applied sciences

MDPI

Article

Correlation between Functional Magnetic Resonance and Symptomatologic Examination in Adult Patients with Myofascial Pain Syndrome of the Masticatory Muscles

Felice Festa [1], Nicla Lopedote [1], Chiara Rotelli [1], Massimo Caulo [2] and Monica Macrì [1,*]

[1] Department of Innovative Technologies in Medicine & Dentistry, University "G. D'Annunzio" of Chieti-Pescara, 66100 Chieti, Italy
[2] Department of Clinical Sciences and Bio-Imaging, University "G. D'Annunzio" of Chieti-Pescara, 66100 Chieti, Italy
* Correspondence: m.macri@unich.it

Abstract: Myofascial pain syndrome is the most common cause of TMD, characterised by trigger points of skeletal muscles in the masticatory region. Patients with myofascial pain suffer from orofacial pain and headaches. Parafunctional activity such as unconscious teeth clenching predisposes a higher possibility of developing myofascial pain. We report the results of a prospective study of 10 patients with a myofascial pain diagnosis related to TMD who underwent treatment with passive aligners and biofeedback exercise. All patients underwent pain assessment (visual analogic scale and muscular palpation test), measurement of masseters thickness with Dolphin Imaging Software, nuclear magnetic resonance of the temporomandibular joint, and functional nuclear magnetic resonance of the brain before and after gnathological treatment. The same patients underwent pain assessment (VAS and palpation test) for the entire duration of their treatment. This study aimed to assess if the results obtained with the therapy were repeatable using functional magnetic resonance imaging. This enabled us to correlate a subjective datum (pain) to an objective one (variation in the functional connectivity of the networks correlated to pain perception). According to the pain assessment, the treatment considerably reduced the pain in 9 out of 10 patients. Furthermore, the functional nuclear magnetic resonance of the brain showed similar modifications in the cerebral pain and default mode networks in these nine patients. The change in the masseter muscle dimensions was not correlated with the modification of pain. Statistical analysis was performed to evaluate the effects of treatment on VAS and trigger point stimulation and on the length and width of the masseter muscle. Linear regression analysis was used to assess a correlation between the modification of the masseter muscle dimension and the amendment of VAS. A paired *t*-test was used to evaluate statistically significant differences in the connectivity of brain areas of the DMN and the pain network. Our results suggest that the proper treatment of myofascial pain can reduce pain and consistently modify the functional activation of the cerebral pain and default mode networks. Overall, the treatment was repeatable because brain network changes were homogeneous in all patients and did not relate to the intracapsular TMJ condition but only to pain symptoms.

Keywords: functional magnetic resonance; myofascial pain; temporomandibular disorder; masseter muscle; TMJ

Citation: Festa, F.; Lopedote, N.; Rotelli, C.; Caulo, M.; Macrì, M. Correlation between Functional Magnetic Resonance and Symptomatologic Examination in Adult Patients with Myofascial Pain Syndrome of the Masticatory Muscles. *Appl. Sci.* **2023**, *13*, 7934. https://doi.org/10.3390/app13137934

Academic Editor: Bruno Chrcanovic

Received: 15 April 2023
Revised: 27 June 2023
Accepted: 28 June 2023
Published: 6 July 2023

1. Introduction

Temporomandibular joint disorders (TMD) can involve the masticatory musculature, the temporomandibular joint (TMJ), or both [1].

TMDs can be classified as an intra-articular or extra-articular disease: (a) Intra-articular TMDs include (1) congenital or developmental disorders, (2) degenerative joint disorders, or (3) any condition involving the articular disc, the condyle, and the glenoid fossa.

(b) Extra-articular TMDs include all masticatory muscle disorders: (1) myofascial pain disorder, (2) local myalgia, (3) myositis, and (4) myospasm [1].

The causes of TMD include micro- and macrotrauma and systemic, iatrogenic, occlusal, and mental health disorders [2]. Unconscious teeth clenching induces constant trauma to the muscles and joints and represents, therefore, one of the major causes of myofascial pain [3].

Myofascial pain syndrome represents one of the most common TMDs and chronic problems in the maxillofacial region. Myofascial pain is classified as a dysfunction of the stomatognathic system that belongs to Axis I pain-related TMDs.

In the new DC/TMD, the term myofascial pain describes two new DC/TMD conditions: (1) myofascial pain, defined as pain spreading beyond the site of palpation but within the boundary of the muscle being palpated, and (2) myofascial pain with referral, defined as pain in a specific site that creates pain in another area beyond the boundary of the muscle being palpated [4].

It is a painful condition characterised by trigger points, which can create local and referred pain, tenderness, autonomic phenomena, anxiety, and depression [5]. A myofascial trigger point (MTrP) is a hyperirritable spot which provokes pain when compressed and can lead to a characteristic referred pain and motor dysfunction. The painful symptoms radiate pain to distant sites [4,6].

Studies report various treatments for MPS, such as exercises, TrP injections, medications, and other alternative therapies [7]. Most symptoms get better with a combination of non-invasive treatments and occlusal devices. Other means are recommended for chronic or refractory cases, such as nonsteroidal anti-inflammatory drugs, muscle relaxants, benzodiazepines, or antidepressants [1].

Tension-type headache (TTH) is the most prevalent primary headache disorder and affects 80% of the population; therefore, the socio-economic consequences are significant. Studies show that the pain elicited by active MTrPs reproduces the headache pattern in TTH patients [8].

