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TESI DI DOTTORATO DI RICERCA

"Role of BDNF-mediated neuroplasticity in patients affected by Fibromyalgia versus other chronic rheumatic diseases"

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Abstract

Background: Fibromyalgia (FM) is still often viewed as a psychosomatic disorder. However, the increased pain sensitivity to stimuli in FM patients is not a phenomena of imagination, but the result of specific abnormalities in the central nervous system (CNS) pain matrix.

Brain-derived neurotrophic factor (BDNF) is an endogenous protein involved in neuronal survival and synaptic plasticity of the central and peripheral nervous systems. Several lines of evidence converge to indicate that BDNF also participates in structural and functional plasticity of nociceptive pathways in the CNS and within the dorsal root ganglia and spinal cord. At these levels, release of BDNF appears to modulate or even mediate nociceptive sensory inputs and pain hypersensitivity.

In the literature few studies evaluated BDNF levels in serum, plasma and cerebrospinal fluid samples of FM patients, finding increased levels of this neurotrophin compared to healthy controls. A number of studies also investigated BDNF levels in synovial fluid and plasma samples from patients affected by rheumatoid arthritis (RA), who are chronically subjected to pain, even though of inflammatory and autoimmune origin. No studies instead have been performed on patients affected by chronic fatigue syndrome (CFS), a condition that frequently overlaps with FM and whose etiopathogenesis, still unclear, is probably different from that of FM.

Objectives: The primary objective of the present PhD thesis project was therefore to investigate BDNF-mediated neuroplasticity, by detecting BDNF levels in FM patients, and therefore by comparing these levels to the ones found in CFS, RA and healthy volunteers.

Secondary objectives were: (i) the relation between BDNF levels and clinical variables, including neurocognitive disorders, which were assessed in FM and CFS patients by means of a computerized system; (ii) the relation between BDNF levels and psychiatric comorbidity in FM and CFS patients; (iii) the relation between BDNF levels and inflammatory status in RA patients; (iv) serum and plasmatic BDNF levels in a subgroup of FM patients before, immediately after, and 3-months after thermal treatments.

Materials and methods: Among the patients recruited in the study, there were 68 FM, 45 CFS, and 46 RA together with 40 healthy controls. BDNF serum levels were determined by enzyme-linked immune-sorbent assay (Promega), and the differences among the various

groups were observed. Demographic and clinical parameters were investigated in relation to BDNF levels. Moreover, a subgroup of FM patients (n=28) also participated to a clinical trial held at the Montecatini Thermal Baths, and another subgroup of FM patients (n=40), together with CFS patients, also completed a computerized test battery for the assessment of neurocognitive disorders.

Results: The main findings of the work can be summarized as follows: (i) the increased BDNF levels in sera of patients affected by FM, CFS and RA (but only those who were positive to rheumatoid factor of all isotypes), compared to healthy volunteers; (ii) the positive correlation between BDNF and rheumatoid factor (IgG and IgM isotypes), and the negative one with C-reactive protein; (iii) the lack of correlation between BDNF and neurocognitive disorders, assessed by the software CNS Vital Signs©; (iv) the higher prevalence of neurocognitive disorders in FM than in CFS patients, despite the more frequent complaint of CFS patients; (v) the tight relationship between neurocognitive impairments and chronic pain, which is independent of psychiatric comorbidity.

Conclusions: The conclusion reached by this study is that BDNF-mediated neuroplasticity in FM, CFS and RA could be interpreted as a protective mechanism against injuries, chronic pain and, more generally, against chronic stress conditions. This hypothesis could explain the elevated BDNF levels found in sera -but not in plasma- samples, and their decrease after thermal treatment. Although BDNF is not specific for FM or chronic pain -the difference here reported between BDNF levels of FM, CFS, RA patients compared to healthy controls are not strong enough to allow the use of BDNF in the diagnostic field- this work on one hand opens the way to new investigations on FM, CFS and RA etiopathogenesis, and on the other could suggest BDNF as a useful biomarker for FM/chronic pain therapy monitoring**.**

Chapter 1

Introduction

1. Fibromyalgia

1.1. Definition and classification criteria

Fibromyalgia (FM) is a common extra-articular rheumatic syndrome characterized by chronic musculoskeletal widespread pain and presence of multiple points tenderness to palpation (tender points). Muscle pain is typically aggravated by inactivity or exposure to cold. This condition is often associated with general symptoms, such as fatigue -particularly post-exertional fatigue not resolved by rest-, sleep disorders, stiffness, headaches, affective and neurocognitive disorders, which can appear together with a plethora of other symptoms, particularly of neurovegetative origin. Fibromyalgia may arise as a primary or secondary disease process. It is most frequent in females aged 20 to 50 years (*Adams et al.,1997*).

FM is considered a severe subtype of a condition termed *chronic widespread pain* (*Croft P et al, 1996; Croft P., 2000*), which is also characterized by generalized pain, but without the additional requirement of tender points. People with FM and chronic widespread pain experience poor subjective health, fatigue, sleep disorder, and physical impairments (*Aaron LA and Buchwald D, 2003*; *Prescott E et al, 1993*).

The American College of Rheumatology (ACR) classification criteria drawn up in 1990 has been used for decades. According to it, the diagnosis of fibromyalgia required tenderness on pressure in at least 11 of 18 specified sites and the presence of widespread pain, defined as axial pain, left and right-sided pain, and upper and lower segment pain (*Wolfe F et al, 1990*). However, in the last years, many objections have been expressed in relation to ACR 1990 criteria, particularly because they stipulated that, in order to make a diagnosis of fibromyalgia, chronic widespread pain should be present for at least 3 months, without specifying that any other disease, accounting for the chronic widespread pain, had to be excluded by the examiner. Furthermore, tender point count was rarely or incorrectly performed in primary care and the importance of symptoms was not considered. Indeed patients whose symptoms

and tender points decreased often failed to satisfy these criteria: approximately 25% of FM patients did not satisfy the ACR 1990 classification criteria although they were considered by their physicians to suffer from this condition.

The previous objections, together with the real need to find a common definition and classification for FM, in 2010 led Wolfe and colleagues (*Wolfe F et al, 2010*) to develop simple, practical criteria for clinical diagnosis of fibromyalgia. These new criteria had been thought to be suitable for use in primary and specialty care and did not require a tender point examination, providing instead a severity scale for characteristic fibromyalgia symptoms.

The authors identified two variables that best defined fibromyalgia and its symptom spectrum: the widespread pain index and the composite symptom severity scale, a composite variable composed of physician-rated cognitive problems, unrefreshing sleep, fatigue and somatic symptoms.

Furthermore in 2011 Wolfe published a modification of the ACR preliminary Diagnostic Criteria for Fibromyalgia, which allowed their use in epidemiologic and clinical studies without the requirement for an examiner. Practically, the author modified the symptom severity scale by substituting for the somatic symptoms item a 0-3 item that represented the sum of 3 items: headaches, pain or cramps in liver abdomen or depression symptoms during the previous 6 months. However, it is important to remark that, although simple to use, the new criteria are not thought to be used for self-diagnosis (*Wolfe F et al, 2011*). The presence of diagnostic criteria based only on clinical evidence let us understand that any objective diagnostic biomarker has not been jet discovered.

1.2. Epidemiology

Fibromyalgia has a different prevalence depending on the population studied and the criteria used, oscillating from 0.7% to 20% (*Wolfe F et al, 1995*). Most FM patients are middle-aged women (73-88%). However it has also been described in children (*Yunus MB and Masi AT, 1985*), as well as in the elderly (*Yunus MB et al, 1988*). In a rheumatology unit, about 10-20% of the patients suffer FM, a proportion that descends to 2.1-5.7% in non-specialized clinics (*Wolfe F et al, 1995*; *Wolfe F, 1989*). In a large cohort of Spanish patients the prevalence of FM in the adult population is estimated at 2.37% (95% CI: 1.53-3.21). Eighty to 90% of fibromyalgia suffers are women (*Mas AJ et al, 2008*).

1.3. Comorbidities and/or overlapping conditions

Fibromyalgia frequently occurs together with other conditions. Indeed it can be secondary to other rheumatic diseases, such as Rheumatoid Arthritis (RA) (*Yunus MB, 2012*), Sjögren Syndrome (*Priori R et al, 2010*) and Systemic Lupus Erythematosus (*Yunus MB, 2012*), or to coexists with other syndromes and disorders, particularly chronic fatigue syndrome (CFS) (*Aaron LA et al, 2000*; *Hollins M et al, 2011*), temporomandibular disorder (*Aaron LA et al, 2000*), restless leg syndrome (*Viola-Saltzman M et al, 2010*), irritable bowel syndrome and diabetic neuropathic pain (*Koroschetz J, et al 2011*).

However, the most frequently FM-associated conditions are probably represented by RA and CFS, which will be described in **paragraphs 2** and **3**.

1.4. Etiology

The etiology of FM has not yet been fully understood. Several researchers (*Kindler LL et al, 2011*; *Yunus MB, 2008*; *Coderre, TJ et al, 1993*; *Nielsen LA and Henriksson KG, 2007*, and other authors) agree on the role of central sensitization as a mechanism of hyperalgesia induction, but unfortunately the mechanism by which the sensitization occurs is less clear. As noted, central sensitization is due to ongoing C-fiber stimulation, or painful input, resulting in sustained increases in the excitability and responsiveness of neurons in the spinal cord (*Zusman M, 2002*). While pain processing abnormalities, such as deficient endogenous pain inhibition (*Julien N et al, 2005*), have been proposed to enhance the intensity of nociception in patients with FM, research is less clear on the mechanism generating the ongoing nociceptive input needed to initiate central sensitization (*Vierck JCJ, 2006*). During the last two decades, a plethora of etiologic causes for fibromyalgia have been explored. The hypotheses generally more accepted are based on the following triggering factors: local chronic pain, genetic polymorphisms, infectious agents, autonomic nervous system (ANS) dysfunction, distress and traumatic events.

1.4.1. Hypothesis of local chronic pain

One of the more accredited hypotheses is that regional or focal chronic pain might produce the sustained noxious input that results in hypersensitivity of the central nervous system (*Bennett R, 2005*; *Lidbeck J, 2002*; *Staud R, 2007*). This hypothesis proposes that longstanding bombardment of spinal cord neurons by A-beta and C fibers, resulting from ongoing focal pain conditions, gives rise to the neuroplastic changes characteristic of central

sensitization (*Meeus M and Nijs J 2007*; *Nielsen LA and Henriksson KG 2007*), making regional pain conditions a possible instigator for the altered pain processing of FM. In fact, it has been proposed that regional pain syndromes precede the development of widespread pain in most patients with FM (*Nielsen LA and Henriksson KG 2007*). This proposal is supported by the fact that FM is frequently associated with several focal pain conditions that also have evidence of being characterized by altered pain processing, including TMD, IBS, IC, headaches, back pain, and neck pain (*Staud R, 2007*). These peripheral pain generators could provide the necessary tonic nociceptive input that leads to abnormal pain processing within the CNS. Indeed, generalized hyperalgesia has been confirmed in several of these disorders (*Kindler LL et al, 2011*).

1.4.2. Genetic hypothesis

Another frequently supported hypothesis is the genetic one. Indeed, about one-third of patients with FM have a close relative who is similarly affected and this relative is usually a woman. A family member of a patient with FM is about 8 times more likely to develop FM, compared to a family member of a patient with RA. It is reasonable, therefore, to predict that genetic predisposition to or more biochemical dysfunctions may be important to the development and/or perpetuation of FM (*Xiao Y et al, 2011*). Genetic associations with FM have been sought with polymorphisms of enzymes, receptors and transporters, as recently reviewed (*Bazzichi L et al, 2010*). However, most of the identified associations have not been confirmed and few if any have been linked to a relevant biological function marker (*Blanco I et al, 2010*; *Ablin JN et al, 2009*).

One of this rare marker is represented by COMT polymorphisms. The enzyme COMT catalyses methylation, which is the major metabolic transformation of catecholamines that occurs throughout the body. The COMT gene has abundant functional polymorphisms, among which the better known transition occurs in codon 158. Indeed, the val/val genotype gives rise to an effective enzyme whereas the met/met genotype produces a “lazy” enzyme unable to effectively clear catecholamines from the system (*Zubieta JK et al, 2003*). Indeed two studies showed that subjects with FM less frequently have the val-158-val genotype of the COMT gene when compared with healthy control individuals, suggesting that females that do not degrade catecholamines properly have higher risk to develop FM (*Gursoy S et al, 2003*; *Garcia-Fructuoso FR, 2006*). Moreover, another research found other frequent polymorphisms, in linkage disequilibrium with the val-158-met transition, that induce an even more defective enzyme. It has been shown that a particular COMT gene haplotype named

HPS gives rise to a 11 times less efficient enzyme, which is interestingly associated with high pain sensitivity in healthy females (*Diatchenko L et al, 2005*).

In a recently published genetic study, a large scale candidate gene approach was used to evaluate over 350 genes known to be involved in nociception, inflammation, and affection (*Smith SB et al, 2012*). Several unsuspected genes, differed in frequency between FM patients and healthy controls, although none of the previously found gene polymorphisms have been found (*Bazzichi L et al, 2010*).

Of particular interest is the association of the GRIA4 polymorphism with FM, which seemed to involve central sensitization mechanisms, providing a further proof of what have been speculated in the previous paragraphs. Indeed GRIA4 encodes the AMPA-sensitive, ionotropic glutamate receptor subunit GluR4, which mediates fast excitatory transmission of nociceptive signals in the CNS. Moreover, spinal AMPA receptors have also been implicated in the production of visceral hyperalgesia. Collectively, these observations suggest that alterations in AMPA receptors are likely to contribute to the complex signs, symptoms, and comorbidities associated with FM (*Smith SB et al, 2012*) (Figure 1).

1.4.3. Infectious hypothesis

It has been proposed that fibromyalgia syndrome could occur after an opportunistic infection, but results from the literature are not consistent (*Ablin JN et al, 2006*). Recently it has been reported that FM patients had higher levels of serum helicobacter pylori IgG than healthy controls (*Akkaya N et al, 2011*), but this was immediately followed by a comment highlighting its limits, which made results appearing unreliable (*Zavos C et al, 2011*).

1.4.4. Autonomic nervous system dysfunction hypothesis: FM in relation to stress and traumatic events

Living organisms survive by maintaining harmonious equilibrium or homeostasis. Stress can be defined as a state of disharmony, or threatened homeostasis. For human beings a stressor could have a psychological origin (ongoing anger, anxiety, or depression) but can also originate from a biological insult (an infection, a burn, or a myocardial infarction). The term stress or stressor should therefore not be restricted to psychological events but should be rather viewed in an ample physiological context (*Martinez-Lavin M, 2007*).

The Autonomous Nervous System (ANS) may be viewed as the interface between mind and body functions, the main regulatory system of the body in charge of maintaining essential

involuntary functions, such as the so called vital signs (blood pressure, pulse, respiration, and temperature). The ANS balances the function of all internal organs, with the heart rate, intestinal motility, urination, and sexual activity, among many other variables, all regulated by the system. It is activated by centers located in the spinal cord, brain stem hypothalamus, and thalamus. These centers also receive input from the limbic area and other higher brain regions. Emotions (fear, anger, and panic) have therefore immediate biological responses (pupil dilation, paleness, and tachycardia). The peripheral autonomic system is divided into two branches; *sympathetic* and *parasympathetic*. These two divisions have antagonistic actions on most bodily functions and thus their proper balance preserves homeostasis. The action of these two branches is mediated by three neurotransmitters named catecholamines: norepinephrine, epinephrine, and dopamine.

