Variations in EEG and motor functions related to COMT gene in patients with fibromyalgia

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Doctor by the Universitat de les Illes Balears
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Para las pacientes

y mis dos amores (Marina y Charles)
El cerebro es más amplio que el cielo colócalos juntos contendrá uno al otro holgadamente y tú también. El cerebro es más hondo que el mar retenlos azul contra azul absorberá el uno al otro como la esponja al balde. El cerebro es el mismo peso de Dios pésalos libra por libra se diferenciarán si se pueden diferenciar como la sílaba del sonido.

Emily Elizabeth Dickinson - El cerebro
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Symbols, Acronyms and Abbreviations

6MWT  Six-minute walking test

val158met  polymorphism of the human catechol-O-methyltransferase (COMT) gene

ACR  American College of Rheumatology

APS  average pain sensitivity

CNS  central nervous system

COMT  catechol-O-methyltransferase enzyme

COP  center of pressure

dB  decibel

FIQ  Fibromyalgia Impact Questionnaire

FM  Fibromyalgia

GHRH  human growth hormone release

HAD  Hospital Anxiety and Depression Scale

HPS  High pain sensitivity

IAPS  International Affective Picture System
IASP  International Association for the Study of Pain Press
LPS   low pain sensitivity
met   methionine amino acid
OSQ   Oviedo Sleep Questionnaire
PSQI  Pittsburgh Sleep Quality Index
SAM   Self-Assessment Manikin
SF-36  Short Form Health Survey
SNPs  single nucleotide polymorphisms
SWS   Slow-wave sleep
TUG   Timed up and go task
val   vanilla amino acid
VR    Virtual Reality
WHYMPI  West Haven-Yale Multidimensional Pain Inventory
Resumen

La fibromialgia (FM) es un síndrome crónico caracterizado por dolor generalizado, fatiga, sueño no reparador, quejas somáticas y alteraciones afectivas y cognitivas. Aunque existe evidencia reciente indicando que las emociones negativas pueden desempeñar un papel modulatorio relevante para el mantenimiento de los síntomas de la FM, poco se conoce de la influencia de los polimorfismos genéticos sobre la función motora, el sueño y el procesamiento afectivo en fibromialgia. El objetivo principal de esta tesis fue analizar la influencia del polimorfismo val158met del gen de la COMT, que se encuentra asociado a la actividad enzimática de la degradación de catecolaminas, sobre la marcha y el equilibrio, el sueño y la regulación emocional. Para ello, se ha adoptado un enfoque multidisciplinar en el que se ha tenido en cuenta parámetros biomecánicos de la función motora, la actividad cerebral durante el sueño y durante la modulación afectiva del reflejo de sobresalto para comparar individuos que muestran bien una baja o una alta actividad de COMT (heterocigotos met y portadores del alelo val, respectivamente). La función motora fue evaluada mediante el análisis de la marcha y el equilibrio con grabaciones de videos en personas sanas y pacientes con FM (estudio 1). Además, dos subgrupos de pacientes con FM basados en el polimorfismo de la COMT participaron en un registro nocturno de polisomnografía (estudio 2) y una tarea experimental con la presentación de estímulos acústicos de sobresalto durante la visualización de imágenes afectivas (estudio 3). El estudio 1 mostró que las pacientes con FM presentan una reducción significativa en los parámetros de la marcha tales como velocidad, longitud del paso y del paso completo, o la cadencia, así como déficits en el control postural y el equilibrio. El estudio 2 reveló que las pacientes con FM y baja actividad de la enzima COMT parecen estar más impactadas físicamente, más deprimidas y con peor calidad de sueño (mayor número de despertares durante la noche, mayor tiempo en la cama y sueño más fragmentado durante la fase REM) que las pacientes con FM con alta actividad de la enzima COMT. Por último, el estudio 3 mostró que las pacientes con FM y baja actividad de la enzima COMT presentan alteraciones significativas de los componentes tempranos de la actividad cerebral desencadenada por estímulos agradables y desagradables comparadas con las pacientes con FM y alta actividad de la COMT. Estos estudios sugieren: 1) que la marcha y el equilibrio se encuentran alterados en las pacientes con...
FM comparadas con personas sin dolor, y 2) que el sueño y el procesamiento afectivo en las pacientes con FM puede ser modulado por el polimorfismo val158met del gen de la COMT que regula la actividad enzimática de las catecolaminas. En resumen, estos hallazgos proporcionan un apoyo adicional a la idea de que los síntomas de la fibromialgia precisarían de una evaluación y de una intervención terapéutica de carácter multidimensional con el objetivo de proporcionar las óptimas condiciones para la mejora de la calidad de vida de estos pacientes. Asimismo, estos hallazgos subrayan la relevancia de considerar marcadores genéticos y neurofuncionales para una compresión más completa del síndrome de fibromialgia.
Resum

La fibromialgia (FM) és una síndrome crònica caracteritzada per dolor generalitzat, fatiga, son no reparador, queixes somàtiques i alteracions afectives i cognitives. Encara que existeixen evidències indicant que emocions negatives poden tenir un paper modulador rellevant en el manteniment dels símptomes de la FM, poc es coneix de la influència dels polimorfismes genètics sobre la funció motora, el son i el processament afectiu en la fibromiàlgia. L’objectiu principal d’aquesta tesi doctoral va ser analitzar la influència del polimorfisme val158met del gen de la COMT, que es troba associat a l’activitat enzimàtica de la degradació de les catecolamines, sobre la marxa i l’equilibri, el son i la regulació emocional. Per això, s’ha adoptat un enfocament multidisciplinari en el qual s’han tingut en compte paràmetres biomecànics de la funció motora, l’activitat cerebral durant el son i durant la modulació afectiva del reflex de sobresalt per comparar subjectes que mostren una baixa o alta activitat de COMT (homozigots met i portadors d’al·lel val, respectivament). La funció motora fou avaluada mitjançant l’anàlisi de la marxa i l’equilibri amb gravacions de vídeo en persones sanes i en pacients amb FM (estudi 1). A més, dos grups de pacients amb FM basats en el polimorfisme de la COMT participaren en un registre polisomnogràfic nocturn (estudi 2), i en una tasca experimental ambla presència d’estímul acústic de sobresalt durant la visualització d’imatges afectives (estudi 3). L’estudi 1 mostra que les pacients amb FM presenten una disminució significativa en paràmetres de la marxa com són la velocitat, la longitud de cada pas i del pas complet o cadència, així com dèficits en el control postural i de l’equilibri. L’estudi 2 desvetllà que les pacients amb FM amb baixa activitat de l’enzim COMT pareixen estar més impactades físicament, més deprimides i amb pitjor qualitat de son (major nombre de despertars durant la nit, major quantitat de temps en el llit i un son més fragmentat durant la fase REM), que les pacients amb FM amb alta activitat de l’enzim COMT. Per últim, l’estudi 3 mostra que les pacients de FM amb baixa activitat de l’enzim COMT presenten alteracions significatives en els components primerencs de l’activitat cerebral desencadenada per estímul agradables o desagradables, comparades amb les pacients de FM amb alta activitat del COMT. Aquests estudis suggereixen: 1) la marxa i l’equilibri es troben alterats en les pacients amb FM comparades amb les persones sense dolor, i 2) que el son i el processament afectiu de les pacients amb FM port estar modulat pel polimorfisme
val158met del gen del COMT que regula l’activitat enzimàtica de les catecolamines. En resum, aquests resultats remarquen encara més la idea de que els símptomes de la fibromiàlgia precisen d’una avaluació i d’una intervenció terapèutica de caràcter multidimensional amb l’objectiu de proporcionar les òptimes condicions per la millora de la qualitat de vida d’aquestes pacients. Així mateix, aquestes troballes subratlen la rellevància de considerar els marcadors genètics i neurofuncionals per a una compressió més completa de la síndrome de fibromiàlgia.
Resumo

A fibromialgia (FM) é uma síndrome crônica caracterizada por dor generalizada, fatiga, sono não reparador, queixas somáticas, bem como alterações afetivas e cognitivas. Embora exista evidência recente indicando que as emoções negativas podem desempenhar um papel modulatório relevante para a manutenção dos sintomas da FM, pouco se conhece sobre a influência dos polimorfismos genéticos sobre a função motora, o sono e o processamento afetivo na fibromialgia. O objetivo principal desta tese foi analisar a influência do polimorfismo val158met do gene da COMT, que se encontra associado à atividade enzimática da degradação de catecolaminas, sobre a marcha e o equilíbrio, o sono e a regulação emocional. Para tanto, adotou-se um enfoque multidisciplinar levando em consideração os parâmetros biomecânicos da função motora, a atividade cerebral durante o sono e durante a modulação afetiva do reflexo de sobresalto para comparar indivíduos que possuem uma baixa ou alta atividade do gene da COMT (homozigotos met e portadores do alelo val, respectivamente). A função motora foi avaliada mediante a análise da marcha e do equilíbrio com gravações de vídeos em pessoas saudáveis e em pacientes com FM (estudo 1). Além disso, dois subgrupos de pacientes com FM baseados no polimorfismo da COMT participaram de um registro noturno de polissonografia (estudo 2) e uma tarefa experimental mediante apresentação de estímulos acústicos de sobresalto durante a visualização de imagens afetivas (estudo 3). O estudo 1 demonstrou que as pacientes com FM apresentam uma redução significativa dos parâmetros da marcha tais como velocidade, tamanho do passo e da passada, ou a cadência assim como, déficits no controle postural e equilíbrio. O estudo 2 revelou que as pacientes de FM que possuem baixa atividade da enzima COMT parecem estar mais fisicamente impactadas e deprimidas, além de apresentarem uma pior qualidade de sono (maior número de despertares durante a noite, maior tempo na cama, e um sono mais fragmentado durante a fase REM) quando comparadas com pacientes de FM que possuem haplótipo associado a alta atividade da enzima COMT. Por último, o estudo 3 demonstrou que as pacientes com FM com baixa atividade da enzima COMT apresentam alterações significativas nos componentes iniciais da atividade cerebral desencadeada por estímulos agradáveis e desagradáveis quando comparadas às pacientes de FM com alta atividade da enzima COMT. Estes estudos sugerem: 1) a marcha e equilíbrio são alterados em
pacientes com FM comparados com pessoas sem dor e 2) que o sono e o processamento afetivo das pacientes com FM podem ser modulados pelo polimorfismo val158met do gene da enzima COMT que regula a atividade enzimática das catecolaminas. Em síntese, estas descobertas proporcionam um apoio adicional à ideia de que os sinais e sintomas da fibromialgia precisariam de uma abordagem e uma intervenção terapêutica de caráter multidimensional com o objetivo de proporcionar ótimas condições para a melhora da qualidade de vida destes pacientes. Além disso, estes resultados destacam a importância de considerar marcadores genéticos e neurofisiológicos para uma compreensão mais completa da síndrome da fibromialgia.
Abstract

Fibromyalgia (FM) is a chronic syndrome characterized by widespread pain, fatigue, un-refreshing sleep, somatic complaints, and affective and cognitive alterations. Although there is recent evidence indicating that negative affect may play a relevant modulatory role for the maintenance of fibromyalgia symptoms, little is known about how genetic polymorphisms may influence motor function, sleep and affective processing in fibromyalgia. The major goal of the present thesis was to analyze the influence of the val158met polymorphism of the COMT gene which is associated with the enzymatic activity level of cathecolamine degradation on gait and balance, sleep and emotional regulation. For this purpose, a multidisciplinary approach taking into account biomechanical parameters of motor function and parameters of the brain activity during sleep and during affective processing was used to compare individuals displaying either low (met homozygotes) or high COMT activity (val carriers). Motor function was assessed by analyzing gait and balance through video recordings in healthy controls and FM patients (study 1). In addition, two subsamples of FM patients based on the val158met polymorphism participated in a night polysomnography recording (study 2) and an experimental task with presentation of startle noise stimuli when viewing affective pictures (study 3). Study 1 showed that FM patients display a significant reduction in gait parameters such as speed, step length and full step, cadence and etc., as well as deficits in postural control and balance. Study 2 revealed that FM patients with low-activity of the COMT enzyme appear to be more physically impacted and depressed, and to have poorer quality of sleep (greater number of awakenings during the night, longer in bed and more fragmented sleep during REM) than FM patients with high-activity of the COMT enzyme. Finally, Study 3 showed that patients with low-activity of the COMT enzyme display significant alterations of the early components of the event-related brain potentials elicited by pleasant and unpleasant stimuli as compared with FM patients displaying high COMT activity. These studies suggest: 1) that gait and balance are altered in patients with FM compared to pain-free controls, and 2) that sleep and affective processing in FM patients may be modulated by the val158met polymorphism of the COMT gene that regulates the enzyme activity of catecholamines. In summary, these findings provide further support for the notion that FM symptoms would require multidimensional assessment and intervention
to provide optimal conditions for improving quality of life in these patients. Moreover, our find-
ings underline the relevance of considering genetic and neurofunctional markers for a complete
understanding of fibromyalgia.
Pain is one of the most basic mechanisms to ensure the survival of an organism. The perception of pain involves anatomical structures and physiological functions to process noxious information in the central nervous system from nociceptors to brain networks. The individual to escape from damaging situations, to protect a damaged body part while it heals, and to avoid similar experiences in the future. Moreover, pain is considered the fifth vital sign and one of the most common symptoms in the context of disease. The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. Pain is, therefore, a multidimensional phenomenon that is strongly influenced by biopsychosocial and genetic factors.

Moreover, persistent pain has a decisive and negative influence on the individual quality of life. In clinical settings, chronic pain has been usually recognized as that pain which persists after the normal time of healing (between one and six months). Nevertheless, for research purposes, chronic pain is usually defined as that pain lasting more than 6 months. The occurrence of chronic pain is growing worldwide, perhaps due to new habits of life, increasing longevity of individuals, environmental changes and new concepts that define pain. In this context, pain relief constitutes an absolute requirement of our modern society and determines a health professional action priority.
assessment of the multiple aspects of the pain experience is an essential and challenging component of any management protocol [1]. Nevertheless, pain is a subjective phenomenon and no satisfactory measures of pain exist.

The negative impact that pain exerts on patients requires an intervention from biopsychosocial and integrative perspectives. From this model of health, pain is a continuous dimension that affects all components of individual’s life. Psychological therapy, neuropsychological rehabilitation and physical therapy can provide valid and effective perspectives in the treatment of various aspects involved in chronic pain, thus improving patients’ quality of life. Figure 1.1 shows how non-pharmacological therapies can contribute to alleviate symptoms and complaints in patients with FM.

