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Research

Neuro-Upper, a Novel Technology for Audio-Visual Entrainment. A Randomized Controlled Trial on Individuals with Anxiety and Depressive Disorders

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Abstract

Disturbances of neural oscillation patterns have been reported in many diseases. Besides the Brain-Computer Interfaces (BCI) initial role as assisting devices, BCIs are suggested for a wider range of applications in that non-invasive BCIs have the advantage of having access to brain activity providing insight into the user's emotional state. The possibility to recognize emotions can be employed in BCIs to provide the user with more natural ways of modulating his/her mood states. Neuro-Upper (NU) is a non-invasive technology, different from usual BCI, and designed for enabling the auto-calibration of neural oscillations. NU through one-channel recordings collects eight EEG frequency bands and sub-bands and delivers flickering lights corresponding to their occurrence in near real time with direct mapping to the monitor. Mathematical algorithms, principally informed by the frequency in successive seconds of time, make choices of lights and timing to permit resonance between neural oscillatory frequencies and the musical playlists used in order to obtain brainwaves entrainment.

The study reported results from a randomized controlled trial carried out on fifteen individuals (aged 23-61 years, 8 females, and 7 males) with anxiety and depressive disorders assigned to an experimental or control group. Procedures were delivered during an average of 52 consecutive sessions of 30 min conducted in a dark environment. Statistical analysis indicated a significant decrease in symptoms (HAM-D scores) and improvements in cognitive functions outcomes. Variability in the HAM-D scores seems explained by the differences found in beta 1, beta 2 and delta while the unexpected increase in the state of anxiety symptom (STAI Y-1) appear predicted by variations of alpha 2 and beta 2 sub-bands. Rise in QIP scores appear influenced by the variations of theta band. Disturbances of neural oscillation have implications for both neuropsychiatric health and downstream peripheral physiology. The possibility of non-invasive optimization of brainwaves through NU suggests potential roles for this technology.

Keywords: Audio-Visual Entrainment; Brainwave Auto-Calibration; Anxiety; Depression; Technology

Introduction

The present study introduces a novel, non-invasive electroencephalography-based device, named Neuro-Upper (NU). The aim of NU is to facilitate the auto-calibration of neural oscillations through audio-visual entrainment. In this section, we review the closest technology used for the diseases associated with neural oscillatory disturbance. Brain-Computer Interface (BCI) is a rapidly emerging field of multidisciplinary research and applications integrating research from neurosciences, psychology, engineering, rehabilitation and other health-care disciplines. A BCI is a communication system that allows a person to convey her/his intention to the external world by merely thinking without depending on the brain's normal output. Of particular relevance for BCIs are electrical potentials recorded through non-invasive techniques. Electroencephalogram (EEG) is the preferred brain monitoring method in current BCIs for its high time resolution, non-invasiveness, ease of acquisition, and cost effectiveness. Power spectrum analysis is usually used for decomposing the EEG signal into different frequency bands. NU, as in a BCI system, use EEG signals; however, these signals are automatically conditioned by audio-visual entrainment.

Intermittent photic stimulation (IPS) can induce in the EEG photic driving, a physiologic response consisting of rhythmic activity time-locked to the stimulus at a frequency identical or

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harmonically related to that of the stimulus [1]. It is currently assumed that the distinguished action of different neural circuits within the brain occurs at different specific frequencies, and the principle of resonance filtering of stimuli underlies the responses to external stimulation and encoding. Photic driving can directly influence psycho-physiological mechanisms. Because of driving at the alpha frequencies produces a calming effect and marked physiological reactions in heart rate or in galvanic skin resistance, these properties have been applied in intervention context [2]. More recently, frequency-following response (FFR) was used as an EEG signal to explore how the auditory system encodes temporal regularities in sounds [3], mainly with a direct right-lateralized contribution from the auditory cortex [4]. External rhythmic stimuli elicit a narrow-band ongoing response at the stimulation frequency, the so-called steady-state response (SSR) or entrainment. It is commonly assumed that, whether were SSRs, or sensory entrainment, rhythmic stimulation evokes local modulation sofpower, e.g., in the visual cortex [5, 6]. A typical setup for SSVEP-BCI is to have subjects observe repetitive visual stimulus (RVS) in the surrounding area; the brain processes that concurrently occur are able to modulate the SSVEP elicited by RVS. Another noteworthy aspect of this field is that different colours of the visual stimuli affect the elicited SSVEP [7]. Research have indicated that patients with a major depressive disorder had low activation in the right temporo-parietal cortex when watching arousing stimuli, although they had normal occipital activation [8]. SSVEPs were also classically considered as a marker of anticipative anxiety and some studies have stated that the associations among psychometric measures of anxiety and depression and EEG spectral power measures were positively correlated to alpha and negatively correlated to delta, regardless of cortical area [9, 10]. In high-anxiety individuals, alpha 2 sub-band seems to be the most reactive.