For many years it has been assumed that abnormal activity of the sympathetic nervous system may be involved in the pathogenesis of chronic pain syndromes. This assumption was based mainly upon the evidence that pain is spatially correlated with signs of autonomic dysfunction, with the fact that blocking the efferent sympathetic supply to the affected region relieves the pain, and with the observation that norepinephrine injections rekindle the pain. The sympathetically maintained pain concept has strong and ample foundations in the animal model. In contrast, the clinical information sustaining this pathogenesis is mostly anecdotal and does not, in most instances, fulfill the strict evidence-based medicine criteria (*Martinez-Lavin M, 2004*).

The defining FM features (widespread pain plus positivity to tender points examination), as well as the paresthesias these patients suffer from, could theoretically be explained by the pathogenetic mechanism known as "sympathetically maintained pain". This type of neuropathic pain is frequently characterized by a post-traumatic onset and by the presence of stimuli-independent pain perception accompanied by paresthesias and allodynia (*Martinez-Lavin M, 2004*), which are precisely FM pain features. Different controlled studies have determined that subjects with FM have higher rates of physical or emotional trauma prior to the onset of their symptoms (*Buskila D and Neumann L, 2000*). FM is clearly a stimulus-independent pain state since there is no underlying structural damage and inflammatory signs are conspicuously absent.

Several groups of investigators have defined that the typical FM tender points reflect a state of generalized allodynia (*Russell J, 1998*). Additional arguments that favor the

involvement of the sympathetic nervous system in the genesis of FM pain are the reports describing such pain as appearing submissive to sympatholytic maneuvers and to be rekindled by norepinephrine injections (*Martínez-Lavín M et al, 2002*). Moreover, as mentioned in paragraph 1.4.2, also genetic studies investigating COMT polymorphisms gave proof of the sympathetic system involvement in FM (*Gursoy S et al, 2003*; *Garcia-Fructuoso FR et al, 2006*).

The role of central and peripheral-stress induced sensitization in the development of FM symptoms is confirmed by pre-clinical studies. Recently, Green et al. (*Green PG et al, 2011*) tested the hypothesis that a rat model of fibromyalgia syndrome, sound stress-induced and at delayed onset, enhanced musculoskeletal and cutaneous mechanical hyperalgesia. Moreover through this model they were able to demonstrate the development of other important FM features, such as comorbidity with irritable bowel syndrome (as indicated by presence of gastrointestinal hypersensitivity), increased anxiety (as indicated by increased Anxiety Index in the elevated plus maze test) and comorbidity with temporomandibular disorder, indicated by hyperalgesia in the masseter muscle.

Thus the conclusion is that an established association between stress and FM could be supported considering the following assertions: (i) acute stress can induce long-term changes in pain sensitivity with delayed onset (e.g., following a motor vehicle accident); (ii) response to acute stress is both the strongest predictor of maintenance of pain symptoms weeks later and increased pain symptoms at a later date in individuals with fibromyalgia and other forms of chronic widespread pain (*Green PG et al, 2011*).

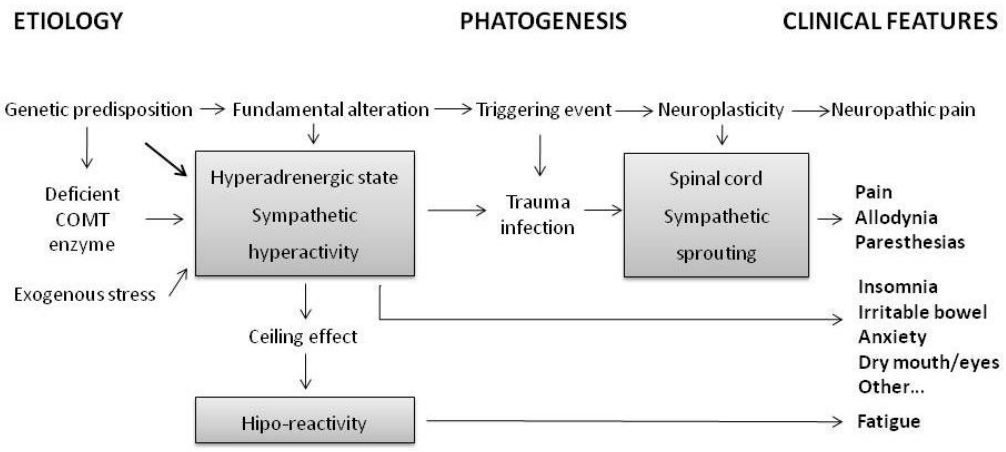

Figure 1 Theoretical etiopathogenetic mechanisms in fibromyalgia (modified from *Martinez-Lavin M, 2007*).

1.5. Pathogenetic mechanisms

In 2011 Kindler and colleagues reviewed the scientific literature concerning the etiopathogenesis of FM, then describing the more accredited pathophysiologic mechanisms underlining chronic pain and hyperalgesia conditions. According to the authors, it is firmly established by now that persistent pain may lead to neuroplastic changes within the peripheral and central nervous system (CNS).

As originally observed in animals, that repetitive C-fiber stimulation exponentially increases dorsal horn stimulation, so that the same level of stimulus produces a progressive increase in activation of second order neurons projecting to the brain (*Mendell LM, 1966; Yunus MB, 2008*). This phenomenon, termed *windup*, represents one major mechanism through which ongoing pain produces a hyperexcitable state within the CNS. When the *windup* occurs, pain impulses originating in peripheral nerve endings –better known as *nociceptors*- activate both Aδ and C-nociceptive fibers; these are the nerve fibers that carry the nociceptive impulse to dorsal horn neurons in the spinal cord. It is of great importance to underline that some of these neurons are multimodal and respond to the sensations of touch, pressure, temperature, and pain and are therefore called wide dynamic range (WDR) neurons. Particularly, when pain is chronic, it causes a persistent activation of Aδ and C fibers, stimulating the release of neurotransmitters and neuromodulators (i.e., **substance P**, **nerve growth factor**, **brain-derived neurotrophic factor**, calcitonin gene - related peptide, glutamate, and aspartate) into the dorsal horn synapse (*Urban MO and Gebhart GF, 1999*). Influx of these neurochemicals sensitizes the WDR neurons so that they become hyperexcitable, responding to lower levels of nociceptive stimuli (i.e., hyperalgesia) as well as some previously non-painful stimuli (i.e., allodynia) (*Eide PK, 2000*).

Another important mechanism through which central sensitization modulates the expression of hyperalgesia is represented by the expansion of receptive fields. This occurs as a result of prolonged excitation of WDR neurons, which in turn activates adjacent neurons, thus expanding their receptive fields beyond the site of the original injury. Clinically, this results in pain being experienced by stimulation of locations that had not previously provoked a pain response (*Coderre TJ, 1993*; *Nielsen LA and Henriksson KG, 2007*).

Although researches on the development of chronic pain states and hyperalgesia has traditionally focused on transmission of pain through ascending pathways (from the periphery

to spinal and supraspinal centers), a growing number of studies is now expanding the knowledge about the descending influences on the generation and maintenance of sensitization (*Heinricher MM et al, 2009*; *Tracey I and Dunckley P, 2004*; *Kindler LL et al, 2011*) (Figure 2).

Concerning studies on fibromyalgia, central sensitization and impaired descending pain modulation are generally accepted as the two major underlying mechanisms causing widespread hypersensitivity to pain (*Perrot S, et al, 2008*; *Staud R and Rodriguez ME, 2006*). Attributes of FM that support the role of central sensitization include: (i) decreased pain thresholds and enhanced sensitivity outside of typical tender point locations, (ii) expansion of pain receptive fields, (iii) **increased levels of substance P and neurotrophic factors in the cerebral spinal fluid**, (iv) abnormal windup, and (v) prolonged pain after cessation of painful input (*Dadabhoy D et al, 2008*; *Giovengo SL et al, 1999*).

FM patients who were subjected to a cold pressor test showed lower pain threshold and tolerance (i), as well as higher ratings of pain intensity and unpleasantness on visual analogue scales, as reported by Reyes del Paso et al. (*Reyes del Paso GA et al, 2011*). Moreover, data from this study suggest impaired autonomic cardiovascular regulation in FM in terms of reduced sympathetic and parasympathetic influences, as well as blunted sympathetic reactivity to acute stress. The association between baroreflex function and pain experience reflects the pain inhibition mediated by the baroreceptor system. Given this reduced baroreflex sensitivity in FM, the authors hypnotized that deficient ascending pain inhibition could arise from the cardiovascular system, which may contribute to the exaggerated pain sensitivity of FM.

The expansion of receptive fields (ii) is an important mechanism through which central sensitization modulates the expression of hyperalgesia. This occurs as a result of prolonged excitation of WDR neurons, which in turn activates adjacent neurons, thus expanding their receptive fields beyond the site of the original injury. Clinically, this results in pain being experienced by stimulation of locations that had not previously provoked a pain response.

As for point (iii), substance P, along with excitatory amino acids, such as glutamate (*Harris RE, 2010*) and aspartate, is known to enhance the transmission of pain through the primary afferent neurons (*Larson AA et al, 2000*). A study demonstrated that substance P levels in FM patients are two- to threefold that of healthy controls (*Russell IJ, 1998*; *Russell IJ and Bieber CS, 2006*). Increased levels of substance P can induce hyperalgesia and allodynia by lowering the firing threshold of spinal cord neurons and extend long distances from the pain locus, resulting in sensitization at sites distant from the pain locus (*Bennett RM, 1999*). Moreover,

the combination of **elevated glutamate** and **substance P** and **reduced serotonin** supports a role for central amplification in the abnormal pain transmission and perception of patients with FM. In fact, in these patients cerebrospinal fluid (CSF) levels of **NGF** and **BDNF** were founded increased compared to healthy non-painful controls (*Giovengo et al SL, 1999*; *Laske C et al, 2007*; *Sarchielli P et al, 2007*), and correlated with increased **glutamate levels**. The authors speculated that NGF probably acts indirectly to increase BDNF expression, which then modulates NMDA receptor activity to increase the excitatory amino acids glutamate and aspartate, supporting the involvement of a central mechanism in the pathophysiology of FM (*Reyes del Paso GA et al, 2011*) (Figure 1).

As for *windup* phenomenon (iv), it is known to describe the changes that occur after ongoing painful input resulting in increased excitability of dorsal horn neurons, enhanced responsiveness to painful and non-painful input, and an increase in spontaneous activity (*Staud R et al, 2004*). This phenomenon is a normal occurrence to painful stimuli, but FM patients demonstrate enhanced *windup* with a greater degree of neuronal excitability and prolonged aftersensations (*Staud R, 2007*). This means that WDR neurons have a lower firing threshold and take longer to resolve following cessation of the stimuli.

Concerning hyperalgesia (v), Alonso-Blanco et al conducted a study focusing on local and referred pain from active myofascial trigger points (MTrPs). They demonstrated that this kind of pain fully reproduced the overall spontaneous clinical pain area in patients with FM and that widespread mechanical pain hypersensitivity was related to a greater number of active MTrPs, suggesting that nociceptive inputs from active MTrPs may contribute to central sensitization in FM (*Alonso-Blanco C, et al 2011*).

Because peripheral sensitization manifests only at the pain locus, whereas central sensitization is detected in healthy tissue distant from the pain locus, it is possible to conclude that hypersensitivity, not localized to the area of injury, indicates underlying changes in the CNS that might be explained by central sensitization (*Kindler LL et al, 2011*). Thus, central sensitization can be seen as the expression of neuroplasticity, rendering central pain a type of neuropathic pain, which in fact respond to the treatment with anticonvulsant drugs indicated for pain of neuropathic origin, such as pregabalin and gabapentin (*Crofford L et al, 2005*).

Nevertheless, central sensitization alone could not be the only responsible for FM pain and related manifestations occurrence; also the involvement of peripheral tissue nociception should be supported. Indeed, results of studies analyzing neural mechanisms of somatic hyperalgesia suggest that FM pain is associated with widespread primary and secondary

cutaneous hyperalgesia, which are dynamically maintained by tonic impulse input from deep tissues and likely by brain-to-spinal cord facilitation. Thus FM pain is likely to be at least partially maintained by peripheral impulse input from deep tissues. This conclusion is also supported by results of several studies showing that injection of local anesthetics into painful muscles normalizes somatic hyperalgesia in FM patients (*Staud R, 2010*).

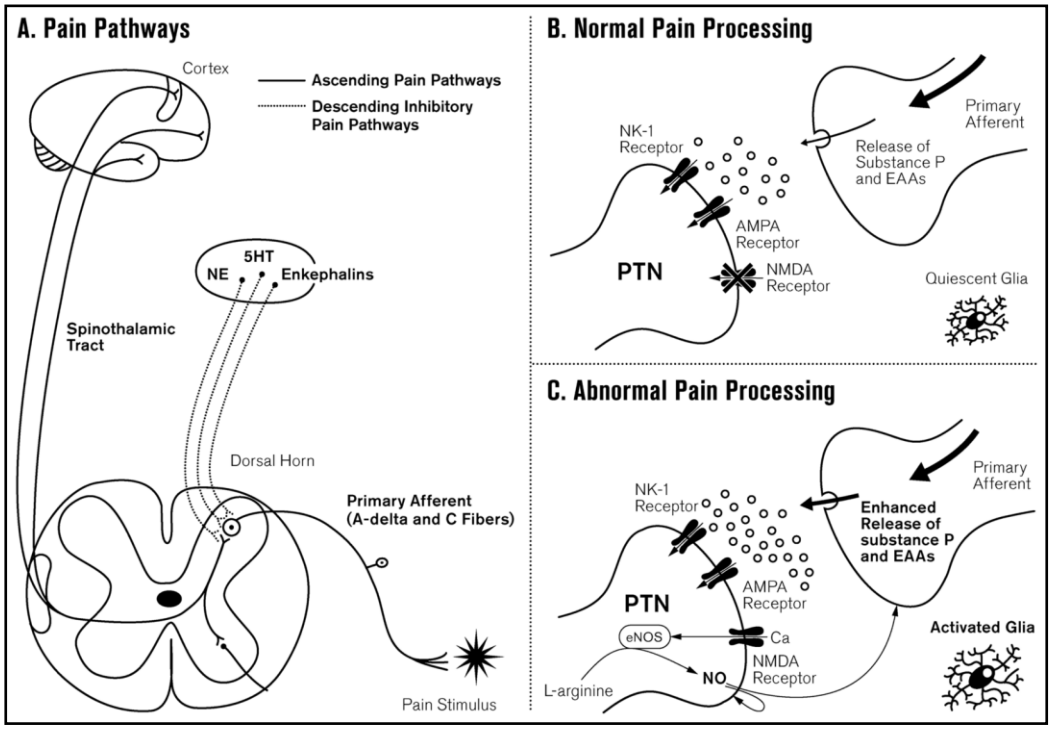

Figure 2 (A) In the classical model of acute pain, painful stimuli are transmitted from the periphery to the dorsal horn via primary afferent fibers (α-δ and C nerve fibers) and from the dorsal horn to the brain via the spinothalamic tract. (B) In the dorsal horn, incoming afferent pain signals cause the release of substance P and excitatory amino acids (EAAs), which bind to activate postsynaptic receptors on the pain transmission neurons (PTNs). Glia are present but quiescent. (C) The intense or prolonged exposure to painful stimuli causes an increasing in incoming afferent signals, and enhances presynaptic release of substance P and EAAs. An influx of Ca^{2+} increases the production of NO, which diffuses out of the neuron, making it hyperexcitable and further enhancing the presynaptic release of EAAs and substance P. The activation of glia causes the release of substances (e.g., nitric oxide, reactive oxygen species, prostaglandins, proinflammatory cytokines, NGF) that further increase presynaptic release and postsynaptic hyperexcitability. (5-HT = serotonin; NE = norepinephrine; NMDA = *N*methyl- D-aspartic acid; AMPA = alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NK-1 = neurokinin; cNOS = constitutive nitric oxide synthase; NO = nitric oxide) (From *Bradley LA, 2009*).