Figure 1.1: Block diagram illustrating the importance of non-pharmacological therapies for a better quality of life in patients with fibromyalgia. This block diagram was adapted from Hausdorff et al. (2001) [4]. CNS, central nervous system; PNS, peripheral nervous system; ROM, range of motion.
This thesis is focused on the syndrome of fibromyalgia, a high-prevalent chronic disease, which includes widespread pain sensitivity and several other symptoms such as negative affect, fatigue, sleep and cognitive disturbances. As it occurs with patients suffering from other chronic pain conditions, clinical standards for the intervention in fibromyalgia should be included to improve physical and psychological functioning, as well as to increase participation in daily life. Nevertheless, little is still known about how chronic pain may affect motor function, sleep and affective processing in these patients. Taking into account that enhanced pain sensitivity is one of the key symptoms in all chronic pain diseases, it is of high relevance to understand how specific genetic polymorphisms are involved in pain sensitivity and how they can modulate clinical symptoms. In the next sections, major clinical and pathophysiological aspects of fibromyalgia will be described in detail.

1.1 Fibromyalgia and its pathophysiological process

Fibromyalgia (FM) is a chronic pain syndrome characterized by enhanced pain sensitivity and fatigue, as well as cognitive and affective symptoms [5]. Fibromyalgia exerts a considerable impact on daily activities and quality of life. In particular, it has been frequently shown that fatigue in fibromyalgia is severe enough to reduce physical activities and lead to a sedentary lifestyle by reducing physical abilities and increasing risk for disabilities [6,7]. Functional limitations in FM patients seem to be similar to those observed in patients with osteoarthritis or rheumatoid arthritis [8,9]. Furthermore, it has been shown that loss of function is strongly associated with work disability in FM [10,11]. Thus, it is argued that fibromyalgia is a complex syndrome that incorporates a wide range of symptoms and functional alterations in many systems, including the central nervous system (CNS). Currently, a major scientific challenge is to clarify which alterations are pathogenic and which ones simply represent epiphenomena of a chronic disease [12].
1.1.1 Chronic pain and central sensitization

Fibromyalgia patients show higher sensitivity to a wide range of painful stimuli like heat, cold, and mechanical pressure [13]. In addition, stimuli delivered at intensity levels that would not cause pain in healthy individuals can trigger pain responses in patients with FM, suggesting the existence of allodynia that could be due to an enhanced brain processing of afferent somatosensory information [14–16].

From a physiological perspective, it is known that prolonged exposition to pain stimuli makes that nociceptive neurons at the dorsal horn undergo a relevant sensitization process. One of the molecular mechanisms involved in this process is the activation of nitric acid by the postsynaptic neuron, which would increase the presynaptic release of excitatory substances (phosphorus and glutamate) and induce postsynaptic hyperexcitability [12,14] (see Figure 1.2).

**Figure 1.2: Pain processing and its modulation.** Activation of peripheral nociceptors by noxious stimuli generates signals that travel to the dorsal horn of the spinal cord via the dorsal root ganglion. From the dorsal horn, these signals are carried along the ascending pain pathway or the spinothalamic tract to thalamus and cortex. Pain can be controlled by nociception-inhibiting and nociception-facilitating neurons. Descending signals originating in the supraspinal centers can modulate activity in the dorsal horn by controlling spinal pain transmission. (Figure from Häuser et al. [12]).
The release of pro-inflammatory cytokines and other amino acids and excitatory substances of the glia within the spinal cord dorsal horn may also influence this process. The final aim of this process is to prolong the postsynaptic neuron hyperexcitability. Thus, the transmission of pain signals can be increased and may result in clinical symptoms as hyperalgesia and allodynia as presented by patients with neuropathic pain [12,14].

Central hyperexcitability have emerged as a prominent hypothesis for pain sensitization and the pathogenesis of chronic pain, as it occurs in fibromyalgia. Basically, central sensitization or hyperexcitability means that the CNS plays the leading role in the augmentation or amplification of pain and, probably, in the development of other comorbid symptoms (such as disturbed sleep, fatigue, memory and depressed mood) [12].

Another closely related mechanism to the development and maintenance of chronic pain could be the reduced ability of the CNS to achieve descending pain modulation (conditioned pain modulation) [17]. In FM patients, the increase of pain sensitivity has been linked to central sensitization mechanisms by changes in the descending pain control pathways [15]. The function of such pathways can be compromised by low levels of serotonin and noradrenaline in the spinal cord dorsal horn, thus increasing the hyperexcitability of the nociceptive system [12] (see Figure 1.3).

Together with an abnormal pain processing, it has been argued that FM patients could display an altered function of the hypothalamic-pituitary-adrenal axis. Thus, for instance, increased basal cortisol levels during the first hours after awakening [18], together with decreases in prolactin levels during sleep [19] and reduced secretion of melatonin during the night [20] have been found. However, no significant relationship between cortisol levels and fatigue has been reported. [14]. Several mechanisms have been described and incorporated into the concept of centralized pain, including mechanisms acting at the spinal level as well as interactions and altered connectivity between pain and non-pain-related brain areas [12].
Figure 1.3: Potential pathophysiological processes in fibromyalgia. Sensitization of the central nervous system (CNS) has been suggested as one of the main pathophysiological changes underlying fibromyalgia. The genetic set point for sensory (including pain) regulation can be modified by psychological factors, such as anxiety, depression and catastrophizing and biopsychosocial stress (for example, trauma, childhood adversities, major life events or infections). Peripheral factors, such as ongoing nociceptive input produced by co-morbidities, can also affect pathogenesis. In the CNS, several changes can be noted, including neurotransmitter imbalances, altered functional connectivity and changes in the hypothalamic–pituitary–adrenal (HPA) axis, which influence the autonomic system. Red arrows represent stressors. GABA: γ-aminobutyric acid; NGF: nerve growth factor. (Figure from Häuser et al. [12])
1.2 Clinical and epidemiological aspects of fibromyalgia

Fibromyalgia is a common chronic pain disorder, which appears in all populations [12]. In general, the prevalence of patients with symptoms that meet diagnostic criteria according to the American College of Rheumatism oscillates between 2% and 4% [12, 21]. It seems to be 10 to 20 times more common in women than in men and usually affects more individuals in the age group between 35 and 60 years [22]. However, although most patients with FM are middle-aged women (73 – 88%) [23], it has also been described in teenagers [24, 25] and in the elderly [26].

According with several surveys [23, 27, 28, 28], fibromyalgia is the second most common rheumatic disease, affecting more than 6 million people in Western European countries [28]. FM is likely an importance source of chronic widespread pain in these countries and, hence, a health and economic burden to the community [28]. Therefore, FM can be considered a major health problem among contemporary women [29]. As a possible explanation, it has been argued that women would be more exposed to stress both for its biological condition and the cultural roles that society historically imposes to them [30, 31]. Moreover, it seems plausible that concerns of daily life, excess of work, lack of physical activity and leisure could have an impact on health condition of body and mind, aggravating the effects of fibromyalgia symptoms on quality of life.

1.2.1 Aggravating factors in fibromyalgia

Although the etiology still remains unclear [12], it has been proposed that some genetic polymorphisms could be related to increased pain sensitivity in FM patients [13, 32, 34]. Moreover, several studies have suggested that FM pathophysiology could be triggered by physical (acute illnesses, surgery, accidents, etc.) and psychological trauma (stress, emotional trauma, sexual violence, childhood trauma and abuse, daily life hassles, exposure to war, catastrophic events and persecution) [12, 35–38]. It has been also observed that patients suffering from other inflammatory joint diseases, such as rheumatoid arthritis, ankylosing spondyloarthritis [39] or joint hyperlaxity [40], may often develop typical fibromyalgia symptoms.
Furthermore, it seems plausible that sedentary lifestyles and low levels of fitness could contribute to the aggravation of underlying pain, fatigue and depression symptoms in these patients. However, the evidence of this contribution is still contradictory. Thus, for instance, several studies have reported that individuals with FM tend to be sedentary [41] and to display below-average levels of cardiorespiratory fitness [41–44]. Nevertheless, individuals with FM were able to perform maximal cardiorespiratory fitness tests, as well as low and moderate intensity aerobic exercises, or flexibility and muscle strengthening exercises [45]. Moreover, it is possible that physical and psychological variables could also modulate the effects of physical activity on FM symptoms. In this sense, it has been shown that high body mass index (BMI) and overweight can increase risk for FM symptoms, especially among women who also reported low levels of physical exercise [46]. The Nord-Tronndelag Health Study was conducted to explore the risk of developing FM in a large, unselected female healthy population (n=15990) after a follow-up of 11 years [46]. The authors found that 380 cases developed FM at follow-up, and that women with overweight or obesity (BMI > 25.0 kg/m²) had a 60 –70% higher risk compared with women with normal weight (BMI: 18.5–24.9 kg/m²).

Various cognitive and affective factors have been involved in pain amplification and chronicity [47, 48]. Thus, for example, it has been shown that individual’s interpretation of stimuli (cognitive factor) is capable of modifying the own painful experience, either by increasing or decreasing pain [49]. The chronic pain patient is in a negative emotional context caused by the impact of sustained pain, as well as, by the personal impact, family, social and labor involved. In fact, numerous studies have found that patients with chronic pain displayed higher scores on depression and anxiety, as well as an exacerbated tendency to catastrophizing when compared with pain-free individuals [50,51].

The bidirectional association between pain and sleep disturbances has also been recognized (Okifuji & Hare, 2011) [52]. Thus, for instance, it is generally accepted that alterations of the sleep-wake cycle are one of the most common comorbid symptoms in chronic pain patients and that sleep can easily become unbalanced by pain-relief medication. Moreover, there is ample evidence showing that disordered sleep may adversely impact pain sensitivity, and that sleep deprivation also seems to attenuate analgesic effects of medications. Sleep can also be affected
by unhealthy habits or lifestyle patterns such as consumption of tobacco and alcohol, or overweight. Amalgamating the research evidence on the factors affecting sleep quality in people with insomnia, Lundh and Broman (2000) [53] proposed an integrative model of the interaction between sleep-interfering and sleep-interpreting processes. The four boxes at the top of Figure 1.4 represents the sleep interfering processes and the three boxes at the bottom highlight the sleep interpretation processes that impact on sleep quality. As shown by the model, these processes are likely to influence each other with beliefs about sleep by directly influencing behavior and cognitive coping strategies. Consequently, as highlighted by the model, individual’s behaviors, thoughts, environment and physical health all have an important influence on sleep quality. Maintaining the sleep equilibrium can therefore be difficult and it has been estimated that up to 45% of the adult population may experience some form of sleep disturbance [54]. The role of these factors on sleep in FMS will be discussed in more detail in the “Human sleep and fibromyalgia” section of the present thesis.

It is important to bear on mind that the regulatory system of pain is mainly in charge of substances such as endogenous opioids and monoamines like serotonin are also the neurochemical substrate of emotions [55]. It is no wonder that psychiatric disorders such as depression and anxiety disorders often coexist with chronic pain and can negatively impact the pain symptoms [56]. Depression and anxiety have been consistently found to be higher in people with chronic pain, possibly due to the demands of living with a chronic condition, and there is evidence that mood may also have an influential role in individual’s perceptions of sleep quality [57] and physical activity. Continuing efforts in both experimental and clinical research are needed to develop a translationally meaningful understanding of how all these factors impact pain. Figure 1.5 shows a representative model for the understanding of the aggravating factors in the development and maintenance of fibromyalgia. Genetic factors, bad habits like daily intake of stimulants or alcoholic drinks before bed, smoking, lack of physical activity, stress and physical or psychological traumas may aggravate FM symptoms, damaging patients’ health and their quality of life.
Figure 1.4: The Integrative Model of the interaction between sleep-interfering and sleep-interpreting processes. This theoretical model introduces a distinction between sleep-interfering and sleep-interpreting processes in insomnia. Moreover, the model uses this distinction as a way of organizing existing empirical research and theoretical approaches to insomnia. The main argument is that these two kinds of processes may combine and interact in various ways in different subvarieties of insomnia. One reason for the distinction between sleep-interfering and sleep-interpreting processes is that they will probably require different forms of treatment approaches. To the extent that a person’s insomnia is due to sleep-interpreting processes, the treatment focus should be on the kind of beliefs, attributions, and personal standards (cognitions) that are involved. To the extent that the insomnia is due to sleep-interfering processes, like emotional conflicts, traumatic events, negative conditioning, or other arousal-producing processes, treatment should be focused on these emotional processes. And, of course, to the extent that both kinds of processes are involved, an optimal treatment may be assumed to involve an integration of both kinds of approaches. (Figure adapted from Lundh and Broman (2000) p. 308 [53]).
Figure 1.5: Model of the aggravating factors in fibromyalgia. Representative model of the factors that can aggravate the signs and symptoms of fibromyalgia. The blue ellipses represent some factors that can aggravate the syndrome of fibromyalgia (genetic, sleep, emotional, and physical factors). Brown boxes represent the genetic factors and how some polymorphisms can increase sensitivity to pain, aggravating the symptoms of fibromyalgia. The purple boxes represent the sleep factors, such as the bad daily habits like watching too much TV, consuming exciting drinks before bedtime, as well as smoking habits, which can trigger sleep disorders. The orange boxes are related to emotional factors (stress, emotional trauma, catastrophic events, for example) and how they may aggravate the symptoms of FM patients triggering psychiatric disorders such as anxiety and depression. The green boxes represent the physical factors and how these can aggravate the symptoms of fibromyalgia. Some illnesses, accidents, lack or excess of physical activity may lead to limitations and functional disability leading to physical impairment in fibromyalgia patients.
1.3 Genetic aspects of fibromyalgia

The vulnerability to the development of FM seems to be influenced by environmental, hormonal and genetic factors. Current research strongly supports genetic underpinnings in the development of fibromyalgia, as occurs in other chronic pain conditions [58]. Thus, for instance, first-degree relatives of patients with fibromyalgia showed an eightfold greater risk of developing the syndrome [59], and family members have more tender points and are at increased risk of having other functional disorders (temporomandibular disorder, headache, regional pain syndromes) than controls [60]. This familial co-aggregation is assumed to represent an overlap between clinical syndromes that are characterized by pain centralization and shared features such as pain, fatigue, cognitive difficulties and affective symptoms.

