it was difficult to show training effects in the present study. Finally, the exploratory nature of this study did not allow for clear conclusions regarding intervention effects and can serve, at this point, as pilot data suggesting further research.

At the end of the intervention protocol all participants had answered a questionnaire about their general opinion on the study. It was observed that most of participants enjoyed the intervention experience. They reported feeling more calm, concentrate and with more focus and enthusiasm in daily tasks. Additionally, most had reported that the training intensity and duration was appropriate. They reported easy to moderate difficulty in the training.

Concerning the cognitive training approach applied to 5 participants in the NF+NC group, most participants seemed to improve in the Corsi Test, increasing either their block span or the number of corrected trials. In the n-Back test, the results are not clear – in the 1-back task most participants had good performance and were able to maintain it throughout sessions. Regarding the 2-back task, most participants seem to have decreased performance from initial to final assessments, although no significant results were obtained. If a big sample size proves these results, we might consider the adjustment of these tasks. First, the time of intervention (8 days) is shorter than interventions in clinics and psychology, which are normally extended for a longer period, with an increased intensity in training sessions. Additionally, the fact the 2-back is implemented immediately after the 1-back, and these two tasks have the exact same visual paradigm, may have been in the origin of the bad performance of most participants in the 2-back task.

Regarding the neurofeedback training intensity, traditionally the number of neurofeedback training sessions is relatively high in clinical studies (up to 30 or 40 sessions), but in more recent studies the individualized neurofeedback in healthy participants has been shown to succeed with substantially less sessions. In our study, 8 individual sessions were applied, but only 4 sessions were dedicated to each rhythm. We are not yet aware of the implications that this modality of Alpha and Theta up-training might have on neurofeedback outcomes. It was observed in the present study slightly differences between the Alpha training and Theta training, but further investigations are needed to assess real effects and associate them with intensity of training or rhythm specific trainability characteristics.

Literature reports the need of appropriate controls in neurofeedback approaches for validating EEG-neurofeedback protocols effects in cognitive enhancement (Enriquez-Geppert, Huster, Scharfenort, Mokom, Zimmermann, et al., 2013; Gruzelier, 2013). Regarding future studies, we anticipate a need for control groups to support any effects we may observe with this intervention protocol. First, a need for a control group for neurofeedback effects is needed – the so-called sham-neurofeedback control group (i.e. participants who receive a pseudo-feedback not related to the actual EEG activity). Additionally, a control group with no particular intervention should be included aiming at assessing test-retest effects on behavior results. We also intend to establish a group performing only behavioral training, which could discriminate effects of the different training approaches in this study. Thus, we could analyze the effects of behavioral training independently of the effects of neurofeedback training, and hence draw conclusions about the potentiation effects in the combined approach group.

4.5. FURTHER INVESTIGATIONS

As already commented, the main limitation in the present study was the number of participants in consideration. Yet more participants are now being recruited and intervened so we expect in the near future to draw conclusions about the presented protocol and the potential enhancement effects of the combined approach of behavioral training and neurofeedback.

The present work addresses training effects on frontal-midline (Fz channel) activity enhancement. However we think that further investigations should explore neurofeedback propagation effects on other brain regions (e.g. posterior brain regions), that are also associated with cognitive functions. Also, specificity of the neurofeedback training, concerning the effects on other frequency bands should also be evaluated, namely concerning beta and gamma frequency bands, which also appear to be involved in memory processes.

Concerning the aforementioned non-responsiveness in neurofeedback, which in the current results accounts for 30% to 40% of participants, some studies have reported a complete lack of behavioral effects or clinical symptoms relief in these participants (Lubar et al., 1995, Hanslmayr et al. 2005). In the present study, even though some analyses were performed with responders and non-responders separately, the training effects on the cognitive battery was not the case. So, to better address the possible behavioral outcomes triggered by the intervention protocol, analysis should be performed without considering participants who were not able to modulate their EEG rhythms.

