Scientific Research Publishing

World Journal of Neuroscience, 2024, 14, 103-119
https://www.scirp.org/journal/wjns
ISSN Online: 2162-2019
ISSN Print: 2162-2000

Clinical Treatment of Functional Pain Syndromes along the Microbiome-Gut-Brain-Axis: Combined Approach with Neuromodulation-Neurofeedback and Multispecies Probiotic

Maddalena Castelletti[1], Gloria Crocetti[2], Michele Masotti[3], Alberto Montagna[3], Annarita Graziani[4]

[1]Magenta Medical Center, Milan, Italy
[2]IPSE Clinical Center, Rome, Italy
[3]GEA Soluzioni, Turin, Italy
[4]Institut AllergoSan, Graz, Austria
Email: info@magentamedicalcenter.com

How to cite this paper: Castelletti, M., Crocetti, G., Masotti, M., Montagna, A. and Graziani, A. (2024) Clinical Treatment of Functional Pain Syndromes along the Microbiome-Gut-Brain-Axis: Combined Approach with Neuromodulation-Neurofeedback and Multispecies Probiotic. *World Journal of Neuroscience*, **14**, 103-119.
https://doi.org/10.4236/wjns.2024.144010

Received: May 18, 2024
Accepted: September 21, 2024
Published: September 24, 2024

Abstract

Functional pain syndromes are very common diseases that negatively impact the quality of life of patients with important socio-economic repercussions. The clinical alterations associated with these pathologies are multiple and have a complex psycho-organic character that moves along the micorobiome-gut-brain-axis. For the present study, 45 patients of both sexes (19 male, 26 female) aged 30 - 59 years were enrolled because of a diagnosis of Functional pain syndromes (FPS) that lasted for more than 6 months. All patients underwent pre-treatment clinical assessments (T0) for anxiety disorder, multidimensional assessment of pain, monitoring of baseline values of Alpha-Theta cerebral rhythm in occipital region and monitoring of salivary cortisol levels. All the patients underwent a clinical treatment combined with central neuromodulation with neurofeedback—Alpha Theta increase protocols (once a week for three months), administration of multispecies probiotic (one dose per day for 3 months) and clinical psychological interviews (once a week for three months). At the end of treatment (T1), patients were re-evaluated. Results show statistically relevant improvements of each feature considered: the Relief from Pain provided by the medication increases on average from 36.6% to

87.3%, the salivary Cortisol level at 11 pm decreases from 6.4 ng/ml to a physiological value of 1.2 ng/ml, and the anxiety rating score is reduced from 28 to 12. Moreover, the 23.9% increase in α-θ relative power shows the positive outcome of the brain autoregulation. This study highlights that the combined approach of Neurofeedback with drugs and multispecies probiotic results in great improvements in the patients' life.

Keywords

Functional Pain Syndromes (FPS), Neurofeedback-Neuromodulation, Hypothalamus-Pituitary Axis (HPA), Multispecies Probiotic, Microbiome-Gut-Brain-Axis (M-GBA)

1. Introduction

Functional pain syndromes (FPS) are a diagnostic group of conditions that remain loosely defined. Pain is often a warning signal from the body indicating an abnormality in structure or function. However, there is a subset of patients who experience pain and are subject to symptoms, suffering, and disability with unclear disease etiology or biomechanical cause. Many patients who experience FPS also experience clusters of symptoms that can affect a wide array of systems, often due to a combination of varying physiological, affective, and cognitive influences [1]. ISS (Italian Higher Institute of Health) data relating to 2023 indicate that chronic functional pain affects ten million people (around 4 million men and almost 6 and a half million women) and is present in 8% of the population aged 18 - 44, with an increase to 21.3%. among 45-54 years old, to 35% among the so-called "young elderly" (65 - 74-year-olds), up to 50% among those aged over 85. Too often, a patient receives a diagnostic label or labels that reflect the specialist's area of expertise as opposed to the patient's overall experience. For example, a patient with excessive bowel symptoms might present to a gastroenterologist who diagnoses her with irritable bowel syndrome (IBS), whereas a patient who has widespread muscle pain and tenderness may go to a rheumatologist who diagnoses him with fibromyalgia [2]. A recent study showed that these different conditions may actually reflect a common entity but have labels that reflect the specialists' area of expertise. The most common diagnoses that reflect this functional overlap include headache, functional gastrointestinal disorders (FGIDs), fibromyalgia, and chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) [2] [3]. The involvement of organ-system specialists in evaluating and diagnosing these complex chronic pain conditions mean that very little research has been done across disciplines to determine if these conditions are truly different or do, in fact, reflect a common entity. Research into the field of FPS is further hampered by the fact that the same symptom cluster is associated with various diagnostic names, for example, somatoform disorder, somatization, functional somatic syndrome, bodily

distress disorder, central sensitization syndrome, amplified pain syndrome, and primary pain disorder [3]-[6]. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) has changed the term to somatic symptom disorder, a name that had not been previously misused. In this iteration of the diagnosis, the manual's authors completely removed any distinctions between medically explained and medically unexplained somatic symptoms [7]. It should also be considered that recent neuroimaging studies have shown neurophysiological and neuropsychological alterations in patients with FPS; such abnormalities would result in a pathogenetic neural mechanism related to central and peripheral sensitization process; in particular, images obtained by functional Magnetic Resonance Imaging (fMRI) of subjects with PAP displayed increased levels of combined glutamine-glutamate (Glx) within the anterior insula and greater anterior insula connectivity to the medial prefrontal cortex (mPFC). Increased connectivity between these regions was positively correlated with anterior insula Glx concentrations and with psychopathological outcomes [8]. Patients who have repeated sensations of pain can develop memory traces on a neuronal level that increase sensitivity to further sensations. In these cases, a typically benign sensation can be interpreted as pain due to the neuronal memory traces. Over time, the memory trace can rewire larger portions of the brain and increase its range until a symptom memory matrix has been sensitized, and several physical symptoms can be triggered at once with increasing intensity; hypersensitivity has been shown in children with FGIDs, Crohn's disease, fibromyalgia, and chronic fatigue [9]. Dysregulation of the hypothalamus-pituitary axis' (HPA) ability to regulate the body's response to stress has been found in patients with FPS. Studies have shown that many patients with FPS have cortisol irregularities [10]. Several studies have explored the dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis in individuals with chronic pain, as well as the crucial role of the glucocorticoid receptor (GCC). The HPA axis is a key regulator of cortisol production, and abnormalities in the axis can lead to alterations in cortisol levels [11]. Moreover, elevated cortisol levels in response to chronic pain may perpetuate the pain cycle by contributing to muscle tension and inflammation.