The genetic basis for these functional disorders has been highlighted by twin studies, demonstrating that different functional somatic syndromes co-aggregate and that genetic factors may contribute up to half the risk of developing them [61, 62]. Several polymorphisms have been identified as specific markers of this genetic risk. Many of these specific markers are related to metabolism and breakdown of neurotransmitters that are involved in pain modulation. Thus, for instance, polymorphisms in the genes encoding catechol-O-methyltransferase, dopamine type 4 receptors, serotonin 5-hydroxytryptamine 2A receptors and serotonin transporters have been significantly involved [33].

1.3.1 Catechol-O-methyltransferase

One of the genetic polymorphisms most involved in the variability of pain perception, as well as in the physiopathology processes of chronic pain is the *val158met* polymorphism (*rs4680*), located in the gene coding for the catechol-O-methyltransferase enzyme (COMT). Such enzyme is responsible for the metabolism of the catecholamines (dopamine, adrenaline, noradrenaline). The *val158met* polymorphism codes one replacement of valine (*val*) by methionine (*met*) at the location 158 of the amino acid sequence [13]. This variation may influence enzyme’s thermostability and, as consequence, catecholamine degradation may be reduced up to 4 times [63]. Chen *et al.* [64] found that the rate of degradation of dopamine by COMT enzyme was depen-
dent on the genotype, with a faster dopamine metabolization in val homozygotes than in met individuals (degradation rate from 1/3 to 1/4 smaller than val homozygotes), and intermediate degradation rates in heterozygous individuals.

The fact that genotypes of the COMT gene can be associated with different metabolic rates leads to the possibility of assessing clinical symptoms in subgroups of patients with different levels of dopamine in the synaptic cleft. Moreover, recent studies have indicated that polymorphisms of the COMT gene could be also linked to different cognitive and emotional profiles. Thus, for instance, it has been found that the methionine allele was associated with better cognitive measures (in particular, executive functions and working memory), while the valine allele was associated with higher levels of emotional regulation [65, 66]. Bodenmann et al. [67] argued that this enzyme could also contribute to interindividual differences in α EEG oscillations during a counting behavioral task.

Regarding pain perception, it has been suggested that COMT gene may increase pain sensitivity either by noradrenergic or dopaminergic activation [68], leading to suppression of endogenous opiates [69] (see figure 1.6). In addition, COMT haplotypes have also been implicated in pain processing. During genetic recombination, closely located SNPs present a very high probability to be inherited together. Thus, for instance, it has been observed that the haplotype combination of val158met polymorphism with three other COMT gene polymorphisms may be linked to the variability in the sensitivity to experimental pain [13] and to the risk of developing chronic pain diseases like temporomandibular pain [70, 71].

Single-nucleotide polymorphisms of the COMT gene and its involvement in pain perception have been extensively examined in patients with FM [29, 71, 73, 74]. Thus, for instance, Martínez-Jauand et al., [13] reported that genotypes of the val158met polymorphism of the COMT gene were associated with pain sensitivity in FM patients, with higher activity of the COMT enzyme being associated with lower pain sensitivity. Diatchenko and collaborators [70] described that the haplotype combination of this COMT polymorphism together with 3 other silent polymorphisms from the same gene (the rs6269, rs4633 and rs4818 polymorphisms) was associated with differences in the enzymatic activity and could explain 11% of the variability in pain sensitivity. The haplotypes were defined as low pain sensitivity (LPS), average pain sen-
Figure 1.6: Relationship between the COMT enzyme and theoretical pathological mechanisms in fibromyalgia. This figure synthesizes the pathogenesis of FM according to Martinez-Lavin [72]. The algorithm is based on emerging genomic evidence supporting the concept of FM as a sympathetically maintained pain syndrome. FM can be viewed as a disease of modern times, in which the main regulatory system of the body unsuccessfully attempts to adapt to contemporary stressful lifestyles. High-risk individuals would be those with defective catecholamine-degrading enzymes. Central to the pathogenesis of the illness is a constant hyperadrenergic state that could lead to a breakdown of the system. In such instances neuroplasticity takes place, establishing abnormal connections between the sympathetic nervous system and the nociceptive fibers. The resulting clinical syndrome would be a neuropathic type of pain (Figure from Martinez-Lavin (2007) [72]).

sensitivity (APS) and high pain sensitivity (HPS). Furthermore, the authors [70] demonstrated that the risk of temporomandibular pain was twice lower in subjects with LPS haplotype compared to subjects with APS and HPS haplotypes.

Nevertheless, the association between genetic factors and the development and maintenance of fibromyalgia symptoms such as pain sensitivity remains inconclusive. Thus, for instance, Häuser and collaborators [12] revealed that current research on genetic factors involved in FM [75,76] present low odds ratios ranging between 1.5 and 5.4 [58]. Similar to other complex conditions, missing heritability remains to be explained through other physiological pathways and mechanisms [77]. It is likely that the final CNS set point for pain processing is determined by a large number of separate genetic markers that interact with lifetime events and behaviors (epigenetic influences). Moreover, genetic influences have also been implicated in several other
fibromyalgia symptoms such as sleep disturbances [67, 78, 79], depression and anxiety [80] and biomechanical disorders [81, 82] see Table 1.1. How all these genetic, epigenetic, behavioral and neurophysiological factors interact to maintain pain over time remains to be elucidated.
**Table 1.1**: Association of fibromyalgia with genetic polymorphisms

<table>
<thead>
<tr>
<th>Genetic polymorphisms</th>
<th>References</th>
<th>Associated with</th>
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<tbody>
<tr>
<td><em>HTR2A</em> (serotonin-2A receptor gene)</td>
<td>Bondy et al. (1999)</td>
<td>personality trait in fibromyalgia [83]</td>
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<tr>
<td><em>5-HTTLPR</em></td>
<td>Offenbaecher et al. (1999)</td>
<td>personality trait in fibromyalgia [86]</td>
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<td><em>5-HTTLPR</em></td>
<td>Gursoy (2002)</td>
<td>personality trait in fibromyalgia [87]</td>
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<td>s-allele of the 5-HTTLPR</td>
<td>Deuschle et al. (2010)</td>
<td>patients suffering from insomnia [88]</td>
</tr>
<tr>
<td><em>Dopamine D4 receptor gene</em></td>
<td>Buskila et al. (2004)</td>
<td>personality trait in fibromyalgia [89]</td>
</tr>
<tr>
<td><em>val158met</em> polymorphism (<em>COMT</em> gene)**</td>
<td>Zubieta et al. (2003), Martinez et al. (2013)</td>
<td>pain [13, 69]</td>
</tr>
<tr>
<td><em>val158met</em> polymorphism (<em>COMT</em> gene)**</td>
<td>Gürsoy et al. (2003)</td>
<td>several pain conditions [90]</td>
</tr>
<tr>
<td><em>val158met</em> polymorphism (<em>COMT</em> gene)**</td>
<td>Bodenmann et al. (2009)</td>
<td>variation in brain alpha oscillations in wakefulness, rapid-eye-movement (REM) sleep, and non-REM sleep in health young men [67]</td>
</tr>
<tr>
<td><em>val158met</em> polymorphism (<em>COMT</em> gene)**</td>
<td>Bartley (2011)</td>
<td>temporomandibular disorders [82]</td>
</tr>
<tr>
<td><em>HLA DQB1</em>0602 genotype</td>
<td>Spitzer et al. (2010)</td>
<td>characteristics of narcolepsy, highly fragmented sleep in conjunction with neuromuscular, fatigue or generalized pain [91]</td>
</tr>
<tr>
<td><em>val66met</em> (<em>rs6265</em> polymorphism)</td>
<td>Martinowich et al. (2007)</td>
<td>depression and anxiety [80]</td>
</tr>
<tr>
<td><em>val66met</em> (<em>rs6265</em> polymorphism)</td>
<td>Peciña et al. (2014)</td>
<td>pain sensitivity [92]</td>
</tr>
<tr>
<td><em>FAAH</em> (<em>C385A</em> polymorphism)</td>
<td>Hariri et al. (2009)</td>
<td>reward-related behavior [97]</td>
</tr>
<tr>
<td><em>OPRM1</em> (<em>A118G</em> polymorphism)</td>
<td>Wang et al. (2012), Fillingim et al. (2005)</td>
<td>changes in libido and insomnia in methadone maintenance patients [98], pressure pain sensitivity [99]</td>
</tr>
</tbody>
</table>

**Genetic polymorphism used in this doctoral thesis**
## 1.4 Human movement and fibromyalgia

Human walking is a form of locomotion that allows the lower limbs to easily accommodate to different surfaces and obstacles along the way. The efficiency of these tasks depends on the mobility of joints and muscular activity that is selective in time and intensity \[100\]. Walking also involves the implementation of specific movement patterns, consisting of repetitive sequences of forward limb movements and, at the same time, the maintenance of body stability [101]. Interestingly, each individual seems to have a unique pattern of motion [100] and the analysis of such individual gait patterns makes possible to determine specific motion deficits and to adjust specific rehabilitation programs [102].

Human locomotion has been described as a series of alternating, rhythmic limb and trunk movements, which determine a forward displacement of the center of gravity. As the body moves on the supporting leg, the other leg swings forward in preparation for the next support [103] (see Figure [1.7]). The term gait cycle is used to describe the complex activity of walking. Each gait cycle is divided into two periods considering the contact of the feet with the ground: support (during which the foot is on the ground) and swinging or oscillation (the foot is in the air for the advancement of the member) [101, 104]. Full step length, or stride length, is defined as the linear distance between successive points of contact of the heel of the same foot. The concept of step length corresponds to the linear distance between the contact points between the two feet [103]. Support represents 60% of the gait cycle (20% for double support and 40% for single limb stance), whereas oscillation or swing represents the remaining 40% (see Figure [1.7]). The muscles play a key role for successful gait. In particular, the muscles of lower limbs play three different roles during locomotion: the segment brakes driven by the kinetic energy, the shock damping, and the vibration and acceleration of the segments [103–105]. Most of the major muscle groups are activated during the initial contact and separation of the feet from the ground. These events correspond to periods of acceleration and deceleration of the limb, that is, the transfer of body weight from one foot to the other [105]. Analysis of the gait cycle provides information on various spatiotemporal gait parameters, kinematics, kinetics and muscle activity. Values of each gait cycle can be used to assess individuals’ improvements.
after intervention [101].

Figure 1.7: Schematic representation of the timing of the gait cycle. The gait cycle is the time interval between the exact same repetitive events of walking. The defined cycle can start at any moment, but it starts when one foot contacts the ground. There are two phases of the gait cycle: stance and swing. The stance phase is the part of the cycle when the foot is in contact with the ground. It comprises 60% of the full gait cycle, beginning with initial foot strike and ending with toe-off. The stance phase of gait can be divided into three additional periods: contact period, when the heel strikes the ground; mid-stance period, when the foot is flat on the ground moving on to heel lift; propulsive period, when the heel is lifted off the ground and the toe off position is taken. The swing phase occurs when the foot is in the air and comprises 40% of the cycle, beginning with toe-off and ending with second (ipsilateral) foot strike.

Several disorders with chronic pain symptoms such as rheumatoid arthritis, cancer, spinal stenosis, or myofascial pain syndrome, have been associated to specific alterations in posture and gait cycle. According with Wolfe and collaborators [5], fibromyalgia may also present joint stiffness, severe fatigue, reduced isometric strength in the lower limbs and bradykinesia [107]. Previous research has also revealed that FM patients displayed deficits in postural stability [7,108,109], a complex task that involves the rapid and dynamic integration of multiple inputs to execute appropriate neuromuscular activity [110]. Impaired balance has been reported as one of the top ten debilitating symptoms in FM (45% of patients) [7]. Moreover, frequency of falls seems to be higher in FM patients (34.4%) [108] than in persons aged 65 years and older (25-35%) [111], or in patients with rheumatoid arthritis (RA) [9]. These changes in the characteristics of gait, balance and muscle recruitment patterns have even been associated with a
reduction in the quality of life of patients with fibromyalgia [112–116]. Moreover, considering the ample evidence suggesting that fibromyalgia is characterized by central hyperexcitability, it seems plausible that an abnormal function of the central nervous system could somehow be also affecting patients’ balance and gait [117], resulting in significant limitations of their daily activities [118].

Nevertheless, balance and activity level in fibromyalgia have been mostly assessed by using retrospective self-reports [108,119], which are strongly influenced by patients’ beliefs about their own physical functioning and pain [120]. In the last decades, different recording devices have been developed to monitor balance and physical activity over long periods of times. Furthermore, it has been demonstrated that accelerometry-based ambulatory monitoring systems provided more objective measurements of variability in physical activities over several days than self-reports [121]. Moreover, recent research has underlined the role of putative genetic markers in explaining gait and balance alterations [122,123]. Thus, for instance, it has been suggested that genetic variations in the catechol-O-methyltransferase (COMT) enzyme such as the val158met single nucleotide polymorphism could be linked to gait disturbances and cognitive impairments in Parkinson patients [123]. In this sense, it has been demonstrated that carriers of the met allele displayed impaired performance in executive function tasks [124].

Given that gait is associated with attention and executive control processes subserved by the prefrontal cortex, it seems plausible that genetic variations in this polymorphism may be responsible not only for cognitive processes but also for gait, sleep and affective processing. Accordingly, a recent research has found that met/met was associated with reduced gait velocity in non-demented older adults [122]. There is however little information about the relationship between motor function and genetic biomarkers in fibromyalgia.

### 1.5 Human sleep and fibromyalgia

Sleep is a dynamic process consisting of well-defined stages, each of them characterized by specific electrophysiological, behavioral, and cognitive changes that differentiate them from each other and from wakefulness [125]. The functions of sleep remain unknown despite our
rapidly increasing understanding of the processes generating and maintaining sleep. There is evidence that both deep and light sleep are necessary for life, having an important role in the restoration and adaptation of the body [126–128]. A number of non-mutually exclusive hypotheses have been proposed [128–134]. Thus, for instance, it has been suggested that sleep is essential to restore the entire organism, physically and mentally. Thus, increased protein anabolism, growth hormone some of testosterone, prolactin and luteinizing hormone, together with decreases of catabolizing hormones such as cortisol during slow sleep would be important for restoring physiological functions [126]. In addition, REM sleep would be essential restoring mental functions. Moreover, immunological processes are stabilized during sleep, and cognitive processes such as learning and memory are consolidated. In conclusion, the role of sleep in adult learning and plasticity and brain development results of high relevance to understand how pain might influence patients’ daily activities.