This prospective study reports on 10 patients with headaches and orofacial pain from myofascial pain syndrome, caused by unconscious clenching [9], treated with gnathological therapy: passive aligners and biofeedback exercise.

This study aimed to evaluate the repeatability of the variation in functional connectivity at rest after treatment, the actual stretch/lengthening of the masseters (taken as a reference for masticatory muscles) after treatment, and their correlation with the development of symptoms (VAS and palpation).

Therefore, we wanted to see if the results obtained with therapy were repeatable using functional magnetic resonance imaging. This enables us to correlate a subjective datum (pain) to an objective one (variation in the functional connectivity of the networks correlated with pain perception).

2. Materials and Methods

The study was performed in the Oral Sciences Department of the University of Chieti G. D'Annunzio, in Italy. Ethics approval (number 23) was obtained by the hospital's Independent Ethics Committee of Chieti—Italy. The study protocol followed the European Union Good Practice Rules and the Helsinki Declaration.

The sample consisted of 10 patients treated at the Orthodontics Department for TMDs, who signed an informed consent form before the study.

The study lasted 18 months: 3 months to enrol the patients, 12 for the follow-up of the recruited subjects, and 3 for the data processing.

All patients underwent quantitative pain assessment (VAS and palpation test), measurement of masseter thickness before and after the treatment with Dolphin Imaging Software [10], and fMRI and TMJ MRI before and after the gnathological treatment. Patients underwent CBCT examinations because the orthodontic treatment was planned after the gnathological one [11]. A quantitative pain assessment was performed every follow-up to evaluate therapy progression.

2.1. Inclusion Criteria

Over 18 and under 60 years of age; diagnosis of TMDs using MR imaging of the TMJ in both closed- and open-mouth positions; diagnosis of chronic myofascial pain syndrome of the masticatory muscles during rest or function (following the taxonomy of the diagnostic criteria for temporomandibular disorders: pain in the jaw, temple, in front of the ear, or in the ear with examiner confirmation of pain location in the masticatory structure; pain alteration with jaw movement; function or parafunction; proof of pain in the temporalis or masseter muscles) [12]; and pain 4+ times a week for at least 12 weeks with average pain severity of 4 on a 10-point scale for at least 1 h per day.

2.2. Exclusion Criteria

Pregnancy; claustrophobia; moderate or severe psychiatric disorder; fibromyalgia; chronic pain disorder; diagnosis of metabolic disease, coagulopathy, neurological disorder, vascular disease, or neoplasia; current opioid or psychiatric medications use.

Participants taking non-steroidal anti-inflammatory drugs or paracetamol treatments stopped those medications at least one day before the study appointment.

2.3. Study Protocol

The prospective study included 10 patients: 5 with intra-articular and extra-articular disorders (myofascial pain and intra-capsular incoordination) and 5 with only extra-articular disorders (myofascial pain). Enrolment was based on the diagnosis of TMD based on a standardised clinical valuation that fulfils the Research Diagnostic Criteria (RDC TMDs) [13].

These patients were affected by headaches and orofacial pain from myofascial pain syndrome, caused by unconscious clenching [9], and were treated with gnathological therapy: passive aligners and biofeedback exercise.

Operators identified 'parafunction' in the awake brain state using direct questions and visual observation of patients' behaviour [14]. During visual observation, we could identify patients with parafunctional habits by diagnosing damaged tooth structures, fractured teeth and restorations, craze lines, or loss of teeth. Other intraoral clinical signs are tongue indentations; bony exostoses or tori; periodontal changes, including widening of the periodontal ligament, tooth mobility, and recessions. If clinicians note this kind of damage, they should discuss the damage with the patients and review their medical history to determine the cause [15].

All patients underwent the following:

- Quantitative pain assessment (VAS and palpation test) during every follow-up.
- Measurement of masseter thickness before and after the treatment.
- Some studies evaluated the actual variation in the activity of some masticatory muscles in patients suffering from TMD. These studies revealed a correlation between TMD pain and an increased tone of masticatory muscles (greater tenderness scores, higher sEMG activity), in particular temporalis and masseter muscles. These studies proved a significant correlation between headaches and mastication muscle tone changes [9,11–16].
- fMRI and TMJ MRI before and after the gnathological treatment.

This study evaluated the functional connectivity (fcMRI) of the pain network (PN) and the default mode network (DMN). Some research showed a stable variation in pain and cerebral behavioural networks in therapeutic procedures through the functional magnetic resonance of the brain [17,18].

The PN is the cortical network of the physiology of pain. In contrast, the DMN manages unconscious processes involving perceived pain.

In the first stage of the study, the subjects underwent CBCT, clinical examination, palpation test, and VAS to diagnose unconscious teeth clenching [19] and myofascial pain TMD. Then, they underwent a TMJ MRI to assess TMJ condition and an fMRI of the brain.

In the study's second phase, all the patients were treated with passive aligners and biofeedback exercises. Patients underwent VAS and palpation tests once a month.

After 12 months, all patients underwent, once again, TMJ MRI, fMRI of the brain, and CBCT.

The same operator followed each patient from the beginning until the end of the study.

2.4. Sample

In total, 18 patients were eligible for the study. Six declined to participate in the study, and two were lost during the follow-up.

The sample included 10 patients with myofascial pain syndrome. Five had only extra-articular TMD, and five had extra-articular and intra-articular TMD.

All patients underwent gnathological therapy to ameliorate orofacial pain and headache. The stage after gnathological therapy was orthodontic.