Frontal activity characterized in terms of decreased power in the alpha band has been reliably found to be associated with emotional states. Indeed, the relatively greater trait left frontal activity is associated with tendencies toward a general appetitive, approach, or behavioural activation motivational system, and the relatively greater right frontal activity is associated with trait tendencies toward a general avoidance or withdrawal system. Delta oscillations are usually considered a correlate of inhibition and some disorders, like depression, obsessive compulsive, and anxiety are associated with an increase of delta power. A substantial evidence linking delta oscillations with the brain reward circuits suggests that the nucleus accumbens reward system is dysfunctional in individuals who suffer from depression. Anhedonia is a vulnerability factor for some psychiatric disorders and in a non-clinical sample was associated with increased resting delta current density in the rostral anterior cingulate cortex. Anhedonic individuals showed selective reduction of activity for music in the nucleus accumbens (NAcc) - but normal activation levels for a monetary gambling task - exhibiting decreased functional connectivity between the right auditory cortex and ventral striatum (including the NAcc). In contrast, individuals with greater than average response to music showed enhanced connectivity between these structures. These findings suggest that specific musical anhedonia may be associated with a reduction in the interplay between the auditory cortex and the sub-cortical reward network [11]. Increased theta and delta power have been associated with poor antidepressant treatment response. Knyazev [12] showed that when subjects expected bad news, delta power and connectivity increased in the orbit-ofrontal and the anterior cingulate cortices mostly for individuals who express higher scores on state anxiety. These findings suggested that frontal medial delta activity rises when this area receives less dopaminergic firing from the nucleus accumbens. Other investigations have related activity within primitive structures (such as the periaqueductal grey region, PAG) to emotional states of 'panic' [13] which are often triggered by somatic sensations, involving rapid onset, intensifying autonomic reactions, feelings of immediate danger, and urge of escape.

The existence of an array of conditions, which share thematic forms of neural oscillatory disturbance [14, 15] suggests a possible positive role for technologies that may profitably influence neural regulation. Psychological disorders can be a product of alteration from typical circuitry in limbic, frontostriatal and prefrontal regions rather than neurochemistry [16], and that rather than specific regions of dysfunction, network involvement is frequent, as showed from common comorbidity, as in DMN (Default Mode Network) and SN (Salience Network) for depression [17].

Anxiety disorder is a generalized term for a variety of abnormal and pathological fear and anxiety states, including GAD, PD, agoraphobia, obsessive-compulsive disorder, phobic disorders, and traumatic stress disorders. Different subtypes of depression have been defined, and the clinical course of depression is acknowledged variable with patients moving in and out the diagnostic subtypes over time. Depressive and anxiety disorders are highly prevalent and associated with high levels of service use, a considerable disease burden [18], substantial economic costs [19], and a significant loss of quality of life [20]. Several effective treatments for depressive and anxiety disorders are available, including antidepressant medication and different forms of psychotherapy. Cognitive behaviour therapy (CBT) is typically considered the gold standard in anxiety treatments. However, due to methodological issues, the magnitude of its effect is currently difficult to estimate. Differences in success between psychotherapy and antidepressant medication were small to non-existent [21], and different kinds

of antidepressants and psychotherapies have varying levels of efficacy in treating depression and anxiety disorders. Finally, pharmacotherapy and psychotherapies have side effects, although at a different grade.

Technology based intervention might provide a valuable treatment method also reducing the presence of therapist [22]. Comparable treatments may be equal in effects, but they may not be equal in terms of costs and individual preferences. Neuro-Upper (NU) is the novel device created in our laboratory set on brain responses to exogenous repetitive visual and auditory stimuli. Music excerpts are able to triggering specific brain waves and physiological responses also recruiting motor regions [23-25]. Baroque music (60 bpm) induces alpha rhythms and slows down heart and respiration rates, whereas music with driving rhythms or fast tempos (e.g., rock) stimulate beta waves and speed up heart and respiratory rate together with global extra-musical responses [26]. Neuromodulators, endorphins, endocannabinoids, dopamine and nitric oxide resulted transformed because of musical experience. Different arrangements appear to activate distributed brain regions in unique ways, so brain responds predictably to different styles, fragments of music or voices [27] suggesting robust interactions between subcortical regions and auditory sensory cortices that establish a potentially rewarding stimulus [28-30] and indicate the involvement of dopaminergic mechanisms [31]. The powerful pleasure associated with music listening is related with dopamine activity into the mesolimbic reward system, including both dorsal and ventral striatum [32]. These effects are not restricted to the harmonic or metrical structure including other music characteristics, as well as timbre, loudness changes, pitch [33] or the integration of verbal content [34]. Entrainment is most likely with periodic stimuli [35] eliciting periodic responses at frequencies compatible with their periodicity and providing indication for a selective neuronal entrainment [36].

A previous study [37] has offered preliminary evidence of audio-visual entrainment effect demonstrating a significant decrease in depressive symptomatology following NU treatment and unexpected improvements in cognitive function. NU collects EEG data through one-channel recordings and delivers flickering light - corresponding to the eight frequency bands and sub-bands measured - through an array of coloured lamps with direct mapping in near real time. In these terms, NU can be conceptualized as a BCI. The device have direct access to the information in brain activity so that the machine can interpret it and, thus, generate to the user a feedback of his/her brainwaves. Furthermore, they may also increase the user's sense of embodiment with the machine; that is, the users sense that the device is an extension of their own body [38]. In the course of running of NU sessions to subjects of the first study, it was estimated to occur changes in emotional symptomatology (not requiring additional clinical intervention) in approximately 85-90%. Probably, NU exposure facilitates healthful reorganization of neural oscillations at some level (s) of primary neural process, with consequences for both neuropsychiatric health and downstream peripheral physiology.