From a physiological point of view, several sleep parameters can be obtained from polysomnographic recordings [135, 136]. Thus, the recordings of brain electrical activity (EEG), eye movements (EOG), muscle activity (EMG) and cardiac activity (EKG) are essential for sleep characterization [137] in both normal and pathological conditions. As a brief summary, the following recording techniques are usually recorded during polysomnographic recordings:

- **Electroencephalogram (EEG)** with electrodes placed on the scalp of the subject to measure electrical brain activity. Usually the C3-A2 and C4-A1 derivations are used [137].

- **Electrooculogram (EOG)** with electrodes placed on the extreme corners of the eyes for measuring eye movements during REM sleep.

- **Electrocardiogram (EKG)** with two or three electrodes usually placed in chest and abdomen.

- **Naso-oral airflow** with a thermistor placed in front of the nostrils and mouth that records temperature differences between inhaled and the exhaled air. Nasal prongs are also used to quantify the nasal flow during polysomnography.

- **Respiratory effort** transducers in thorax and abdomen to assess respiration.
• *Oxygen saturation* by pulse oximetry (SpO2).

• *Recording of snoring sounds* with a sensor to observe snoring and sleep apnea.

• *Recording of body position* recorded by sensors to observe changes of decubitus.

• *Recordings of lower limb movements* by electromyograms of the left and right tibial to assess restless leg syndrome.

According to these criteria, classification of adult human sleep can be summarized in the Table 1.2.
Table 1.2: Characteristics of physiological variables during different sleep phases. Stages 1 and 2 are also known as *Light Sleep*, and Stages 3 and 4 are also known as *Deep Sleep*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Waking</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>REM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>Wakefulness</td>
<td>It is defined by the presence of relatively low voltage EEG and mixed frequencies. Alpha reduction (less than 50% of the route). Appearance of Beta waves (greater than 14 Hz) and theta (4-7 Hz).</td>
<td>Known as a light sleeper. Presence of sleep spindles and K complexes.</td>
<td>Presence of slow waves of 2 Hz or less with amplitudes greater than 75 µV</td>
<td>Over 50% of the interval is occupied by waves of 2 Hz with amplitudes higher 75 µV</td>
<td>Defined by mixed frequencies and low amplitude</td>
</tr>
<tr>
<td>of the EEG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wave</td>
<td>Predominance of alpha rhythm (8-12 Hz) and / or low voltage EEG waves with mixed frequencies.</td>
<td>Mixed waves between 2-7 Hz. At the end of this phase usually observed the vertex sharp waves in combination with such mixed waves and can reach up to 200 microvolts</td>
<td>Sleep spindles bands with a frequency of about 14 Hz (sigma band), K complexes and delta waves present lasting &lt;20% of the plot</td>
<td>Delta discontinuous activity (20-50% traced)</td>
<td>Delta continuous activity (over 50% of the route)</td>
<td>Presence of beta, alpha and “saw tooth” waves (4-6 Hz)</td>
</tr>
<tr>
<td>EMG (chin)</td>
<td>High tonic activity</td>
<td>Tonic activity at lower levels of waking</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Muscle atonia</td>
</tr>
<tr>
<td>EOG</td>
<td>Rapid eye movement and blinking</td>
<td>Presence of slow eye movement (pendulous)</td>
<td>Lack of eye movements</td>
<td>Lack of eye movements</td>
<td>Lack of eye movements</td>
<td>Rapid eye movement more frequently grouped in bursts (rapid eye movements are phasic phenomena)</td>
</tr>
<tr>
<td>Breathing and</td>
<td>Irregular, dependent activity</td>
<td>Regular</td>
<td>Regular</td>
<td>Regular</td>
<td>Regular</td>
<td>Irregular</td>
</tr>
<tr>
<td>EKG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEG signal</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>example</td>
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<td></td>
</tr>
</tbody>
</table>

22
The total daily sleep time gradually diminishes with age as well. Sleep in the newborn comes to be about 17 to 18 hours, during adolescence reaches the 7 to 8 hours typical at adulthood. From this age daily sleep time progressively declines until 5 or 6 hours at elder age. The newborn sleep is characterized by a large proportion of REM stage and EEG delta activity; circadian organization of sleep and wakefulness begin to be acquired from 12 weeks. As the years pass, the proportions of deep sleep (stages III-IV and REM) decreases, so that the old man hardly presents stages III-IV during sleep; the number of episodes of nocturnal awakenings also increases, a circumstance which may be aggravated by diseases or disorders typical at elder ages; and elder people also loses the circadian characteristics, so older people tend to nap during daytime hours, events that often result in a loss of sleep at night (see figure 1.8).

Figure 1.8: The relationships between sleep stages changes with age. Figure redraw from Roffwarg et al. (1966) in Nicolau et al. (2000), emphasizing how the waking increases while the sleep (most specially REM) is reduced, in coincidence with development of the brain. The figure showing changes, with age, in total amounts of daily sleep, daily REM sleep, and in percentage of REM sleep. Note sharp diminution of REM sleep in the early years, in contrast the quantity of NREM sleep is undiminished for many years. Although total daily REM sleep falls steadily during life, the percentage rises slightly in adolescence and early adulthood. The color light grey represent of slow wave sleep, dark grey represent of REM stage and white represent of waking.

In human adults, sleep follows a particular cyclic pattern that was already described by Rechtschaffen and Kalesin. Basically, it can be divided into two main states: slow sleep (non-REM) and paradoxical sleep (REM). Non-REM sleep is characterized by the presence of
synchronized high amplitude EEG bands (delta bands). It is divided into four stages, the last of which is deep sleep stage. REM sleep is characterized by low-amplitude desynchronized EEG bands. The majority of sleep time is characterized as non-REM (about 75-85%), while the rest corresponds to REM (about 20-25%) and awake (about 5%). The duration of non-REM cycles is progressively reduced throughout the night (in particular, stages III and IV), whereas the duration of REM cycles is progressively increased.

Figure 1.9 displays an schematic representations (hypnograms) of the human sleep through the night in three ages group.

Sleep disorders can be broadly categorized into insomnias, hypersomnias, circadian rhythm disorders, sleep-breathing disorders, narcolepsy, parasomnias, and sleep movement disorders [140]. Studies show that at least 50% of patients with chronic pain report significant sleep disturbance [141-143]. Several clinical studies have consistently found that sleep disturbance is positively associated with pain severity [141, 144-146]. Katz and colleagues [147], found that hip impairment, back problems, and arthritis were significant risk factors for mild and severe insomnia, even after controlling for health habits, such as exercise, alcohol consumption, smoking, and sociodemographic variables. Morin and co-workers [146] classified 105 pain patients as either good or poor sleepers. They found that poor sleepers reported greater pain intensity and unpleasantness than good sleepers, despite there were no group differences on depression and anxiety. Moreover, studies controlling for the effects of psychological distress and/or behavioral factors suggest that sleep disturbance in chronic pain is not likely to be entirely explained by these factors [144,146,148].

Patients with fibromyalgia often have problems with sleep [149-152] and complaints about sleep-related daytime symptoms are quite frequent in patients with chronic pain. For example, it has been estimated that 54-70% of these patients have difficulty sleeping and insomnia, poor quality of sleep, numerous night awakenings, daytime sleepiness and fatigue [153]. This alterations in sleep architecture, and sleep efficiency, with reductions around of 72% and 76% [154,156]. Other studies also showed an increase in the number of awakenings and stage I of the non-REM sleep [156-159], reduced amount of slow band sleep (quantity of phases III and IV), sleep spindles and REM sleep percentages [157,159], as well as an increased number
Figure 1.9: Examples of sleep hypnogram in children, young adults and elderly. The x-axis represents the time of the record, the y-axis represents the sleep stages. The total amount of daily sleep gradually diminishes with age, sleep tends to be lighter, less deep sleep stage and a larger number of awakenings during the night. (Figure adapted from Broughton (1987) 139).

Experimental studies on the effects of painful stimuli on sleep have consistently shown pain to cause microarousal as measured by increased high frequency EEG activity in the alpha and...
beta ranges at the expense of slow frequency EEG activity \([166–168]\). For example, painful stimuli delivered to muscle and joint reduces delta band activity and enhances high frequency activity in the alpha, beta, and sigma ranges \([156, 167]\). These authors concluded that pain lightens sleep and diminishes the putative restorative effects of SWS. Similarly, thermal nociceptive stimuli produce cortical arousal without frank awakening \([166]\) and transient tachycardia in all sleep stages \([169]\).

Several key domains impacted by FM have been identified by Mease \textit{et al.} \([170]\), in an extension of the American College of Rheumatology (ACR) criteria \([5]\). They reported that sleep alterations (difficulty falling sleep, staying asleep or getting up in the morning) are one of the key outcome domains and one of the highest ranked domains (although not as highly ranked as pain). On the other hand, it has been suggested that the fear of pain and the common difficulties faced by chronic pain patients in the handling of the persistent pain in daily activities can lead to an important reduction of movement and, as a consequence, to the reinforcement of a vicious cycle of pain and disability \([121, 171]\). Affleck \textit{et al.} \([172]\) showed that a painful day is
followed by a night of poor sleep, and conversely, a night of poor sleep is followed by a significantly more painful symptoms in FM patients during the day. Additionally, this work suggested that changes in slow-wave sleep (SWS) corresponding to the period of GHRH hormone might influence the pain intensity experienced during the day. Moreover, musculoskeletal complaints were directly related to non-repaired sleep patterns and changes in the normal pattern of cortisol secretion [173]. In this sense, it has been suggested that low levels of serotonin could be linked to sleep problems [157].

The neurochemistry of sleep is a complex process involving several neurotransmitters. Moldofsky and Warsh [174] suggested that patients with FM may have a deficiency of serotonin, since that neurotransmitter affects both deep restorative sleep and pain perception. Serotonin is a recognized chemical mediator of deep sleep and of pain perception by both the thalamus and the peripheral nervous system [175]. It is known to alter the function of substance P [176, 177], particularly with reference to the interpretation of sensory stimuli. It has been reported that patients with FM have a higher serum concentration of serotonin autoantibodies not only when compared with healthy controls [178], but when compared with patients with other rheumatic diseases [179]. Also low serum levels of both serotonin [180, 181] and its precursor tryptophan [182] prevailing in patients with FM. Since serotonin affects not only restorative sleep but also pain perception [177, 183], low serum levels of these compounds might have be involved in some of the above-mentioned symptoms. Tryptophan and serotonin are melatonin precursors [177, 184], which have sleep-promoting properties [20, 177, 185]. Surveys show decreased levels of tryptophan and other amino acids, in addition to an increase of concentration of substance P, and endorphins acid 5-hydroxy-indoleacetic in the blood and cerebrospinal fluid of patients with FM [177]. Regarding dopamine, it has a warning effect and therefore a reduced serum levels seems to promote sleep [186]. Neurotransmitters such as acetylcholine, norepinephrine, histamine and several neuropeptides (such as substance P and corticotropin) also have an influential role in the promotion of wakefulness [187].

According to Diekelmann and Born [128] the specific neurochemical milieu of neurotransmitters and hormones differs strongly between slow wave sleep and REM sleep. Some of these neuromodulators contribute to memory consolidation. Interestingly, the most prominent con-
tributions to memory processing seem to originate within the cholinergic and monoaminergic brainstem systems that are also involved in sleep regulation. Noradrenergic and serotonergic activity reaches a minimum during REM sleep, but it is unclear whether this contributes to memory consolidation. The release from inhibitory noradrenergic activity during REM sleep enables the re-activation of procedural and emotional aspects of memory (in corticostriatal and amygdalar networks, respectively), thus supporting memory consolidation [188].

Sleep disorders are complex and highly variable phenotypes regulated by many genes, gene interactions, environment, and gene-environment interactions [67, 78, 79, 189, 190]. Evidence indicates that polymorphic variants in a number of candidate genes such as circadian locomotor output cycles kaput (CLOCK), period, adenosine deaminase, monoamine transporter families, and genes involved in the catabolism of monoamine neurotransmitters affect several characteristics of sleep-wake regulatory processes in both normal and pathological conditions [189, 190]. Although the COMT gene is not directly involved in the etiology or maintenance of a particular disease, it may have some critical effects on cognitive performance, as well as sleep architecture, sleep EEG, and vulnerability to sleep loss [186]. Drug response varies between subjects and may relate to body composition and weight, age and gender, but also to inter-individual differences in the constitutive pathways involved [186]. Genetic factors are now being recognized as key determinants of inter-individual and inter-ethnic differences in drug metabolism.

Another important issue is related to the quality of sleep in fibromyalgia. Nevertheless, there is nowadays some concerns regarding the study of quality of sleep in patients with chronic pain, probably because it is an elusive construct [191]. Despite sleep quality is a common criterion used to evaluate sleep, there is no authoritative definition of what it is and how it is being interpreted by the sleeper [192]. Researchers and clinicians have developed different methods to operationalize the construct [191]. Some use multicomponent questionnaires that ask information about sleep patterns, presence of sleep disturbances, and use of sleep medications to generate a global index of sleep quality, such as the self-report questionnaires - Pittsburgh Sleep Quality Index (PSQI) [193], Insomnia Severity Index (ISI) [194], Epworth Sleepiness Scale (ESS) [195] and Oviedo Sleep Questionnaires (OSQ) [196]. However, many of these subjective measures limit the assessment of sleep to pain-related sleep disturbance, and few
ones incorporate sleep diaries over a sufficient period of time to provide relevant diagnostic information in patients with chronic pain. A qualitative study recently conducted by Hamlee, Afolalu and Tang \[191\] demonstrated that sleep quality judgment is complex and involves retrospective decision making influenced by not only memories of the night but also how we feel and what we do during the day. This authors recruited six patients with fibromyalgia, five with back pain and six health individual, aged between 18 and 65 years in which participated in a telephone interview where they answered a series of questionnaires pain, sleep and fatigue and a series of subjective questions about sleep. They noted that sleep quality is not solely determined by nighttime parameters but also by daytime processes through retrospective judgment, and particularly, people with chronic pain see pain experience and sleep quality as two linked entities that influence their ability to engage in daytime activities as planned.