2. Chronic Fatigue Syndrome

Chronic Fatigue Syndrome (CFS) patients report a plethora of symptoms**,** summarized as follows: persistent or recurrent fatigue, diffuse musculoskeletal pain, sleep disorders, and subjective cognitive impairment of 6 months duration or longer. Symptoms are not caused by ongoing exertion, are not relieved by rest and result in a substantial reduction of previous levels of occupational, educational, social, or personal activities. A somewhat unusual secondary symptom, considered a key illness characteristic by some prominent researchers (e.g., Hawk C et al, 2006), is postexertional malaise (PEM) lasting at least 24 hours. PEM refers to sustained symptom flare-ups after routine physical or mental activities, such as housework, brief walks, or even reading for short periods. These postexertional symptoms may be delayed by as much as 24 hours or more after the triggering activity (Yoshiuchi K et al, 2007).

This syndrome principally affects young women (male/female ratio ¼; age between 30-40) (*Jason LA et al, 1999*; *Steele L et al, 1998*). Minor alterations of immune, neuroendocrine and autonomic function may be associated with this syndrome. A considerably larger illness category, unexplained chronic fatigue (UCF), is also defined by 6 months of unexplained debilitating fatigue but without the requirement of additional symptoms (*Sharpe M et al, 1997*). In both clinical conditions (UCF and CFS), the fatigue symptoms are medically "unexplained", which indicates that they are not primarily attributable to identifiable medical conditions, such as untreated thyroid illness, anemia, severe sleep apnea, or morbid obesity. Six to thirteen percent of the population is estimated to have UCF (*Ranjith G, 2005*).

Although the case definitions of CFS and FM appear quite different, these illnesses show considerable overlap in symptoms: 30-70% of patients with CFS also meet diagnostic criteria for FM and *vice versa* (*Goshorn RK, 1998*; *Fukuda K et al, 1994*). In this regards, the 20-70 % of FM patients has been esteemed to also meet CFS diagnostic criteria and that the 35-70% of CFS patients also fulfills the FM ones (*Aaron LA et al, 2000*).

3. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is the most frequent form of chronic inflammatory arthritis, with a prevalence of 0.5–1% in the adult world population (*Kvien, 2004*). Despite intense research and great advances in patient management and therapy development in recent years, still little is known about the underlying pathogenesis and etiology of the disease.

RA is commonly defined as a systemic autoimmune disease mainly characterized by an autoimmune attack of the synovial membrane of joints, but extra-articular tissues also can be affected. The identity of the autoantigens that are targeted by this autoimmune response remains elusive. Autoreactive T cells, as well as B type ones, are thought to play a role in the aberrant immune response; both cell types have in the past been analyzed for the identification of autoantigen targets in RA. Up until now, most candidate RA autoantigens have been identified by analysis of autoantibodies and B cells in RA patients. Such research has provided evidence for the presence of numerous antibody specificities in RA (*Blass S et al, 1999*). Joint-specific as well as non-joint-specific, ubiquitous, and non-self RA antigenic targets have been reported in previous studies (*Mewar D and Wilson AG, 2006*; *van Boekel MA et al, 2002*; *Magalhaes RP et al, 2002*). Unfortunately, none of the previously identified antigenic targets can fully explain the etiology and pathogenesis of RA. Of consequence, the diagnosis of RA, currently based on ACR classification criteria of 1987 (*Arnett FC et al, 1988*) or based on the ACR/EULAR revised criteria of 2010 (*Aletaha D et al, 2010*), depends primarily on clinical manifestations. The only serologic tests routinely used in RA assessment are the determinations of rheumatoid factor (RF) and anti-citrullinated peptides antibodies (ACPA). RF can be detected in 60–80% of RA patients. However, its specificity is limited because RF is also detected in other diseases, such as connective tissue diseases, Sjögren syndrome, chronic infections, and malignancy, as well as in healthy individuals (*Somers K et al, 2009*). More recently ACPA, which bind antigenic determinants containing the unusual amino acid citrulline, emerged as sensitive and specific serological markers of rheumatoid arthritis (RA), providing superior alternative of the rheumatoid factor (RF) test in the laboratory diagnostics of RA. Several studies have described the occurrence of anti-CCP in 41–68% of patients with early RA. However, up to 90% of anti-CCP positive patients were also positive for RF and co-occurrence of both antibodies was not more specific for RA than occurrence of either antibody alone (*Nell VP et al, 2005*).

Therefore, FM and RA are both rheumatic diseases of unknown etiology, but with different pathogenetic mechanisms, which however are mainly characterized by chronic pain. That in fact could be the reason why a higher prevalence of patients with RA (12.2 – 19.8%) also developed a FM syndrome. Indeed, as early as 1983, Wolfe and Cathey recognized the concomitant occurrence of FM among RA patients, observing that many features of FM syndrome (e.g. pain at multiple sites, fatigue, a feeling of malaise and tenderness at multiple spots, including joint areas) may also be present in RA itself (*Yunus MB, 2012*).

4. Brain-derived neurotrophic factor

Brain-derived neurotrophic factor **(**BDNF) is an endogenous protein of 14 kDa belonging - together with NGF, and neurotrophins (NT) 3 and 4/5 - to the family of neurotrophic factors. It is known to be involved in neuronal survival and synaptic plasticity of the central and peripheral nervous system (CNS and PNS). Several lines of evidence converged to indicate that BDNF also participates in structural and functional plasticity of nociceptive pathways in the CNS and within the dorsal root ganglia and spinal cord. Moreover, release of BDNF appears to modulate or even mediate nociceptive sensory inputs and pain hypersensitivity. The gene encoding for BDNF is induced by cortical neurons and it is necessary for survival of striatal neurons in the brain (*Mattson MP et al, 2004*; *Laske C et al, 2007*). Expression of this gene is reduced in both Alzheimer's and Huntington disease patients (*Zhang H et al, 2006*; *Zuccato C et al, 2007*).

4.1. Biosynthesis

BDNF is produced as a 32-kDa precursor (pro-BDNF) that undergoes N-glycosylation and glycosulfation on residues located within the pro-domain region. N-terminal cleavage of the precursor generates mature BDNF as well as a minor truncated form of the precursor (28 kDa) that arises by a different processing mechanism than mature BDNF. Data also demonstrate that pro-BDNF could be biologically active, as determined by its ability to promote TrkB autophosphorylation. Because biosynthesis of neurotrophins normally occurs at low levels in neurons and non-neuronal cells, it is impossible to analyze endogenous neurotrophin processing with currently available techniques. Therefore, Mowla S.J. and colleagues used a vaccinia virus expression system to overexpress pro-BDNF and to study its processing in a variety of cell lines as well as in primary cultures of mouse hippocampal neurons. They observed a significant amount of unprocessed pro-BDNF being released into conditioned medium by AtT-20 cells and hippocampal neurons, cells that can release proteins both by the regulated and constitutive secretory pathways (*Mowla SJ et al, 1999*).

Recent data show that cells with a regulated secretory pathway, including central nervous system neurons, release mature (i.e. fully processed) NGF (*Mowla SJ et al, 1999*) and NT-3 (*Farhadi HF et al, 2000*) via the constitutive secretory pathway, whereas mature BDNF is packaged in vesicles and released through the regulated pathway (*Mowla SJ et al, 1999*).

Furthermore, BDNF is contained in a microvesicular fraction of lysed brain synaptosomes consistent with its anterograde transport in large dense core vesicles (*Fawcett JP et al, 1997*; *von Bartheld CS et al,. 1996*).

4.2. BDNF-mediated neurotransmission: evidence from animal models

In the sensory system BDNF is produced by a subset of primary sensory neurons (nociceptors) that respond to tissue injury. The cell bodies of these bipolar neurons are located in the dorsal root ganglia (DRG) where BDNF is synthesized and packaged in large dense-core vesicles.

BDNF is then transported anterogradely to the central terminals of sensory neurons in the dorsal horn where it can be released and activate its receptors. Concerning receptors, it is important to highlight that neurotrophins interact with two cell-surface receptors, the low affinity p75 receptor and the high-affinity tyrosine kinase receptors (trk). Whereas all neurotrophins bind the p75 receptor with similar affinity, the other receptors are bound with different affinity by each family member, as shown in Figure 3. Indeed NGF shows higher affinity for trkA receptors, while BDNF - as well as neurotrophin 4/5 - preferentially binds trkB, and NT-3 binds both trkC and, although to a lesser extent, trkA receptors. BDNF is produced at presynaptic level (in the cell bodies of sensory neurones that project into the dorsal horn) and at the level of postsynaptic dendrites (within the hippocampus); the activation of trkB that occurs in both types of synapse gives rise to glutamate releasing, with consequent potentiation of glutamatergic transmission (*Malcangio M et al, 2003*).

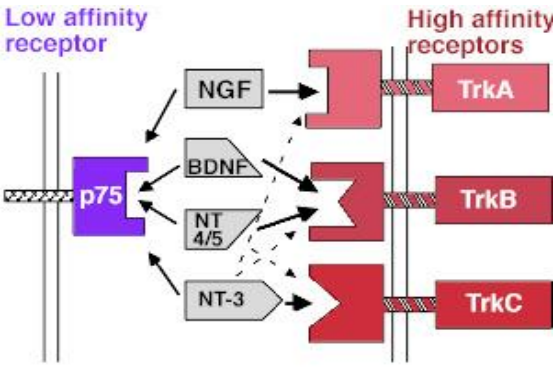

Figure 3 Neurotrophic factors and their receptors

In 2002 Pezet and colleagues (*Pezet S et al, 2002*) reviewed the literature concerning BDNF neurotransmission on *in vitro* preparations and animal models, providing the evidence

that antagonism of BDNF attenuates the second phase of hyperalgesia induced by formalin (in nerve growth factor-treated animals) and the thermal hyperalgesia induced by carageenan. By electrically stimulating isolated dorsal horn-with dorsal roots following different protocols (continuous, titanic or burst/capsaicin stimulations), as illustrated in figure 4, they showed that BDNF release from sensory neurons could be evoked only by burst stimuli at C-fibre intensity of stimulation or by capsaicin superfusion of dorsal horn slices isolated *in vitro*. Thus they concluded that BDNF is involved in some aspects of central sensitization, particularly in conditions of peripheral inflammation.

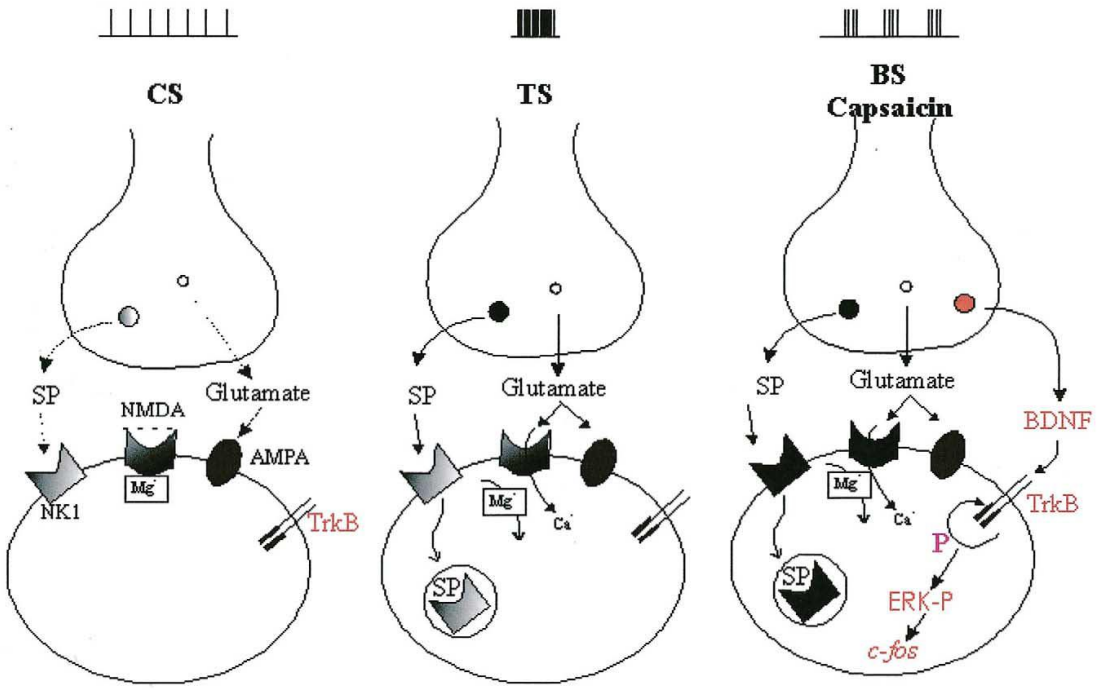

Figure 4 Glutamate, SP and BDNF are released from nociceptors to activate second order dorsal horn neurons. Distinctive firing patterns of primary afferents encode the release of different transmitters and subsequent neuronal activation in the spinal dorsal horn. Continuous stimulation (CS) leads to the activation of NK and AMPA receptors on post-synaptic neurons. Tetanic stimulation (TS) recruits AMPA, NMDA, NK receptors. After burst stimulation (BS) or capsaicin treatment, in addition to NK, AMPA and NMDA receptors, TrkB receptors are recruited due to BDNF release. This leads to down-stream phosphorylation of ERK and transcription of *c-fos* (from *Pezet S et al, 2002*)

As illustrated in figure 4, also the enhancement of N-methyl-D-aspartate (NMDA)-evoked responses could represent a mechanism of hyperalgesia (*Staud R, 2004*): indeed the phosphorylation on subunits 1 and 2B increases the probability that these channels are in an

open state (*Chen L et al, 1992*; *Sarchielli P et al, 2002*). This mechanism is clearly demonstrated in animal models of hyperalgesia and pain. The factors leading to this altered state of phosphorylation, however, are not clear. There is compelling evidence from another area of the CNS, the hippocampus, that BDNF is a modulator of central neuronal activity, since the activity-dependent release of BDNF is related to the expression of hippocampal long-term potentiation (LTP). LTP is defined as a characteristic activity-dependent increase in synaptic potency within the CNS (*Kang H et al, 1995*), and several evidence now suggest that BDNF may play a role in the spinal cord similar to that in the hippocampus; as BDNF high-affinity to trk-B receptors induces long-term potentiation at hippocampal synapses, which are involved in learning and memory, it is possible that a similar phenomenon can occur also at the first pain synapse, between primary sensory neurons and dorsal horn neurons (see Figures 5 and 6).