The initial occlusal status of patients was heterogeneous.

Two presented superior and inferior crowding with first-class molars and canines, with norm-divergent biotypes.

Three presented superior and inferior crowding with first-class molars and canines, with a hypodivergent biotype.

Two of them presented third-class molars and canines, with norm-divergent biotypes.

One of them presented third-class molars and canines, with a hypodivergent biotype.

One of them presented third-class molars and canines, with hyperdivergent biotypes.

One of them presented second-class molars and canines, with norm-divergent biotypes.

2.5. Measurements

VAS: visual analogue scale (VAS); it is a graphical representation where the subject has to highlight painful areas, specifying the severity (value from 0 to 10) and the disturbance frequency [20].

PALPATION: The MTrP can be evaluated by palpating the painful areas perpendicular to the direction of the muscle fibres. [4,21]. Different modalities have been used to assess myofascial trigger points, among which ultrasound is the most promising. Still, a gold standard using these techniques must be detailed [8].

The palpation was performed bilaterally with constant pressure. The aim was to find trigger points in the masticatory muscles and TMJ that, once stimulated, provoked a pain sensation through a central excitatory effect.

The pain sensation was graded (0–3):

0: absence of pain;
1: mild pain or discomfort;
2: moderate pain or discomfort;
3: intense pain (patient withdrawing or tearing) [22].

Masseter muscle thickness: All patients' masseter muscle thicknesses were calculated bilaterally, before and after the treatment. Dolphin software (Version number is 12.0 64 bit) was used for the measurement.

The early analysis of the DICOM files was executed in the 3D mode of Dolphin Imaging software. This research incorporates the proper orientation of the skull with the Frankfort Horizontal Plane (reference plane). It identifies the origin and insertion points of the anterior masseter border on both the right and left sides of the skull with coronal sections.

The optional measures in Dolphin Image Software evaluated the changes in the length and width of the patients' masseter muscles before and after treatment with passive aligners.

The length of the masseter muscle was evaluated from the zygomatic arch to the lower limit of the mandible.

The width of the masseter muscle was assessed by measuring its thickest point.

MRI TMJ: Magnetic resonance of TMJ was used to evaluate the integrity of the temporomandibular joint, diagnose the intra-articular or extra-articular disorder, and assess any modifications in the condyle–disc relationship. All the subjects underwent TMJ MRI with open and closed mouths before and after treatment.

fMRI: The fMRI can detect the blood oxygenation level-dependent (BOLD) signals through the neural activity that follows changes in intra-tissue oxyhaemoglobin and deoxyhaemoglobin (a sign of metabolic activity) [23]. The BOLD signal is measured both during the execution of a task (task-evoked fMRI) and during rest as a measure of functional brain connectivity (fcMRI).

This study used functional magnetic resonance of the brain to analyse the functional resting connectivity of the pain network (PN) and default mode network (DMN).

The DMN areas analysed were posterior cingulate cortex (DMN-PCC), praecuneus (DMN-PRECUNEUS), medial prefrontal cortex (DMN-MPFC), right occipital lobe (DMN-RIGHT-OCC), left occipital lobe (DMN-LEFT-OCC), right temporal lobe (DMN-RIGHT-TEMP), and left temporal lobe (DMN-LEFT-TEMP).

The PN studied regions were anterior cingulate cortex (PAIN-ACC), somatosensory cortex 1 right (PAIN-RIGHT-S1), somatosensory cortex 1 left (PAIN-LEFT-S1), somatosensory cortex 2 right (PAIN-RIGHT-S2), somatosensory cortex 2 left (PAIN-LEFT-S2), insula right (PAIN-RIGHT-INSULA), and insula left (PAIN-LEFT-INSULA).

Functional magnetic resonance of the brain indicated, through the functional connectivity matrix differences, the average functional connectivity in the pain and default mode networks.

The functional connectivity matrix differences were obtained from each patient's connectivity matrix at t2 and t1. Each node of the matrices matches the numerical value of the interactivity of two specific ROIs (regions of interest in the brain network): the row's ROI and the column's ROI.

The average functional connectivity of the difference matrix of each subject was the algebraic sum of all nodes in each network.

A positive value for average functional connectivity matched with increased functional connectivity at rest in that network after treatment. In contrast, a negative value corresponded to decreased functional connectivity at rest after treatment, and this does not indicate increased function but a high correlation between areas.

MR data acquisition and processing: MRI data were collected through a GE Medical Systems 3.0 Tesla system with an 8-channel brain receiving coil.

The protocol used a fast 3D-SPGR sequence with the following parameters:

TR = 6.9 ms;

TE = 1.6 ms;

TI = 450 ms,

Flip angle = 15°;

Matrix = 256 × 256;

Field of view = 25.6 × 25.6 cm.

A total of 156 axial slices with 1 mm thickness, yielding a voxel size of 1 × 1 × 1 mm.

The scanning parameters covered the brain, midbrain, pons, and cerebellum.

CNS irregularities associated with M-TMD were evaluated with various MRI tools.

The first phase was brain extraction and parcellation using Freesurfer [24]; the 1000 Functional Connectomes Project was used to obtain residuals from BOLD images [25]. The residuals were registered to the MNI template [26], and the FSL toolbox [27] was used to draw a time course for the selected ROI [28]. Functional connectivity matrices and T2–T1 differences were computed using an in-house Python script [29].