The glucocorticoid receptor (GCC), a crucial component of the HPA axis, plays a significant role in the regulation of chronic pain [12]. The GCC is essential for modulating the anti-inflammatory and immunosuppressive actions of cortisol. Dysfunctional GCC signaling is implicated in chronic pain disorders, such as fibromyalgia and neuropathic pain, where alterations in GCC expression or sensitivity may influence the body's ability to control inflammation and modulate the nociceptive response [11]. Corticotropin-releasing hormone (CRH) production in the hypothalamic paraventricular nucleus (PVN) constitutes a key neuronal mechanism underlying HPA stress response. Chronic stress can activate the HPA axis through heightened stress responses, persistent basal hypersecretion, and adrenal depletion, with the manifestation of this reaction dependent on various factors [11]. Investigating the intricate interactions among chronic stress, the

GCC/HPA axis, and chronic pain holds promise for identifying novel targets in pain treatment. The intricate relationship between cortisol and chronic pain is crucial for developing effective pain management strategies [11]. The HPA axis plays an important role in the control of inflammation, that is, innate immunity, through cortisol. Indeed, GCs are potent anti-inflammatory agents and act by inducing apoptosis in monocytes, macrophages, and T cells and suppressing the NF-κB pathway [13]. This control on inflammation occurs in basal condition, whereas under other circumstances a different set of mechanisms can emerge, leading to HPA axis-related increase in inflammation. This process was named as glucocorticoid resistance, whereby it appears that immune cells become less sensitive to the effects of glucocorticoids. When glucocorticoid resistance develops, "fight or flight" responses to social threat are altered and determines exaggerated inflammation, particularly if these responses occur frequently. Thus, GC resistance provoked by chronic stress may determine a reduction in anti-inflammatory and pro-resolving actions of GCs and a prolonged inflammatory process. Different authors retain that these mechanisms could affect mental and physical health [13]. The biopsychosocial model of pain presents physical symptoms as the denouement of a dynamic interaction between biological, psychological, and social factors. While predisposing factors and consequences of chronic pain are well known, the flip-side is that factors promoting resilience, such as emotional support systems and good health, can promote healing and reduce pain chronification. Quality of life indicators and neuroplastic changes might also be reversible with adequate pain management. Clinical trials and guidelines typically recommend a personalised multimodal, interdisciplinary treatment approach, which might include pharmacotherapy, psychotherapy, integrative treatments, and invasive procedures [14]. Pain is a multidimensional experience with sensory-discriminative, affective-motivational, and cognitive-evaluative components. Pain aversiveness is one principal cause of suffering for patients with chronic pain, motivating research and drug development efforts to investigate and modulate neural activity in the brain's circuits encoding pain unpleasantness [15]. The efficacy of psychosocial interventions in the treatment of chronic pain of both organic and psychogenic origin is known in the literature [16]. Researchers have identified that different cognitive strategies to modulate pain evoke distinct brain activity patterns. For example, during focused attention, brain activity localizes to the pre- and postcentral gyrus (the primary motor and somatosensory cortices, respectively), middle occipital gyrus, and inferior parietal lobe, whereas reappraisal of the pain (imaging the painful stimulus alternating between harmful or nonharmful) engaged the thalamus, amygdala, ventral lateral PFC, MCC, and parahippocampal gyrus. Together, these findings and others suggest that combining specific strategies with targeted brain stimulation or neurofeedback enhances treatment efficacy [15]. Chronic pain often leads to depression, anxiety, and deconditioning, which are targets for treatment. Interventions have their place in the treatment of chronic pain and should be a part of a multidisciplinary treatment plan, personalized medicine

approaches based on good practice principles of chronic disease management [17] [16]. Neuromodulation is an expanding area of pain medicine that incorporates an array of non-invasive, minimally invasive, and surgical electrical therapies [18]. Chronic pain is a significant global health issue. For most individuals with chronic pain, biomedical treatments do not provide adequate relief. Given the evidence that neurophysiological abnormalities are associated with pain, it is reasonable to consider treatments that target these factors, such as neurofeedback (NF) [19]. Given the crucial role of the brain in the experience of pain and its modulation, researchers have hypothesized that direct manipulation of one or more brain regions could enhance pain modulatory systems and thereby reduce the underlying central nervous system (CNS) abnormalities associated with chronic pain. Researchers have developed neurofeedback techniques that teach individuals to self-regulate brain functionality. Neurofeedback is a noninvasive therapy that directly targets brain activity and/or connectivity patterns and uses either electroencephalograph (EEG) recordings or fMRI signals to provide individuals with real-time visual and/or auditory feedback reflective of the targeted brain functionality [14]. As a result of the huge improvement in the understanding of genomics, metagenomics and metabolomics, the human microbiome has been increasingly taken into consideration, in particular the gut microbiome, as highly relevant to systemic human health [20], enabling the definition of several functional axes such as the gut-brain, gut-liver and gut-lung axes. Furthermore, modulation of the host gut microbiome has been proposed as a potential treatment or prophylaxis for many disorders of the human body [21]. Depending on the composition of the gut microbiota, this can lead to protective effects when it is in balance or promote the development of disease in the case of dysbiosis [22]. Probiotics are defined as live microorganisms which, when administered in adequate amounts confer a health benefit on the host [23]. These bacteria and yeasts primarily support gut health by maintaining, or restoring, a balanced gut microbiota. Probiotics are commonly found in fermented foods like yogurt, kefir, kombucha, and in dietary supplements [24]. Probiotics function through a wide range of mechanisms, including modulation of the immune system, maintenance of the gut barrier integrity, production of antimicrobial substances and overall balancing of the gut microbiota [25]. Moreover, some probiotics, such as Bifidobacterium and Lactobacillus, have been clinically studied, and their potential for disease treatment has been investigated [26]. Currently, chronic pain remains one of the world's most persistent and unsolved clinical challenges. With the introduction of the microbiota–gut–brain axis, numerous scientists are paying attention to the regulatory role of gut microbiota in chronic pain. This research hotspot has also opened up a new frontier for understanding the mechanism of chronic pain. The host–microbiota interactions suggest that microbiota metabolites could activate the "pain-sensing" neurons to mediate pain. The alterations in the gut microbiota and its metabolites can directly or indirectly affect neuroinflammation and the neuroimmune system in the occurrence and signal transduction of pain [27]. The bidirectional commu-