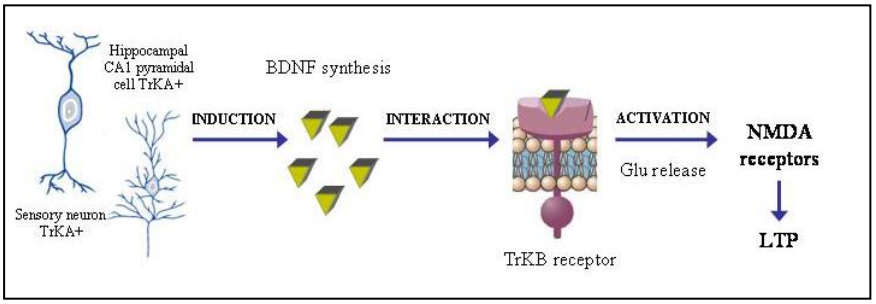

Figure 5 Long term potentiation BDNF-mediated within hippocampus

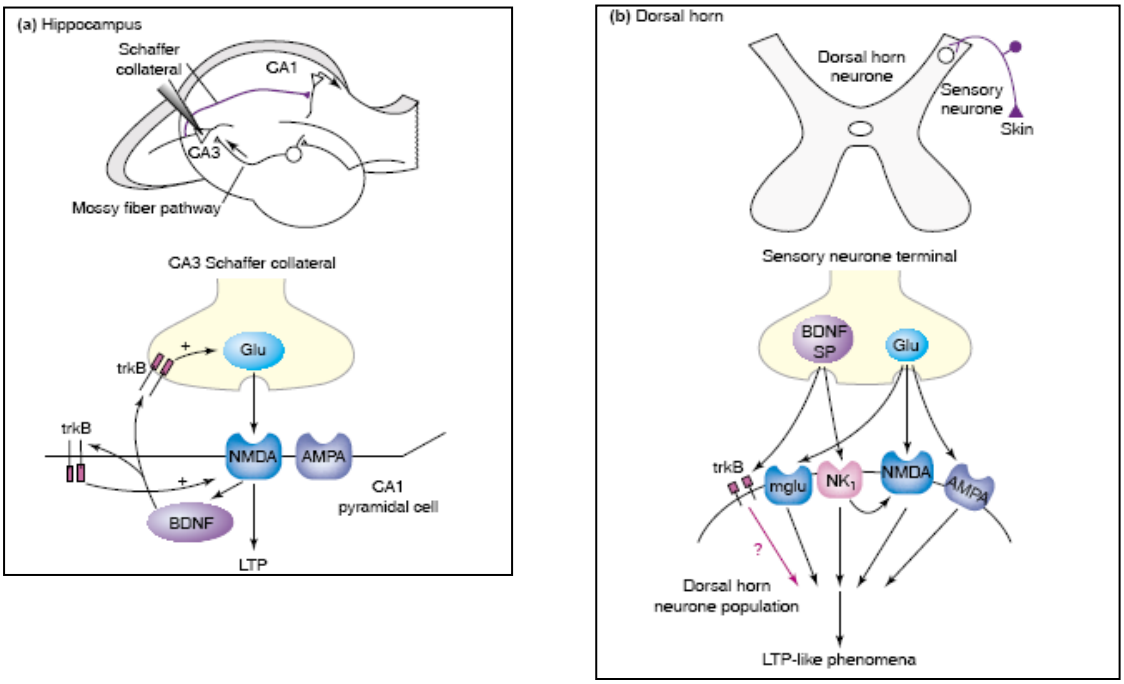

Figure 6 (a) Long term potentiation (LTP) BDNF-mediated at hippocampal level, and (b) theoretical mechanisms of LTP-like phenomena BDNF-mediated induction within the dorsal horn.

4.3. BDNF levels in plasma, serum and platelets

The first evidence for the presence of BDNF in human serum and plasma emerged a decade ago (*Rosenfeld RD et al, 1995*). The average serum BDNF levels are known to be more than 100-fold higher than the plasma BDNF levels (*Radka SF et al, 1996*) and this difference is due to the degranulation of platelets during clotting process (*Fujimura H et al, 2002*). Indeed, human platelets contain large amounts of BDNF (*Pliego RF et al, 1997*; *Yamamoto H et al, 1990*) and it has been also demonstrated that the amount of BDNF in serum is similar to that in washed platelet lysates (*Fujimura H et al, 2002*). Thus, the difference between serum and plasma BDNF levels appear to reflect the quantity of BDNF stored in circulating platelets.

Activation of platelets may contribute to nociceptor excitation and pain, since platelets store and, upon stimulation, release potential algogenic substances such as serotonin, histamine, precursor molecules of bradykinin and BDNF (*Ringkamp M et al, 1994*). Since

platelets are a major source of BDNF in serum, it may be reasonable to assume that platelets are also activated in fibromyalgia, which could explain the significant increase of BDNF serum concentration in these patients.

The cellular sources of BDNF in human plasma have not yet been clearly defined. Potential sources of BDNF are vascular endothelial and smooth muscle cells (*Nakahashi T et al, 2000*; *Donovan MJ et al, 1995*), in addition to activated macrophages or lymphocytes (*Braun A et al, 1992*; *Kerschensteiner M et al, 1999*). Moreover, there is experimental evidence that BDNF can cross the blood-brain barrier (*Poduslo JF et al, 1996*; *Pan W et al, 1998*) and a pre-clinical study found a positive correlation between serum and cortical BDNF levels (*Karege F et al, 2002a*). According to these results, BDNF changes within the CNS may be the expression of BDNF changes within plasma and serum. Nevertheless, data suggest that the majority of BDNF in plasma and serum may be of peripheral origin.

4.4. BDNF and cytokines

Blood levels of the cytokines interleukin (IL)-6 and IL-8 are increased in FM patients (*Wallace DJ et al, 2001*). Schulte-Herbruggen et al. suggest that BDNF secretion by monocytes can be specifically enhanced by IL-6 and TNFa. Since there is only a slight up regulation of BDNF mRNA in these cells after TNF-a or IL-6, researchers presume that the increase of BDNF in supernatants is based also on a release of preformed BDNF in monocytes (*Schulte-Herbruggen et al, 2005*). As IL-6 induces hyperalgesia, fatigue and depression, and IL-8 promotes sympathetic pain, it may be supported that they play a role in the etiopathogenesis of FM and in modulating FM symptoms (*Laske et al., 2007*).

4.5. BDNF, depression and antidepressants

Beyond the relationship between stress and BDNF, changes in neurotrophins and BDNF in depression have been a focus of interest. In addition to the monoamine theory of depression, Popoli and colleague (*Popoli M et al, 2002*) suggest that intracellular pathways have a major role in regulating neuroplasticity and neurodegeneration in the etiology of mood disorders. According to this hypothesis, stress results in neuronal atrophy and decreased neurogenesis, and then depression occurs. By stimulating intracellular pathways, antidepressants lead to up-regulation of the cAMP response element binding (CREB) protein and to an increase in the expression of neurotrophic factors, particularly BDNF. This hypothesis was the precursor of a new understanding of the etiology of depression and has been the inspiration for many

subsequent studies, which reported low serum levels of BDNF in patients with major depressive disorder (MDD) not treated with antidepressants, when compared with patients treated or with control subjects, as well as a negative correlation between the severity of depression or Hamilton Depression Rating Scale (HAM-D) score and serum BDNF level (*Karege F et al, 2002*; *Shimizu E et al, 2003*; *Huang TL et al, 2008*).

Since low-level serum BDNF in MDD is reported, it is suggested that some polymorphisms of the BDNF gene may be significant. Particularly, studies on Val66Met polymorphism of the BDNF gene – that is the most studied polymorphism and has the largest body of data- sought to identify the relationship between BDNF and stress-related mood disorders. It is suggested that healthy controls with the met allele have poor performance in episodic memory and that the Val66Met allele of BDNF is associated with decreased hippocampal volume, possible morphological anomalies in the frontal, parietal, and temporal cortexes, and increased activation of the hippocampus during learning and memory tasks in normal volunteers (*Egan MF et* al, 2003; *Frodl T, 2007*; *Eker Ç et al, 2005*; *Post RM, 2007*). Nevertheless, in 2 larger studies no significant difference was observed between patients with MDD and healthy controls, in terms of Val66Met polymorphism (*Hong CJ et al, 2003*; *Tsai SJ et al, 2003*). However, in a study on citalopram, even though there was no difference between depressed patients and healthy controls, in terms of polymorphism, Met-BDNF allele carriers had better responses to antidepressant treatment (*Choi MJ et al, 2006*).

Therefore, even though BDNF polymorphism does not consistently discriminate between depressed patients and healthy controls, it can surely discriminate between some clinical and therapeutical features of depression. Indeed several studies (*Gönül AS et al, 2003*; *Aydemir Ö et al, 2005*) showed that antidepressant treatment, while leading to MDD remission, significantly increased serum BDNF level, reaching levels that were comparable to the ones found in healthy subjects. Furthermore, two meta-analyses (*Sen S et al, 2008*; *Brunoni AR et al, 2008*) highlighted that serum BDNF level, significantly lower in patients with MDD than in healthy controls, was increased by antidepressant treatment. In particular, Brunoni et al. suggested in their meta-analysis that the level of serum BDNF is associated with clinical changes in depression and that improvement after treatment is due to change in neuroplasticity. As clinical improvement obtained with antidepressant treatment persists, the level of serum BDNF remains unchanged. A 1-year follow-up study reported that while after the first month of treatment patients' plasma BDNF levels did not differ significantly from

those observed in healthy control subjects, serum BDNF levels in patients remained significantly high at all times (*Piccinni A et al, 2008*).

4.6. BDNF and Fibromyalgia

In a recent study by Laske and colleagues (*Laske C et al., 2007*), serum BDNF levels of FM patients were statistically higher compared to healthy controls and, unexpectedly, the comparison of BDNF levels between FM patients with and without recurrent major depression, as well as with or without antidepressant medications in low analgesic doses, revealed no statistically significant differences. This finding indicates that BDNF serum concentrations in FM patients are independent of pre-existing MDD or antidepressant low dose medication. In addition, none of the FM patients and control subjects included in the study had a clinically relevant depressive episode. More recently similar results were also reported on FM plasma samples by Haas and colleagues (*Haas L et al, 2010*).

In order to deepen BDNF involvement in chronic pain conditions and hyperalgesia, several studies and reviews investigated and speculated on the relation between FM and chronic migraine (CM) (*Sarchielli P et al, 2007*; *Sarchielli P et al, 2002*; *Sarchielli P et al, 2007 Review*; *Malcangio M et al, 2003*). Indeed, from a pathophysiological point of view, chronic migraine and fibromyalgia are both considered the consequence of a tonic state of increased excitability, in which neuroplastic changes within spinal horn neurons (as previously described) and the trigeminal nucleus caudalis respectively occur. Baranauskas and colleagues suggested that supraspinal structures, involved in pain processing, could result in spontaneous pain due to a reduced threshold for activation (*Baranauskas G et al, 1998*; *Staud R, 2004*). Researchers also speculated that this specific type of synaptic plasticity can mediate the maintenance of pain in both CM and FM (*Malcangio M et al, 2003*). Indeed, in a first study concerning chronic daily headache, a significant correlation emerged between the cerebro-spinal fluid (CSF) levels of NGF, BDNF, and glutamate, suggesting the putative intervention of both neurotrophins in enhancing glutamatergic transmission underlying chronic sensitization (*Sarchielli P et al, 2002*). In a second study from the same research group, significantly higher levels of BDNF and NGF were found in the CSF of FM ($p<0.001$, $p<0.001$) and chronic migraine (CM) patients ($p<0.0001$, $p<0.0005$), compared with control subjects. No correlation was found, however, between NGF and BDNF levels as well as glutamate levels, and VAS values and pain intensity, pain threshold, and tender point number examinations both in patients with CM and FM (*Sarchielli P et al, 2007*).

4.7. BDNF and Rheumatoid Arthritis

A number of *in vitro* and also *in vivo* studies have suggested that neurotrophins are also involved in arthritic processes (*Manni L et al, 2003*; *Aloe L et al, 1992*); BDNF is reported to be implicated in inflammatory reactions (*Toma H et al, 2002*; *Kerschensteiner M et al 1999*), and its production is increased in response to pro-inflammatory cytokines (*Jornot L et al, 2007*). Interleukin-6 and tumour necrosis factor-alpha (TNFα) in particular enhance BDNF secretion by human peripheral blood monocytes (*Schulte-Herbruggen O et al, 2005*). Thus, one possibility is that the effects of these pro inflammatory cytokines in arthritic processes could be mediated by modulating synthesis of BDNF. Although information concerning the importance of BDNF in joints, including arthritic joints, is sparse, it has been shown that BDNF is detectable in human synovial fluid (*Rihl M et al, 2005*), that macrophages and fibroblasts in synovial tissue from patients with RA stain positively for BDNF (*Weidler C et al, 2005*) and that BDNF mRNA was found in the inflammatory synovium in two samples of RA patients (*Rihl M et al, 2005*). BDNF, apart from being synthesized in inflammatory cells, fibroblasts and various other cell types (for a review see *Nockher WA and Renz H, 2005*), is also a neurotransmitter (*Pezet S et al, 2002*). Therefore, an alternate possibility is that the major role of BDNF in joints is related to influences via the nervous system. Such a suggestion is supported by the interpretation that neurotrophins may show survival-promoting, healing and protective effects on neurons (*Patapoutian A and Reichardt LF, 2001)*. Indeed, neurotrophic factors have been associated with neuroprotective roles in, e.g., brain lesions (*Kerschensteiner M et al 1999*) and inflamed gut tissue (*Reinshagen M et al, 2000*).

Whether or not BDNF is expressed in the nerve structures of joints is unknown. Several lines of evidence favor an effect of anti-TNFα treatment on nerve structures. It has been shown that infliximab, one of the most commonly prescribed anti-TNFα, attenuates the BDNF levels in the dorsal root ganglion and spinal cord in a rat model of herniated *nucleus pulposus* (*Onda A et al, 2004*) and that injection of infliximab seemed to prevent hyperalgesia that is caused by a combination of disk incision and nerve displacement (*Murata Y et al, 2005*).

A study by Grimsholm and colleagues (*Grimsholm O et al, 2008*) showed that baseline levels for BDNF in plasma from RA patients were clearly higher compared with those of healthy controls. Following anti-TNF treatment, there was a decrease in BDNF levels in plasma. Thus, blocking the pluripotent cytokine TNFα had an influence on circulating levels

of BDNF. This finding was supported in part by a previous preliminary report (*Del Porto F et al 2006*) in which it was shown that the plasma levels of BDNF decreased, but not significantly.

Based on these observations, one speculative suggestion may be that anti-TNF treatment, apart from having effects on circulating and local cells such as fibroblasts, has an effect on the expression of BDNF not only centrally in the nervous system (*Onda A et al, 2004*), but also in joint nerves, as a mechanism for pain relief. It may be that BDNF is not only related to neuroprotective but also pain-mediating effects; it is indeed a well-known fact that the nerves of joints become sensitized during inflammation (*McDougall JJ, 2006*).

Chapter 2

Objectives

The principal aim of the present PhD thesis project was to investigate BDNF-mediated neuroplasticity, by detecting BDNF levels in FM patients, and therefore by comparing these levels to the ones found in CFS, RA and healthy volunteers.

Secondary objectives were: (i) the relation between BDNF levels and clinical variables, including neurocognitive disorders, which were assessed in FM and CFS patients by means of a computerized system; (ii) the relation between BDNF levels and psychiatric comorbidity in FM and CFS patients; (iii) the relation between BDNF levels and inflammatory status in RA patients; (iv) serum and plasmatic BDNF levels in a subgroup of FM patients before, immediately after, and 3-months after thermal treatments.

Chapter 3

Materials and Methods

Sixty-eight patients affected by FM, together with 45 patients with CFS, 46 with RA and 40 healthy controls, were recruited at the Rheumatology Division of Santa Chiara Hospital (University of Pisa), from March 2010 to January 2012. On each participants' group the following evaluations were performed.

Clinical parameters

- **FM group** (*Study A* and *Study B*): Patients recruited (n=68) already had a FM diagnosis according to ACR criteria of 1990 (*Wolfe F et al, 1990*). As established by the clinical practice, a rheumatologic visit, including Tender Points (TP) count, and a psychiatric interview - the *Structured Clinical Interview* (SCID) according to DSM-IV- were conducted. Patients were also asked to complete the *Fibromyalgia Impact Questionnaire* (FIQ), the *Functional Assessment of Chronic Illness Therapy-Fatigue Scale* (FACIT), the *Health Assessment Questionnaire* (HAQ), the *Medical Outcomes Study 36-Item Short Form Health Survey* (SF-36), the *Self-raiting anxiety scale* and the *Self-raiting depression scale* (SAS and SDS respectively). Finally, another subgroup of 40 patients also performed the CNS vital signs® neurocognitive test battery (*Gualtieri CT et al, 2006*).