2.6. Treatment Protocol

The treatment protocol involved using two passive polycarbonate aligner splints (PAS) (upper and lower) and a four-step biofeedback exercise, during which the patient had to assume a standing position or lie on a hard flat surface.

- Use of PAS description

The PAS was characterised by uniform and simultaneous contact on all teeth. Acrylic prominences labial to the mandibular canines defined the eccentric guidance to obtain posterior teeth disclusion.

Each patient wore the lower splint during the day and the upper splint at night, removing them only during mealtimes and oral hygiene procedures. Patients never wore both splints at the same time. The patients had to alternate splints (wearing the upper one at night and the lower one during the day). Hence, the clinician was able to understand and differentiate whether the parafunction activity occurred at night, during the day, or both. Consequently, the clinician was able to monitor the therapy efficiently.

The patients performed the biofeedback exercise with the splint they wore during the day, the lower one. Then, they removed it for meals and oral hygiene procedures and put the daytime splint back on. After the last oral hygiene procedures, the patient wore the night splint (without the daytime one) in the evening and during the night.

- Biofeedback exercise description

The biofeedback exercise lasted 2 min and was performed thrice daily (before breakfast, lunch, and dinner) with at least three hours between each execution.

It consisted of 4 steps:

The first step involved a bilateral masseter muscle contraction, executed while the patient wore the LPAS. During the maximum contraction, a light touch with the forefinger was applied (common in all the steps). The patient had to visualise the muscle volume for five seconds as a swollen rugby ball.

Then, the masseter was half-toned bilaterally, and the muscle volume had to be visualised as a semi-deflated rugby ball for five seconds.

After that, the masseter muscle relaxed; the jaw was opened by around 1 mm during the minimum contraction. The muscle volume had to be visualised for five seconds as a deflated rugby ball.

In the last step, the patient had to touch the palatine vault with the tip of the tongue for five seconds.

- Monthly follow-up

The treatment lasted 12 months on average.

Once a month, a follow-up evaluation was performed through VAS and palpation test of the temporal, masseter, sternocleidomastoid, digastric, and pterygoid muscles.

Patients continued to record headache diaries during the whole study period. The same operator checked the cooperation of the patients every month and the progress of the therapy.

- Check-up after the therapy

At the end of therapy, the evaluation was performed through VAS, palpation test, MRI, and fMRI control to evaluate the therapy outcome in TMJ and the cerebral network. The thickness of the masseters before and after the treatment was assessed.

2.7. Statistical Analysis

All statistical analysis was performed using IBM SPSS version 26.0 (Armonk, NY, USA) and evaluated at a two-sided alpha level of 0.05.

- Repeated measures ANOVA was used to evaluate the effects of treatment on VAS and trigger point stimulation.
- The post hoc effect of the treatment protocol on pre versus post parameters assessing pain and the length and width of the masseter muscle was evaluated using a Wilcoxon Signed-Ranks Test.
- Linear regression analysis was used to evaluate a correlation between changes in the masseter muscle dimension and the amendment of VAS. A false discovery rate correction for multiplicity was independently applied to the primary and secondary outcome measures to reduce the risk of a type 2 error (i.e., accepting a false null hypothesis or excluding the presence of a statistically significant result when present).
- A paired *t*-test was used to assess statistically significant differences in the connectivity of brain areas of the DMN and pain network.

3. Results

Ten patients with myofascial pain syndrome, diagnosed according to the International Classification of Headaches Disorders 3rd edition (ICHD-III beta), without migraines, were enrolled in this study after they gave their written informed consent.

The demographic characteristics of the patients are reported in Table 1.

Table 1. Demographic characteristics of the patients and the effect of the gnathological treatment on referred pain.

	Age	Gender	TMD: Intra-Articular (IA) or Extra-Articular (EA)	VAS at T1	VAS at T2	Painful Areas at T1	Painful Areas at T2	Length of Symptoms	Change in Symptoms
PT 1 (CS)	41	F	IA	8	4	Neck, under eyes, shoulders, TMJ, mandible maxilla	Neck, shoulders	2 years	The symptoms exacerbated during this period
PT 2 (SS)	22	F	IA	8	1	TMJ around eyes nape trapezoids	TMJ	1 year	The symptoms exacerbated during this period
PT 3 (RF)	26	M	EA	5	1	Mandible, neck shoulder, lumbar area, head	Neck	2–3 years	Symptomatology remained constant during this period
PT 4 (AN)	41	F	IA	7–8	4	Sinusitis-like symptoms, TMJ, neck, shoulders, pelvis	TMJ	15 years	The symptoms exacerbated during this period
PT 5 (CT)	55	F	EA	6	0	Masseter, mandible, maxilla		5 years	The symptoms exacerbated during this period
PT 6 (NM)	24	F	IA	8	4	Head, mandible, neck, shoulders	Head, neck, shoulders	2 years	The symptoms exacerbated during this period
PT 7 (RE)	22	F	EA	7	2	Head, mandible, masseter, neck, shoulders	Head, neck, shoulders	About 1 year	The symptoms exacerbated during this period
PT 8 (LM)	22	F	EA	8	3	Head, mandible, neck, shoulders	Head, mandible	2–3 years	Symptomatology exacerbated during this period
PT 9 (FA)	48	M	IA	7–8	0	Head, neck, mandible, around eyes, shoulders, lumbar		9–8 years	The symptoms exacerbated during this period
PT 10 (SG)	34	M	EA	5	1	Head, masseter, mandible, neck, shoulders	Neck, shoulders	1 year	The symptoms exacerbated during this period

PT: patient, IA: intra-articular, EA: extra-articular, VAS: visual analogue scale, F: female, M: male, T1: time 1, T2: time 2.