nication signalling pathway between the gut microbiota and the brain has garnered extensive attention, and the bidirectional communication signalling pathway is described as the "gut microbiota—brain axis". Many studies explored the correlation between psychological disorders and the gut microbiome, finding specific gut dysbiosis patterns in patients suffering from depression and anxiety [28]. Clinical studies focusing on the administration of a specific multispecies probiotic containing the probiotic strains *Lactobacillus casei W56, Lactobacillus acidophilus W22, Lactobacillus paracasei W20, Lactobacillus salivarius W24, Lactobacillus plantarum W62, Lactococcus lactis W19, Bifidobacterium lactis W51* und *W52,* und *Bifidobacterium bifidum W23* showed relevant anti-inflammatory properties and reductions of inflammation monitored by the IL-6 gene expression in the participants to the study [29]. On the one hand, the brain can regulate various functions of the intestine through the sympathetic and parasympathetic branches of the autonomic nervous system (ANS), hypothalamic-pituitaryadrenal (HPA) axis, and endogenous pain regulation system [30]. Neuropathic pain (NP) is a persistent and irreversible condition that is usually chronic and occurs frequently. NP manifests as persistent or recurring pain, which gravely affects the quality of life of patients. Gut microbiota can act on a pivotal intersection between the neuroimmunoendocrine and gut-brain axes, establishing a complex network that is a key factor that directly or indirectly affects [31]. In summary, chronic pain is a pathology resulting from the interplay of many complementary factors: central or peripheral neurological alterations, endocrinological alterations, mental disorders such as anxiety and depression, alterations along the gut-brain axis and psychosocial factors [2] [5] [10] [12] [15] [20].

2. Materials and Methods

For the present study, 45 patients of both sexes (19 male, 26 female) aged 30 - 59 years were enrolled who turned to Magenta Medical Center, Milan and IPSE Clinical Center, Rome, because of a diagnosis of Functional pain syndromes (FPS) that lasted for more than 6 months with partial benefit from pharmacological therapies prescribed by physicians of the territorial primary care services. After approval of the ethics committee, compilation of the informed consent as per the European Community guidelines, all patients underwent pre-treatment clinical assessments (T0); it is important to underline that, when dealing with FTS, it is necessary to simultaneously evaluate both biological parameters and psychological and psychosocial factors *i.e.*, psychometric assessment for anxiety disorder through Hamilton anxiety rating scale (HAMA) [32], multidimensional assessment of pain with Brief Pain Inventory short version (BPI) [33], monitoring of baseline values of Alpha-Theta cerebral rhythm in occipital region and monitoring of salivary cortisol levels. All the patients underwent a clinical treatment combined with central neuromodulation with neurofeedback-Alpha Theta increase protocols (once a week for three months), administration of multispecies probiotic (one dose per day for 3 months) and clinical psychological interviews (once a week for three

months). At the end of treatment (T1) patients were re-evaluated with HAMA, BPI, values of Alpha-Theta cerebral rhythm in occipital region and monitoring of salivary cortisol levels.

2.1. Hamilton Anxiety Rating Scale (HAMA)

Evaluation of clinical anxiety referred to a population of adults and adolescents. A correlation between SSD and anxiety disorders is known in the literature [34] [35]. The scale is made up of 14 points, each of which is defined by a series of symptoms, measures of both psychological anxiety (mental agitation and psychological stress) and somatic anxiety (physical disorders related to anxiety). The score is obtained by evaluating the sum of the items. Each item is scored on a scale from 0 (not present) to 4 (severe). Score > 17 is considered to be of clinical relevance [32].

2.2. Brief Pain Inventory (PBI)

Multidimensional pain scales allow for a better structured assessment of pain that is not limited to measuring pain intensity and localization but also assesses the impact of pain on quality of life, psychological well-being and social activities; the BPI is a questionnaire that measures pain severity and interference with the patient's daily living activities. The localization of pain, drugs intake and pain relief are also evaluated. This test can be self-reported or investigated with a structured interview. The PBI short form is validated for clinical trials in the Italian population [33].

2.3 Salivary Cortisol Levels

Hypothalamic-Pituitary-Adrenal (HPA) axis dysregulation has been implicated in chronic widespread pain [12]. Also, elevated morning and nocturnal cortisol are risk factors for depression and anxiety disorders; high evening cortisol levels are often related to chronic pain conditions with associated anxiety and insomnia [36]. Salivary samples were performed by nursing staff using a *Salivette* swab and they were immediately analysed by the laboratory. The normal reference values for evening salivary cortisol (11.00 pm) are 0.6 - 1.6 ng/ml [37].

2.4. Clinical Psychological Interviews

The efficacy of psychosocial interventions in the treatment of chronic pain of both organic and psychogenic origin is known in the literature [16]. It is crucial to consider that psychological distress is one of the main mediators between chronic pain and disability. Psychological treatment is an important part of multidisciplinary care and a potential alternative to medication depending on the severity and nature of pain [38]-[41]. With regard to chronic pain, the Italian Consensus Conference of Pain in Rehabilitation assigns primary recommendation of psychological intervention compared to all other approaches, especially recommended in combination with neuromodulation treatments for pain management [16].