In the last years, there has been a significant growth of new sleep assessment tools for clinical practice. Thus, for instance, it is acknowledge that sleep is a complex process that involves fluctuations of autonomic functions such as blood pressure, temperature and brain activity. These fluctuations may change their properties through different sleep stages showing specific relationships among different systems \[197\]. Application of non-linear dynamics methods to physiological signals demonstrated that non-linear models are useful for understanding complex psychophysiological phenomena. The EEG signals are highly subjective and the information about the various states may appear at random in the time scale \[197,198\]. Therefore, non-linear EEG parameters are highly useful in diagnosing diseases. The non-linear analysis of sleep parameters can also provide relevant information such as those highlighted in studies with healthy people \[198,199\], schizophrenia \[200\] and Parkinson’s disease \[201\]. However, there is no information about non-linear brain activity parameters during sleep in patients with fibromyalgia related to the COMT enzyme.
1.6 Emotion and fibromyalgia

Emotion is a subjective experience that involves the whole person, mind and body. It is a complex reaction triggered by stimuli or thoughts, which involves personal feelings, and behavioral, cognitive, motor and physiological responses experience [202]. Emotion is involved in all reactions to stimuli that, by their intensity or relevance, move the organism to some kind of action [203]. Basically, emotions are psychophysiological phenomena of short duration and represent efficient ways of adaptation to requirements of the environment [204]. From a psychological point of view, emotions are able to alter attention and perception, as well as to facilitate learning and to activate memory. From a physiological point of view, an emotion quickly organizes responses from different biological systems, such as facial expression, muscle tone, voice, and responses from endocrine and nervous systems, in order to achieve an optimization of the internal resources for an effective response [205]. In this sense, affective experience is believed to emanate from evolutionarily old and centrally mediated motivational systems. The defensive system is activated by threatening stimuli and associated with the subjective experience of negative affect, whereas the appetitive system is activated by reward stimuli and associated with the subjective experience of positive affect [206].

Several studies have shown that psychological factors such as depression, emotional distress, negative affective mood, vulnerability to stress, catastrophizing and hypervigilance as well as environmental factors could contribute to the heightened pain sensitivity observed in patients with chronic pain like fibromyalgia [207, 208]. Although little is still known about the influence of psychosocial variables on processing of painful and non-painful information among FM patients, there exist empirical data emphasizing the role of emotions and psychological stressors in the origin and maintenance of FM [207]. In this sense, it seems that anxiety and depression are the most common negative emotions linked to chronic pain diseases [209]. Anxiety eliciting stimuli may increase pain perception and pain-irrelevant anxiety will decrease the subjective experience of pain [210]. In a similar way, several studies have revealed a high prevalence of depression in FM [207]. Moreover, it has been repeatedly found that depression may exacerbate or perpetuate the experience of pain after chronic pain has been estab-
lished \[57,208,211,212\]. Similarly, common depressive symptoms such as apathy and sleep disorder are also very frequent in patients with chronic pain and fibromyalgia. In particular, sleep disturbances in FM and depression appear to be very similar with a typical alpha EEG sleep anomaly consisting of alpha wave intrusion into predominantly delta wave sleep \[152\].

It has been also suggested that several symptoms of fibromyalgia such as depression, anxiety, sleep problems and fatigue, as well as pain medication could contribute to enhanced pain sensitivity \[51,208\]. However, the relationship between these symptoms and pain sensitivity in FM is still far from clear. Thus, although some studies have provided direct evidence about the role of depression, anxiety and fatigue in pain perception \[208,213-215\], other authors have suggested that the close relationship between negative affect and pain could be mediated by heightened attention to bodily signals \[207\]. Moreover, the influence of negative affect on pain perception could be modulated by third variables such as pain-related fear or catastrophizing. In this sense, it is known that fear of pain may lead to avoidance behavior as a maladaptive response with negative consequences like further weakening of the musculoskeletal system, disability in daily life, social isolation and depression \[216\]. Thus, it seems plausible that chronic pain patients could be caught in a vicious circle of augmenting avoidance, disability and pain over time. Additionally, pain-related fear may induce misinterpretations of bodily sensations in a catastrophic manner (wrong beliefs and expectations about pain) thus increasing pain-related anxiety and contributing to the persistence of pain \[216\].

Nevertheless, a very promising perspective for the experimental study of the influence of emotion on pain perception is provided by the so-called priming motivational hypothesis \[217\]. According to this hypothesis, emotion is governed by two opponent motivational systems (appetitive/approach and aversive/avoidance systems) and pain could be modulated depending on which motivational system is currently activated. Thus, negative emotions would activate the aversive motivational system thus leading to an enhancement of pain perception, whereas positive emotions would lead to an activation of the appetitive motivational system and to pain inhibition. Consequently, enhanced pain sensitivity in FM patients could be due to either high levels of negative affect or low levels of positive affect, especially during stressful events \[16,50,218,222\]. In this sense, previous studies have indicated that clinical pain and
brain processing of nonpainful somatosensory information were significantly enhanced in FM when innocuous somatic stimuli were presented together with aversive pictures [50, 219]. It has also been found that patients with FM displayed increased brain responses to faces expressing pain and anger, whereas pain-free controls were characterized by enhanced brain responses to happy faces [220]. Moreover, patients with FM seem to elicit more increased subjective ratings of unpleasantness and corrugator EMG activity in response to aversive pictures than healthy controls, whereas no group differences appeared in response to pleasant pictures [223].

The activation of these two motivational systems has been also analyzed by eliciting startle reflexes during the simultaneous induction of positive and negative affective states. Basically, it has been demonstrated that viewing slides depicting scenes of negative emotional valence potentiates the amplitude of startle reflexes, whereas viewing pleasant pictures inhibits these reflexes [224]. Nevertheless, although the affective modulation of startle reflexes provides experimental evidence for an abnormal activation of the appetitive and aversive motivational system in clinical populations such as FM, research is still scarce and contradictory results have been found. Thus, some studies have shown that patients with FM and healthy controls did not differ on startle eyeblink reflex elicited by acoustic or somatosensory probes [16, 50], whereas recent studies have observed a reduced affective modulation in patients with FM as compared with healthy controls [218, 225]. Although the latter finding may suggest an inability to engage modulatory affective systems during the processing of startle probes in FM, the adequacy of startle probes to mimic clinical pain or the appropriateness of affective pictures to evoke sustained and personally relevant mood states in patients with FM cannot be ruled out [16]. In addition, further studies have demonstrated that FM patients have also deficits in the processing of positive or pleasant stimuli, suggesting a significant inability to mobilize sufficient affective resources to neutralize the experience of pain and the associated negative affect [226]. Thus, it could be that FM patients were more susceptible to the effects of negative emotions due to deficits in positive affect and the use of more avoidance coping strategies (e.g., resignation, passivity, social withdrawal) [207]. Moreover, it has been argued that stress-related loss of positive affect might be responsible for slow recovery from stressful events, increased stress-related fatigue, and central sensitization in FM [226]. In summary, all these
findings demonstrate that affective disturbances in FM may be reflecting a significant emotional dysregulation which could be elicited as consequence of an enhanced activation of the aversive motivational system, a reduced activation of the appetitive motivational system or both.

As it was mentioned previously, there is increasing neurobiological evidence that the pathogenesis of FM is at least partially related to an abnormal brain processing of nociceptive stimuli [227]. Increased temporal summation responses to nociceptive stimuli, expansion of nociceptive receptive fields, hyperalgesia, and altered functioning of nociceptive modulating system and pain-related brain areas have been frequently reported [228–234]. Moreover, it is assumed that these peripheral and central sensitization events may also lead to an exaggerated reorganization of limbic circuitries (affective pain processing) and prefrontal cortices (pain evaluation) providing the necessary learning strategies for the maintenance of chronic pain over time [235]. In particular, central sensitization of nociceptors and brain regions involved in chronic pain could be at least partially related to a malfunction of the monoamine system [236, 237]. Enzymes like catechol-O-methyl-transferase (COMT) are involved in the metabolization of endogenous substrates, such as noradrenaline, dopamine, histamine, and aminoacids [227]. The polymorphism of the COMT gene that codes for the substitution of valine (val) by methionine (met) at codon 158 (val158met) has been associated with a fourfold reduction in the activity of the enzyme [227]. Moreover, typical pharmacological treatments in FM, including painkillers (such as nonsteroidal anti-inflammatory drugs) [14, 15, 238, 239], antidepressants (such as amitriptyline and nortriptyline) [240], anxiolytics (benzodiazepines) [241], antiepileptic drugs (gabapentin and pregabalin) [242, 243], and sleep drugs [244, 245] (see Annex B for medications most commonly used by patients with FM), point towards a disruption of neurotransmitter balance. Nevertheless, there is no information about how negative mood state and sleep disturbances are modulated by neurotransmitter systems involved in pain processing. Furthermore, there is no information of how genetic variants of the COMT gene could influence affective processing in FM patients.
Objective and Hypothesis

The theoretical introduction of the present thesis has provided an overview of the evidence supporting the study of in patients with fibromyalgia. Moreover, it has shown that patients with fibromyalgia complain of sleep problems quite often and that these sleep deprivation problems might be associated with an enhanced pain sensitivity and the presence of affective disorders. Thus, the difficulty in dealing with pain can lead to a significant reduction in physical activity and consequently to a vicious cycle of pain, sleep problems, affective disorders and disability.

In an attempt to provide further information on these issues, this thesis examines the effects of chronic pain on gait and balance (study 1), sleep quality (study 2) and response to affective pictures (study 3) by comparing fibromyalgia patients and healthy controls (study 1) and two groups of fibromyalgia patients based on a functional polymorphism of the COMT gene (studies 2 and 3). In particular, the following general aims are addressed:

Aim 1: To analyze deficits in gait and balance in patients with fibromyalgia (FM)

Although many studies have demonstrated that patients with fibromyalgia displayed alterations in motor function and a high risk of falls, there are no studies analyzing the spatiotemporal patterns of micro-movements that are produced during the gait and balance cycle, as well as
the relationship of these changes with complaints of pain. Accordingly, study 1 of this thesis was carried out to address the two following specific aims:

1. To measure gait and balance performance in patients with fibromyalgia by using video records and a computer vision software for movement analysis.

2. To apply nonlinear analysis to evaluate the dynamic of these balance fluctuations in pain-free controls and FM patients.

Initially, the present thesis was aimed to compare motor function in FM patients by grouping participants according to the genotypes of the val158met polymorphism of the COMT gene. For this purpose, it was hypothesized that FM patients with low COMT activity (met homozygotes) would display impaired gait and balance than FM patients with high COMT activity (val carriers). Nevertheless, this part of the thesis work is not reported here due to technical difficulties in genetic determination which led to a significant reduction of the sample size (4 patients with low COMT activity and 12 patients with high COMT activity). In any case, no statistically significant results were observed in this small sample.

**Aim 2: To compare the sleep macrostructure in fibromyalgia patients with high and low COMT activity**

Previous literature on fibromyalgia has shown that sleep disorders are a prevalent symptom and that there is a significant relationship between altered sleep and pain. Currently, the use of nonlinear analysis techniques, such as multiscale entropy, has provided new tools for exploring sleep architecture in neurological patients with Parkinson and Alzheimer diseases. In addition, there are no known studies that compare sleep patterns with EEG data among patients with fibromyalgia associated with high and low activity of the COMT enzyme. Accordingly, study 2 of this thesis was carried out to address the two following specific aims:

1. To characterize the sleep architecture of patients with fibromyalgia who have high and low COMT activity enzyme, by performing polysomnographic recordings.
2. Characterize and compare the dynamics and complexity of the sleep EEG data signal by using linear and non-linear analysis tools between FM patients with different COMT haplotypes during polysomnography record.

It was hypothesized that patients with higher activity of the COMT enzyme would have better sleep quality than those with low activity. Moreover, it was hypothesized that the EEG entropy would provide information on the pathophysiology underlying FM associated to genetic haplotypes of the COMT enzyme.

Aim 3: To compare the affective processing in fibromyalgia patients with high and low COMT activity

According to previous literature, a genetic variation in the catechol-O-methyltransferase (COMT), such as the val158met polymorphism, may influence performance in cognitive and emotional tasks. Polymorphisms in the gene encoding for the COMT enzyme are also associated with pain sensitivity in healthy controls and fibromyalgia patients. Taking into account that FM patients display an altered brain processing of affective stimuli, it should be clarified if the val158met polymorphism of the COMT gene could be associated with these alterations. Accordingly, study 3 of this thesis was carried out to address the two following specific aims:

1. To compare linear and nonlinear parameters of EEG signals between FM patients with different COMT haplotypes during a dual-task specifically designed to assess the affective modulation of brain processing.

It was hypothesized that FM patients with low COMT would display reduced entropy as compared to FM patients with high COMT, as well as reduced EEG alpha power and evoked responses to non-painful body information under negative mood conditions.
**Methodological aspects of the research**

The following methodological aspects are discussed in the section: ethical issues in research and materials and methods used in the experimental part of this work. The next table summarizes the sample and experimental methodology used in each of the studies.
Table 3.1: Table with summary of methodological approaches for each study described in this thesis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Biomechanics Study</th>
<th>Sleep Study</th>
<th>Emotions Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 FM and 16 Pain-free women</td>
<td>Biomechanical gait and balance analysis</td>
<td>13 FM high COMT and 11 FM low COMT activity</td>
<td>Presentation of affective pictures (pleasant, neutral and unpleasant). Rating valence and activation caused by each image.</td>
</tr>
<tr>
<td>13 FM high COMT and 11 FM low COMT activity</td>
<td>Sleep Polysomnographic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 FM high COMT and 10 FM low activity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experimental paradigm</th>
<th>Technical measure</th>
<th>Type of analysis</th>
<th>General parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomechanical gait and balance analysis</td>
<td>Motion analysis tool with Cv-Mob</td>
<td>Assessment questionnaires and physical tests; Hurst exponent;</td>
<td>The Hurst exponent (H) was obtained by fitting a power-law curve (fractal Brownian motion model) to the scaling function between the deviation of the time series (position in X-axis) and different time window sizes ([246, 247]).</td>
</tr>
<tr>
<td>Sleep Polysomnographic</td>
<td>PSG and EEG Sleep record</td>
<td>Assessment questionnaires; and polysonomographic variables; PSA and MSE analysis of EEG signals.</td>
<td>Segments for PSA and MSE are composed by the relative proportion of each wave-length along each 30 seconds subset of EEG data analyzed (frequencies of signal for each 5 seconds window of data, with an overlap of 2.5 seconds between subsequent windows). For PSA: the frequency of EEG records of electrodes C3-A2, and C4-A1 for the 4 first frequencies bands: (\delta (0.5 – 4 \text{ Hz}), \theta (4.5 – 8 \text{ Hz}), \alpha (8.5 – 12 \text{ Hz})) and (\beta (12.5 – 30 \text{ Hz})) was analyzed. For MSE, 20 scales were analyzed.</td>
</tr>
<tr>
<td>Presentation of affective pictures (pleasant, neutral and unpleasant). Rating valence and activation caused by each image.</td>
<td>EEG record</td>
<td>Assessment questionnaires; PSA and MSE analysis of EEG signals.</td>
<td>Segments from -100 to 900ms: PSA (\delta (2 – 3.9 \text{ Hz}), \theta_1 (4 – 5.9 \text{ Hz}), \theta_2 (6 – 7.9 \text{ Hz}), \alpha_1 (8 – 9.9 \text{ Hz}), \alpha_2 (10 – 11.9 \text{ Hz}), \beta_1 (12 – 17.9 \text{ Hz})) and (\beta_2 (18 – 22 \text{ Hz})). For MSE (5 scales factors (1, 5, 10, 15, 20). For ERP, the components N100 (100 – 200 ms, Cz), P200 (200 – 300 ms, Cz), and P300 (300 – 450 ms, Pz) were analyzed.</td>
</tr>
</tbody>
</table>

3.1 Ethical Issues

According to the resolution of the Boletín Oficial del Estado number 159, Law 14/2007, of July 4, 2007, for Biomedical Research, research involving human subjects has ethical characteristics and must offer the participating individuals anonymity and respect regarding their cultural, ethical, social, moral and religious values, as well as their habits and customs, minimizing any risk that is inherent in the research process \([248]\).