Ten subjects aged between 18 and 60 were selected. The sample was not uniform in gender, as there were three men and seven women. Five subjects were affected by intra-articular and extra-articular TMD, and five subjects were affected by extra-articular TMD. The reduction in pain after treatment, the painful areas before and after treatment, and the duration and evolution of the symptoms from the onset until the end of the treatment are described in Table 1.

The outcomes of the palpation test are reported in Tables 2 and 3. Palpation of the masticatory muscles was performed to search for and highlight active or latent trigger points before and after treatment.

Table 2. Masseter, temporal, and sternocleidomastoid palpation test after treatment compared with baseline.

	Masseter Palpation at T1	Masseter Palpation at T2	Temporal Palpation at T1	Temporal Palpation at T2	Sternocleidomastoid Palpation at T1	Sternocleidomastoid Palpation at T2
PT 1 (CS)	3	1	2	1	3	1
PT 2 (SS)	3	1	3	1	3	0
PT 3 (RF)	2	0	2	0	3	1
PT 4 (AN)	3	2	2	0	3	1
PT 5 (CT)	2	0	2	1	2	0
PT 6 (NM)	3	1	3	0	3	1
PT 7 (RE)	3	0	2	0	2	0
PT 8 (LM)	3	1	2	0	2	1
PT 9 (FA)	3	1	2	0	2	1
PT 10 (SG)	2	0	2	1	2	1

PT: patient, T1: time 1, T2: time 2.

Table 3. Digastric and pterygoid palpation test after treatment compared with baseline.

	Digastric Palpation at T1	Digastric Palpation at T2	Pterygoid Palpation at T1	Pterygoid Palpation at T2
PT 1 (CS)	1	0	3	1
PT 2 (SS)	2	0	3	0
PT 3 (RF)	2	0	3	1
PT 4 (AN)	0	0	3	2
PT 5 (CT)	1	0	2	0
PT 6 (NM)	1	0	3	1
PT 7 (RE)	0	0	3	1
PT 8 (LM)	0	0	3	0
PT 9 (FA)	1	0	3	1
PT 10 (SG)	0	0	2	0

PT: patient, T1: time 1, T2: time 2.

As compared with baseline (T1), after the treatment (T2), the pain symptomatology was reduced both in intensity and in the number of painful regions in each subject (Table 1), and the pain elicited by the masseter, temporal, and sternocleidomastoid trigger points was lowered in all patients (Tables 2 and 3). Repeated measures ANOVA with a Huynh–Feldt correction indicated a statistically significant effect of treatment ($F(1.000, 54.000) = 454.820$, $p < 0.001$), independent of pain assessment, and an interaction between treatment and VAS/trigger points ($F(53.567, 54.000) = 31.613$, $p < 0.001$). The results of the post hoc analyses using a Wilcoxon Signed-Ranks Test for pre- vs. post-treatment VAS and individual trigger points (Tables 1–3) indicated that a highly significant improvement was obtained in all scores.

Following therapy, a significant decrease in the length of the left ($p = 0.021$) and width of the right ($p = 0.041$) masseter muscles was observed. The changes in the masseter muscle dimensions did not correlate with the change in the VAS score.

The functional connectivity values of DMN and PN are reported in Table 4. The algebraic sum was calculated from the functional matrices resulting from the functional magnetic resonance performed before and after the treatment for each patient. The value obtained defines the average functional connectivity within the analysed brain networks.

Table 4. Average connectivity of the DMN and PN.

	DMN Average Connectivity: T2–T1	PN Average Connectivity: T2–T1
PT 1 (CS)	15.83	1.14
PT 2 (SS)	−6.86	2.03
PT 3 (RF)	−7.34	20.24
PT 4 (AN)	−6.76	13.41
PT 5 (CT)	−8.93	0.20
PT 6 (NM)	−1.96	31.18
PT 7 (RE)	−0.79	17.95
PT 8 (LM)	10	2.2
PT 9 (FA)	−8. 58	21.82
PT 10 (SG)	5.13	−9.77

PT: patient, T1: time 1, T2: time 2, DMN: default mode network, PN: pain network.

Seven out of ten patients observed a negative average functional connectivity in the DMN. At the same time, an positive average functional connectivity in PN was observed in nine of ten patients (Figures S1–S10).

Variations in fMRI within a network for each patient were uniform. The DMN of patient 1 showed different values, probably due to previously detected spinal disc herniation. This patient had first-class molars and canines, mild superior and inferior crowding, and a norm-divergent biotype.

In the tenth patient, the differences were probably due to the lack of consistency in following the therapeutic protocol, as reported by the patient.

The connectivity correlations for the DMN and PN were evaluated with a paired samples *t*-test and Pearson's Correlation Coefficients. The observed decrease in the DMN connectivity (−7.4%) was not statistically significant, while the increase in the pain network (20.3%) connectivity was highly statistically significant (Table 4). A strong negative correlation was observed for the DN and a strong positive correlation for the pain network.

This result suggests that the benefit of the therapy is not linked to physical modifications, but to changes in the brain seen with the fMRI. We are opening the possibility of further publications in this area.

Figures S1–S10: Matrix difference between t2 and t1 with average connectivity value.