2.5. Alpha-Theta Neurofeedback Training Increase

The study of brain rhythms is fundamental in determining the mental state of a subject. EEG signal is divided into so called "frequency bands", where lower frequencies mark for more relaxed mental conditions. In particular, the following protocol (called alpha-theta training) is meant to help the subject learn how to force its own brain to move towards more calm brain activity, characterized by prevalence of alpha and theta rhythms. For EEG acquisition, a setup of four Ag or Ag-Cl electrodes was used to obtain two derivations: C3-O1 and C4-O2, following the 10-20 international system. A reference electrode was placed on the back of the non-dominant hand. The placement of the electrodes is crucial for the success of the procedure: it is necessary to achieve a low impedance between the electrodes (a suggested maximum of 10 kΩ) because the EEG signal has a much-limited amplitude (in the order of μV) and is therefore highly susceptible to external noise. The electrodes must, therefore, ensure adequate quality of the electrode-skin contact. The alpha-theta training protocol consisted in one session per week for three months, resulting in a total of 25 sessions, each one articulated as follows. First, a preliminary phase during which the initial brain activity of the subject, called baseline, is set: peaceful visual and auditive stimuli are given to the patient to induce a neutral/relaxed state of mind, which will serve as a reference for the training phases. The baseline is representative of the brain activity of the subject before the treatment and is needed to set a customized standard for each person approaching this procedure. This is the most delicate part of the session, because all the subsequent training phases are set upon the baseline. During this period, the EEG signal must be constant, and no specific events must occur. Relative power of the alpha and theta bands are recorded, and meaningful statistical values (average and standard deviation) are obtained and stored. These values are used to set specific ranges and thresholds for the training phases. There are three different training phases, each of them lasting between three and five minutes. The trainings are separated by resting periods of one minute each. During the trainings the subject's relative power of alpha and theta bands are acquired and used to give a visual and/or auditive feedback: if the relative power of the band of interest increases, images will be clearer and music louder as a positive reinforcement. If the relative power lowers, images will be blurrier and music volume will decrease. Also, some noise, such as rain noise, will increase in volume to cover the music as a not rewarding stimulus. The three trainings are ordered following an increase in difficulty, with the first one lasting three minutes and concerning alpha rhythms, which are easier to control. The second training lasts around four minutes and shifts towards theta rhythms only, while the third is the longest (almost five minutes) and asks the patient to control a specific frequency band between 6 and 9 Hz, called alpha-theta band. This pushes the subject to try its best to succeed, hence helping learn new strategies to stay calm and avoid or ignore stressors. During the process, the patient can monitor the relative power of the band of interest thanks to the help of digital indicators, which allow the patient to gain greater awareness of their own state. The more sensorial feedbacks are given to the patient, the better it enables good management of their alertness or relaxation state. The founding principle of this technique is what is

called autoregulation: using bio-neurofeedback techniques, the subject learns how to control and reproduce a certain physiological function. This is achieved by transducing cerebral states the patient is not aware of, into external stimuli that can be recognized and hence guide the subject into a deeper awareness and an enhanced ability to control physiological mechanisms. Biofeedback can be intended as a way to make an involuntary process voluntary [42] [43].

2.6. Multispecies Probiotic

The patients received a multispecies probiotic, with the following composition: corn starch, maltodextrin, inulin, potassium chloride, vegetable protein (rice), 9 human bacterial strains, magnesium sulphate, fructooligosaccharides (FOS), enzymes (amylases), manganese sulphate. The 9 human bacterial strains were: *Lactobacillus casei W56, Lactobacillus acidophilus W22, Lactobacillus paracasei W20, Bifidobacterium lactis W51, Lactobacillus salivarius W24, Lactococcus lactis W19, Bifidobacterium lactis W52, Lactobacillus plantarum W62 and Bifidobacterium bifidum W23*. One sachet of multispecies probiotic (7.5×10^9 CFU for 3 g) was mixed into approx. 1/8 L water once daily on an empty stomach.

3. Results

The violin plots presented in **Figure 1.** show the comparison between pre- and post-treatment scores. The considered scores are hereby explained in detail: **BPI Interference with Life score →** this score is obtained by computing the average of the scores provided for each of the last 7 items of the BPI questionnaire, which specifically focus on the interference the pain has on the daily activities of the patient, on a scale 0 - 10.

1) BPI Pain score → this score is obtained by computing the average of the 4 scores regarding the intensity of the pain felt, specifically the mean intensity, the pain felt at the moment of the questionnaire, the maximum and minimum intensities in the 24 hours previous to the questionnaire, on a scale 0 - 10.

2) BPI Relief from Pain score → this score specifies the level of relief obtained from the medication therapy, on a scale 0 - 10.

3) HAMA score → this score, as explained in section 2.2, is a metric for psychometric assessment for anxiety disorder, on a scale 0 - 56.

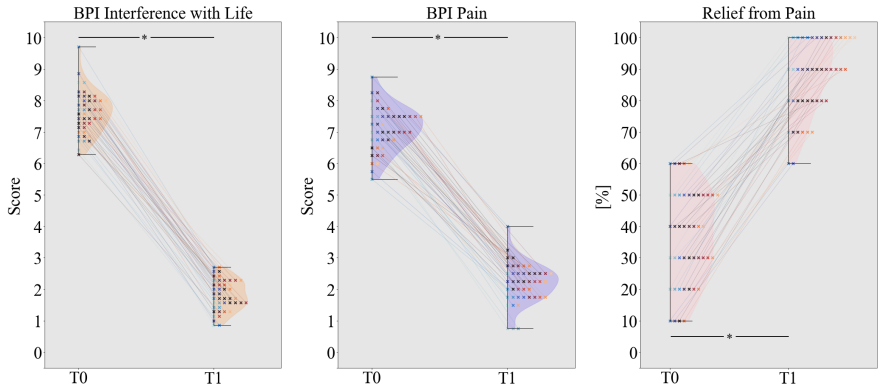
4) Cortisol h23 → the value of salivary cortisol, expressed in ng/ml, was collected for each patient at the same time of the day (11 pm) to grant the comparability of the metric between patients.