- **CFS group:** Patients recruited (n=45) already had a CFS diagnosis according to Fukuda criteria of 1994 (*Fukuda K et al, 1994*). As established by the clinical practice, a rheumatologic visit, including Tender Points (TP) count, and a psychiatric interview (SCID) were conducted. Like FM, also CFS patients completed FIQ, FACIT, HAQ, SF-36, SAS, and SDS questionnaires. Moreover, all the patients performed the CNS vital signs® neurocognitive test battery (*Gualtieri CT et al, 2006*).

- **RA group:** Patients recruited (n=46) already had a RA diagnosis according to ACR classification criteria of 1987 (*Arnett FC et al, 1988*). We only selected patients without psychiatric comorbidity (no use of substances active on the CNS was allowed), who also had never assumed biotechnological drugs (*biologics-naïve* patients), such as rituximab, anti-TNFα inhibitors, abatacept, tocilizumab, and any other experimental drug, in order to eliminate a possible confounder; only *disease-*

modifying antirheumatic drugs (DMARDs), i.e. steroids -e.g. 6-metylprednisolone (6MP)-, leflunomide, methotrexate, hydroxyclorochine and few others, were allowed. As established by the clinical practice, a rheumatologic visit, which included the joint assessment and calculation of the *Disease Activity Score* on 28 joints (DAS28), was performed. In order to calculate the DAS28 score, patients were also asked to refer their *general health status* (GH, on a 0-100 visual analogical scale, were 100 is the worse ever experienced health status).

- **Health control (HC) group:** 40 healthy volunteers were recruited among the people working at the Rheumatology Division, excluding everybody who was taking drugs acting on the CNS, and who had any disease diagnosed.

Blood parameters

All patients and controls were subjected to blood sampling to evaluate serum BDNF levels. In each group the following blood tests were also performed, according to the good clinical practice:

- **FM (*Study A*) and CFS groups:**
    1. cytokines (IL2, IL6, IL8, IL10, TNFα and INFγ)
    2. cortisol
    3. growth hormone (GH)
    4. serotonin (5HT)
    5. adrenocorticotropic hormone (ACTH)
- **FM (*Study B*) group:**
    - BDNF plasmatic levels
- **RA group:**
    1. erythrosedimentation rate (ESR)
    2. C-reactive protein (CRP)
    3. rheumatoid factor (RF) - IgM, IgA and IgG isotypes
    4. anti-citrullinated peptide antibodies (ACPA)

Study B overview

Title: "Serum and plasmatic BDNF levels in patients affected by Fibromyalgia in treatment with balneo- or balneo-fangotherapy".

A subgroup of 28 patients included in Study A was at the same time recruited to take part in the *Study B*. This second study involved the patients' randomization into balneo- (n=14) or balneo-fangotherapy (n=14), both performed at Montecatini Thermal Baths, in order to compare the two different treatments.

After standard rheumatologic visit and psychiatric interview (as previously described), patients were subjected to T0 (baseline) blood withdrawal and they were asked to complete FIQ, FACIT, HAQ, SF36 questionnaires. After a cycle of 10 daily treatments (lasted two weeks totally) at the thermal baths, patients were asked again to perform a second visit (T1), which included blood withdrawal and questionnaires. Finally patients came back after 3 months from the previous visit, repeating the same visit procedures (T2).

BDNF levels measurement

Blood samples were obtained by venipuncture of participants fasted, at 9.00 am. As for serum samples, 9 ml of blood were collected in a test tube without anticoagulant, and centrifuged at 2000 x g for 10 minutes. Concerning plasma, 12 ml of the blood were collected in tubes with EDTA anticoagulant, and then centrifuged at 200 x g for 20 minutes to obtain platelet-rich plasma. This was then centrifuged at 2000 x g for 15 minutes to separate platelets and thus obtaining platelet-poor plasma.

For quantitative assays of BDNF in serum and plasma the kit BDNF Emax® Immunoassay Systems (Promega) was used. This commercial kit is based on the Enzyme-Linked Immunosorbent Assay (ELISA) method. Tests were performed according to the manufacturer's instructions. A 96-multiwell polystyrene plate (Nunc Maxisorp) was coated with 100 µl of monoclonal anti-human BDNF antibody at a dilution of 1:1000. After overnight incubation at 4° C and washing of the plate with Tris buffer pH 7.6, supplemented with 0.05% Tween-20 (TBS-Tween), we proceeded by blocking the wells with 200 µl of the Block and Sample 1x buffer. After 1 hour incubation at room temperature and subsequent washing, 100 µl of serum (diluted 1:75 with Block and Sample 1x) or plasma (pre-treated according to manufacturer's instructions[1], in order to break the bond of BDNF with plasmatic proteins and then increasing the concentration of free circulating BDNF, and finally diluted 1:2 with Block and Sample 1x buffer) samples were incubated together with standard BDNF subjected to serial dilutions, realizing a 0-500 pg/ml standard curve. After 2 hours incubation

[1] Plasma samples were diluted 1:4 in DPBS and then treated with HCl 6N (to a pH of 3.0). After 15 minutes of incubation samples were neutralized with NaOH 3N (to a pH of 7.0-8.0).

under agitation (400 rpm) and after 5 washes with TBS-Tween buffer, we proceeded with the incubation of 100 µl primary polyclonal anti-human BDNF antibody, diluted 1:50 with Block and Sample 1x buffer. The plate was therefore left to incubate for 2 hours, then the sequence of washing was repeated and 100 µl of anti-IgG secondary antibody horseradish peroxidase-conjugated (HRP), diluted 1:20, were added to each well. After one hour incubation and subsequent washing, the substrate 3.3', 5.5'-tetramethylbenzidine (TMB) was added. After 10 minutes incubation under stirring and in the absence of light, the development of blue color was observed. The enzymatic reaction was thus immediately quenched by addition of 100 µl of HCl 1N. Finally, we proceeded with the spectrophotometric reading at 450 nm, and then to the calculation of the of the sample concentrations (ng/ml), by means of the standard curve.

Measurement of RF IgM, IgG and IgA levels

Blood samples were obtained by venipuncture of RA patients fasted, at 9.00 am. Nine ml of blood were collected in a test tube without anticoagulant, and centrifuged at 2000 x g for 10 minutes to obtain serum. RF levels of IgM, IgG and IgA isotypes were measured by Aeskulisa Rf-AGM® (Aesku Diagnostics). This commercial kit is based on a sandwich ELISA technique. Tests were performed according to manufacturer's instructions. Briefly, a 96-well microplate, precoated with a patented specific antigen, were incubated for 30 minutes with RA patients serum samples diluted 1:101 with the provided buffer, together with standards (range 0-300 U/ml) and two controls (positive and negative). After 3 washes with the wash buffer provided by the kit, a ready-to-use solution consisting of a secondary antibody anti-IgM, IgG or IgA, conjugated with HRP, was added. After 30 minutes of incubation and 3 washes, TMB was added and, after 30 minutes, the reaction was stopped with an acid solution provided by the kit. The adsorbance was read at 450 nm and the concentrations (U/ml) of samples calculated by means of the standard curve.

CNS Vital Signs©

CNS Vital Signs© is a computerized neurocognitive health assessment platform that enables the objective evaluation of cognition, by providing clinicians a valid, reliable, and affordable "research quality" tool. Indeed it helps to accurately measure and characterize a patient's neurocognitive function based on his or her status (age, level of education, and skills in the use of computer) or effort. CNS Vital Signs© evaluates the neurocognitive abilities, as described in Table 1. The software calculates a score for each neurocognitive domain, and a general neurocognitive index (NCI). These scores are auto-scored using an algorithm based

on a normative data set of 1900+ subjects, ranging from ages 8 – 90, that represents the "average" score (*Gualtieri CT and Johnson LG 2006*). In order to compare our patients to healthy subjects living in more similar socio-environmental conditions than the ones provided by the software owners, also 20 healthy subjects performed CNS Vital Signs©. Since results from these subjects overlapped the average scores provided by the software, the statistical analysis was realized only taking into account the standard scores and ranges calculated by the software itself for FMS or CFS patients.

The test battery lasts approximately 30 minutes. The exercises included in the software are:

Verbal memory (VBM). Fifteen words are presented, one by one, on the screen every two seconds. For immediate recognition, the participant has to identify those words nested among fifteen new words. Then, after six more tests, there is a delayed recognition trial. This test measures verbal learning, memory for words, word recognition and immediate and delayed recall.

Visual memory (VIM). This test is analogue to the previous, except for the fact that geometric figures are presented. This test measures visual learning, memory for geometric shapes, geometric shapes recognition, immediate and delayed recall.

Finger tapping test (FTT). This test requires athletes to press the Space Bar with their right index finger as many times as they can in 10 seconds. They do this once for practice, and then there are three test trials. The test is repeated with the left hand. This test measures motor speed and fine motor control.

Symbol Digit Coding (SDC). This test consists of serial presentations of screens, each of which contains a bank of eight symbols above and eight empty boxes below. The participant types in the number that corresponds to the symbol that is highlighted. With this test information processing speed, complex attention, visual-perceptual speed and information processing speed are evaluated.

Stroop test (ST). It consists of three parts. In the first part, the words RED, YELLOW, BLUE, and GREEN (printed in black) appear at random on the screen, and the participant presses the space bar as soon as the athlete sees the word. In the second part, the words RED, YELLOW, BLUE, and GREEN appear on the screen, printed in color. The participant is asked to press the space bar when the color of the word matches what the word says. In the third part, the words RED, YELLOW, BLUE, and GREEN appear on the screen, printed in color. The participant is asked to *press the space* bar when the color of the word does not match what the word says. With this test it is possible to assess executive function, simple and

complex reaction time, speed-accuracy trade-off, information processing speed and inhibition/disinhibition.

Shifting Attention (SAT) test. It is a measure of ability to shift from one instruction set to another quickly and accurately. Participants are instructed to match geometric objects either by shape or by color. Three figures appear on the screen, one on top and two on the bottom. The top figure is either a square or a circle. The bottom figures are a square and a circle. The figures are either red or blue (mixed randomly). The participant is asked to match one of the bottom figures to the top figure. The rules change at random (i.e., match the figures by shape, for another, by color). This test evaluates executive function (shifting sets), reaction time, information processing speed, and speed-accuracy trade-off.

Continuous Performance (CPT). It is a measure of vigilance or sustained attention or attention over time. The athlete is asked to respond to the target stimulus "B" but not to any other letter. The stimuli are presented at random. With this test sustained attention, choice reaction time, and impulsivity are measured.

CLINICAL DOMAINS	CLINICAL DOMAIN DESCRIPTION
Neurocognitive Index (NCI)	*Measure:* An average score derived from the domain scores or a general assessment of the overall neurocognitive status of the patient. *Relevance:* Summary views tend to be most informative when evaluating a population, a condition category, and outcomes.
Composite Memory	*Measure:* How well subject can recognize, remember, and retrieve words and geometric figures. *Relevance:* Remembering a scheduled test, recalling an appointment, taking medications, and attending class.
Verbal Memory	*Measure:* How well subject can recognize, remember, and retrieve words. *Relevance:* Remembering a scheduled test, recalling an appointment, taking medications, and attending class.
Visual Memory	*Measure:* How well subject can recognize, remember and retrieve geometric figures. *Relevance:* Remembering graphic instructions, navigating, operating machines, recalling images, and/or remember a calendar of events.
Processing Speed	*Measure:* How well a subject recognizes and processes information i.e., perceiving, attending/responding to incoming information, motor speed, fine motor coordination, and visual-perceptual ability. *Relevance:* Ability to recognize and respond/react i.e., fitness-to-drive, occupation issues, possible danger/risk signs or issues with accuracy and detail.
Executive Function	*Measure:* How well a subject recognizes rules, categories, and manages or navigates rapid decision making. *Relevance:* Ability to sequence tasks and manage multiple tasks simultaneously as well as tracking and responding to a set of instructions.
Psychomotor Speed	*Measure:* How well a subject perceives, attends, responds to visual-perceptual information, and performs motor speed and fine motor coordination. *Relevance:* Ability to perform simple motor skills and dexterity through cognitive functions i.e., use of precision instruments or tools, performing mental and physical coordination i.e., driving a car, playing a musical instrument.
Reaction Time	*Measure:* How quickly the subject can react, in milliseconds, to a simple and increasingly complex direction set. *Relevance:* Driving a car, attending to conversation, tracking and responding to a set of simple instructions, taking longer to decide what response to make.
Complex Attention	*Measure:* Ability to track and respond to information over lengthy periods of time and/or perform mental tasks requiring vigilance quickly and accurately. *Relevance:* Self-regulation and behavioral control.
Cognitive Flexibility	*Measure:* How well subject is able to adapt to rapidly changing and increasingly complex set of directions and/or to manipulate the information. *Relevance:* Reasoning, switching tasks, decision-making, impulse control, strategy formation, attending to conversation.

Table 1 CNS Vital Signs© domains and their descriptions.

Statistical analysis

The statistical analysis of differences between the two groups was performed using the two-tailed T-test. The correlations between two variables were determined by using linear regressions and Pearson correlations. Also a multivariate analysis was performed in order to adjust comparisons for independent variables (age, sex, illness duration and pain perception), that varied between the groups of patients. The comparison between dichotomous variables was performed by means of the Chi-squared test. In order to establish a coefficient of concordance between neurocognitive disorders complained and established by the CNS Vital Signs©, the contingency analysis was performed. Significance for the results was set at $p < 0.05$. All statistical analyses were carried out using the Graph Pad Prism 5.0 and SPSS 14.0 softwares.

Chapter 4

Results

1. BDNF levels in FM, CFS, RA patients and healthy volunteers

Demographic and clinical characteristics

Subject's demographic characteristics are summarized in Table 2. The 4 groups of participants were statistically different for age (p<0.0001) and sex (p<0.0001) but not for race. Particularly, CFS patients were younger compared to FM (p<0.0001) and RA (p<0.0001), RA patients were older than any other group (p<0.0001 for each comparison). Female sex was prevalent in FM group compared to healthy control (p<0.0001) and CFS group (p<0.0001), in agreement with the literature (*Neumann L et al, 2003*; *Jason LA et al, 1999*). Moreover, illness duration was found to be longer in FM patients compared to CFS (p=0.0024) and RA patients (p=0.014). This is probably due to: (i) CFS patients were younger, and (ii) all RA patients were biologics-naïve.

	FM	**CFS**	**RA**	**HC**	***p***
N	68	45	46	40	-
age (y); mean (SD)	48.4 (9.8)	37.5 (11.5)	60.2 (11.8)	40.7 (10.4)	<0.0001
gender (% women)	97.1	51.1	87.0	73.3	<0.0001
race/ethnicity	100% Caucasian	100% Caucasian	100% Caucasian	100% Caucasian	-
Illness duration (y); mean (SD)	10.3 (7.2)	6.1 (5.0)	6.3 (6.7)	-	0.0006

Table 2 Demographic and clinical characteristics (HC, *healthy controls*). *p* values here reported derived from ANOVA test.

There were not significant differences in therapies assumed by FM and CFS; similar percentages of non-steroidal anti-inflammatory drugs (NSAIDs), antidepressants (selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and tricyclic compounds), benzodiazepines, muscle-relaxants, anticonvulsants at low doses for neuropathic

pain, opioids and steroids were found between the two groups (see also paragraph 3 of this chapter).

Clearly, RA patients were assuming different therapies; in particular the 43.2% of patients was in treatment with low doses of 6-metylprednisolone (6MP), the 24.3% with intramuscular or oral methotrexate, the 18.9% with hydroxycloroquine, the 8.1% with NSAIDs, and the 2.7% with leflunomide.

From a psychiatric point of view, only FM and CFS patients were subjected to psychiatric interview. However, since no RA patient was taking psychiatric drugs and any psychiatric consultation has never been asked for these patients, we can affirm that in this group of patients psychiatric comorbidity was negligible.