The main goal of the present investigation was to evaluate the intervention effects with a randomized controlled trial in individuals affected of anxiety and depressive disorders sampled from the general population focusing on the relationships between brainwaves changes and psychometric data. Another aim was to investigate the relationship between neuroendocrine measures and NU training. Several studies have reported reduced *5-hydroxyindoleacetic acid* (5-HIAA) in individuals who have a history of depression, impulsivity, such as suicidal attempts and violent acts, irrespective of their diagnosis [39]. A wide variety of stress has been associated with an activation of the HPA axis, and accordingly to quantify the dysregulation of the HPA axis cortisol levels are measured and were shown to be increased in depressed patients [40]. Hypo function of the serotonin system, indicated by lower levels of 5-HIAA and higher cortisol levels tend to co-segregate [39, 41] -for instance in borderline personality disorder, a disease that commonly presents with comorbid depression and may share neurobiology. We anticipated higher cortisol and lower 5-HIAA levels in participants at the baseline evaluation; consequently, cortisol and 5-HIAA responses to the NU treatment are longitudinally investigated predicting a concurrent change with the symptoms remission.

Method

Participants

Recruitment was carried out in the general population of the city of Parma, in the northeast of Italy. The individuals who responded to a call requesting voluntary people with depressive and or anxiety symptoms are invited to a face-to-face screening. Individuals who exhibit any behavioural and psychological symptoms of dementia (MMSE scoring <23) were excluded. Subjects were also excluded if they had severe medical conditions. Concurrent psychotropic medication were allowed but any change in prescriptions over the course of the treatment period was scrutinized. Eligible adults aged ≥18 years were screened using the Hamilton Rating Scale for Depression (HAM-D) [42] and the State Trait Anxiety Inventory Form Y (STAI-Y) [43]. The following severity ranges were used for the HAM-D scoring: no depression (≤7); mild depression (8-17); moderate depression (18-24); and severe depression (≥25) suggesting that the threshold to define remission (i.e., the absence of depression) should be equal or lowerthan7. The STAI-Y is divided into two sections, each composed of twenty four-point Likert items: STAI-Y1 assesses state anxiety while STAI-Y2 assesses

trait anxiety. Individuals who score between eight and more at the HAM-D and positioned themselves above the 95 percentile at the STAI-Y2were enrolled for the study.

Seven subjects completed on average fifty-two experimental sessions of NU training (mean age 47.29 years, SD=14.98). Subjects assigned to control group were eight (mean age 51.25, SD=6.96). All subjects reported having normal hearing, no neurological conditions and all had no musician training. All subjects declared the absence of neurological illnesses, and were screened for the photosensitive epilepsy. They were right-handed as indicated by the Edinburgh Inventory [44]. All participants did not significantly differ in age and educational level (Table 1). All participants had single or multiple comorbidities along with the Diagnostic Statistical Manual of Mental Disorders criteria for personality disorders.

The research was conducted in the Cognitive Psychology Laboratory of the Department of Medicine and Surgery at the University of Parma. All the details of the experimental procedures and the research targets of the paradigm were explained to the subjects, who agreed to voluntarily participating in the study. Informed, written consent was obtained from all of the participants. The research was carried out in accordance with the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. Given that the experiment did not involve clinical tests, use of pharmaceuticals or medical equipment or involve participant discomfort in any other way, in agreement with the regulation of the local ethical committee of the University of Parmathis study was exempt from approval of Ethics Committee for Clinical Research of the University of Parma. All procedures were non-invasive and the subjects were free to withdraw at any time without any penalty.

Material and Apparatus

Each participant underwent to a complete assessment involving first the Structured Clinical Interview for DSM-IV-R Axis II Disorders (SCID-4-RV) [45]. Then they received the following tests: Spielberger State Trait Anxiety Inventory (STAI) [43], Hamilton Rating Scale for Depression (HAM-D) [38], Wechsler Adult Intelligence Scale Revised (WAIS-R) [46], Raven's Progressive Matrices (SPM) [47] and Mini Mental State Examination (MMSE) [48]. The WAIS-R has retained the original WAIS format: it contains the same 11 subtests that are used to derive Full Scale, Verbal, and Performance IQs. All tests were administered and scored according

Table 1. Demographic data, concurrent treatments of participants, and group assignment.