This study followed the principles of the above-mentioned resolution, which was submitted to the Ethics Committee and Clinical Research of the Balearic Islands with the reference number IB-1284/09.

Participants were informed about all aspects of the research, authorizing the dissemination of the results obtained by signing a consent form.
3.2 Materials and methods common to all studies

Data collection was performed at the University of the Balearic Islands and in two Fibromyalgia Associations between April 2010 to March 2013.

In this section, we briefly describe the techniques of recording and data analysis used for all experiments in this thesis.

3.2.1 Participants

The present thesis is divided into three studies. In the first study, a sample of 26 FM and 16 healthy control subjects were compared for differences in movement pattern. The comparison between participants with low vs. high COMT activity could not performed in this study due to the small sample of FM patients that could be genotyped (4 with low, 16 with high COMT activity, and 6 undetermined). A second and different sample of FM patients participated in studies 2 and 3. In both studies, the sample was composed of 26 FM patients which were genotyped and classified into low and high COMT activity subgroups. This resulted in 2 subgroups: 11 FM patients with low COMT activity and 13 FM patients with high COMT activity. Data from two FM patients could not be genotyped and were not included in these studies.

The groups consisted only of women due to the higher prevalence of FM in females \([249]\) and to avoid possible differences related to gender in physiological variables analyzed in different jobs \([250]\). The two groups of FM patients were diagnosed by specialists following the 1990 criteria of the American College of Rheumatology \([251]\). The healthy volunteers did not show any symptoms related to pain.

Exclusion criteria for all three studies were: presence of cardiovascular disorders, neurological disorders, severe organic diseases (i.e. cancer), mental disorders as a primary disorder (i.e. psychotic disorders, bipolar disorders or serious personality disorder), abuse disorders or substance dependence.
3.2.2 Genetic markers and haplotypes

The genetic data presented in this thesis was previously analyzed and published by Martínez et al. [13]. In all studies, polymorphisms and haplotypes of the COMT gene were analyzed. Nevertheless, genetic data were reported only in studies 2 and 3. Due to the small sample size (the low COMT activity subgroup was composed of less than 5 participants), statistical comparisons could not be performed in study 1 and only the comparison between FM participants and healthy controls was reported. In table 3.2, genetic markers and methodologies used in each case are outlined.

Table 3.2: Study of markers and genetic haplotypes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>DNA sample</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNPs functional</td>
<td>COMT (val158met, and no-functional rs6269, rs4633, and rs4818)</td>
<td>• PCR (real-time), determining profiles of fluorescence (LightCycler® 480).</td>
</tr>
<tr>
<td></td>
<td>buccal cells</td>
<td>• Sequencing (Big Dye® Terminator Cycle Sequencing Kit v3.1 and ABI PRISM® 3100 Genetic Analyzer),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• RFLP method</td>
</tr>
<tr>
<td>Haplotypes</td>
<td>COMT</td>
<td>Pairwise linkage disequilibrium estimations (LD) between SNPs and haplotypes reconstruction were performed with Haploview software 4.2.</td>
</tr>
</tbody>
</table>

See section “Genotyping” from the paper by Martínez and co-workers [13].

3.2.3 Clinical Interview and questionnaires

In the three studies of this thesis, an semi-standardized interview was conducted to characterize the participants from a socio-demographic point of view and to assess general health and obtain information regarding medication intake. In addition, in order to evaluate the clinical, emotional, and biopsychosocial factors, sleep patterns, as well as development and maintenance of pain, different questionnaires, tasks and scales were used (see Table 3.3). The following section gives a brief explanation of these self-report measures.
3.2.3.1 Fibromyalgia Impact Questionnaire (FIQ)

The FIQ is a self-administered instrument composed of 10 questions [252]. The first question contains 11 items related to the ability to perform daily routines and activities - each question is rated on a 4 point Likert type scale. Items 2 and 3 ask the patient to mark the number of days they felt well and the number of days they were unable to work (including housework) because of fibromyalgia symptoms. Items 4 through 10 are horizontal linear scales marked in 10 increments on which the patient rates work difficulty, pain, fatigue, morning tiredness, stiffness, anxiety and depression.

3.2.3.2 Hospital Anxiety and Depression Scale (HAD)

The HAD is a brief questionnaire (containing 14 items), and was originally designed to detect emotional disturbances in non-psychiatric patients treated at hospital clinics. The scale measures both anxiety and depression on two separate subscales, each containing 7 items. A score varying from 0 to 3 is assigned to each item. The total score varies between 0 and 21. For each of the HAD subscales, a high score corresponds to a greater severity of symptoms [253].

3.2.3.3 Health and Quality of Life Questionnaires

• Espiditest: This questionnaire was developed by Zambon Institute [254] to assess quality of life in patients experiencing acute and chronic pain and validated in a sample of 1390 patients. The questionnaire consists of 21 items and total sum scores range from 18 (high quality of life) to 99 (low quality of life).

• SF-36 Health Surveys: The Spanish version consists of 36 items that detect both positive and negative health states, which consider eight dimensions: physical functioning (10 items), social function (2 items), role physical (4 items), role emotional (3 items) mental health (5 items), vitality (4 items), bodily pain (2 items) and general health (6 items). Response options are Likert scales that assess the intensity or frequency, ranging between 3 and 6 depending on the item. The score / value of each item is encoded and transformed into a scale that has a route from 0 (the worst state for that dimension) to 1 (best state).
It is an instrument for measuring the overall health status and outcomes of medical interventions. It can be combined with specific tools to evaluate the patient’s quality of life. It can be self-administered or completed by an interviewer.

There are twelve health questions with three, five or six possible answers that are quantified from zero to one hundred: the possible scores for the three alternative questions are 0, 50, 100; for the five alternative questions, they are 0, 25, 50, 75, 100; and for the 6 alternative questions are 0, 20, 40, 60, 80, 100. Scores grouped into eight dimensions, the most favorable responses have higher scores: physical functioning, role limitations due to physical health problems, bodily pain, social functioning, mental health, emotional, role limitations due to emotional problems, vitality, energy or fatigue and general health perception.

3.2.3.4 Pain Questionnaires

- **McGill Pain Questionnaire** [257]: It consists primarily of 3 major classes of word descriptors — sensory, affective and evaluative — that are used by patients to specify subjective pain experience. It also contains an intensity scale and other items to determine the properties of pain experience. The 3 major measures are obtained: (1) a pain rating index, based on two types of numerical values that can be assigned to each word descriptor, (2) the total number of words chosen for the description of pain; and (3) the current pain intensity based on a 1–5 intensity scale.

- **West Haven-Yale Multidimensional Pain Inventory (WHYMPI)** [258]: To assess the impact of pain on their lives through 5 subscales (pain severity, pain interference, affective distress, social support, life control), the self-perceived responses of others to patients’ own pain behavior (solicitous, punishing, and distracting responses), and the extent to which patients participate in common daily activities (household chores, social and recreational activities).
Table 3.3: References for the Instruments and Questionnaires used along this thesis

<table>
<thead>
<tr>
<th>References</th>
<th>Instruments and Questionnaires</th>
<th>Aspects Evaluated</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lang et al., 1980</td>
<td>Self-Assessment Manikin (SAM)</td>
<td>Nonverbal pictographic measures: Valencia (pleasure-displeasure), and Arousal (Calm-On)</td>
<td>3</td>
</tr>
<tr>
<td>Bennet et al., 2005</td>
<td>Fibromyalgia Impact Questionnaires (FIQ)</td>
<td>Functional impact caused by fibromyalgia</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Kerns et al., 1985</td>
<td>West-Haven-Yale Multidimensional Pain Inventory (Whympi)</td>
<td>Section I: intensity of pain and its impact on the patient’s life. Section II: Patient perception on the responses of those close to their pain behaviors. Section III: Participation of patients in activities.</td>
<td>2, 3</td>
</tr>
<tr>
<td>Melzack, 1975</td>
<td>McGill</td>
<td>This questionnaire provides quantitative information that can be treated statistically, and is sufficiently sensitive to detect differences among different methods to relieve pain. The McGill Pain Questionnaire consists primarily of 3 major classes of word descriptors – sensory, affective and evaluative – that are used by patients to specify subjective pain experience.</td>
<td>2, 3</td>
</tr>
<tr>
<td>Ware et al., 1992; Alonso et al., 1995</td>
<td>SF-36 Health Surveys</td>
<td>Measure the same eight health domains, and each survey provide psychometrically based physical component summary (PCS) and mental component summary (MCS) scores.</td>
<td>2, 3</td>
</tr>
<tr>
<td>Zambon Institute</td>
<td>Espiditest</td>
<td>Test quality of life of patients with pain adapted to Spanish reality</td>
<td>2, 3</td>
</tr>
<tr>
<td>Zigmond and Snaith, 1983</td>
<td>Hospital Anxiety and Depression Scale (HAD)</td>
<td>Evaluates anxiety and depression, has been widely used to evaluate mood disorders in patients with physical disorders.</td>
<td>2, 3</td>
</tr>
<tr>
<td>Buysse et al., 1989</td>
<td>Pittsburgh Sleep Quality Index (PSQI)</td>
<td>It is an effective instrument used to measure the quality and patterns of sleep in the older adult. It differentiates “poor” from “good” sleep by measuring seven domains: habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction over the last month subjective sleep quality, sleep latency, sleep duration.</td>
<td>2, 3</td>
</tr>
<tr>
<td>Garcia et al. (1998, 2000)</td>
<td>OSQ</td>
<td>It is a questionnaire hetero-administered, and serves as a diagnostic aid for disorders such as insomnia and hypersomnia sleep because of the DSM-IV and ICD-10 criteria.</td>
<td>2, 3</td>
</tr>
<tr>
<td>Enright, 2003</td>
<td>Six-minute walking test (6MWT)</td>
<td>There is a full range of tests that you could perform to assess a patient’s functional capacity.</td>
<td>1</td>
</tr>
<tr>
<td>Shumway-Cook et al., 2000</td>
<td>Timed up and go task (TUG)</td>
<td>It is a test of balance that is commonly used to examine functional mobility</td>
<td>1</td>
</tr>
<tr>
<td>Khasnis et al., 2003</td>
<td>Modified version of the Romberg’s balance test</td>
<td>Romberg’s test is a commonly performed test during neurological examination to evaluate the integrity of dorsal columns of the spinal cord. The importance of the test increased as the basis of abnormal findings was understood. Gradually it came to be used for excluding dorsal column abnormalities in patients presenting with unsteadiness of gait.</td>
<td>1</td>
</tr>
<tr>
<td>Berg et al., 1991</td>
<td>Berg Balance Scale</td>
<td>The Berg Balance Scale is directly related to other tests of balance and mobility, with a test reliability of 98%.</td>
<td>1</td>
</tr>
</tbody>
</table>

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3.3 Specific Materials and Methods

Given the multidisciplinary characteristic of this thesis, we will detail in the following sections the methodological aspects of each of our different approaches: biomechanical, sleep and emotional studies.

3.3.1 Biomechanical study

In this section the motor tasks performed by the patients are described.

3.3.1.1 Motor Tasks

Gait and balance parameters were obtained in FM patients and pain-free controls by using the following functional tasks:

- **Berg Balance Scale** [259]: This is a performance-based assessment tool developed to measure balance during functional activities. The test is often used for patients exhibiting reduced functioning, self-reporting loss of balance, or having unexplained falls [259]. The scale consists of 14 functional tasks (e.g. sitting unsupported, change of sitting to standing position and vice-versa, standing with both feet together, standing on one leg, turning 360 degrees) with scores ranging from 0 (unable to perform) to 4 (normal performance). Total scores range from 0 (severely impaired balance) to 56 (excellent balance). Scores below 46 are good predictors for the occurrence of multiple falls [260].

- **Six-minute walking test (6MWT)** [261]: This is a functional test in which subjects are instructed to walk for 6 minutes as quickly as possible. It has been used to assess individuals with mobility deficits [262] and FM patients [263–265]. The 6MWT is considered a good indicator of exercise tolerance and aerobic capacity, since it causes a physiological stress without demanding maximum aerobic capacity [264]. Ratings of perceived exertion were obtained after the 6MWT by using the Borg Effort Scale [266], a 15-point scale ranging from 4 (complete lack of effort) to 20 (maximum effort or exhaustion).
• Timed up and go task (TUG): This is a standardized test for assessment of functional mobility. It is performed by using an ordinary armchair (45 cm in height) and a stopwatch. Subjects are seated with their back against the chair and instructed to stand up, walk three meters, turn around, walk back to the chair and sit down at an ordinary comfortable speed [267] (Figure 3.1).