The only significant difference found between FM and CFS patients was the prevalence of lifetime bipolar disorder of type II (BD II) (p=0.005). However the difference did not remain significant considering the current disorder. Prevalence of axis-I psychiatric comorbidities in the two groups of patients are summarized in Table 3 (see also paragraph 3 in this chapter).

	LT			**current**		
	FM	**CFS**	***p***	**FM**	**CFS**	***p***
N	68	45		68	45	
GAD/PD %	33.82	20.00	0,167	5.88	6.67	0,819
MDD %	14.71	8.89	0,531	2.94	2.22	0,715
BD II %	**1.47**	**17.78**	**0,005**	0.00	2.22	0,835
ED %	1.47	4.44	0,715	0.00	0.00	-
OCD %	1.47	0.00	0,835	1.47	0.00	0,835

Table 3 Prevalence of axis-I psychiatric comorbidities, assessed by SCID-I (DSM-IV). (GAD, *generalized anxiety disorder*; PD, *panic disorder*; MDD, *major depressive disorder*; BD II, *bipolar disorder type II*; ED, *eating disorders*; OCD, *obsessive-compulsive disorder*).

Comparison between BDNF levels of FM, CFS and RA versus HC

By means of univariate analysis, serum BDNF levels (concentrations shown in Table 4) were found statistically different among the 4 groups (p=0.0009), in particular they were higher in FM compared to healthy controls (HC) (p=0.012), and in CFS patients compared to healthy

controls (p<0.0001). No statistically significant differences were found between FM and CFS, and between RA and HC (see Figure 7).

	FM	**CFS**	**RA**	**HC**
Number of values	68	45	46	40
Mean	**9,522**	**9,912**	**9,106**	**7,622**
Std. Deviation	**2,149**	**2,532**	**2,519**	**2,208**
Std. Error	0,2730	0,3774	0,4140	0,3536
Minimum	4,750	2,840	4,910	2,560
25% Percentile	8,278	8,830	7,240	6,220
Median	9,510	10,55	8,790	8,200
75% Percentile	11,11	11,53	10,18	9,410
Maximum	13,78	14,28	14,87	10,89
Lower 95% CI of mean	**8,976**	**9,151**	**8,266**	**6,907**
Upper 95% CI of mean	**10,07**	**10,67**	**9,945**	**8,338**

Table 4 Column statistics of serum BDNF levels (ng/ml)

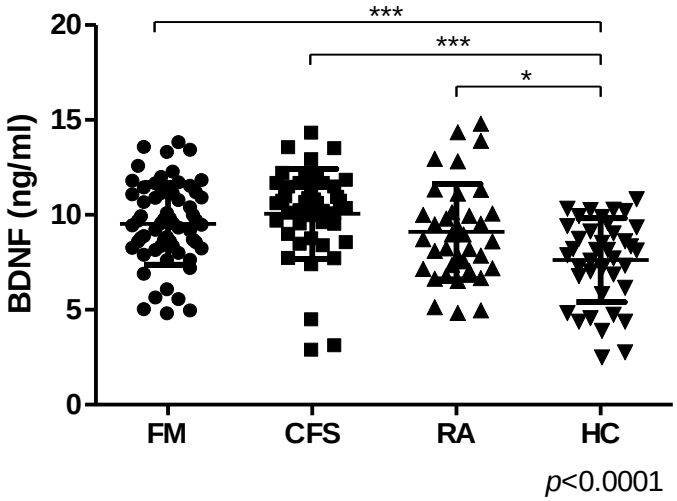

Figure 7 Scatter plot of serum BDNF levels (one-way ANOVA test and Bonferroni's post hoc test; ***, p<0.0001; *, p<0.05).

Since we found that age and illness duration were different between FM and healthy controls group, a multivariate analysis was also performed. The *p* adjusted for age and sex still remained significant (p=0.011).

No statistically significant difference in BDNF levels was found between patients (FM and CFS considered together) with a lifetime psychiatric disorder versus patients with a still active (current) disorder, and also versus patients who had never suffered from a psychiatric disorder (data not shown).

By examining CFS from a clinical point of view, we could differentiate between CFS with and without overlapping FM (CFS patients who also meet FM criteria had TP number ≥ 11, and VAS pain score ≥ 5). CFS/FM patients had higher BDNF levels compared to CFS patients alone, although the difference was not statistically significant. With ANOVA analysis, CFS/FM had higher levels of BDNF compared to healthy controls (p<0.0001), and CFS difference compared to healthy controls was, however, confirmed (p=0.006), as shown in Figure 8.

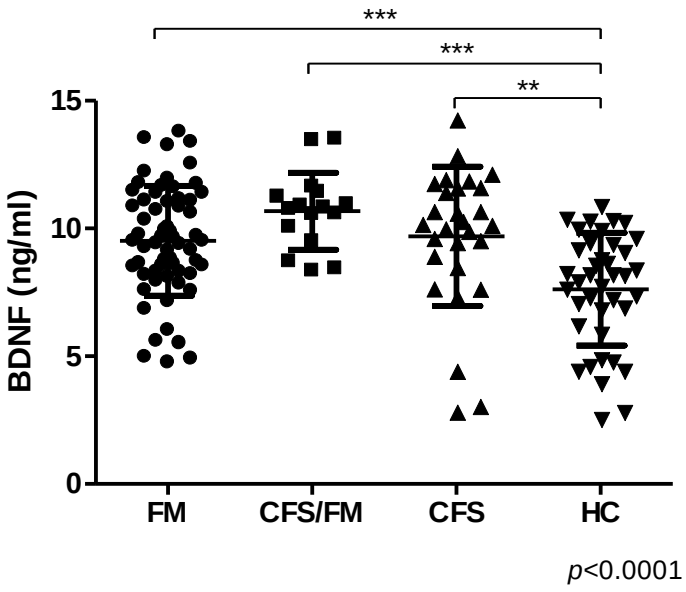

Figure 8 Scatter plot of serum BDNF levels (one-way ANOVA test and Bonferroni's *post hoc* test): CFS divided into CFS in overlap with FM (CFS/FM) and *pure* CFS.

Correlations of BDNF levels with clinical parameters

In Table 5 questionnaire scores and TP count for FM and CFS patients are summarized.
The relation between serum BDNF and clinical parameters -such as pain, fatigue, anxiety, depression, health and physical and mental status- in FM and CFS patients was examined by using the TP number and index[2] (for pain) and scores obtained by the questionnaires completed by all the patients. These questionnaires were the FIQ (for pain, fatigue, anxiety and depression), the FACIT for fatigue, the HAQ for the health assessment, the SAS for anxiety, the SDS for depression, and the SF36 for physical and mental status. The only and more relevant difference highlighted between FM and CFS patients was related to pain; TP number, TPi, and VAS pain were indeed the variables that differed the most in the two cohorts of patients (p<0.0001 for each item), with higher scores for FM compared to CFS.

	FM	**CFS**	***p***
N	68	45	-
TP number	**16.12 (2.89)**	**8.07 (7.41)**	**<0.0001**
TPi	**7.10 (1.56)**	**2.86 (2.71)**	**<0.0001**
FIQ	**63.59 (14.68)**	**55.54 (20.38)**	**0.018**
FACIT	27.38 (9.37)	30.48 (9.97)	0.1
VAS pain	**7.15 (2.27)**	**3.54 (3.01)**	**<0.0001**
VAS fatigue	8.03 (2.29)	7.74 (2.03)	0.5
VAS anxiety	**6.11 (2.42)**	**5.03 (2.56)**	**0.026**
VAS depression	**4.97 (2.78)**	**3.81 (3.33)**	**0.05**
SAS	44.63 (9.44)	41.64 (8.16)	0.134
SDS	46.51 (8.16)	45.13 (8.44)	0.464
HAQ	**0.76 (0.44)**	**0.49 (0.43)**	**0.012**
SF36 physical status index	30.32 (6.89)	32.26 (7.26)	0.321
SF36 mental status index	36.49 (11.87)	36.74 (11.59)	0.936

Table 5 Clinical characteristics, questionnaire scores and visual analogue scales – mean (SD).

[2] TP index was calculated by dividing the TP total score (sum of the 0-10 scores reported by the patient for each TP) for the number of positive TPs.

Also FIQ, VAS anxiety, VAS depression and HAQ were slightly higher in FM group than in CFS, probably meaning that FM patients were generally more compromised by the disease in from a mental (mood) and functional point of view. However no correlation was found between BDNF and each of the above mentioned questionnaires.

In RA patients, BDNF levels did not correlate with the severity of disease, calculated with DAS28 (data not shown).

Correlations of BDNF levels with routine blood tests

The determined blood parameters (cytokines IL-2, IL-6, IL-8, IL-10 and TNFα; cortisol; GH; 5HT; ACTH) were not found to correlate with BDNF levels, both in FM and CFS patients (taken together or separately). Only INF-γ positively correlated with BDNF levels when patients were considered together (r=0.34; *p*=0.01).

Examining RA group, 90% of patients were RF positive (RF+). In particular, we found that patients with positive RF IgM and IgG isotypes had higher BDNF levels compared to RF- patients (*p*=0.03 for IgM and *p*=0.01 for IgG). RA patients with RF+ of all isotypes also had higher BDNF levels compared to healthy controls (*p*=0.0051 for IgA, *p*=0.0021 for IgG and *p*=0.0042 for IgM) (data not shown). Moreover RF IgM and RF IgG (but not IgA) were found to positively correlate with BDNF levels (r=0.42, *p*=0.0094 and r=0.42, *p*=0.0097 respectively), as shown in Figure 9.

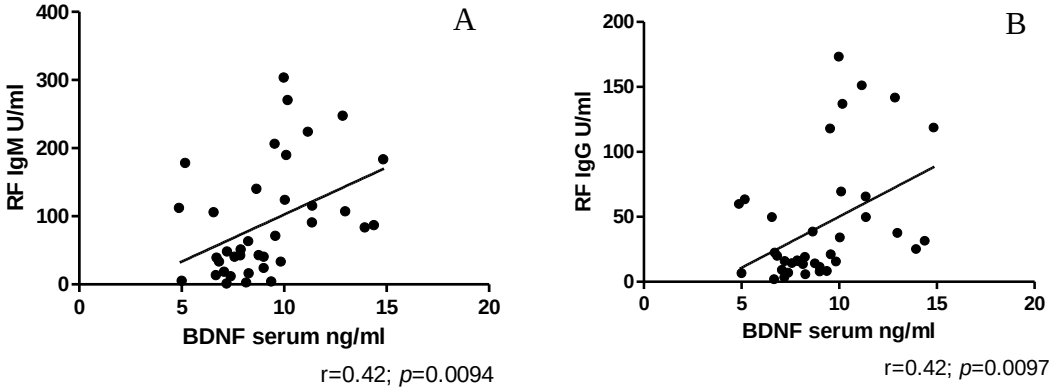

Figure 9 Linear regression of serum BDNF levels and IgM (A) or IgG (B) RF levels (U/ml).

Furthermore, CRP was found to negatively correlate with BDNF concentrations (r=-0.33; p=0.04), RF+ patients had lower CRP levels (p=0.04), and patients taking 6MP had lower BDNF (p=0.002) but higher CRP levels (p<0.0001). Finally, 37.5% of RA FR+ patients was taking 6MP, versus 53.9% of RA RF- patients, although the difference did not reach the statistical significativity. No other difference was found between RA RF+ and RA RF- patients in terms of drug use, disease severity and duration (data not shown).

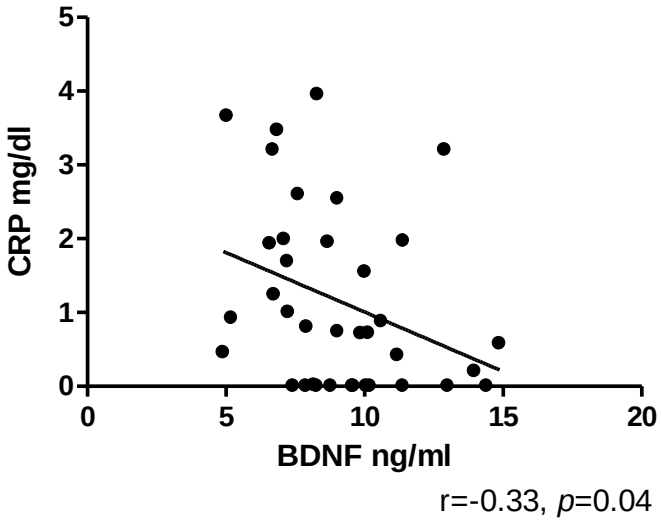

Figure 10 Linear regression of serum BDNF levels (ng/ml) and CRP (mg/dl)

2. BDNF levels in FM patients before and after thermal baths (*Study B*)

Clinical characteristics of patients who participated to thermal treatments are summarized in Table 5. Fourteen out of 28 patients were randomized to balneo-therapy while the other 14 to balneofango-therapy. At the baseline the two groups of patients showed similar demographic and clinical characteristics (data not shown).

Serum BDNF levels -measured at baseline (T0), after the treatments (T1) and 3 months after the end of the treatments (T2)- were found to significantly decrease at T2, as shown in Figure 11. The same trend was not observe for plasma samples.

No difference was observed at the baseline between patients who did balneo- and balneofango-therapy. Moreover, also in this subgroup of patients, at each visit, BDNF levels

did not correlate with illness duration, pain perception, or with other clinical scores (data not shown). However, at T1 VAS pain and FIQ were decreased compared to T0, although the difference did not reach the statistical significance (p=0.06 and p=0.08 respectively).

	FM
N	28
age (y)	50.96 (8.19)
sex	96.4%
race	% Caucasian
TP number	16.25 (3.30)
Tpi	7.54 (1.65)
FIQ	64.09 (17.09)
FACIT	27.89 (9.74)
VAS pain	7.11 (2.31)
VAS fatigue	8.18 (2.11)
VAS anxiety	6.21 (2.92)
VAS depression	5.58 (2.98)
HAQ;	0.75 (0.45)
SF36 physical status index	30.66 (14.42)
SF36 mental status index	66.87 (23.07)

Table 6 Clinical characteristics and questionnaire scores –mean (SD)- of patients who participated to *Study B.*

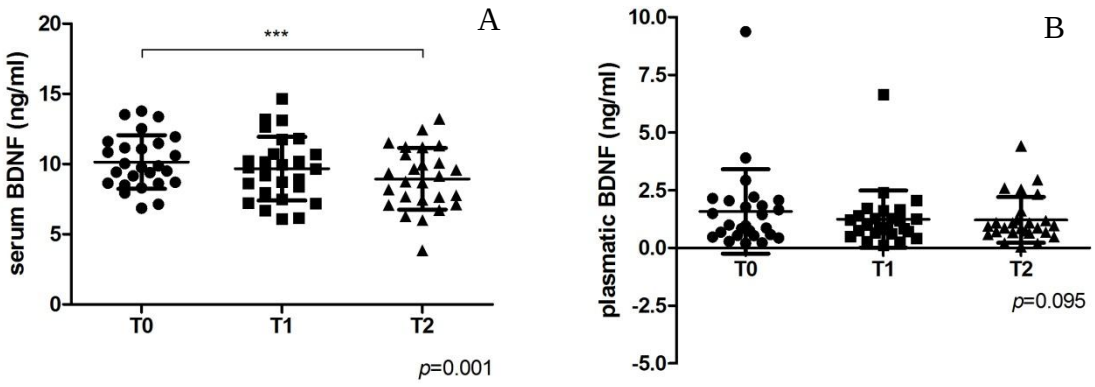

Figure 11 A) Serum BDNF levels (ng/ml) at T0, T1 and T2 of thermal treatment. B) Plasmatic BDNF levels (ng/ml) (ANOVA analysis for repeated measures).