4. Discussion

Approximately 70 per cent of the general population experiences at least one of the symptoms of a temporomandibular disorder (TMD) [30]. Temporomandibular disorders are generally present in early adulthood.

TMD can have many causes, among which the most common are occlusal disharmony, mandibular instability, parafunctional habits, stress, and anxiety [31]. Unconscious teeth clenching causes constant trauma to the masticatory region and represents myofascial pain's main etiological factors. The latter is characterised by trigger points, which cause continuous aches and possible central excitatory effects, usually described by patients as headaches [3]. Studies show that the referred pain elicited by active MTrPs reproduces the headache pattern in TTH patients [8]. The TrPs in the muscles are involved in chronic tension-type headaches (CTTH) [32,33].

A recent study evaluated the effects of four different therapies on TMD patients: night splint, betamethasone, sodium hyaluronate, or platelet-rich plasma injections. All four groups had improved mouth opening and pain reduction [34]. Another study compared the degree of stretching of the temporal muscles and masseters through post-isometric

relaxation methods and treatments for myofascial relaxation. A decrease in the electrical activity of the muscles examined in both study groups was noted [35].

Myofascial pain is the most frequent TMD found in patients. There is a correlation between TMD and muscle activity and the tone of the chewing muscles.

In this study, we report the effect of gnathological therapies on pain and the correlated changes in masseter thickness, pain, and the default mode neural network in subjects with myofascial pain in the masticatory region due to unconscious clenching. Parafunctional activities, such as bruxism and teeth clenching, cause continuous microtrauma to the TMJ. This condition stimulates the fibres of the masticatory muscles for a long time, causing pain in the affected areas.

The study protocol consisted of three key points concerning clenching and, therefore, myofascial pain: The splint's thickness did not exceed 0.7 mm, because the brain does not recognise this as a foreign body between the teeth; therefore, the patient did not grind on it. One splint was used during the day and another during the night. There were also stretching exercises for paravertebral musculature (mainly through a lat machine) to reduce the unconscious clenching reflex that activates the paravertebral musculature.

Biofeedback exercises for the tongue give the patient an awareness of the spatial positioning of the arches so that they can become aware of the teeth clenching and stop it.

The biofeedback technique allows patients to recognise, correct, and prevent the physiological alterations underlying pathological conditions with consequent reduction or elimination. Qualified panels and meta-analyses assessed biofeedback-related approaches to headache treatment. These reviews indicate that various forms of biofeedback were effective for migraine and tension-type headaches [36].

After the treatment, the patients reported significantly decreased symptomatology (Tables 1–3), pain, and detected trigger points (VAS and palpation), and decreased unconscious teeth clenching and muscular tension. Regardless of the type of joint disorder, modifications in the disc–condyle relation (TMJ resonances) were not observed in all patients.

Functional magnetic resonance of the brain revealed that in 9 out of 10 patients, the average increased fcMRI of the pain network was statistically significant, whereas, after the treatment, the average fcMRI of the DMN decreased in 8 patients out of 10 (Table 4, Figures S1–S10). A strong negative correlation was observed for the DNM, and a strong positive correlation for the PN. The results of two patients differed from the other ones: one patient had abnormal fMRI behaviour because of residual pain in the cervical area due to herniated disc; another one had reverse fMRI compared to the other subjects, probably due to the lack of consistency and cooperation in following the therapeutic protocol, as reported by the patient.

A recent study showed that DMN and PN are functionally related but have an inverse temporal modulation [37]. This study supports our results: lower functional connectivity of the DMN is related to a much better functional connectivity of the PN after the treatment. The decrease/increase in functional connectivity at rest within a neural network does not correspond to an increase or decrease in the physiological activity of that network, indicated by an increase or decrease in the task-evoked activity instead.

Hence, since the treatment changed the network's rest function, the data suggest that it was associated with a functional reorganisation of the brain, correlated with clinically detectable changes in symptoms and morphology. A lack of correlation between physical changes in muscle dimension with VAS modification implies that the transformation of the brain network plays a more critical role. All ten patients had uniform functional changes in pain and default mode networks.

This result allowed us to correlate a subjective datum (pain) with an objective datum (modifications in the brain networks). Interestingly, the response of the brain networks was homogeneous in all patients, regardless of the heterogenous occlusal status.

These data and results allow us to assume that the analysed protocol is objectively effective in reducing the pain of myofascial pain syndrome and repeatable in different patients of disparate ages, genders, occlusal status, and other joint conditions. We found

an inverse correlation between PN and DMN (already demonstrated) and a homogeneous modification of the two networks after treatment in the analysed patients. The patients could not confound the result of the functional magnetic resonance since it was performed at rest.

The limitations of the study are the low sample size, non-uniform gender, and the wide range of ages. However, we believe that the results are essential and substantial because the assessments are complex and uncommon. This project will continue to increase the sample size and perform new assessments of the same patients.

Studies report several ways to treat MPS: muscular exercises, TrP injections, medications, and a combination of non-invasive treatments and occlusal devices. Other means are suggested for chronic or refractory cases, such as nonsteroidal anti-inflammatory drugs, muscle relaxants, benzodiazepines, or antidepressants [8,9].

The treatment with a passive splint and biofeedback exercises described in this study is non-invasive and does not involve the use of drugs. Nevertheless, the patients did not show a recurrence of symptoms. This result showed the effectiveness of the treatment, and this was demonstrated by the fact that the brain networks underwent homogeneous modifications, consistent with each other and with the results of other studies [3,8,22–35].