5) α-θ max → during the third stage of the Neurofeedback training the goal is to increase the relative power of the α-θ band [6 - 9 Hz], as explained in section 2. The chosen metric to determine the changes of this parameter during the experiment is the maximum relative power recorded during the third stage, expressed as a percentage of the total power.

3.1. Scores Comparison between Pre- and Post-Treatment

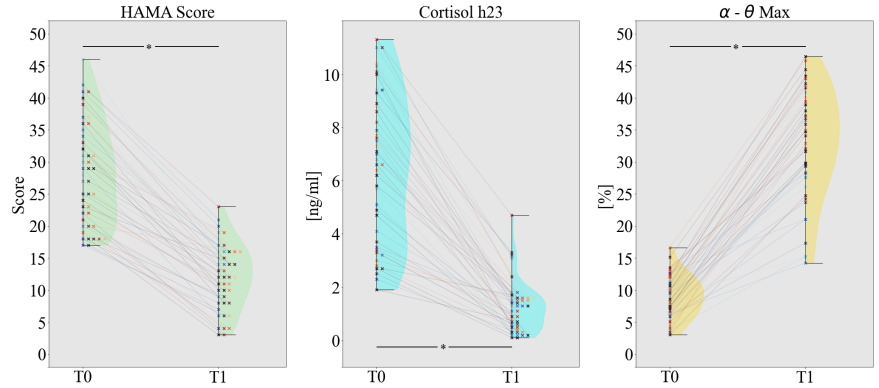
The violin plots were chosen as the best way to represent the data due to their

ability to highlight the distribution of the population pre- and post-treatment. These plots highlight how the samples are distributed and allow to understand the changes both at the patient level, by linking each subject pre- and post-treatment values, and at a higher level by showing the distribution change after the treatment. In **Figure 1.** the three extracted features from the Brief Pain Inventory questionnaire are presented, showing an average reduction of −5.8 on Interference with Life score, −4.9 on Pain score and an increase of 51.0% on the Pain reduction due to the treatment. In **Figure 2.** the Hamilton Anxiety Rating Scale, the Cortisol level at 11 pm and the α-θ maximum relative power are presented, showing an average reduction of -16.0 on HAMA score, −5.2 on Cortisol level and an increase of 23.9% on the α-θ value. In **Table 1.** these results are summed up.



*The Wilcoxon Signed-Rank test between the 2 distributions showed a statistically relevant difference with p << 0.001

Figure 1. Violin plots showing the distribution of the scores pre- and post-treatment. Interference with Life score (a), Pain score (b) and Pain Reduction (c).



*The Wilcoxon Signed-Rank test between the 2 distributions showed a statistically relevant difference with p << 0.001

Figure 2. Violin plots showing the distribution of the scores pre- and post-treatment. HAMA score (a), α-θ max percentage (b) and Cortisol level at 11 pm (c).

Table 1. Comparison between pre- and post-treatment.

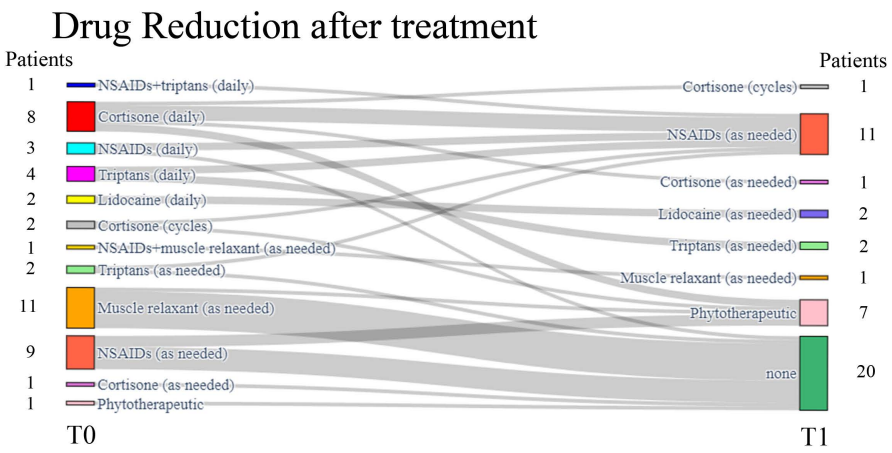
	Mean [$P_{0.05}$, $P_{0.95}$]**					
Time	HAMA*	Cortisol h23* [ng/ml]	α-θ max* [%]	Pain*	Reduction*	Interference*
T0	28.0	6.4	8.9	7.1	36.6	7.6
T1	12.0	1.2	32.8	2.2	87.3	2.2
T1 - T0	−16.0 [−26, −9]	−5.2 [−8.8, −1.4]	23.9 [8.2, 33.6]	−4.9 [−6.3, −3.5]	50.7 [30, 72.5]	−5.8 [−7, −5]

*The Wilcoxon Signed-Rank test between the 2 distributions showed a statistically relevant difference with p << 0.001; **$P_{0.xx}$ being the xx-th percentile.

To determine the statistical relevance of the results, an Anderson-Darling normality test was firstly performed on each distribution at T0 and T1 to determine whether to use a t-test or a Wilcoxon Signed-Rank test. Since only a limited number of features are normally distributed, hence breaking the normality assumption for a t-test, the choice was to perform a non-parametric Wilcoxon Signed-Rank test on each pair of distribution T0 and T1. Each considered aspect showed a statistically relevant variation on average (either reduction or increase) between pre- and post-treatment with a p-value < 0.001.

3.2. Drug reduction after Treatment

Due to the intrinsic complexity of the subject, many different customizable drug treatments are available to help the patient during the therapeutic path. For this reason, great attention was given to the evolution of the drug assumption after the treatment. To take into consideration the great variability between different drugs and doses, **Figure 3.** shows a Sankey Diagram specifically chosen for its great ability



NSAIDs = Non-Steroidal Anti-Inflammatory Drugs.

Figure 3. Sankey Diagram showing the flux of the patients' therapeutic path from pre-treatment to post-treatment. The height of each rectangle is directly proportional to the number of patients undergoing the labeled therapeutic path.

to highlight the change from one therapeutic treatment to another. The entire population (45/45 patients) was able to reduce the drug intake after treatment, either by reducing the dose, or by switching to a lighter therapeutic iter (phytotherapeutic drugs) or even by completely interrupting the drug assumption.