4.6. CONCLUDING REMARKS

The presented work aimed at investigate the effectiveness of the frontal-midline Alpha and Theta up-training protocol on working-memory performance of healthy ageing participants.

For that purpose, a protocol for neurorehabilitation covering two different methodologies has been developed. One methodology supports neurofeedback modulation, concerning the power enhancement either of Alpha or Theta rhythms. The other comprises neurocognitive tasks, namely the n-Back Test (the 1-back and the 2-back versions) and the Corsi Block-Tapping Test (either in Forward or in Backward order).

In general, the protocol established appear to induce an enhancement of Alpha and Theta power in frontal-midline as an enhancement in working-memory overall state. However, the small sample size and the inter-individual differences hinder the real effects of the intervention and render possible multiple explanations for the origin of these results.

With an increase in number of participants and further analysis concerning specificity, test/re-test effects and sham-neurofeedback, we hope to contribute to a better understanding of the effects of neurofeedback as well as the potentiation that cognitive training might provide in enhancing cognitive abilities of healthy ageing participants.

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6.ANNEXES

Cognitive Intervention Protocol for Age-Related Memory Impairments

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Abstract— **With the number of elderly people increasing tremendously worldwide, comes the need for effective methods to maintain or improve older adults' cognitive performance. Using continuous neurofeedback, through the use of EEG techniques, people can learn how to train and alter their brain electrical activity. A software platform that puts together the proposed rehabilitation methodology has been developed: a digital game protocol that supports neurofeedback training of alpha and theta rhythms, by reading the EEG activity and presenting it back to the subject, interleaved with neurocognitive tasks such as n-Back and Corsi Block-Tapping. This tool will be used as a potential rehabilitative platform for age-related memory impairments.**

Keywords— Neurofeedback; EEG; Cognitive intervention; Healthy aging; Serious Games; Brain-Computer Interface, Alpha, Theta, Memory.

I. INTRODUCTION

With the growing life expectancy, the number of elderly people is increasing tremendously worldwide. As a consequence, the burden of age-associated disorders, such as Alzheimer and other kinds of dementia, is also exponentially growing, affecting about 50% of all elderly patients with a high cost to society and a major impact on family and caregivers [1]. Impairment of cognitive ability is associated with a progressive decrease of synaptic plasticity and neuronal inter-connectivity, which are neurophysiological characteristics of the aging brain [2]. Neuroplasticity is characterized by neural redundancy and plastic remodeling of brain networking, that can be secondary to mental or physical training.

Cognitive processes like executive and associative functions are commonly trained on behavioral neurocognitive (NC) tasks, since it has been reported their effectiveness and durability on trained and near transfer tasks [3]. Some studies have described the functional benefits of cognitive training (memory, reasoning, speed of processing) in the elderly population [4], and more specifically, studies on healthy aging have been providing growing evidence on the improvement or maintenance of cognitive abilities when targeting memory domain [5,6].

However, these studies also showed poor results on transferability of tasks for real-life activities and suggest combination with other approaches to reach higher levels of rehabilitation, like cognitively, physically or socially stimulating activities of everyday life [3]. Additionally, novel tools to guide training strategies, as neuroimaging techniques, seem to be a promising field.

In this context, recent studies in patients of Alzheimer Disease [7] and mild cognitive impairment [2] suggest that patients' temporal and spectral EEG features significantly differ from healthy subjects'. Simultaneously, noticeable attention has been paid to Brain-Computer Interface (BCI) applications that address the functional recovery of the central nervous system to improve the cognitive and behavioral performance of children with attention deficits [8], enhance memory retrieval [9] and reduce pain perception [10]. These studies employ neurofeedback (NF) training on their protocols, where the subject is instructed to modulate his own brain rhythms whose feedback is provided by a BCI. Although these studies demonstrate an application context significantly different from the classic BCIs [11], the NF-oriented BCI perspective has been suggested as a promising tool to enhance plasticity and able to provide new outcomes for cognitive functional recovery [12]. Based on real-time EEG recordings, its processing and classification, NF BCI protocols might be able to guide neuroplasticity to promote recovery of brain functions.