5. Conclusions

This study indicated that using passive splints in combination with jaw exercises may be an efficient treatment choice for subjects with myofascial pain TMDs and can induce modifications in the functional activation of the cerebral pain and default mode networks.

The same patients will undergo orthodontic treatment and then a functional resonance again. It will be interesting to see if the changes are still maintained.

Permanent changes in the pain network may represent a new perspective for upcoming investigations and clinicians approaching similar conditions. They have opened the possibility of further publications in this area with higher sample numbers.

This work also extends the frontier to more specific assessments in all pain treatments. They can be based on data that cannot be modified or misunderstood by patients and operators.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app13137934/s1, Figures S1–S10: Matrix difference t2–t1 with average connectivity value.

Author Contributions: Conceptualisation, M.C., M.M. and C.R.; methodology, M.M.; formal analysis, N.L.; investigation, M.C. and N.L.; resources, F.F.; data curation, M.M.; writing C.R.; writing—review and editing, M.M.; visualisation, F.F.; supervision, F.F.; project administration, F.F.; funding acquisition, F.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of D'Annunzio, Chieti (protocol code 23).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gauer, R.L.; Semidey, M.J. Diagnosis and treatment of temporomandibular disorders. *Am. Fam. Physician* **2015**, *91*, 378–386. [PubMed]
2. Okeson, J.P. *Management of Temporomandibular Disorders and Occlusion*; E-Book; Elsevier Health Sciences: Amsterdam, The Netherlands, 2019.
3. Lupoli, T.A.; Lockey, R.F. Temporomandibular dysfunction: An often-overlooked cause of chronic headaches. *Ann. Allergy Asthma Immunol.* **2007**, *99*, 314–318. [CrossRef] [PubMed]