	SERUM			PLASMA		
	T0	**T1**	**T2**	**T0**	**T1**	**T2**
Mean	**10,15**	**9,67**	**8,94**	**1,59**	**1,25**	**1,22**
Std. Deviation	**1,90**	**2,27**	**2,19**	**1,83**	**1,24**	**0,99**
Std. Error	0,37	0,44	0,43	0,36	0,24	0,19
Minimum	6,86	6,09	3,86	0,21	0,13	0,06
25% Percentile	8,64	7,86	7,35	0,54	0,65	0,67
Median	9,82	9,72	8,95	0,98	0,94	0,91
75% Percentile	11,50	11,00	10,80	2,05	1,46	1,33
Maximum	13,78	14,67	13,23	9,37	6,66	4,43
Lower 95% CI of mean	**9,38**	**8,76**	**8,06**	**0,85**	**0,75**	**0,82**
Upper 95% CI of mean	**10,92**	**10,59**	**9,83**	**2,33**	**1,75**	**1,62**

Table 7 Column statistics of serum and plasma BDNF levels (ng/ml) at T0, T1 and T2 of thermal treatment.

3. Neurocognitive disorders in FM and CFS patients

No correlation was found between BDNF serum levels and neurocognitive disorders, neither complained, nor assessed by means of CNS Vital Signs© software. However, interesting results came from the analysis of CNS standard scores and the clinical parameters examined in the FM and CFS patients, as well as from the comparison between the two groups in terms of *complained* and *real* impairments.

Demographic data

Subject demographic characteristics of patients recruited for neurocognitive observations are summarized in Table 7. FM and CFS were significantly different in terms of age and sex (p=0,001 and p=0,0001 respectively), in accordance with the literature (*Neumann L et al, 2003*; *Jason LA et al, 1999*). No difference was found in CFS patients between women and men.

	FM	**CFS**	***p***
N	40	45	-
gender	**97.5% women**	**46.7% women**	**0.0001**
***age (y)*; mean (SD)**	**46.6 (10,5)**	**37.5 (11.5)**	**0.001**
race/ethnicity	100% Caucasian	100% Caucasian	-
education (y); mean (SD)	12.7 (4.2)	14.2 (3.1)	0.062
employment status (%)			
managerial/office	25.0	35.6	0,413
skilled labor	12.5	15.6	0,927
semiskilled labor	10.0	11.1	0,852
students	2.5	15.6	0,092
homemaker	10.0	0.0	0,097
retired	**22.5**	**4.4**	**0,031**
unemployed	17.5	17.8	0,801

Table 8 Demographic characteristics

FM and CFS patients did not differ by race, years of education and employment status (except for the prevalence of retired people in FM group, that depended of the higher mean age). As shown in table 2, more almost 18% of both FM and CFS patients was unemployed and referred to have left work because of the disease.

Clinical characterization

Clinical characteristics, questionnaire scores and scales are shown in **Table 9**.
FM patients had also a longer duration of illness compared to CFS patients (p=0.004).

	FM	**CFS**	***p***
***illness duration (y);* mean (SD)**	**9.8 (6,8)**	**6.4 (5.1)**	**0.004**
Tender Points; mean (SD)			
N	**16.2 (2.5)**	**8.1 (7.4)**	**<0.0001**
TPi	**6.8 (1.5)**	**2.9 (2.7)**	**<0.0001**
FIQ; mean (SD)	62.8 (13.3)	55.5 (20.4)	0.064
FACIT; mean (SD)	27.6 (8.6)	30.5 (10.0)	0.167
***VAS pain;* mean (SD)**	**7.1 (2.3)**	**3.5 (3.0)**	**<0.0001**
VAS fatigue; mean (SD)	8.0 (2.3)	7.6 (2.1)	0.474
VAS anxiety	5.9 (2.0)	5.0 (2.5)	0.102
VAS depression	4.3 (2.4)	3.8 (3.2)	0.461
SAS; mean (SD)	44.8 (9.5)	41.4 (8.3)	0.110
SDS; mean (SD)	46.5 (8.3)	44.4 (8.2)	0.465
cognitive impairments complained (%)	85.0	75.6	0.842
concentration yes/no (%)	80.0	73.3	0.921
memory yes/no (%)	57.5	55.6	0.932
Sleep disorders complained			
sleep disorder yes/no (%)	85.0	71.1	0.704
VAS restful sleep; mean (SD)	7.7 (2.5)	7.2 (2.2)	0.368

Table 9 Clinical characteristics, questionnaire scores and visual analogue scales.

In agreement with the classification criteria, FM patients showed a higher number of TP and a higher TP index (p<0.0001). This difference is also supported by the difference found between patients' VAS pain (p<0.0001). No difference between the two groups was instead identified with respect to fatigue, neither by means of FACIT questionnaire, nor with VAS fatigue. No statistically significant difference was also found for auto-referred feelings of anxiety and depression, which were both assessed with visual scales and questionnaires (SAS and SDS), as shown in Table 9.

Concerning complained cognitive disorders, any difference was found in terms of concentration or/and attention between the two groups of patients. Also sleep disorders, referred by "yes" or "not" and through a VAS "rested/tired" upon awaking, were not

statistically different between FM and CFS patients. No difference was found within CFS group between male and female sex.
Thus, the only variables differing between the two groups were age, sex (prevalence of women), duration of illness and pain perception (VAS pain and TP count).

Pharmacological therapies

Analyzing drug assumption, no statistically significant differences were found between FM and CFS patients, as shown in Table 10. Thus, differences of neurocognitive function between the two groups were independent of drug intake.

Drug	**FM %**	**CFS %**	***p***
NSAIDs	15,0	8,9	0,592
Benzodiazepines	22,5	13,3	0,411
SSRI	20,0	15,6	0,801
SNRI	7,5	11,1	0,844
tricyclic antidepressants	2,5	4,4	0,917
Opioids	10,0	0,0	0,097
Steroids	0,0	4,4	0,527
muscle- relaxants	22,5	11,1	0,263
Anticonvulsants	22,5	11,1	0,263
*antidepressants**	30,0	28,9	0,9
*drugs***	57,5	44,4	0,325

Table 10 Comparison of drug assumption between FM and CFS patients (NSAIDs, *non steroidal anti-inflammatory drugs*; SSRI, *selective serotonin reuptake inhibitors*; SNRI, *serotonin noradrenalin reuptake inhibitors*). * SSRI + SNRI + tricyclic antidepressants; ** patients who assumed at least one drug.

Psychiatric comorbidity

No difference was observed between FM and CFS patients in terms of axis-I psychiatric disorders, assessed by means of SCID-I (Table 11). The prevalent psychiatric condition in both groups was a lifetime (LT) disorder characterized by generalized anxiety (GAD) and panic attacks (PD), which was present in the 37.50% and in the 20.00% of FM and CFS respectively. The same condition persisted only in 1 out of 15 patients (11.10%) and in 1 out of 3 patients (6.67%) in the FM and CFS group respectively (current psychiatric disorder). Bipolar type-II disorder (BD II) was instead observed in a larger number of CFS patients

(17.78%) as a lifetime condition compared to FM patients (2.50%), although the difference was not statistically significant (p=0.086). Only one CFS patient and none of the FM patients, still had a BD II disorder at the observation time. Small percentages of patients from both groups resulted to have psychiatric LT or current disorders such as the major depressive disorder (MDD; 10.00% LT and 5.56% current in FM versus 8.89% and 2.22% in CFS), LT eating disorders such as anorexia and bulimia (ED; 2.50% in FM and 4.44% in CFS), and current obsessive-compulsive disorder (OCD; present only in 1 FM patients, 2.50%). Thank to these observations we can suppose that, at least in our cohort of patients, neurocognitive functioning is independent of psychiatric comorbidity.

	LT (%)			**current (%)**		
	FMS	**CFS**	***p***	**FMS**	**CFS**	***p***
GAD/PD	37.50	20.00	0,12	11,11	6.67	0,817
MDD	10.00	8.89	0,79	5.56	2.22	0,917
BD II	2.50	17.78	0,053	0.00	2.22	0,953
ED	2.5	4.44	0,92	0.00	0.00	-
OCD	2.5	0.00	0,95	2.5	0.00	0,953

Table 11 Lifetime (LT) and current psychiatric comorbidity assessed by SCID-I (DSM-IV)

Neurocognitive functioning assessment

As previously shown, FM and CFS patients similarly complained about neurocognitive problems of concentration or/and attention. Nevertheless, through the use of CNS Vital Signs© test battery, FM patients resulted to have more compromised neurocognitive functions compared to CFS patients. Indeed the neurocognitive index (NCI) was lower in FM than in CFS patients (p=0.0032). The NCI of fibromyalgic patients was lower because also singular items such as visual memory (ViM; p=0.0039), processing speed (PrS; p=0.02), executive function (EF; p=0.001), psychomotor speed (PsS; p<0.0001) and cognitive flexibility (CF; p=0.0011) were lower in these patients compared to CFS. These results were obtained by means of univariate analysis (see Figure 12). Since FM and CFS patients differed in terms of age, sex, disease duration and pain perception (VAS pain and TPi), also multivariate analysis was performed in order to adjust results for all possible confounders. Mean (SD) scores from CNS Vital Signs©, p and adjusted p for age, sex, duration of illness and pain perception (VAS pain and TPi) are shown in Table 12. In CFS patients, no difference was found between female and male sex.

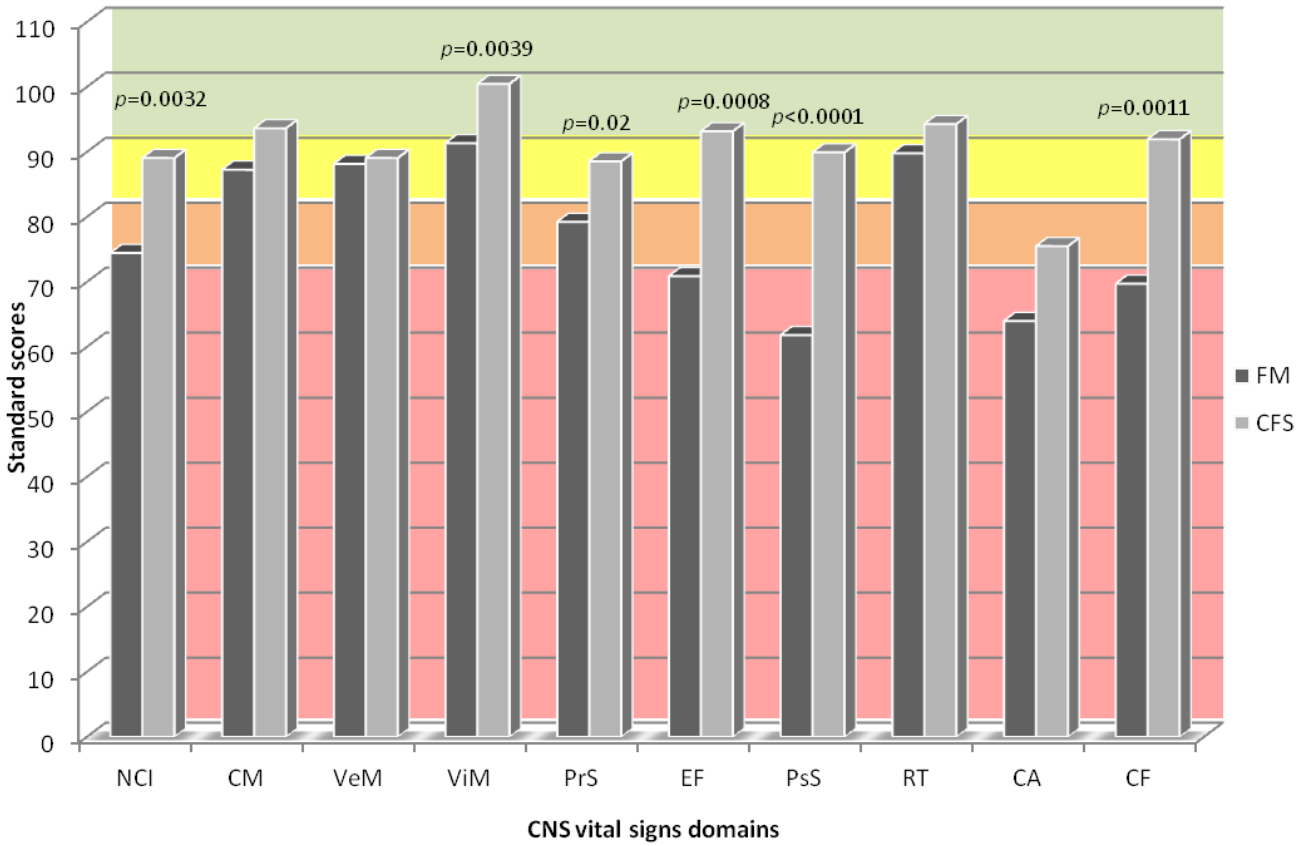

Figure 12 Comparison of CNS Vital Signs© mean standard scores between FM and CFS patients (standard scores ranges: >110=above; 110-90 (light-green)=average; 89-80 (yellow)=low average; 79-70 (orange)=low; <70 (red)=very low).

	FM	CFS	*p*	adjusted *p*
Neurocognitive index (NCI)	**74,48 (21,60)**	**89,09 (22,65)**	**0.0032**	**0.036**
Composite memory (CM)	87,30 (18,33)	93,67 (17,13)	ns	ns
Verbal memory (VeM)	88,18 (20,84)	89,09 (21,26)	ns	ns
Visual memory (ViM)	**91,38 (14,38)**	**100,5 (13,88)**	**0.0039**	**0.027**
Processing speed (PrS)	**79,28 (19,88)**	**88,58 (16,37)**	**0.02**	**0.047**
Executive function (EF)	**70,93 (33,62)**	**93,22 (25,20)**	**0.001**	**0.036**
Psychomotor speed (PsS)	**61,85 (34,69)**	**89,96 (22,75)**	**<0.0001**	**0.012**
Reaction time (RT)	89,83 (19,42)	94,31 (21,88)	ns	ns
Complex attention (CA)	64,03 (45,55)	75,53 (74,02)	ns	ns
Cognitive flexibility (CF)	**69,75 (34,10)**	**91,98 (26,27)**	**0.008**	**0.032**

Table 12 mean CNS vital signs scores (SD). (ns, *not significant*).

Neurocognitive function correlated with the duration of illness. In particular, duration of illness negatively correlated with NCI (r=-0.23; *p*=0.034), ViM (r=-0.28; *p*=0.0099) and PsS (r=-0.45; *p*<0.0001) (see Figure 13 A).

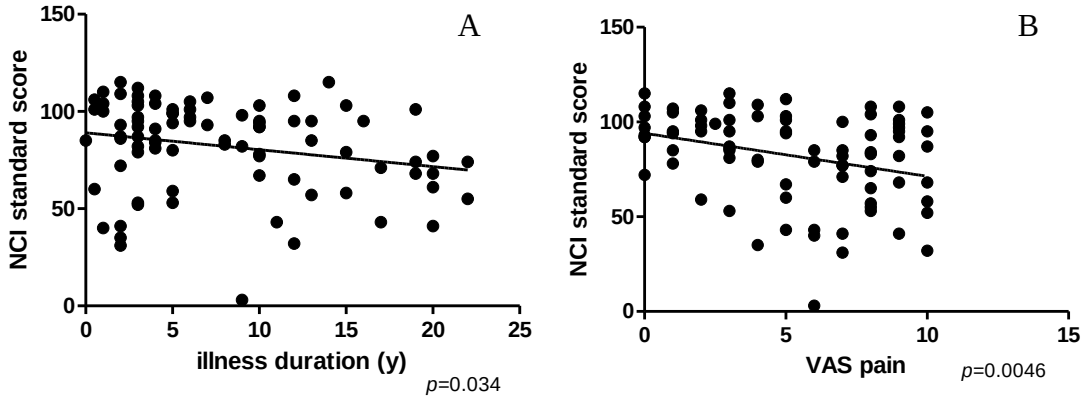

Figure 13 A) Negative correlation between illness duration and NCI. B) Negative correlation between VAS pain and NCI.