The development of a therapeutic tool that stimulates neuronal plasticity mechanisms in individuals with cognitive deficits resultant from brain aging is being pursued. The neurorehabilitation plan combines NF training [13] with common NC tasks (working memory), in a training protocol that interleaves trials of both approaches. This intervention strategy relies on an operant conditioning BCI that prompts the subject to modulate a particular rhythm of the EEG in order to improve performance on NC tasks. In contrast with either NC or NF training single-methodology, we believe that the complementary methodology affords further rehabilitation potential by providing self contained feedback to the subject:

NF training provides the current and target cognitive states and NC training provides the accuracy on test performance addressing specific cognitive functions. Besides promoting larger functional recovery, this combined approach may also increase transfer and durability effects of the training.

II. BACKGROUND

Performance-related electroencephalogram (EEG) markers are currently being studied in the group, by acquiring EEG data during the execution of the Wisconsin Card Sorting Test (WCST) in elder subjects.

EEG power, ERD/ERS (event related desynchronization/synchronization) [14] and coherence, were evaluated and correlated with performance measures. Preliminary results suggest that these EEG features may be regarded as phenotypes of good performance on memory and executive function tasks. These 'phenotypes' may be useful for the developing of therapeutic tools for age-related cognitive impairment using NF and will be published somewhere else.

The selection and identification of EEG phenotypes is deeply important in the design and outcome assessment of neurofeedback, since they will be guiding the neurofeedback protocol design and indicating the target "EEG phenotype" to pursue during cognitive intervention.

III. REHABILITATION PLATFORM AND NEUROFEEDBACK TRAINING PROTOCOL

The software system that put together the proposed rehabilitation platform includes: a digital game protocol that presents to the user his/her brain state in a simple way, and stimulate him/her to improve. This tool will be used as a rehabilitative platform for neurofeedback (NF). The platform has the structure of a NF-oriented BCI –acquisition and storage of EEG signals in real-time, followed by processing and translation of the signals. This classification is presented to the user, in a way of promoting him to modulate his signals, and then a new cycle begins (Fig. 1) [15].

Additionally, the rehabilitation platform includes a set of neurocognitive tasks. In the same way, there will be acquisition and storage of the EEG signals, during the course of the tasks; however, in this case, the user will not receive any feedback from the processing and translational algorithms.

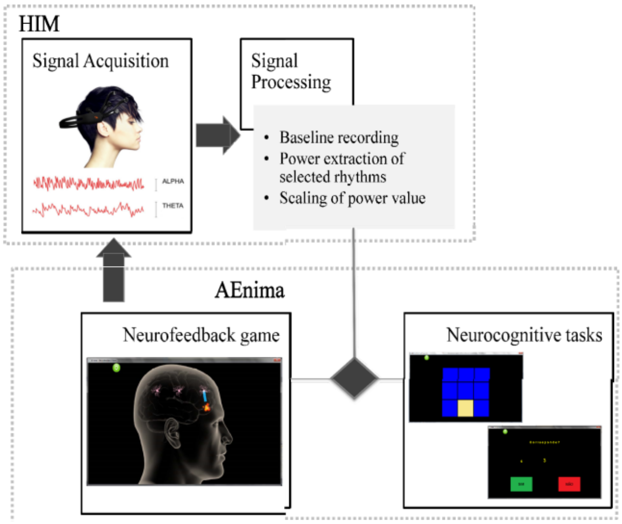

Figure 1. Diagram of the proposed rehabilitation platform, that includes neurofeedback and neurocognitive training.

A. *BCI++*

A BCI platform supports the NF training by reading the EEG activity and presenting it back to the subject. For this propose it will be used a software developed by Sensibilab, the BCI++ platform [16]. BCI++ is divided in two main modules interconnected: a first one called *HIM*, oriented for the real- time acquisition and processing of the EEG signals and the translational algorithm, and a second one called *AEnima* who is responsible for providing visual or auditory feedback about the current brain state of the subject, in a form of a digital game.