4. Discussion

The results show that α-θ Neurofeedback training performed once a week combined with daily assumption of multispecies probiotic and joined with drug therapy leads to an overall statistically relevant improvement in every considered feature. The pain and interference with life caused by the pathology are strongly reduced, while the relief from pain increases more than 50% on average. Moreover, on average, the HAMA score linked to anxiety state drops to 12, which is below the limit of 17, which characterizes anxious state of the patient and the Cortisol level at night, on average, gets back to 1.2, in the range of normal reference values of 0.6 - 1.6 ng/ml. Additionally, the increased α-θ relative power at T1 shows that patients were able to correctly regulate their brain rhythms in order to obtain a more relaxed state of mind. These findings reinforce the evidence that synergic therapies, which combine Neurofeedback and drug assumption with daily intake of multispecies probiotic result in strong improvements on different aspects of life, related to both drug intake and overall pain of the patient. Another strong aspect of this study lies in the consistency of the results on a relatively big sample, which counts 45 patients of both sexes, and therefore allows to extract conclusions to a certain degree of confidence. The results of the present study are in line with what was observed by Da Silva *et al.* that is, the best clinical treatment for chronic pain appears to be the combined approach between pharmacotherapy, psychological support and neuromodulation [44]. On the other hand, one weak aspect of this research that needs to be highlighted is the lack of control groups to be able to clearly establish the contributes of single treatments. Many different control groups should have been considered to evaluate the effects provided by Neurofeedback, probiotics and drugs taken singularly or in pairs and establish which of these treatments (or which combination) has the highest impact on the therapeutic path of the patients. Finally, it is necessary to consider a further critical issue relating to the great variety of drug therapies taken by different patients, which makes it less clear to evaluate how the combined therapy described in this study impacts with each drug.

5. Conclusions

Pain is an unpleasant experience with both organic and psychological characteristics and has a great impact on the quality of life of patients. [13]-[16]. In particular, the condition of functional pain of central origin correlates with anxiety disorders and alterations of the neural circuits involved in the perception and processing of painful stimuli with a tendency to chronicization and a decline in autonomy and quality of daily life activities [13]-[16]. It is also necessary to mention

the social cost of these diseases also linked to the intake of many drugs (often only partially effective) and the use of continuous medical consultations. Researchers have identified that different cognitive strategies to modulate pain evoke distinct brain activity patterns. For example, during focused attention, brain activity localizes to the pre- and postcentral gyrus (the primary motor and somatosensory cortices, respectively), middle occipital gyrus, and inferior parietal lobe, whereas reappraisal of the pain (imaging the painful stimulus alternating between harmful or nonharmful) engaged the thalamus, amygdala, ventral lateral PFC, MCC, and parahippocampal gyrus. Together, these findings and others suggest that combining specific strategies with targeted brain stimulation or neurofeedback enhances treatment efficacy [15]. Literature shows that chronic pain syndromes of central origin are often associated with alterations in biomarkers such as cortisol levels and microbiome status along the gut-brain axis [25]-[28]. Patients who have repeated sensations of pain can develop memory traces on a neuronal level that increase sensitivity to further sensations. In these cases, a typically benign sensation can be interpreted as pain due to the neuronal memory traces. Over time, the memory trace can rewire larger portions of the brain and increase its range until a symptom memory matrix has been sensitized, and several physical symptoms can be triggered at once with increasing intensity; hypersensitivity has been shown in children with FGIDs, Crohn's disease, fibromyalgia, and chronic fatigue. [9]. Dysregulation of the hypothalamus-pituitary axis' (HPA) ability to regulate the body's response to stress has been found in patients with FPS. Studies have shown that many patients with FPS have cortisol irregularities [10]. The host-microbiota interactions suggest that microbiota metabolites could activate the "pain-sensing" neurons to mediate pain. The alterations in the gut microbiota and its metabolites can directly or indirectly affect neuroinflammation and the neuroimmune system in the occurrence and signal transduction of pain [27]. The bidirectional communication signalling pathway between the gut microbiota and the brain has garnered extensive attention, and the bidirectional communication signalling pathway is described as the "gut microbiota—brain axis". Many studies explored the correlation between psychological disorders and the gut microbiome, finding specific gut dysbiosis patterns in patients suffering from depression and anxiety [28]. In light of all these considerations, it seems correct to treat chronic functional pain pathologies of central origin with an integrated approach that involves the association of different therapies such as gut microbiome modulation, neuromodulation with neurofeedback and clinical psychological interviews [16] [17].

The results obtained in the present research study seem to corroborate the hypothesis that integrated treatment is the approach with the most effective impact in terms of pain reduction, reduction of anxious states, reduction of drug intake (both in quantity and frequency), remodulation of cortical rhythms towards physiological parameters, normalization of cortisol levels and better quality of life assessed in individual psychological, relational, work and social aspects. Although the results obtained in the present clinical trial are encouraging, some critical

issues should be highlighted, such as the lack of control groups that could have better highlighted the contributes of single treatments and establish which of these treatments (or which combination) has the highest impact on the therapeutic path of the patients. A desirable future direction for the evaluation of the efficacy of the combined therapeutic protocol we propose could consist in the further expansion of the sample with the possibility of having control groups.

Acknowledgements

The authors would like to thank all the patients who took part in the present clinical study and who placed their trust in the researchers and all the collaborators involved. This paper is dedicated to all researchers in the world who, due to wars and conflicts, are unable to carry out their valuable work. With the hope that science will always be a means of peace.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Bourke, J.H., Langford, R.M. and White, P.D. (2015) The Common Link between Functional Somatic Syndromes May Be Central Sensitisation. *Journal of Psychosomatic Research*, **78**, 228-236. https://doi.org/10.1016/j.jpsychores.2015.01.003

[2] Fantoni, F., Salvetti, G., Manfredini, D. and Bosco, M. (2007) Current Concepts on the Functional Somatic Syndromes and Temporomandibular Disorders. *Stomatologia*, **9**, 3-9.