Participant	Age	Gender	Marital status	Education level	Treatment		Group	
					Medical	Psychotherapy	Experimental	Control
S1	55	M	Married	Pre high school	No	N	x	
S2	29	F	Single	College	No	N	x	
S3	60	F	Married	Post-graduate	Xanax	N	x	
S4	57	M	Married	Pre high school	Xanax - Xiprilex	N	x	
S5	52	F	Divorced	High school	Xanax	N	x	
S6	23	F	Single	College	No	Y	x	
S7	55	M	Married	Post-graduate	Xeris	N	x	
S8	51	M	Single	Post-graduate	No	N		x
S9	48	F	Married	Pre high school	No	N		x
S10	59	F	Divorced	High school	Zoloft	Y		x
S11	46	M	Single	College	Zoloft - Lorazepam	N		x
S12	43	F	Married	Pre high school	No	N		x
S13	63	M	Married	High school	Xanax	N		x
S14	46	M	Married	College	Remeron - Escitalopram	N		x
S15	54	F	Single	Post-graduate	Thymanax - Citalopram	N		x

M: male, *F:* female, *Y:* yes, *N:* no

to standardised procedures. Cortisol concentration in 24 hours and 5-hydroxyindoleacetic acid (5-HIAA) urine test were also measured in two times (pre-test and post-test).

NU is already employed in a previous study with a clinical sample [37]. NU used a Mindwave® headset measuring raw EEG activity data at a 512 Hz sampling rate. Mindwave® headset is one of the most user-friendly, simple-to-use and low-cost a portable EEG. It detects user's electrical brain activity and decomposes the signal into eight outputs according to their frequency: delta (0.5 - 2.75 Hz), theta (3.5 - 6.75 Hz), alpha 1, the "low alpha" band (7.5 - 9.25 Hz), alpha 2, the "high alpha" band (10 - 11.75 Hz), beta 1, the "low beta" band (13 - 16.75 Hz), beta 2, the "high beta" band (18 - 29.75 Hz), gamma 1, the "low gamma" band (31 - 39.75 Hz), and finally gamma 2, the "mid gamma" band (41 - 49.75 Hz). Despite the EEG data were measured through a single electrode, the classification outcome resulted not far behind than multi-electrode BCI [49]. In NU these outputs are amplified and transferred, through aproprietary hardware-software system, to an array of coloured lamps producing flickering light with direct mapping to the monitor of a personal computer. Even though red has revealed to obtain an attentional advantage, blue light increases subjective alertness and performance, we used also green and yellow because these colours are indicated as potential candidates for SSVEP-BCI for their association with a positive content [50, 51]. The array of light lamps, using a multi-colours paradigm (eight PAR 56 Omnilux® lamps, 300 Watts, 26 x 23,5 x 22 cm each covered by coloured gelatine sheets), is controlled by the hardware-software system providing the frequency of each brainwave and translating EEG feedback into flashing stimuli. The lamps array is positioned about at 210 cm of distance from the participants' location and at height of 180 cm from the floor. The hardware-software system translates the EEG frequencies detected every second to a flash, which is received by the subject through different lamps. Depending on algorithm calculations, the delay between measurement and analysis of neural oscillatory activity and consequent presentation of corresponding flash can be as narrow as an estimated between 0.2 and 0.4 msec. The process then iterates. Microsoft Visual C # software is employed to displaying in real-time brainwave patterns modifications. The feedback application is the user's interface that translates data coming from the signal-processing unit of NU into a visual representation on the computer screen to provide a second visual feedback to the user. Brainwaves' occurrence was recorded every second for all the participants.

Play-lists, without limitations about the genre of music, including classical, folk, jazz, electronica, rock or tango (see http://www.zlab.mcgill.ca/supplements/supplements_intro.html for samples) are arranged and maintained constant for each participant and changed only two times along the training period. In a repetitive musical experience, the temporal cues signalling that a potentially rewarding auditory sequence is coming can trigger expectations of euphoric emotional states and create a sense of waiting. The peak emotional response evoked by hearing the known sequence would represent the fulfilled expectations and the correct reward prediction. Each of these phases [32] may involve dopamine release, but in different sub-circuits of the striatum with dissimilar connectivity and functional roles.

NU has been establish as a safe procedure. Based on experience with provision of case management support, feedback from research's participants, the developer and researcher are not aware of any serious adverse events resulting from undergoing NU. On an anecdotal basis, individuals undergoing NU may report an apparent "release of emotions" or paradoxical effects especially in the beginning, which can manifest as brief periods of increased awareness of emotional states, both positive and negative. These experiences are typically transient, that is, lasting intermittently over the course of one to several days.

Procedure

Subjects assigned to the experimental group were invited to participate in daily sessions (from Monday to Friday) scheduled at the same time. They were told to sit relaxed wearing the Mindwave® headset and were instructed to look at the flickering lights with eyes open in a darkened room hearing the playlists through headphones. Musical excerpts are binaurally presented at a comfortable hearing level to each subject in daily sessions of 30 minutes until reaching a total of consecutive sessions comprised between 40 and 60. Participants were required do not to listen to those pieces anymore during the study to ensure the same number of exposure for all the subjects. They are invited to relax avoiding any superfluous head or body movement. Visual stimulation of the recorder EEG pattern was delivered using the flashing effector. Graphic waves' patterns are rendered on the computer screen in the form of bar histograms showing the data for different frequency bands and sub-bands in real-time. Such complex feedback signal is motivated by the assumption that it may lead to a higher commitment of participants, thereby optimizing training outcome. But, it was also been argued that a continuous updating of the feedback signal and the thereby introduced cognitive load may eventually interfere with the main task; furthermore, during the first study it was observed that subjects tended to no longer look at the computer screen [37]. It may fail to profit from continuous feedback because of the delay between the mental modulation of neural events and feedback signalling the outcome. Following the last session (post-test), the same tests as the baseline were administered to provide useful post-treatment assessment of participants' progress.