Moreover, considering the two groups together, also VAS pain was found to negatively correlate with NCI standard score (r=-0.31; *p*=0.0046) and with single items of the CNS vital signs, such as ViM (r=-0.30; *p*=0.0066), PrS (r=-0.29; *p*=0.008), EF (r=-0.28; *p*=0.01), PsS (r=-0.30; *p*=0.007), and CF (r=-0.30; *p*=0.007) (see Figure 13 B). On the contrary, VAS fatigue was not found to correlate with any item of the CNS Vital Signs©. However, when the two groups were considered separately, the significant negative correlation of VAS pain and neurocognitive disorders was lost, both in FM and in CFS groups. This finding is probably due to the fact that, within each group of patients, VAS pain scores did not spread on the entire scale, while when patients were taken together, we could observe a wider range of VAS pain scores. Furthermore, while in FM patients VAS pain correlated with VAS fatigue (r=0.47; *p*=0.003;), in CFS patients did not. NCI did not correlate with any clinical parameters, nevertheless other correlations were found in CFS group: executive function and cognitive flexibility negatively correlated with FIQ (r=-0.36; *p*=0.016 and r=-0.318; *p*=0.035, respectively) and FACIT (r=-0.30; *p*=0.048 and r=-0.374; *p*=0.011, respectively). In FMS group instead ViM negatively correlated with VAS pain (r=-0.475; *p*=0.008) and with FIQ (r=-0.374; *p*=0.042) (data not shown).

Neurocognitive standard scores were also analyzed by comparing both FM and CFS patients with a psychiatric disorder versus those who had not. In particular, we found statistically significant differences between patients who had or had not a lifetime psychiatric disorder in terms of NCI (mean ± SD: 75.0±25.8 vs 90.3±16.8; p=0.002), PrS (mean ± SD: 80.4±19.1 vs 88.5±17.3; p=0.043), EF (mean ± SD: 76.0±34.6 vs 90.2±25.7; p=0.036), PsS (mean ± SD: 68.2±35.8 vs 86.3±24.3; p=0.008), CF (mean ± SD: 74.1±35.4 vs 89.9±25.7; p=0.022).

The more impaired function in patients with a lifetime psychiatric disorder was psychomotor speed, whose mean standard score was well under the range of normality (*very low* score), while the other functions seemed to be less impaired. As for presence of a current psychiatric disorder, no difference was instead observed between patients who had and patients who had not.

Further analysis showed that neurocognitive function, measured with the NCI, also differ between patients assuming or not antidepressants (p=0.015), particularly in terms of complex attention (p=0.008), which seemed to decrease well under the normality range in patients taking antidepressants (mean ± SD: 44.07 ± 94.81 and 82.24 ± 33.58 respectively). Considering instead patients under one or more pharmacological treatments versus patients not treated at the observation time, the only difference highlighted was related to processing speed (PrS), which was slightly lower in patients assuming drugs (p=0.04).

Finally we calculated the k-coefficient of concordance between complained (*subjective*) and *objective* neurocognitive disorders in both group of patients. We found in both groups a lack of concordance between complained and real deficits, although in CFS the difference resulted more marked, as shown in Table 13.

	FM	**CFS**
NCD subjective %	85.0%	84.4%
NCD objective %	42.5%	22.2%
concordant	21/40	15/45
k-coefficient	0.0082	-0.009

Table 13 Analysis of *subjective* versus *objective* neurocognitive disorders (NCD).

Chapter 5

Discussion

One of the most interesting results highlighted by this PhD thesis project was the comparison of BDNF levels in FM versus CFS and RA patients, and healthy controls. The literature concerning BDNF and FM reported elevated levels of this neurotrophin in sera, plasma, and cerebrospinal fluid samples (*Laske et al., 2007*; *Haas L et al, 2010*; *Sarchielli P et al, 2007*). Thus, with the present study we confirmed the data, also adding a further information: also in CFS patients, who also had a overlapped FM, BDNF serum levels were increased compared to healthy volunteers. Unexpectedly, also CFS patients presenting only with persisted fatigue, therefore without a musculoskeletal pain complaint, had elevated BDNF levels.

Several questions spontaneously arise:

- Could be chronic stressors, to which both patients are subjected, the main cause explaining the reason why the CNS activate its neurotransmitters (especially those belonging to nociceptive and neuroplasticity pathways) to cope with the threat?
- Is BDNF-mediated neuroplasticity the biological defense weapon through which our system faces a chronic pain or, more generally, a chronic stressor?

These speculation, supported by several researchers and studies (*Popoli M et al, 2002*; *Urban MO and Gebhart GF, 1999*; *Giovengo SL et al, 1999*; *Pezet S et al, 2002*), are also confirmed by the result found in FM patients who participate to thermal therapies program, whose BDNF serum levels, together with VAS pain and FIQ score (although these last did not reach the statistical significance), significantly decreased after the end of the study. Of great importance is the fact that only serum -and not plasmatic- BDNF decreased after the cycle of thermal treatments; this finding seems to suggest that BDNF stored in platelets (that is the peripheral amount of BDNF), and not circulating BDNF (that is the one coming from the CNS) is somehow involved in FM and CFS pathogenesis, as well as in RA, even if in this case the role of autoimmune system needs further investigations.

Fibromyalgia and chronic fatigue syndromes are still often viewed as psychosomatic disorders. In the past years several authors (*Raphael KG et al, 2006*; *Epstein SA et al, 1999*; *Ercolani M et al, 1994*) has speculated that the psychiatric background of FM and CFS could be the main cause triggering for altered central perceptions, leading in this way to chronic

pain and fatigue without a clinically explainable reason. However, our results showed -and also confirmed previous results (*Laske C et al, 2007*)- that BDNF levels are independent of psychiatric comorbidity (assessed by SCID-I as axis-I disorders), psychiatric spectra (as highlighted by the lack of correlation between BDNF and SAS/SDS scales, and also with VAS pain and anxiety), and psychiatric drugs substances, in both syndromes.

Also data coming from the analysis of RA group are encouraging. RA represents indeed another model of chronic pain, but despite FM and CFS, less subjected to psychiatric disorders (*Walker EA et al, 1997*). Particularly RA patients recruited for this study were not using drugs acting on CNS and were never diagnosed for a psychiatric disorder. The finding that RA patients positive for RF IgA, IgG and IgM had higher serum levels of BDNF, and that BDNF concentrations positively correlated with RF IgG and IgM U/ml, suggest a possible involvement of a previous inflammation status to which the system was reacting increasing BDNF levels. Indeed, positivity to RF, that normally coincide with a worse severity of disease, autoimmune activation and inflammation, is here resulted linked to higher BDNF levels, and probably as a consequence of this, with a decrease of CRP and thus with a reduced use of 6MP, which is consistent with the literature (*Kerschensteiner M et al, 1999*). The use of 6MP certainly reduces the symptoms of inflammation and pain, but have no effect on autoimmune dysregulation. Probably BDNF peripheral release could be interpreted as a continuous attempt of the system to face and reduce pain and inflammation. These suppositions are also reported in other several studies (*Kerschensteiner M et al, 1999*; *Reinshagen M et al, 2000*; *Onda A et al, 2004*; *Murata Y et al, 2005*; *Grimsholm O et al, 2008*; *Del Porto F et al, 2006*), which found that plasma BDNF levels in RA patients were increased at the baseline compared to healthy control, but they get lower after anti-TNFα therapy. The strong point of the present study was to have excluded patients who were taking -or had taken- biologic drugs; this choice now allows us to reach more sustainable conclusions. Further investigations on larger cohort of RA patients are necessary to confirm our findings, and the evaluation of BDNF levels before and after the treatment with different biologic drugs will soon performed.

Another important aspect highlighted by the present research, even if it is not the only and neither the first to deal with the subject (*McCracken LM et al, 2001*; *Westoby CJ et al, 2009*; *Suhr JA, 2003*), is the one concerning the relation between chronic pain and neurocognitive impairments. The fact that CNS Vital Signs© standard scores did not correlate with BDNF levels, in any way and in any group of patients, lead us to consider them as independent

variables, or at least not directly influenced one by the other, although the following evidence seems to suggest this relationship: (i) BDNF high-affinity to trk-B receptors is known to induce long-term potentiation at hippocampal synapses, which are involved in learning and memory (*Kang H et al, 1995*), and (ii) in human, serum BDNF increased together with improvement in cognitive function after acute cycling exercise (*Ferris LT et al, 2007*). Thus, neurocognitive impairments in FM and, although to a less extent in CFS patients, are principally dependent of chronic pain – as resulted by the correlation of neurocognitive index and other CNS Vital Signs© domains with VAS pain and illness duration, as well as by a worse impairment in FM compared to CFS patients- and, as a consequence, of central sensitization, even though they seem not to be linked to BDNF-mediated neuroplasticity. Unlike other studies (*Roth RS et al, 2005*; *McCracken LM et al, 2001*; *Suhr JA, 2003*), we did not find any correlation between neurocognitive impairment and depression, nor fatigue, although some neurocognitive items –in particular psychomotor speed- were lower in patients with a lifetime psychiatric disorder compared to patients without. Moreover, concerning the frequently found prevalence of neurocognitive impairments in female sex - reported by previously mentioned authors - our data suggested instead that in FM and CFS patients sex did not influence neurocognition, since the sex-adjusted analysis still remained significant, and no difference was highlighted by comparing male and female patients within CFS group. However, unlike these studies, in the present one neurocognitive functioning was assessed by means of computerized system, more objective and accurate in evaluate every single ability.

Accumulating evidence now indicates that a number of chronic pain and stress-related disorders, including chronic low back pain, FM and CFS are characterized by gray matter reductions (*Apkarian AV et al, 2004*; *de Lange FP et* al, 2005; *Kuchinad A et al, 2007*). A possible explanation for the decreased gray matter density in these disorders might be atrophy secondary to excitotoxicity and/or exposure to inflammation-related agents, such as cytokines (*Apkarian et al., 2004*). Kuchinad and colleagues (2007) found that FMS patients have brain gray matter atrophy with a yearly decrease in gray matter volume more than three times than that of age-matched controls. Gray matter loss occurs mainly in regions related to stress (parahippocampal gyrus) and pain processing (cingulated, insular and prefrontal cortices). A recent study found that FM patients had significantly less gray matter volumes than healthy controls in pain-related brain areas but not gray matter changes associated with depression (*Robinson ME et al, 2011*). Structural changes in these systems could contribute to the maintenance of pain, while gray matter atrophy in areas such as parahippocampal and frontal

cortices appears consistent with cognitive deficits but also with affective disturbances reported in FM patients.

We have already shown in Chapter 4 (named *Results*, Figure 12) how evident is the neurocognitive function impairment in FM patients, also compared to CFS patients who had instead almost all the CNV Vital Signs© domains within the normal range. In particular, FM patients, aside from the NC index, which is only a composite index that globally indicate how compromised are cognitive faculties, reported **very low** standards score – here listed in order of severity- in psychomotor speed, complex attention (low also in CFS), cognitive flexibility, executive functioning, and processing speed (low).
An impaired psychomotor speed involves difficulties in performing simple motor skills i.e., use of precision instruments or tools, performing mental and physical coordination i.e., driving a car, playing a musical instrument. A deficit in complex attention make instead problematic the performance of mental tasks that require vigilance, quickly and accurately. Cognitive flexibility is interpreted as the ability to adapt to rapid changes and to increasingly complex set of directions. Thus, an impaired cognitive flexibility can cause difficulties in reasoning, switching tasks, decision-making, etc…, similar to difficulties caused by a deficit in executive function, which is the ability to sequence tasks and manage multiple tasks simultaneously, as well as tracking and responding to a set of instructions. It is not surprising that these two capabilities go hand in hand. Finally, processing speed, that is the ability to recognize and respond/react, when impaired it can causes occupational issues and difficulties in identifying possible danger/risk signs.

Of great interest are the negative correlations of peculiar NC domains (EF, CF and ViM) with clinical parameters which typically describe the severity of both FM and CFS (FIQ, FACIT and VAS scales). According to these results, CFS patients who had a more severe disease (higher FIQ and FACIT scores) also had more impaired executive function and cognitive flexibility, confirming the possibility of a dysfunction at prefrontal level, as reported by neuroimaging studies (*MacHale SM et al, 2000*). FM patients who had more severe disease (higher FIQ and VAS pain) reported instead poor performance in terms of visual memory, suggesting the possible involvement of brain areas such as medial temporal lobe and occipital cortex in the pathophysiology of the syndrome (*Khan ZU et al, 2011*).

Globally considering the reported findings, in FM patients attention and concentration generally seemed more compromised than memory. This finding could be due to chronic pain, as also observed by Glass (Glass JM, 2006). In particular complex attention was found reduced by the use of antidepressants (SSRI, SNRI and/or tricyclic compounds), as previously reported also by McCracken and colleagues (*McCracken LM et al, 2001*). Processing speed instead seemed to be more impaired in patients who were assuming at least one drug among those which are normally prescribed for the treatment of FM and CFS. Further investigations on the relationship between neurocognitive disorders and drugs commonly prescribed for the treatment of FM -and also on the link among the various affective disorders- could be useful to understand if the impairment of neurocognitive function could be at least partially ascribed to the use of some of these ones. However that fact that no difference was found in pharmacological therapies between FM and CFS patients let us suppose that the neurocognitive impairment in FM is not due to assumed drugs.

Interestingly, the analysis between *complained* attention and memory disorders and *objective* neurocognitive impairment showed that, while FM patients were more compromised but less frequent aware of their cognition problems, CFS patients usually complained without having a real deficit. A possible explanation of the last observation could be related to personality traits of CFS patients, such as self-criticism and perfectionism (*Kempke S et al, 2011*), which can lead to excessive worries about their performance (e.g., concern over mistakes and doubt about actions). Since difficult to demonstrate, the reason of discrepancy between subjective and objective neurocognitive disorders in CFS and FM will probably remain explained only by mere suppositions.

Chapter 6

Conclusions

The main findings of the present PhD thesis work have been: (i) the increased BDNF levels in sera of patients affected by FM, CFS and RA (but only those who were positive to rheumatoid factor), compared to healthy volunteers; (ii) the positive correlation between BDNF and rheumatoid factor of IgG and IgM isotypes, and the negative one with C-reactive protein; (iii) the lack of correlation between BDNF and neurocognitive disorders, assessed by the software CNS Vital Signs©; (iv) the more elevated prevalence of neurocognitive disorders in FM than in CFS patients, despite the more frequent complaint of the latter; (v) the tight relationship between neurocognitive impairments and chronic pain, which is independent of psychiatric comorbidity.

The BDNF-mediated neuroplasticity is somehow involved in the pathogenesis of FM and related condition, particularly in CFS, which shares with FM the symptoms of chronic pain. Moreover, both disease are known to be chronic stress conditions, being characterized by dramatic reduction of physical and mental energy, vitality, as well as neurocognitive performances, independent of the presence of psychiatric disorders. Thus it is likely that BDNF-mediated neuroplasticity in these syndromes is a protective mechanism. This hypothesis could explain the elevated BDNF levels found in sera -but not in plasma- samples, and their decrease after thermal treatment.

Even if BDNF it is not specific for FM or chronic pain, and the difference here reported between BDNF levels of FM or CFS patients compared to healthy controls are not strong enough to use BDNF in the diagnostic field, BDNF -as also proved by this work- has smoothed the way to new investigations on FM, CFS and RA etiopathogenesis on one hand, and could turn into being a useful biomarker for FM/chronic pain therapies monitoring on the other.

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