[3] Dell, M.L. and Campo, J.V. (2011) Somatoform Disorders in Children and Adolescents. *Psychiatric Clinics of North America*, **34**, 643-660. https://doi.org/10.1016/j.psc.2011.05.012

[4] Nimnuan, C., Rabe-Hesketh, S., Wessely, S. and Hotopf, M. (2001) How Many Functional Somatic Syndromes? *Journal of Psychosomatic Research*, **51**, 549-557. https://doi.org/10.1016/s0022-3999(01)00224-0

[5] Rief, W. and Isaac, M. (2014) The Future of Somatoform Disorders: Somatic Symptom Disorder, Bodily Distress Disorder or Functional Syndromes? *Current Opinion in Psychiatry*, **27**, 315-319. https://doi.org/10.1097/yco.0000000000000089

[6] Schechter, N.L. (2014) Functional Pain: Time for a New Name. *JAMA Pediatrics*, **168**, 693-694. https://doi.org/10.1001/jamapediatrics.2014.530

[7] American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. https://doi.org/10.1176/appi.books.9780890425596

[8] Castelletti, M., Berné, E., Castagnoli, E.D.C., Montagna, A. and Tonon, G. (2024) Galvanic Skin Response—Extinction Biofeedback Training for Psychogenic Abdominal Pain: A Validation Protocol. *Journal of Biosciences and Medicines*, **12**, 190-201. https://doi.org/10.4236/jbm.2024.125015

[9] Simons, L., Basch, M., Chow, E., Logan, D. and Schechter, N. (2015) Perspectives on the Clinical Significance of Functional Pain Syndromes in Children. *Journal of Pain Research*, **8**, 675-686. https://doi.org/10.2147/jpr.s55586

[10] Rief, W. and Barsky, A.J. (2005) Psychobiological Perspectives on Somatoform

Disorders. *Psychoneuroendocrinology*, **30**, 996-1002. https://doi.org/10.1016/j.psyneuen.2005.03.018

[11] Knezevic, E., Nenic, K., Milanovic, V. and Knezevic, N.N. (2023) The Role of Cortisol in Chronic Stress, Neurodegenerative Diseases, and Psychological Disorders. *Cells*, **12**, Article 2726. https://doi.org/10.3390/cells12232726

[12] Begum, N., Taylor, J.R., Brown, C., Rajan, J., Keevil, B., Pye, E., *et al*. (2021) Morning and Evening Salivary Cortisol Levels in Patients with Chronic Widespread Pain and Those at High Risk. *European Journal of Pain*, **26**, 197-206. https://doi.org/10.1002/ejp.1854

[13] Zefferino, R., Di Gioia, S. and Conese, M. (2020) Molecular Links between Endocrine, Nervous and Immune System during Chronic Stress. *Brain and Behavior*, **11**, e01960. https://doi.org/10.1002/brb3.1960

[14] Cohen, S.P., Vase, L. and Hooten, W.M. (2021) Chronic Pain: An Update on Burden, Best Practices, and New Advances. *The Lancet*, **397**, 2082-2097. https://doi.org/10.1016/s0140-6736(21)00393-7

[15] Mercer Lindsay, N., Chen, C., Gilam, G., Mackey, S. and Scherrer, G. (2021) Brain Circuits for Pain and Its Treatment. *Science Translational Medicine*, **13**, eabj7360. https://doi.org/10.1126/scitranslmed.abj7360

[16] Kohrt, B.A., Griffith, J.L. and Patel, V. (2018) Chronic Pain and Mental Health: Integrated Solutions for Global Problems. *Pain*, **159**, S85-S90. https://doi.org/10.1097/j.pain.0000000000001296

[17] Dale, R. and Stacey, B. (2016) Multimodal Treatment of Chronic Pain. *Medical Clinics of North America*, **100**, 55-64. https://doi.org/10.1016/j.mcna.2015.08.012

[18] Moisset, X., Lanteri-Minet, M. and Fontaine, D. (2019) Neurostimulation Methods in the Treatment of Chronic Pain. *Journal of Neural Transmission*, **127**, 673-686. https://doi.org/10.1007/s00702-019-02092-y

[19] Roy, R., de la Vega, R., Jensen, M.P. and Miró, J. (2020) Neurofeedback for Pain Management: A Systematic Review. *Frontiers in Neuroscience*, **14**, Article 671. https://doi.org/10.3389/fnins.2020.00671

[20] Hernández-Calderón, P., Wiedemann, L. and Benítez-Páez, A. (2022) The Microbiota Composition Drives Personalized Nutrition: Gut Microbes as Predictive Biomarkers for the Success of Weight Loss Diets. *Frontiers in Nutrition*, **9**, Article 1006747. https://doi.org/10.3389/fnut.2022.1006747

[21] Gebrayel, P., Nicco, C., Al Khodor, S., Bilinski, J., Caselli, E., Comelli, E.M., *et al*. (2022) Microbiota medicine: towards clinical revolution. *Journal of Translational Medicine*, **20**, Article No. 111. https://doi.org/10.1186/s12967-022-03296-9

[22] Hou, K., *et al*. (2022) Microbiota in Health and Diseases. *Signal Transduction and Targeted Therapy*, **7**, Article No. 135.

[23] Schlundt, J. (2012) Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. FAO/WHO.