Participants assigned to the control group are placed in waiting

list and did not receive any treatment, with the exception of the participation to a self-help group (at an ONLUS association not involved in the study) which was on a fifteen-day basis. The procedure followed in control condition consisted exclusively in the administration, in two different times, of the same tests battery as the experimental subjects. The first measurements were obtained during the screening phase. After a period of about 4 months, equivalent to the time during which the experimental group had completed treatment sessions with NU, all participants included in the control group were invited to undergo the test battery again in order to assess the occurrence ofany changes.

Adverse Events

The experimental intervention was well tolerated. The only side effect reported was headache, which affected one participant. The severity of the headache was reported to be slight and did not stop him from continuing with treatment.

Results

To address research question about possible differences due to the NU training, we first evaluated the comparisons of pre- and post-treatment scoring on the psychological battery (WAIS-R and RPM of HAM-D, STAI Y-1 and Y-2, QIV, QIP and QIT) to detect possible differences between the two measurements within of the groups. The Wilcoxon rank-sum test was used to define differences between pre and post-treatment measures (p values <.05 were considered significant). Since most of subjects refused to perform the second cortisol and 5-HIAA evaluations, these data were excluded from the analysis.

Figure 1. shows all mean scores and standard deviations obtained from the experimental and control groups in the two assessment phases. In order to evaluate the differences in test scores between the two groups, and thus a possible effect attributable to the treatment, Wilcoxon - Mann - Whitney (WMW) test for independent samples was used. Specifically, once verified the assumptions of the normal distributions to be compared and the homoscedasticity of their variances (with Shapiro-Wilks and Levine test), U tests were first applied to the mean scores obtained from the two groups in the first measurements and, if no significant difference was found, they were applied to the scores for the second measurements. Due to the small sample size, these analyses were preferred rather than mixed ANOVAs to repeated measurements.Wilcoxon-Mann Whitney's U tests applied to the pre-test data of the two groups revealed significant baseline differences for STAI Y-2, representing a mean score of 40.71 for the experimental group and a mean score of 55.12 for the control group (U=49.5, p=.014). Consequently, data for this post-test scale have been excluded from the subsequent analyses. The mean pre-test scores in other scales did not differ significantly (STAI Y-1: 42.14 *vs.* 53.25, U=42, p=.117; HAM-D: 19.71 *vs.* 14, U=15.5, p=.122; QIV: 104.1 *vs.* 114.4, U=34.5, p=.487; QIP: 104.1 *vs.* 115.8, U=30, p=.862; QIT: 117.9 *vs.* 116.1, U=27, p=.953; RPM: 45.57 *vs.* 42, U=21, p=.451), thus allowing the applicability of the analyses also for the corresponding post-test.

In the present study, we do not focused on the distinction between different levels of disorders severity because of the small number of subjects. Although there were no significant differences in the pre-test for the two groups in HAM-D scores, participants in the experimental group showed a more severe depression (3 participants a severe level, 1 a moderate level, and other 3 a mild level) than subjects in the control group (3 subjects a moderate and 5 a mild level). As mentioned above the two groups differ significantly for the trait anxiety (STAI Y-2), with the control group having in this case scores higher than the experimental group.

The statistical analyses performed on the data obtained from experimental group subjects suggested significant differences between baseline and post-treatment measurements for the depressive symptoms and cognitive functions expressed in QI. Specifically, a significant decrease in the mean score of HAM-D was found (x=19.71 and x=7.71, W=28, p=.022) suggesting a remission of depressive symptoms, and a significant increase in the three QIs obtained at the WAIS-R.

Unexpectedly, the mean Verbal QI (QIV) significantly increases (x=104.14 and x=130.57, W=0, p=.016), as the Performance QI(QIP=104.1 and QIP=145.6, W=0, p=.016) and the Total QI (QIT=117.9 and QIT=143.9, W=0, p=.016) indicating an heavy progress. RPMs scores showed a slight improvement that does not reach statistical significance (x=45.57 and x=48.14, W=4, p=.209). STAI Y-1 and Y-2 scores indicated a significant increase in anxiety levels (State anxiety x=42.14 and x=53, W=3, p=.14; Trait anxiety x=40.71 and x=48.14, W=5, p=.15 respectively).