4. Schiffman, E.; Ohrbach, R.; Truelove, E.; Look, J.; Anderson, G.; Goulet, J.-P.; List, T.; Svensson, P.; Gonzalez, Y.; Lobbezoo, F.; et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: Recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. *J. Oral Facial Pain Headache* **2014**, *28*, 6–27. [CrossRef] [PubMed]
5. Escobar, P.L.; Ballesteros, J. Myofascial pain syndrome. *Orthop. Rev.* **1987**, *16*, 708–713. [PubMed]
6. Sabatke, S.; Scola, R.H.; Paiva, E.S.; Kowacs, P.A. Injecction of trigger points in the temporal muscles of patients with miofascial syndrome. *Arq. Neuropsiquiatr.* **2015**, *73*, 861–866. [CrossRef]
7. Urits, I.; Charipova, K.; Gress, K.; Schaaf, A.L.; Gupta, S.; Kiernan, H.C.; Choi, P.E.; Jung, J.W.; Cornett, E.; Kaye, A.D.; et al. Treatment and management of myofascial pain syndrome. *Best Pract. Res. Clin. Anaesthesiol.* **2020**, *34*, 427–448. [CrossRef]
8. Do, T.P.; Heldarskard, G.F.; Kolding, L.T.; Hvedstrup, J.; Schytz, H.W. Myofascial trigger points in migraine and tension-type headache. *J. Headache Pain* **2018**, *19*, 84. [CrossRef]
9. Vavrina, J.; Vavrina, J. Bruxismus: Einteilung, Diagnostik und Behandlung [Bruxism: Classification, Diagnostics and Treatment]. *Praxis* **2020**, *109*, 973–978. [CrossRef]
10. Becht, M.P.; Mah, J.; Martin, C.; Razmus, T.; Gunel, E.; Ngan, P. Evaluation of masseter muscle morphology in different types of malocclusions using cone beam computed tomography. *Int. Orthod.* **2014**, *12*, 32–48. [CrossRef]
11. Elshebiny, T.; Bous, R.; Withana, T.; Morcos, S.; Valiathan, M. Accuracy of Three-Dimensional Upper Airway Prediction in Orthognathic Patients Using Dolphin Three-Dimensional Software. *J. Craniofacial Surg.* **2020**, *31*, 1098–1100. [CrossRef]
12. Peck, C.C.; Goulet, J.P.; Lobbezoo, F.; Schiffman, E.L.; Alstergren, P.; Anderson, G.C.; de Leeuw, R.; Jensen, R.; Michelotti, A.; Ohrbach, R.; et al. Expanding the taxonomy of the diagnostic criteria for temporomandibular disorders. *J. Oral Rehabil.* **2014**, *41*, 2–23. [CrossRef]
13. Schiffman, E.; Ohrbach, R. Executive summary of the Diagnostic Criteria for Temporomandibular Disorders for clinical and research applications. *J. Am. Dent. Assoc.* **2016**, *147*, 438–445. [CrossRef]
14. Lavigne, G.J.; Khoury, S.; Abe, S.; Yamaguchi, T.; Raphael, K. Bruxism physiology and pathology: An overview for clinicians. *J. Oral Rehabil.* **2008**, *35*, 476–494. [CrossRef]
15. Goldstein, R.E.; Auclair Clark, W. The clinical management of awake bruxism. *J. Am. Dent. Assoc.* **2017**, *148*, 387–391. [CrossRef]
16. Khawaja, S.N.; McCall, W., Jr.; Dunford, R.; Nickel, J.C.; Iwasaki, L.R.; Crow, H.C.; Gonzalez, Y. Infield masticatory muscle activity in subjects with pain-related temporomandibular disorders diagnoses. *Orthod. Craniofac. Res.* **2015**, *18* (Suppl. S1), 137–145. [CrossRef]
17. Andreescu, C.; Aizenstein, H. Predicting treatment response with functional magnetic resonance imaging. *Biol. Psychiatry* **2016**, *79*, 262–263. [CrossRef]
18. Festa, F.; Rotelli, C.; Scarano, A.; Navarra, R.; Caulo, M.; Macrì, M. Functional Magnetic Resonance Connectivity in Patients with Temporomadibular Joint Disorders. *Front. Neurol.* **2021**, *12*, 629211. [CrossRef]
19. Aguilera, S.B.; Brown, L.; Perico, V.A. Aesthetic Treatment of Bruxism. *J. Clin. Aesthet. Dermatol.* **2017**, *10*, 49–55.
20. Nishiyama, A.; Otomo, N.; Tsukagoshi, K.; Tobe, S.; Kino, K. The True-Positive Rate of a Screening Questionnaire for Temporomandibular Disorders. *Open Dent. J.* **2014**, *8*, 236–240. [CrossRef]
21. Fernández-de-Las-Peñas, C.; Dommerholt, J. International Consensus on Diagnostic Criteria and Clinical Considerations of Myofascial Trigger Points: A Delphi Study. *Pain Med.* **2018**, *19*, 142–150. [CrossRef]
22. Nixdorf, D.R.; John, M.T.; Wall, M.M.; Fricton, J.R.; Schiffman, E.L. Psychometric properties of the modified Symptom Severity Index (SSI). *J. Oral Rehabil.* **2010**, *37*, 11–20. [CrossRef] [PubMed]
23. Glover, G.H. Overview of functional magnetic resonance imaging. *Neurosurg. Clin. N. Am.* **2011**, *22*, 133–139. [CrossRef] [PubMed]
24. Fischl, B.; Salat, D.H.; Busa, E.; Albert, M.; Dieterich, M.; Haselgrove, C.; van der Kouwe, A.; Killiany, R.; Kennedy, D.; Klaveness, S.; et al. Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron* **2002**, *33*, 341–355. [CrossRef]
25. Mennes, M.; Biswal, B.B.; Castellanos, F.X.; Milham, M.P. Making data sharing work: The FCP/INDI experience. *Neuroimage* **2013**, *82*, 683–691. [CrossRef] [PubMed]
26. Brett, M.; Johnsrude, I.S.; Owen, A.M. The problem of functional localization in the human brain. *Nat. Rev. Neurosci.* **2002**, *3*, 243–249. [CrossRef] [PubMed]
27. Jenkinson, M.; Beckmann, C.F.; Behrens, T.E.; Woolrich, M.W.; Smith, S.M. FSL. *Neuroimage* **2012**, *62*, 782–790. [CrossRef]
28. Nebel, M.B.; Folger, S.; Tommerdahl, M.; Hollins, M.; McGlone, F.; Essick, G. Temporomandibular disorder modifies cortical response to tactile stimulation. *J. Pain* **2010**, *11*, 1083–1094. [CrossRef]
29. Van Rossum, G. *Python Tutorial; Technical Report CS-R9526*; Centrum voor Wiskunde en Informatica (CWI): Amsterdam, The Netherlands, 1995.
30. Yadav, S.; Yang, Y.; Dutra, E.H.; Robinson, J.L.; Wadhwa, S. Temporomandibular Joint Disorders in Older Adults. *J. Am. Geriatr. Soc.* **2018**, *66*, 1213–1217. [CrossRef]
31. Bitiniene, D.; Zamaliauskiene, R.; Kubilius, R.; Leketas, M.; Gailius, T.; Smirnovaite, K. Quality of life in patients with temporomandibular disorders. A systematic review. *Stomatologija* **2018**, *20*, 3–9.
32. Chatchawan, U.; Thongbuang, S.; Yamauchi, J. Characteristics and distributions of myofascial trigger points in individuals with chronic tension-type headaches. *J. Phys. Ther. Sci.* **2019**, *31*, 306–309. [CrossRef]

33. Moraska, A.F.; Stenerson, L.; Butryn, N.; Krutsch, J.P.; Schmiege, S.J.; Mann, J.D. Myofascial trigger point-focused head and neck massage for recurrent tension-type headache: A randomized, placebo-controlled clinical trial. *Clin. J. Pain* **2015**, *31*, 159–168. [CrossRef]
34. Sousa, B.M.; López-Valverde, N.; López-Valverde, A.; Caramelo, F.; Fraile, J.F.; Payo, J.H.; Rodrigues, M.J. Different Treatments in Patients with Temporomandibular Joint Disorders: A Comparative Randomized Study. *Medicina* **2020**, *56*, 113. [CrossRef]
35. Urbański, P.; Trybulec, B.; Pihut, M. The Application of Manual Techniques in Masticatory Muscles Relaxation as Adjunctive Therapy in the Treatment of Temporomandibular Joint Disorders. *Int. J. Environ. Res. Public Health* **2021**, *18*, 12970. [CrossRef]
36. Andrasik, F. Biofeedback in headache: An overview of approaches and evidence. *Cleveland Clin. J. Med.* **2010**, *77* (Suppl. S3), S72–S76. [CrossRef]
37. Mantini, D.; Caulo, M.; Ferretti, A.; Romani, G.L.; Tartaro, A. Noxious somatosensory stimulation affects the default mode of brain function: Evidence from functional MR imaging. *Radiology* **2009**, *253*, 797–804. [CrossRef]