![Figure 3.1: Timed up and go test (TUG). The subject has to rise from the chair, walk a distance of 3 meters turn around and sit down again.](image)

The stopwatch is started on the word “Go” and stopped as the subject sits down. The TUG time is measured in seconds, ranging from 5.4 to 40.8 seconds in healthy individuals [268]. TUG time appears to be associated with gait speed, balance, functional level and the ability to go out [267]. After the TUG, overall subjective perception of physical effort was measured by using the Borg Effort Scale [266].

• Modified version of the Romberg’s balance test: This is an objective measure of patient’s standing balance [269]. Participants were asked to remain in orthostatic position with their feet in parallel and separated, arms extended along the body and with eyes closed during one minute. The test is based on the fact that balance comes from the combination of several neurological systems (proprioception, vestibular input and vision) and that
maintaining balance while standing in the stationary position with closed eyes should rely on intact sensorimotor integration centers and motor pathways. Thus, the essential feature of the test is that the patient should become unsteady with eyes closed. In the present study, we analyzed the oscillatory body movements during the test performance.

The test was repeated twice and motion on the frontal and sagittal planes was captured by using a digital video camera at 30 frames per second (Casio Exilim EX-FS10). For motion detection analysis, a plumb line hanging on the ceiling at a distance of 3 meters was used as a reference. Participants were also asked to wear a cap with sticks positioned in the vertical and horizontal planes. For the analysis of body sway in the medial-lateral direction, sticks were aligned with the anatomical position of the glabella of the frontal bone. For the analysis of body sway in the anterior-posterior direction, sticks were aligned with the anatomical position of the pinna (tragus).

- **Gait task**: Subjects were instructed to walk on a 3 meters carpet at their normal walking speed, without shoes and with flexed arms positioned on the abdomen. Optical markers were attached to the following body positions: anterior superior iliac spine, posterior superior iliac spine, area between the lateral condyle of the femur and the fibular head, bottom of the patella, lateral and inner malleolus, heel (between the first and second metatarsal), and on the tip of the hallux. Subject’s motion was digitally recorded with a video camera at 210 frames per second (Casio Exilim EX-FS10). The camera was positioned at a distance of 3 meters from the carpet to visualize changes in position, velocity and acceleration of anatomical points along the x-axis. Gait velocity (cm/second), walking duration (seconds), cadence (number of steps/minute), percentage of time in the two phases of the gait cycle (stance and swing phase), and percentage of time with single and double support were computed.
Figure 3.2: Anatomical markers used in the biomechanical study
3.3.2 Methods of studying body activity - CvMob

The parameters of gait (Figure 3.3) and balance (Figure 3.4) were analyzed by a free software program that uses computer vision techniques to analyze the flow of pixels in videos, for location and tracking of image patterns.

Figure 3.3: Screen of the program CvMob during a gait test. The green dot marks the speed gait and the blue stretchers mark the step size during the gait cycle. The graphs represent the speed and acceleration of the point during the gait test.

The CvMob is a gauge of mechanical parameters of motion (trajectory, velocity and acceleration) based on computer vision algorithms applied to videos of moving objects. It was developed in the Physics Institute of the Federal University of Bahia, in Brazil (UFBA/Brazil). It was implemented in C++, using the QT4 framework and computer vision library OPENCV [270].

It has been compared with other technologies used to evaluate gait and balance performance. Balance performance data was collected using the Vicon motion capture system (10 cameras, 120 Hz) synchronized with Advanced Mechanical Technologies - force platforms (AMTI) force plate (1800 Hz) used to collect ground reaction forces. For CvMob measures, a conventional HD camera was used (1024p, 30 Hz). The trajectory of a marker positioned on the right shoulder was measured using both Vicon and CvMob and compared with the center of pressure - COP trajectory, collected by the AMTI force plate. The quiet standing eyes closed balance task was conducted while subjects stood on the force platform for 40 s. A cor-
relation was found in the anterior-posterior direction ($r = 0.927$) and medial-lateral direction ($r = 0.873$) between CvMob and Vicon, as well as between the anterior-posterior direction ($r = 0.883$) and medial-lateral direction ($r = 0.685$) between CvMob and the force plate (see figure 3.5, unpublished data from our lab).

Gait performance was analyzed by comparing Vicon system cameras and CvMob. A sample composed of 56 healthy individuals who walked on a 9-meter long walkway and were simultaneously filmed by CvMob and Vicon system cameras was used. Linear trajectories and angular measurements were compared to validate the CvMob system, and inter and intrarater findings of the same measurements were used to determine reliability. A strong correlation ($r = 0.988$) of the linear trajectories between systems and inter and intrarater analysis was found [271].

These tests prove that CvMob is a reliable tool for the analysis of linear motion and lengths in two-dimensional evaluations of human movement, as well as gait and balance characteristics. The CvMob is available at [https://sites.google.com/site/CvMobufba/](https://sites.google.com/site/CvMobufba/)
3.3.3 Sleep study

3.3.3.1 Sleep Questionnaires

- **Pittsburgh Sleep Quality Index (PSQI)**: The Pittsburgh Sleep Quality Index (PSQI) is a self-reported measure of sleep quality and sleep disorders [272]. The questionnaire consists of 10 items scored between 0 and 3. The overall score is determined by the sum of the single component scores. Higher scores indicate worse sleep quality.

- **Oviedo Sleep Questionnaire (OSQ)** [196, 273]: The OSQ is a questionnaire to assess insomnia, hypersomnia and sleep disorders [273]. The questionnaire consists of 15 items grouped into the following subscales: subjective sleep satisfaction (1 item), insomnia (9 items), and hypersomnia (3 items). The insomnia subscale also examines several dimensions of sleep (sleep latency, duration, efficiency and daytime dysfunction) and provides information about its severity. The two other subscales provide information on the use of any help to sleep or the presence of adverse events during sleep. Overall raw scores range between 9 (no sleep disorder) and 45 (severe sleep disorder).
3.3.4 Emotion study

At the end of this experiment, these patients completed the Self-Assessment Manikin (SAM) [274] to rate pleasantness and arousal elicited by affective pictures. This instrument consists of two sets of humanoid figures representing the dimensions of valence (pleasantness) and arousal. Each rating scale includes nine levels of intensity, ranging from a smiling to a frowning figure for valence and from an apparently agitated to a sleepy-looking figure for arousal [275]. Participants were instructed to assess how they felt while viewing each picture by using these scales.

Emotion-induction task

Participants were asked to sit comfortably when viewing affective pictures from a self-perspective. Sixty-six digital pictures were used from three picture contents (pleasant, unpleasant, and neutral) (22 pictures per content) selected from the International Affective Picture System (IAPS) [206,276,277]. The IAPS constitutes a standardized and exhaustively investigated set of affective pictures containing more than 600 items [50].

The presentation of the affective pictures was accompanied by a startle sound (white noise, 105 dB, 50 ms).

3.3.5 Methods of studying brain activity: EEG

EEG was used as a technique in studies 2 and 3 of this thesis to record brain activity. The EEG records the difference in potential power produced by the pyramidal neurons of the cerebral cortex [278]. Modern techniques for locating sources of brain activity allow to map the brain in physiological and pathophysiological processes in a noninvasive manner, using mathematical models introduced by physiological and anatomical assumptions about the nature and behavior of brain sources [279].

For the polysomnography recording in the study 2 (Sleep), two electrodes were located at C3 and C4 positions according to the International 10-20 System [280,281]. In addition, a bipolar channel for the left and right electrooculogram (EOG), and two bipolar channels for the electromyogram (EMG), one for the submentonian muscle, and the other for the tibialis ante-
rior muscle were used. Respiratory parameters included a channel for abdominal and thoracic respiratory movements and a bipolar channel for electrocardiographic recording (See Figure 3.6). The settings for the EEG recording were 0.5 Hz for the high-pass filter, 50 Hz for the low-pass filter, time constant of 0.4 seconds, and sensitivity of 50 $\mu$V/cm.

Figure 3.6: The electrode montage used in the sleep study. The pink and red colors, represents two central electrodes and referential mastoids (C3A2 and C4A1); the blue color, represents EOG; the yellow color, represents EMG and the light and dark green colors, represents the EKG, used in this study.
For the study of emotions (Study 3), 32 EEG electrodes placed according to the International 10-20 System and two electrodes for the recording of eye movements (EOG) were used (Figure 3.7). The EEG signal was sampled at 1000 Hz, and filtered with a bandpass filter (0.05 to 70 Hz) and a notch filter (50 Hz). All impedances were kept below 10 Ω during recording. Signals were edited offline for visual inspection. In addition, signals were segmented, baseline corrected, digitally filtered (low pass filter at 30 Hz) and corrected from artifacts.

**Figure 3.7: Electrode locations used during the emotion study.** The light grey with blue color represents the 32 electrodes used in EEG recording. The light grey with red color represents the ground.
This section shows the major findings from the three studies of the thesis. The study 1 was submitted to the journal Frontiers in Neuroscience and it is currently under revision. The last available version of this paper is included. The findings from studies 2 and 3 are presented following the format of standard scientific journals and they are intended to be submitted after final approval of the rest of co-authors (Pedro Montoya Antoni Gamundi).

Each study can be read separately and independently of the other; they are, however, united by their analyses of relevant symptoms in patients with fibromyalgia.
4.1 Study 1: Altered functional performance in patients with fibromyalgia

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*This study is under review in the journal Frontiers of Human Neuroscience (2016-09-19) (see Annex A).
Abstract

Fibromyalgia is a common chronic pain condition that exerts a considerable impact on patients’ daily activities and quality of life. Objectives: The main objective of the present study was to evaluate kinematic parameters of gait, functional performance and balance in women with fibromyalgia syndrome. Methods: The study included 26 female patients with fibromyalgia (49.2 ± 8.0 yrs) according to the criteria of the American College of Rheumatology, as well as 16 pain-free women (43.5 ± 8.5 yrs). Gait and balance parameters were extracted from video recordings of participants performing several motor tasks. Non-linear dynamic of body sway time series was also analyzed by computing the Hurst exponent. In addition, functional performance and clinical pain were obtained by using standardized motor tests (Berg’s balance scale, six-minute walking test, timed up and go task, Romberg’s balance test) and self-report questionnaires. (Fibromyalgia Impact Questionnaire). Results: Gait parameters evaluated by video recording parameters showed that walking speed was significantly diminished ($p < .001$) in FM patients as compared to pain-free controls, probably due to significant reductions in stride length ($p < .001$) and cycle frequency ($p < .001$). Analyses of balance by using video recordings also revealed significant differences between fibromyalgia and pain-free controls on body sway in the medial-lateral and anterior-posterior axes (all $ps < .01$). Deficits in gait and balance were significantly correlated with pain, fatigue, and stiffness in fibromyalgia, as well as depression or anxiety. Conclusion: Our results show that the balance time series in patients with FM are uncorrelated, suggesting changes in the feedforward process of balance control. Our analysis revealed that FM patients displayed balance and gait characteristics similar to those observed during aging processes. These results suggest that a precise evaluation of impairments and strategies underlying functional performance in postural stability and gait is necessary for optimal rehabilitation and fall prevention in fibromyalgia.

Keywords: fibromyalgia, chronic pain, gait, balance, biomechanics, Hurst exponent, computer vision software
Introduction

Fibromyalgia (FM) is a chronic syndrome characterized by widespread pain sensitivity and fatigue, as well as by cognitive and affective symptoms [5]. Fibromyalgia also exerts a considerable impact on daily activities and quality of life. In particular, it has been frequently shown that fatigue in fibromyalgia may be severe enough to reduce physical activities and lead to a sedentary lifestyle by reducing physical abilities and increasing risk for disabilities [6,7]. FM patients often reported functional limitations that were quite similar to those reported by persons with osteoarthritis or rheumatoid arthritis [8,9]. Furthermore, it has been shown that loss of function could be strongly correlated with work disability in these patients [10,11].

Previous research has also revealed that FM patients may also display deficits in balance or postural stability [7,108,109], a complex task that involves rapid and dynamic integration of multiple sensory, motor, and cognitive inputs to execute appropriate neuromuscular activity [110]. Impaired balance has been reported as one of the top ten debilitating symptoms in fibromyalgia with prevalence rates around 45% [7]. Moreover, frequency of falls seems to be higher in FM patients (34.4%) [108] than in persons aged 65 years and older (25-35%) [111], and patients with rheumatoid arthritis (RA) [9]. Nevertheless, balance and activity level in fibromyalgia have been mostly assessed by using retrospective self-reports [108,119], which are strongly influenced by patients’ beliefs about their own physical functioning and pain [120]. In the last decades, different types of recording devices have been developed to monitor and to assess balance and physical activity over long periods of times, providing valid information about subjects’ daily activities. Thus, it has been demonstrated that accelerometry-based ambulatory monitoring systems provide more objective measurements of variability in physical activities and pain over several days than self-reports [121].

Biomechanical analysis of gait also constitutes a useful tool for the assessment of motor function, functional capacity and muscle fatigue [113,282]. Previous studies have observed that fibromyalgia women display a reduced walking speed, which could be a consequence of decreases in stride length and cycle frequency, as well as bradykinesia [112,114]. Furthermore, it has been suggested that gait at normal speed in these patients may be preferentially achieved
by using their hip flexors instead of their ankle plantar flexors, thus increasing metabolic de-
mands and fatigue in comparison to pain-free controls [113].

The aim of the present study was to analyze gait and balance parameters in fibromyalgia and
to examine the possible relationship between subjective and objective measures of motor func-
tion with subjective complaints. In particular, we hypothesized that FM patients would display
significant gait and balance deficits as compared with pain-free controls, and that these mo-
tor disturbances would be correlated with increased patients’ ratings of pain, fatigue, morning
tiredness, stiffness and physical impairment (as measured by the FIQ questionnaire). Considering
that activity and balance fluctuations have well defined fractal properties in a wide range of
time scales, we also aimed to apply nonlinear analyses to evaluate the dynamic of these balance
fluctuations in pain-free controls and FM patients. This nonlinear approach allows an evalu-
atation of the autocorrelation in successive displacements, giving us information about possible
disturbances in motor control mechanism to correct balance.