Data analysis by Wilcoxon from the control group shows one significant difference between pre and post-treatment assessment in STAI Y-1 score which dropped from an average of 53.25 to 43.38 (W=36, p=.014) suggesting a decrease in State anxiety, probably due to the improved familiarity with the researcher during the second evaluation. Moreover, scores for the Y-2 form of the same scale demonstrated a significant decrease (x=55.12 andx=51.5, W=29, p=.141) suggestive of a diminution in Trait anxiety possibly attributable to the medical treatment or to theparticipation of self-help group activities. Nevertheless, scores for depressive symptoms and cognitive functioning, fail to suggest any significant difference between pre and post-test measurements (HAM-D 14 and 11.62, W=21, p=.270; QIV 114.4 and 118, W=8, p=.195; QIP 115.8 and 121.8, W=5, p=.079; QIT 116.1 and 120.1, W=7.5, p=.161; RPM 42 and 45.25, W=4.5, p=.126 respectively).

At the post-test no statistically significant differences were detected

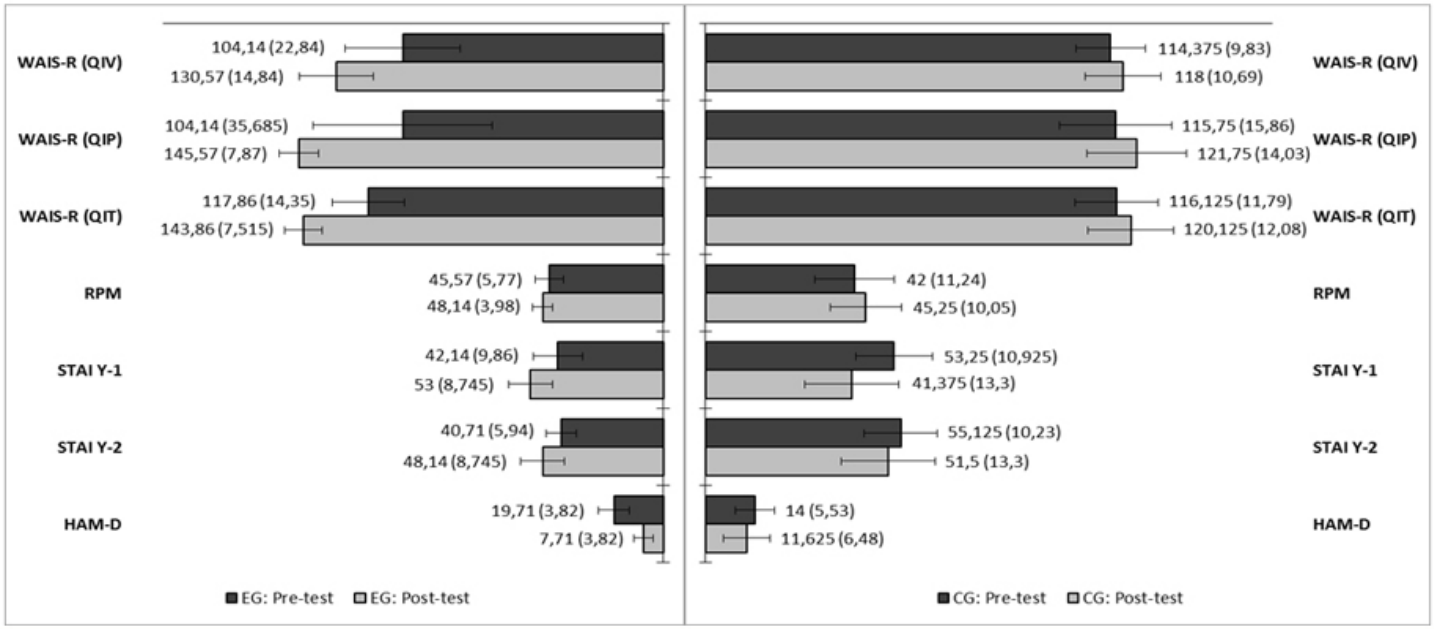

Figure 1. Mean scores and standard deviations (in parenthesis) for each test used during pre and post-treatment measurements. The left panel shows data for participants of the Experimental Group (EG) and the right panel reports data from the participants of the Control Group (CG).

between the experimental and control groups in most of measures (STAI Y-1 53 *vs.* 41.38, U=14, *p*=.117; HAM-D 7.71 *vs.* 11.62, U=38.5, *p*=.245; RPM 48.14 *vs.* 45.25, U=25.5, *p*=.816), the only noteworthy difference is obtained for the QIs. Specifically, the QIP mean scores is significantly higher in the experimental group compared to those of the control group (QIP 145.6 *vs.* 121.8, U = 4, *p*= 0.006; QIT 143.9 *vs.* 120.1, U = 3, *p* =0.004).

The Figure 2. displays both mean scores and standard deviation (in parenthesis) for each WAIS-R Verbal (Information, Digit Span, Vocabulary, Arithmetic, Comprehension, and Similarities) and Performance IQ subtests (Pictures Completion, Pictures Arrangement, Block Design, Object Assembly, and Digit Symbol) obtained from participants in the Experimental Group. It can be seen from Figure 2 that scores in which the experimental group individuals showed better gains were Vocabulary (x=12.28 and x=16.71 for pre- and post-test measurements, respectively), and Arithmetic (x=8.28 and x=12.57 for pre- and post-test measurements) in the WAIS Verbal IQ. Block Design (x=8 and x=12 for pre- and post-test measurements), and Object Assembly (x=9.43 and x=14 for pre and post-test measurements) were best improvements for the Performance Scale IQ.