B. *Translational Algorithm*

The central piece of a BCI is the translational algorithm implemented. It has to combine the EEG features from various electrodes and translate them into a brain state representation, allowing a classification in real-time of the subject's current condition compared with the target one.

In the proposed algorithm, the focus will be on two brain rhythms, theta and alpha, that are crucial on memory-related NF protocols, [17, 18]. The power of theta and alpha rhythms is calculated on 1s segments and is updated 10 times per second. In the beginning of each session, a baseline record will be performed during 60 s. The subject has to be as relaxed as possible, while being exposed to the graphic environment of the game (Fig. 2). The medium and standard deviation of values of power in this segment will be the parameters of a z-score for the classification algorithm. Accordingly with the level of difficulty, the minimum and maximum values will be 2x, 3x or 4x the standard deviation. Every time the algorithm is updated, the power values will be scaled on the new range defined by new minima and maxima values.

Within each difficulty level, the values for minimum and maximum power can also be re-evaluated to be more adequate to the user performance. So, every time the user reaches a value higher than the maximum defined or a value lower than the minimum defined, these values will became the actual maximum and minimum values.

In order to adjust the game difficulty level, and consequently, the minimum-maximum range, the proposed algorithm evaluates regularly the latest power values, by raising or decreasing the game difficulty when the power z-score is near the extreme values.

C. *Neurofeedback Game*

The neurofeedback game is being developed in *AEnima* which is based on the Irrlicht game engine[1]. The game is subdivided in two game modes: one where the feedback is calculated from the theta rhythm power; and another one where the feedback is calculated from alpha power. Essentially it will accept the feedback value of the power for one of the rhythms from the translation algorithm, and presenting it to the player, in a form of a bar whose length increases or decreases depending on the power value. The higher the value of the power, the better the subject performance.

The game is designed with a therapeutic meaning: the goal of the game is to extinguish a fictional fire, either nearby the hippocampus (theta NF) or in the prefrontal cortex (alpha NF), with a neuron that carries an axon full of water that has to reach the fire region. This axon is animated as a bar that increases or decreases its length (approaching or withdrawing of the fire focus) according to alpha or theta power, calculated on the translational algorithm (Fig. 2). The sum of the power values yield during the game will set the score of the player. The fire in the hippocampus will also diminish as the bar increases towards it (and the score increases), and will be completely extinguished if the bar reaches maximum value.

Additionally, the adjustment of the difficulty level of the game by the algorithm is represented by a change in the location of the fireman neuron, for a further region in the case of a level upgrade. The power values range will be larger, and it will be more difficult for the player to reach the fire.

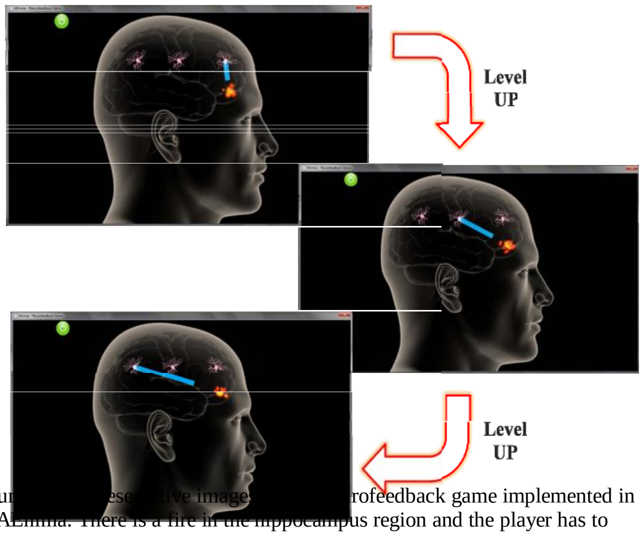

Figure 2. Representative images of the neurofeedback game implemented in AEnima. There is a fire in the hippocampus region and the player has to increase the blue bar, that starts in the neuron, to reach this fire and extinguish it. Three levels of difficulty are presented here, that vary in the distance from the neuron to the fire.