[24] Maftei, N., Raileanu, C.R., Balta, A.A., Ambrose, L., Boev, M., Marin, D.B., *et al*. (2024) The Potential Impact of Probiotics on Human Health: An Update on Their Health-Promoting Properties. *Microorganisms*, **12**, Article 234. https://doi.org/10.3390/microorganisms12020234

[25] Wang, X., Zhang, P. and Zhang, X. (2021) Probiotics Regulate Gut Microbiota: An Effective Method to Improve Immunity. *Molecules*, **26**, Article 6076.

https://doi.org/10.3390/molecules26196076

[26] Vlasova, A.N., Kandasamy, S., Chattha, K.S., Rajashekara, G. and Saif, L.J. (2016) Comparison of Probiotic Lactobacilli and Bifidobacteria Effects, Immune Responses and Rotavirus Vaccines and Infection in Different Host Species. *Veterinary Immunology and Immunopathology*, **172**, 72-84. https://doi.org/10.1016/j.vetimm.2016.01.003

[27] Ustianowska, K., Ustianowski, Ł., Machaj, F., Gorący, A., Rosik, J., Szostak, B., *et al*. (2022) The Role of the Human Microbiome in the Pathogenesis of Pain. *International Journal of Molecular Sciences*, **23**, Article 13267. https://doi.org/10.3390/ijms232113267

[28] Anand, N., Gorantla, V.R. and Chidambaram, S.B. (2022) The Role of Gut Dysbiosis in the Pathophysiology of Neuropsychiatric Disorders. *Cells*, **12**, Article 54. https://doi.org/10.3390/cells12010054

[29] Reiter, A., Bengesser, S.A., Hauschild, A., Birkl-Töglhofer, A., Fellendorf, F.T., Platzer, M., *et al*. (2020) Interleukin-6 Gene Expression Changes after a 4-Week Intake of a Multispecies Probiotic in Major Depressive Disorder—Preliminary Results of the PROVIT Study. *Nutrients*, **12**, Article 2575. https://doi.org/10.3390/nu12092575

[30] Mayer, E.A., Tillisch, K. and Gupta, A. (2015) Gut/brain Axis and the Microbiota. *Journal of Clinical Investigation*, **125**, 926-938. https://doi.org/10.1172/jci76304

[31] Liu, L., Wu, Q., Chen, Y., Ren, H., Zhang, Q., Yang, H., *et al*. (2023) Gut Microbiota in Chronic Pain: Novel Insights into Mechanisms and Promising Therapeutic Strategies. *International Immunopharmacology*, **115**, Article ID: 109685. https://doi.org/10.1016/j.intimp.2023.109685

[32] Hamilton, M. (1959) The Assessment of Anxiety States by Rating. *British Journal of Medical Psychology*, **32**, 50-55. https://doi.org/10.1111/j.2044-8341.1959.tb00467.x

[33] Caraceni, A., Mendoza, T.R., Mencaglia, E., Baratella, C., Edwards, K., Forjaz, M.J., *et al*. (1996) A Validation Study of an Italian Version of the Brief Pain Inventory (breve Questionario per La Valutazione Del Dolore). *Pain*, **65**, 87-92. https://doi.org/10.1016/0304-3959(95)00156-5

[34] Dengler-Crish, C.M., Bruehl, S. and Walker, L.S. (2011) Increased Wind-Up to Heat Pain in Women with a Childhood History of Functional Abdominal Pain. *Pain*, **152**, 802-808. https://doi.org/10.1016/j.pain.2010.12.032

[35] Ehlert, U., Heim, C. and Hellhammer, D.H. (1999) Chronic Pelvic Pain as a Somatoform Disorder. *Psychotherapy and Psychosomatics*, **68**, 87-94. https://doi.org/10.1159/000012318

[36] Zajkowska, Z., Gullett, N., Walsh, A., Zonca, V., Pedersen, G.A., Souza, L., *et al*. (2022) Cortisol and Development of Depression in Adolescence and Young Adulthood—A Systematic Review and Meta-analysis. *Psychoneuroendocrinology*, **136**, Article ID: 105625. https://doi.org/10.1016/j.psyneuen.2021.105625

[37] Mezzullo, M., Fanelli, F., Fazzini, A., Gambineri, A., Vicennati, V., Di Dalmazi, G., *et al*. (2016) Validation of an LC-MS/MS Salivary Assay for Glucocorticoid Status Assessment: Evaluation of the Diurnal Fluctuation of Cortisol and Cortisone and of Their Association within and between Serum and Saliva. *The Journal of Steroid Biochemistry and Molecular Biology*, **163**, 103-112. https://doi.org/10.1016/j.jsbmb.2016.04.012

[38] Chou, R., Deyo, R., Friedly, J., Skelly, A., Hashimoto, R., Weimer, M., *et al*. (2017) Nonpharmacologic Therapies for Low Back Pain: A Systematic Review for an

American College of Physicians Clinical Practice Guideline. *Annals of Internal Medicine*, **166**, 493-505. https://doi.org/10.7326/m16-2459

[39] Eaton, S., Roberts, S. and Turner, B. (2015) Delivering Person Centred Care in Long Term Conditions. *BMJ*, **350**, h181. https://doi.org/10.1136/bmj.h181

[40] Williams, A.C.d.C., Eccleston, C. and Morley, S. (2012) Psychological Therapies for the Management of Chronic Pain (Excluding Headache) in Adults. *Cochrane Database of Systematic Reviews*, No. 11, CD007407. https://doi.org/10.1002/14651858.cd007407.pub3

[41] Wong, J.J., Côté, P., Sutton, D.A., Randhawa, K., Yu, H., Varatharajan, S., *et al*. (2016) Clinical Practice Guidelines for the Noninvasive Management of Low Back Pain: A Systematic Review by the Ontario Protocol for Traffic Injury Management (Optima) Collaboration. *European Journal of Pain*, **21**, 201-216. https://doi.org/10.1002/ejp.931

[42] Marzbani, H., Marateb, H. and Mansourian, M. (2016) Methodological Note: Neurofeedback: A Comprehensive Review on System Design, Methodology and Clinical Applications. *Basic and Clinical Neuroscience Journal*, **7**, 143-158. https://doi.org/10.15412/j.bcn.03070208

[43] Koryukalova, Yu.I., *et al*. (2021) Alhpa-Wave Synchronization Observed in Subjects Practicing Relaxation Revealed an Individual Hyper Neural Network in Cognitive Activities. *Journal of Psychiatry*, **11**, 1-7.

[44] Da Silva, O.M., Bautitz, B., Benedeti, F., Pauly, L. and Rabelo-Silva, E.R. (2016) Chronic Pain and Pharmacotherapy in Chronic Renal Patients on Hemodialysis. *Open Journal of Nursing*, **6**, 741-750. https://doi.org/10.4236/ojn.2016.69075