A frequency-domain-based method was implemented for the statistical analysis of brainwaves patterns. We estimated the power spectra of *M* seconds of data, and the power values at the eight frequencies were extracted. The power spectrum analysis is a kind of analysis technique that is used when the time-series signals change over time are transformed into the frequency field, and the signal aspect is evaluated by the degree of change in the frequency. Time-series analysis carried out on median values of spectral power for each sub-band showed that some of the quantitative (median) values are rather stationary, exactly delta (R^2=0.0069) and theta (R^2=0.0049) bands, while the others exhibited a downward trend, confirming only in part data from other studies [52]. To some extent consistent with the entrainment hypothesis, data failed to demonstrate the increase in alpha band, which usually correlates with improved relaxation and increased mental alertness or clarity. On the contrary, beta waves reductions (between 20 and 30 Hz) correlate with decreases in anxiety, ruminative thoughts, and obsessive/compulsive-like behaviours.

Finally, with the aim to evaluate the relationships between brainwave patterns and test scores both the median differences for each band or sub-band between the pre and post-treatment measurements and the tests differences between the pre and post-treatment data for the experimental group were calculated. These data were included in multiple regression models, which were reduced following a stepwise back procedure. Seven separate models were obtained, in which the dependent variable is represented by each test score (WAIS-R QIV, QIP and QIT, RPM, HAM-D, STAI Y-1, and STAI Y-2). For each model, were considered as independent variables only the differences in those brainwaves that, depending on the hypothesis of research, should affect the dependent variable: differences in mean of alpha 1, alpha 2, beta 1, beta 2 and delta for the clinical measures, and differences in gamma-1, gamma 2 medians and theta for the neuropsychological tests. Stepwise

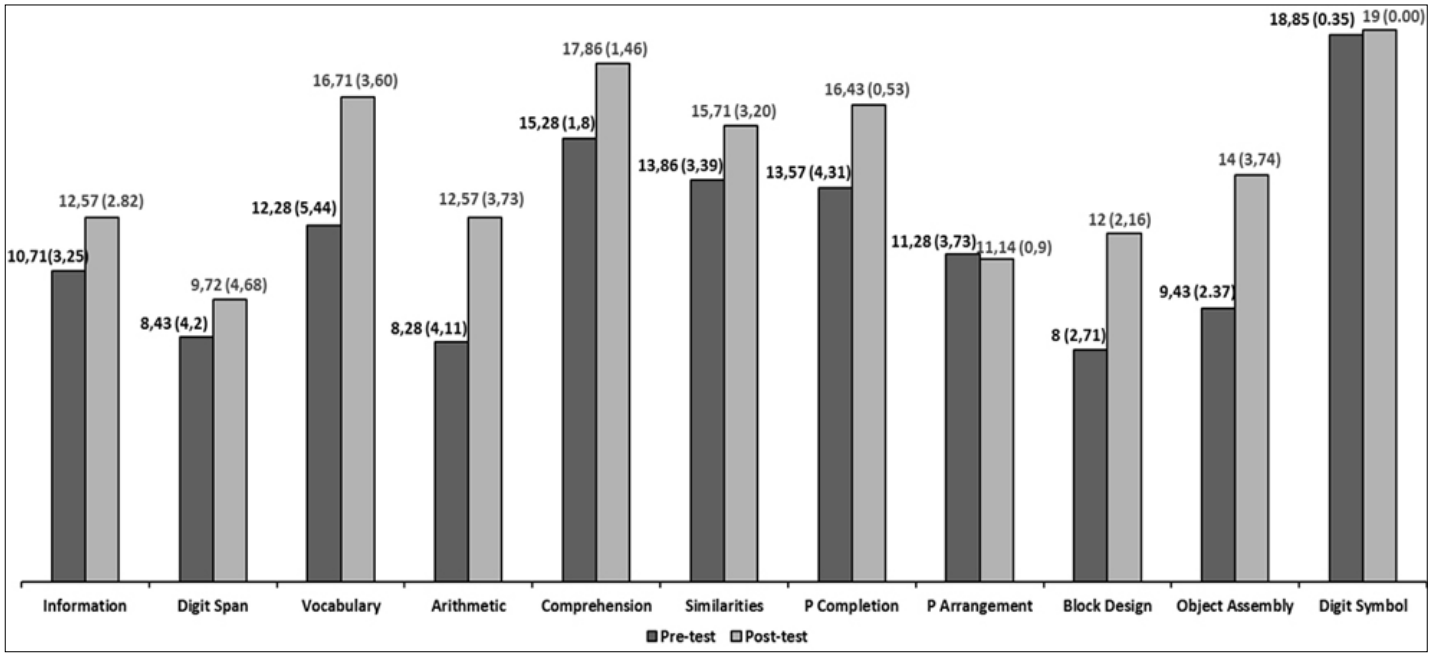

Figure 2. Mean scores and standard deviations (in parenthesis) at WAIS-R Verbal (Information, Digit Span, Vocabulary, Arithmetic, Comprehension, and Similarities) and Performance IQ subtests (Pictures Completion, Pictures Arrangement, Block Design, Object Assembly, and Digit Symbol) obtained from subjects of the Experimental Group during pre and post-test measurements.

regressions, designed to reduce the seven models created, suggested that variations in QIT and RPM scores were not predicted by any of the factors included in the two models (gamma, gamma, and theta differences).