D. *Neurocognitive Tasks*

Two different neurocognitive tasks, both assessing working memory domain, were implemented. The cognitive tests that will be used are the Corsi Block-Tapping Test, adapted from PEBL Psychological Test Battery [19], and the N-back Test, both implemented in the *AEnima* game protocol. They will not receive any feedback or classification from *HIM* and the translational algorithm.

1) Corsi Block-Tapping Test (forward and backward): is a classic test to assess visual-spatial short term working memory. The player has to track a sequence of up to nine identical blocks being highlighted and reproduce it, either in forward or backward order (Fig. 3).

The sequence starts with only two blocks, but when the player repeats correctly one sequence, the game upgrades the level, increasing the number of blocks in the next sequence. On the other hand when the subject repeats wrongly in one sequence, the game downgrades the level and decreases the number of blocks in the next sequence. At the end, measures of block span, accuracy, reaction time and mean of number of blocks of the sequence are computed, as trial reports for every game block performed. .

The game will start with the lower difficulty level, which is the forward Corsi task, and after 2 blocks of the game it will shift to the backward Corsi task.

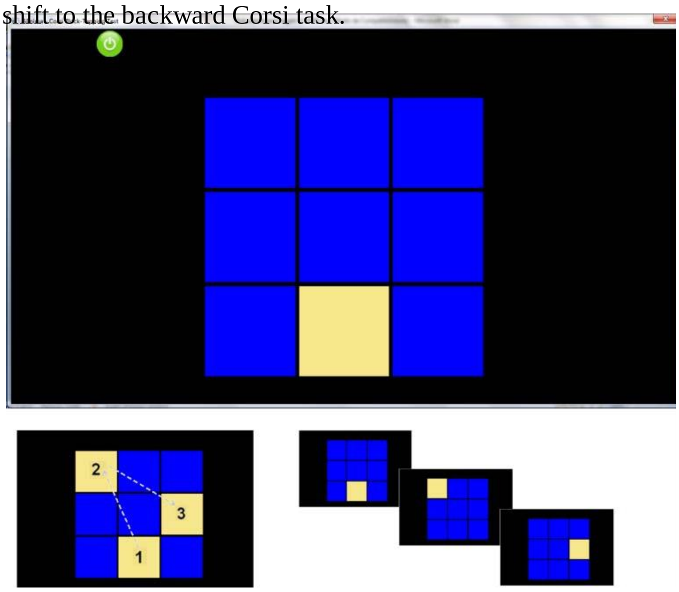

Figure 3. Representative image of the Corsi Block-Tapping Task implemented in *AEnima*. There are 9 blue blocks and a sequence of them is presented to the player, who has to reproduce it. When the player reproduces correctly, the number of the next sequence is increased (up to nine), and when it reproduces it incorrectly the number is decreased.

2) N-Back Test: is a commonly accepted task to measure cognitive performance in working memory domain. The subject is presented successively with digits and has to indicate whether or not the current digit matches the *n* instances before (1-back – the previous digit; 2-back – the digit that appeared two times earlier) (Fig. 4).

The game will start with the lower dificulty level, which is the 1-back task. The subject only has to evaluate if the current digit is the same as the previous one. Measures of accuracy and reaction time are computed at the end, as trial reports for every block performed. If the subject is able to perform successfully two blocks, then there will be a shift to the 2-back task. In this case, the subject has to remember and compare the current digit to the digit that appeared twice behind. The subject will perform two blocks and then the game stops.

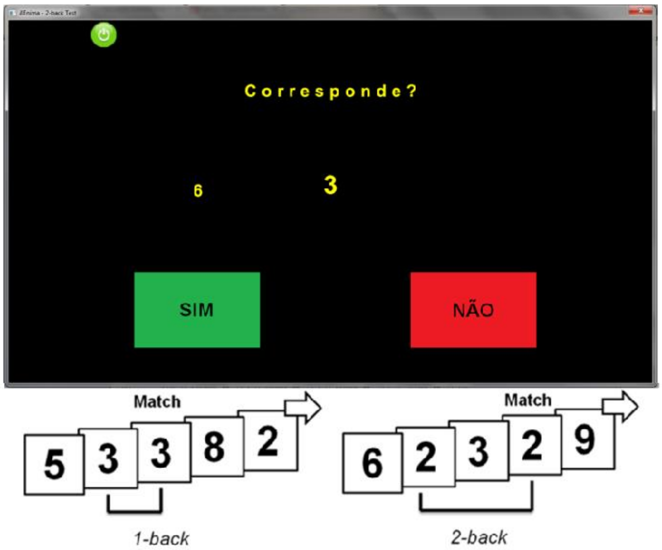

Figure 4. Representative image of the 2-Back Task implemented in AEnima.

A digit is consecutively appearing in the screen, and the player has to warn if it matches the digit presented two times earlier in the game.

3) Arrow Flanker Test: Additionally, the Arrow Flanker Test was implemented as an *AEnima* protocol, for attention domain assessment, and was also adapted from PEBL Psychological Test Battery [19]. In each trial of the game, the subject is presented with a set of arrows. The player has to pay attention to the central target arrow and give a directional response - left or right keys – within a maximum of 1500 milliseconds. The target arrow can be flanked by stimuli in the same direction (congruent), in the opposite direction (incongruent), or not be flanked (neutral) (Fig. 5). Measures of accuracy and reaction time for each stimuli condition are computed in the end of the game, as trial reports for every block performed.

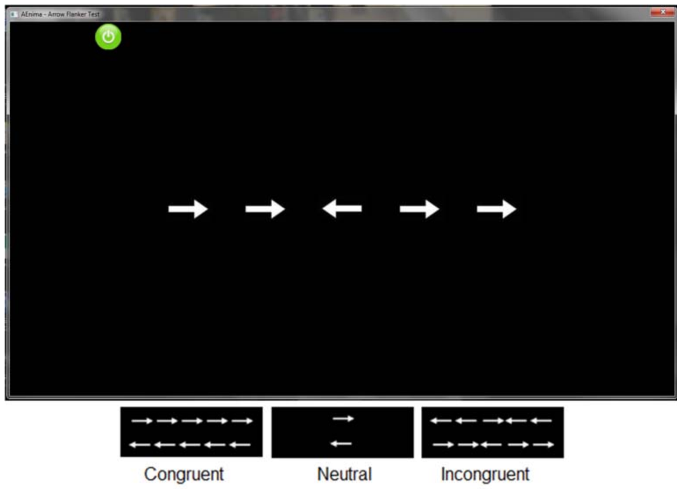

Figure 5. Representative image of the Arrow Flanker Test implemented in *AEnima*. A set of arrows is presented and the player has to give a directional response to the central arrow (target), in a determined set of time. The type of stiimulus that can flank the target arrow are also represented at the bottom of figure.

IV. Cognitive Intervention Prottocol

A pilot study consisting of a rehabilitative intervention with elder subjects will be performed. These subjects belong to an already existing healthy ageing study cohort containing subjects (males and females) with age above 55 years old, without any diagnosed dementia, disabling pathologies or disease previously tested and known to have average performance on different neuropsychological tests, with respect to memory and executive function (including the Mini–Mental State Examination (MMSE), the Stroop Color and Word Test (Stroop test), the Selective Reminding test (SRT) and the Block Design sub-test of the Wechsler Adult Intelligence Scale) [20].

Depending on the cognitive intervention experienced, subjects will be randomly divided in 4 sets, with 10 subjects each, gender balanced. Statistical power was calculated supporting significant differences with the proposed sample size.