Variability in the HAM-D scores seems to be better explained by the differences found in beta 1, beta 2 and delta, with a model in which the probability value associated with F [3,3] =8.268 is at the threshold of significance (p=.058). Variations in STAI Y-1 scores reveal acorrelation with the predictors "alpha 2 differences" and "beta 2 differences" for which the model is significant

(F [2,4]=10.07, p=.027). Furthermore, for the scores changes in STAI Y-2 seem to be predicted by all the factors included in the model, but in this case the probability value associated, F [5, 1] = 2.359, is not significant (p=.456). Finally, the increases in QIV and QIP scores appear influenced by only the variations of the gamma 2 sub-band and of theta band, respectively. However, in the first case the model does not appear significant (F [1, 5] = 2.162, p=.201), while a significant effect of the independent variable on QIP changes (F [1, 5] = 11.76, p=.018) was detected in the second case.

The finding that the remission of depressive symptoms measured with HAM-D after treatment was significantly associated with the variations in beta 1, beta 2 sub-bands and delta seem in contrast with prevailing evidences. During treatment, a downward trend in beta activity is observed, whereas research argues that is the increase in this activity that positively affect mood. Likewise, the correlation observed between delta activity variations and changes in depressive symptoms observed here is not found in other studies. It is documented that in depressive disorders, the slower waves such as delta are the most predominant, although confined to the symptoms of anhedonia or the reduced ability to experience pleasure: in the present study, however, the delta trend continuedunchanged along sessions.The increase in the state of anxiety symptoms (STAI Y-1) seems predicted by variations of alpha 2 and beta 2 sub-bands. If the reduction of alpha 2sub-band could explain the increase in anxiety at the second assessment, as the prevalence of alpha activity is often associated with a state of relaxation, on the other hand a greater beta activity would have to be observed, and this does not occur.

The only evidence that seems to confirm the hypothesis about the relationships between brain-patterns and test changes is the correlation between the increase in performance IQ at WAIS-R at post-test and the variations achieved by theta band following NU training. Usually, this brain activity prevails in frontal medial areas reflecting, in addition to contextual memory processes [9], a cognitive control mechanism for processing performance information. Perhaps it is not casual that during the last treatment sessions theta waves, afterward delta and alpha 1, were predominant and probably affecting the cognitive improvement.

Conclusions

The present results lend further support to the hypothesis that audio-visual stimulation can entrain the EEG frequencies with a certain

impact on anxiety and depressive symptoms. Real-time neuro-feedback allows simultaneous acquisition, analysis, visualization, and feedback of brainwaves. A limitation of neuro-feedback devices is the conscious associative learning required to obtain the EEG operant conditioning. On the contrary, NU technology does not aim to consciously teach the user through signals of reward or inhibition. The core technical aim of NU is to resonate with dynamically changing (obtained with audio entrainment) frequencies in the spectral EEG while the flickering lights provide to the establishment of following response. The underlying biological mechanism is still not completely understood, and is supposed to bea hebbian learning. Several limitations of the study should be acknowledged. The major limitation was the extremely small sample size. Participants showed different levels of depression/anxiety and some of them, in addition to the clinical disorders, had one or more personality disorders diagnoses in comorbidity. The adjunct use of medical treatment or psychotherapy is another interacting variable which effect cannot be easily distinguished. A third limitation is the absence of a follow-up, a placebo and/or control group with healthy subjects. Delivery of NU entails a consistent number of sessions in a quiet environment and instruction to relax listening music; these conditions might produce a placebo effect. In order to establish that clinical improvements associated with NU are attributable to the device, a placebo-controlled trial is crucial. Other research need to explore whether brain self-regulation persists once the training is discontinued, and if the cognitive improvement occurs with healthy individuals. Finally, the lack of biomarker parameters strongly limits the conclusions.

Despite the above-mentioned limitations and some unexpected result (the anxiety scores increase), the main findings support the idea that a dis-regulation of brainwave patterns may account for most of the symptoms (and cognitive dysfunction) in psychological disorders, and that some symptoms can be alleviated by audio-visual entrainment. The behavioural change produced by NU mainly in depressive symptomatology is probably due to a modulation of the insular activity, which plays a central role in sensory integration, emotion, and cognition or to a generalized brain activation, as shown by the improvement in cognitive ability. The idea that dopamine release may occur during audio entrainment has crucial implications, although other data provide neuro-chemical evidence that intense emotional responses to music involve ancient reward circuitry. Many psychiatric symptoms (anxiety, insomnia, and attention deficit) are intensified by excess cortical activation. At the end of NU sessions, many participants reported to feel more relaxed, yet alert, and have a greater sense of well-being. As with the neuro-feedback [53], it is possible that these first results open the door for future applications of NU as a research tool and a new approach to therapy.

Conflict of Interest Statement

The author declares that the research was carried out in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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