The intervention will took place in Health Care Centers in Guimarães and will be carried on 2 weeks in this way:

TABLE I. DAILY TIME LINE FOR REHABILITATIVE INTERVENTION

				EEG + cognitive assessment[a]							EEG + cognitive assessment
N=10	control		ATT[b]					contact with researchers			
N=10	NC		ATT					Corsi[c]/N-Back[d]			
N=10	NF		ATT					Theta/Alpha			
N=10	NF + NC		ATT					Corsi/N-back + Theta/Alpha			
	days	1	2	3	4	5	6	7	8	9	10

a. 32-channel EEG acquisition during performance of a set of cognitive test assessing memory and executive functions.

b. Arrow Flanker Test

c. Corsi Block-Tapping Task

d. N-Back Task

During the training, subjects will be sited in an illuminated and acclimatized room distancing 50-80 cm from a 17 inch computer screen, with touch technology. They will respond to the neurocognitive tasks using touch screen, except for the Arrow Flanker Test, where they will use keyboard keys.

In the first 3 days all subjects will be submitted to the Arrow Flanker test during 10 minutes (5 blocks of 42 trials). This step aims to guarantee that all the subjects present minimum attention level in order to be able to perform the subsequent tasks. It is a pre-requisite in order to give a baseline from where all the subjects will start, independently from the intervention protocol they will follow.

After the third day, a 32-channel EEG will be acquired from all the subjects while performing a battery of cognitive tests (Stroop task, Trail-making test, Digit Span and Matrix Rotation), that assess predominantly executive function and memory state of the patient. At this time, subjects that did not reach the expected results in attention test will be excluded from the experiment.

During the last seven days the subjects will be submitted to a 30-minute intervention protocol each day, since this training period seems to have effects on behavior and electroencephalogram measures [21-23] accordingly to their experimental group:

- Neurocognitive (NC) experimental group – patients will be submitted to sessions of working-memory tasks: Corsi Block-Tapping (4 blocks of 15 trials) and N-Back (4 blocks of 36 trials).
- Neurofeedback (NF) experimental group – patients will perform sessions of neurofeedback game: modulation of theta or modulation of alpha rhythm, with 8 minutes each game.
- Neurofeedback and neurocognitive (NF+NC) patients will perform interleaved sessions of a neurocognitive test and a neurofeedback game. The test and the game will change each day.
- Control – subjects will contact with clinicians and researchers, however, they will not perform training at all.

After the 10th day, subjects will repeat the previously described cognitive battery while acquiring EEG, for a post-training reassessment, in order to document any effects of this intervention. This session is also aimed at acquire a follow-up of the subjects during training time and have feedback of the protocol developed.

The experiments being proposed are already approved by the ethics commission of the cohort of the European Switchbox Project.

V. Future Perspectives and Conclusions

In the end of the cognitive intervention, the behavioral performance for all subjects will be assessed: group analysis for mean corrected z-scores (post-training reassessment minus pre-assessment behavioral performance) with non-parametric test will be applied in order to observe performance differences among intervention protocols , as well as longitudinal analysis of the subjects within the groups for Theta and Alpha power values and performance measures for the neurocognitive tasks (accuracy, RTs, block span and mean memory span), during the days of intervention. Later on, we want to analyze the EEG profiles from the subjects, between groups and within groups, to assess intervention effects on EEG phenotypes.

Our hypothesis is that a training protocol with interleaved sessions of NF training and NC tests will have a greater impact on cognitive performance in comparison with either the NF or the NC training alone. By alternating NF training, which provides quantitative measures of the brain state to be modulated, with NC tests, the subject obtains a broader perspective of his own cognitive recovery. Additionally, the subject can adapt his strategy to optimize behavioral performance based on his own EEG state.

As future work, the intent is to develop a rehabilitative intervention, based on the pilot experiment described herein, in order to assess the therapeutic effects of the proposed protocol. This way, it will be possible to verify if the potential effects of intervention are mainly due to a real modulation of the patient brain signals or otherwise, these effects can have other sources such as the stimulation provided by a rich graphic environment or the social contact with clinicians and researchers.

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