**Material and Methods**

**Participants**

Twenty-six women diagnosed with fibromyalgia (FM) and 16 pain-free women with compara-
ble age and sociodemographic characteristics (Table 4.1) were recruited from different health
centers and patients’ associations in Majorca (Spain). Patients were included in the study if they
fulfilled the 1990 classification criteria of the American College of Rheumatology for fibromy-
algia. Participants were excluded from the study if they reported any other musculoskeletal
rather than fibromyalgia, or any neurological disorder. For medical and ethical reasons, sub-
jects were asked to keep their medication during the study. At the time of recruitment, par-
ticipants were verbally informed about the details of the study and provided written consent.
The study was in accordance with the Declaration of Helsinki (1991) and was approved by the
Ethics Committee of the Balearic Islands (Spain) (reference IB-1284/09).

–Table 4.1–
Self-report questionnaire

FM patients completed the Fibromyalgia Impact Questionnaire (FIQ) [283]. The FIQ is a standardized instrument designed to quantify the overall impact of fibromyalgia over many dimensions (e.g. function, pain level, fatigue, sleep disturbance, psychological distress, etc.). This questionnaire has shown excellent responsiveness to change in clinical studies and a good correlation with other similar questionnaires, such as the SF-36 [252].

Motor function tasks

Gait and balance parameters were obtained in FM patients and pain-free controls by using the following functional tasks:

- **Berg Balance Scale** [259]: This scale is a performance-based assessment tool developed to measure balance during functional activities such as reaching, bending, transferring, and standing. The test is often used for patients who exhibit a decline in function, self-report a loss of balance, or have unexplained falls [259]. The Berg Balance Scale consists of 14 functional tasks (e.g., sitting unsupported, change of sitting to standing position and vice-versa, standing with both feet together, standing on one leg, turning 360 degrees) with scores ranging from 0 (unable to perform) to 4 (normal performance). Total scores range from 0 (severely impaired balance) to 56 (excellent balance). Scores below 46 are good predictors for the occurrence of multiple falls [260].

- **Six-minute walking test (6MWT)**: The 6MWT is a functional walking test in which subjects are instructed to walk for 6 minutes as quickly as possible. This test has been used to assess individuals with mobility deficits [262] and FM patients [263–265]. The 6MWT is considered a good indicator of exercise tolerance and aerobic capacity, since it causes a physiological stress without demanding maximum aerobic capacity [264]. Ratings of perceived exertion were obtained after the 6MWT by using the Borg Effort Scale [266], a 15-point scale ranging from 4 (complete lack of effort) to 20 (maximum effort or exhaustion).

- **Timed up and go task (TUG)**: This task is a standardized test for assessment of functional mobility. The task is performed by using an ordinary armchair (45 cm in height) and
a stopwatch. Subjects are seated with their back against the chair and instructed to stand up, walk three meters, turn around, walk back to the chair and sit down at an ordinary comfortable speed \[267\]. The stopwatch is started on the word “Go” and stopped as the subject sit down. The TUG time is measured in seconds and normal TUG time ranges from 5.4 to 40.8 seconds (mean=15 seconds, SD=6.5) \[268\]. TUG time appears to be correlated with gait speed, balance, functional level and the ability to go out \[267\]. After the TUG, overall subjective perception of physical effort was measured by using the Borg Effort Scale \[266\].

- Modified version of the Romberg’s balance test: The Romberg’s test is an objective measure of patient’s standing balance \[269\]. The original test requires that participants remain in orthostatic position with feet together and eyes closed. In the present study, we modified the procedure by asking the participants to keep the erect position with eyes closed during 1 minute. In addition, they were allowed to keep the orthostatic position with feet in parallel and separated and arms extended along the body to avoid that participants fell when they closed their eyes during data collection. The test is based on the fact that maintaining balance while standing with closed eyes should rely on intact sensorimotor integration and motor pathways. The test was repeated twice and motion on the frontal and sagittal planes was captured by using a digital video camera at 30 frames per second (Casio Exilim EX-FS10). For motion detection analysis, a plumb line hanging on the ceiling at a distance of 3 meters was used as reference. Participants were also asked to wear a cap with sticks positioned in the vertical and horizontal planes. For the analysis of body sway in the medial-lateral direction, sticks were aligned with the anatomical position of the glabella of the frontal bone. For the analysis of body sway in the anterior-posterior direction, sticks were aligned with the anatomical position of the pinna (tragus). Unfortunately, we were not able to analyze the Romberg’s test videos of eleven FM patients and two pain-free subjects due to poor recording quality.

- Gait task: Subjects were instructed to walk on a 3 meters carpet at their normal walking step, without shoes and with flexed arms positioned on the abdomen. Optical markers were attached at the following body positions: anterior superior iliac spine, posterior superior iliac spine, area between the lateral condyle of the femur and the fibular head, bottom of the patella, lateral and inner malleolus, heel (between the first and second metatarsal), and on the tip of the
hallux. Subject’s motion was digitally recorded with a video camera at 210 frames per second (Casio Exilim EX-FS10). The camera was positioned at a distance of 3 meters from the carpet to visualize changes in position, velocity and acceleration of anatomical points along the x-axis. Gait velocity (cm/second), walking duration (seconds), cadence (number of steps/minute), percentage of time in the two phases of the gait cycle (stance and swing phase), and percentage of time with single and double support were computed.

Data reduction and pre-processing

Three groups of variables were analyzed in the present study:

- Raw scores obtained from self-report questionnaire (FIQ).
- Performance scores on standardized motor function tasks (TUG, 6MWT, Berg Balance Scale, Borg Effort Scale).
- Kinematic parameters extracted from video recordings: gait velocity (cm/sec), gait duration (sec), cadence (steps/min), stride and step lengths (cm), percentage of time in the stance/swing phase, and body sway variability in the anterior-posterior and medial-lateral planes (cm). A free open-source software for computer vision analysis of human movement (CvMob) was used [270, 271, 275]. This software computes kinematic parameters by using computer vision techniques. The software has also a high degree of accuracy for calculating body position and movement in the X and Y coordinates recorded by conventional cameras [270].

The non-linear dynamic of time series obtained during the balance test was assessed by computing long term correlations and the Hurst (H) exponent [246]. The H exponent usually ranges between 0 and 1, and describes the tendency of a time series either to cluster in one direction or to regress strongly to the mean. Thus, it has been assumed that H exponents between .5 and 1 would be characteristic of time series with long-term positive autocorrelation (high values will be followed by high values a long time in the future), whereas H exponents between 0 and .5 would suggest long-term switchings between high and low values in adjacent
pairs of datapoints. By contrast, H exponents would be around .5 if time series describe a pure random oscillation (e.g., Brownian noise or accumulated white noise). Moreover, it has been assumed that $H < .5$ would reflect a non-persistent pattern, whereas $H > .5$ would rather reflect a persistent pattern within the time series [246].

The H exponent was obtained in two steps. First, the deviation of the time series relative to their mean values was computed in a sliding window of size $n$ by using the Root Mean Square (RMS) method [247]. The RMS used a scaling function $\overline{W}(n)$ defined as follows:

$$\overline{W}(n) = \frac{1}{N_n} \sum_{u=1}^{N_n} \left\{ \frac{1}{m_n} \sum_{i \in n} \left( Z(x_i) - \bar{Z}_m \right)^2 \right\}^{1/2}$$

(4.1)

with the factor $N_n$ representing the number of windows with $n$ elements, $m_n$ the number of measures within each window, and $\bar{Z}_m$ the average value for those measures. Second, the values for $\overline{W}(n)$ were evaluated for different scales $n$. The Hurst exponent ($H$) is obtained fitting a power-law curve (fractal Brownian motion model) to the scaling function [246,247] as follow:

$$\overline{W}(n) \sim n^H$$

(4.2)

**Statistical analyses**

The null hypothesis that data were sampled from a normally distributed population was examined by using Shapiro-Wilk tests, and differences between patients and pain-free controls were analyzed by using parametric Student t-tests for independent samples, or non-parametric two-sample Kolmogorov-Smirnov tests. Pearson correlations were also used to analyze the relationship between kinematic parameters and clinical symptoms in fibromyalgia. A p-value of .05 was used for statistical significance. The effect size was calculated with effect size Calculators [284]. The effect sizes $d$ were interpreted using the classification of Cohen (1988) [285]: $0.20 \leq d < 0.50$ small effect, $0.50 \leq d < 0.80$ moderate effect, $d \geq 0.80$ large effect. Data are presented as M (SD) in the tables. If appropriate, data are reported as mean difference (MD).
Results

Fibromyalgia (FM) patients and pain-free controls were comparable on age, weight, height and body-mass index (all ps > .05) (Table 4.1). Table 4.2 displays mean and standard deviation of gait parameters in fibromyalgia and pain-free controls during performance on several motor tasks. FM patients walked less distance in 6 minutes (6MWT) ($t_{[29]} = -8.3$, $p < .001$), and took more time to stand-up and to walk a distance of 3 meters (TUG) as compared with pain-free controls ($t_{[40]} = 6.7$, $p < .001$). Moreover, ratings on self-perceived effort (Borg Effort scale) after performance on 6MWT ($K - S = 1.5$, $p < .05$) and TUG tests ($K - S = 3.02$, $p < .001$) were significantly higher in fibromyalgia than in pain-free controls. Finally, FM patients reported increased risk of falls (measured by the Berg Balance Scale) in comparison with pain-free controls ($K - S = 2.9$, $p < .001$). The effect sizes were medium-to-large for all group comparisons.

Annotations: Tables 4.1 and 4.2

Analyses of kinematic parameters further indicated that FM patients had significant deficits in gait and balance. Again, the effect sizes were medium-to-large for all group comparisons. FM patients displayed significant reductions in gait velocity. FM patients displayed significant reductions in gait velocity ($t_{[31]} = -8.3$, $p < .001$), cadence (steps/minute) ($t_{[31]} = -6.2$, $p < .001$), stride ($t_{[31]} = -5.1$, $p < .001$), and step lengths ($t_{[31]} = -4.9$, $p < .001$), and the percentage of single support ($t_{[31]} = -4.3$, $p < .001$) and percentage of swing phase ($t_{[31]} = 4.2$, $p < .001$), as well significant increased gait duration ($t_{[31]} = 5.7$, $p < .001$) in comparison with pain-free participants. Same effects were also yielded when values were referenced to each subject’s legs (distance between the greater Trochanter and the lateral Malleolus) (Table 4.2). Moreover, FM patients displayed greater body sway in the anterior-posterior ($t_{[27]} = 4.6$, $p < .001$) and medial-lateral directions ($t_{[27]} = 5.8$, $p < .001$) as compared to pain-free controls (Table 4.3).
The non-linear analysis of balance time series also revealed significant differences between FM patients and pain-free controls on the Hurst exponents for the anterior-posterior ($t[27] = 2.3, p < .05$) and the medial-lateral axes ($t[27] = 5.1, p < .001$). In both cases, the H exponents were close to .5 in fibromyalgia patients and around .3 in pain-free controls (Figures 4.1 and 4.2 and Table 4.3). The effect sizes were medium-to-large for all group comparisons.

In order to further assess if altered motor function was related to clinical symptoms in fibromyalgia, Pearson correlations were computed between motor performance scores and FIQ scores (Table 4.4). Results indicated that high pain ratings were significantly correlated with higher risk of falls (Berg Balance Scale), increased time to perform the TUG test, reduced gait velocity, increased gait duration, reduced stride and step lengths, and increased body sways in the anterior-posterior and medial-lateral directions. Also, highly subjective complaints, about fatigue and stiffness for the FIQ questionnaire, were related to reduced percentages of single support and swing phase of the gait cycle. In addition, stiffness was positively correlated with increased performance time of the TUG task and enhanced perceived effort after completion of 6MWT test. Moreover, high depression and anxiety scores were correlated with high risk of falls, and performance time of the TUG whereas self-perceived effort after TUG was positively correlated with depression and physical impairment.

Discussion

We analyzed kinematic parameters of gait and balance, as well as subjective complaints (ratings of perceived exertion, pain, fatigue - as measured by the FIQ questionnaire) during performance on several motor and balance tasks in fibromyalgia (FM) patients and age-matched pain-free
controls. Our results indicated that both gait and balance were severely impaired in FM, and the motor performance may be correlated with the clinical symptoms caused by FM.

Gait parameters such as speed, cadence, stride and step lengths, percentage of stance and swing phases, and support base were significantly impaired in FM patients. These findings are in accordance with previous studies showing that FM patients displayed slower cadence during gait compared to pain-free controls [112, 114, 115, 286]. Furthermore, it has been observed that FM women spent more time in double support than in single support, showing reduced muscle endurance and strength in patients with FM, both isometric and isokinetic strength are decreased when compared with healthy persons, particularly in knee joint flexion and extension [112, 287, 288]. In addition, it has been suggested that generalized pain and overweight could inhibit the single support of body and increase the time of double support in FM [112, 287]. This is of special relevance because the preferential use of hip flexors in comparison to plantiflexors of the ankle in FM patients would also indicate an altered mechanism for maintaining balance during gait [113, 286, 289].

Previous studies have also suggested that factors such as level of physical activity, bradykinesia, reduced isometric strength of the lower limbs muscles and overweight, together with fatigue and pain could be also responsible for relevant alterations in muscle recruitment patterns during gait in FM [112-114]. A common assumption is that reduced physical activity might result from fear of pain and subsequent avoidance of activities that are known to exacerbate pain (fear-avoidance model) [216, 290]. Our data indicated that gait deficits in FM patients were mainly correlated with pain intensity, fatigue and stiffness (as measured by the FIQ questionnaire), rather than with depression or anxiety. In this sense, our findings are consistent with previous studies showing that patients with chronic pain displayed a reduced level of activity during the morning and the evening compared to pain-free controls [291]. It was also noteworthy that observed alterations of gait parameters in FM (for instance, a reduction of more than 30% in gait velocity and stride length compared to age-matched healthy individuals) were similar or even greater than those previously reported during aging (for instance, a reduction of 20% in older as compared to young individuals) [292, 293]. Thus, it seems plausible that an altered pattern of gait could also contribute to the characteristic reductions of daily functioning.
A further support for the notion that FM may affect many subsystems responsible for postural control and balance was provided by the results from performance on the Timed up and go (TUG) test. Here, we observed that FM patients took significantly more time to complete the task (around 17 seconds) than pain-free controls (8 seconds). These values were similar to those obtained in a previous study [267] showing that those older people performing the TUG in more than 13.5 seconds were more likely to have suffered a fall in the previous 6 months than those performing the TUG in less than 13.5 seconds. The analyses of balance during functional activities (reaching, bending, transferring, and standing) further indicated that FM patients displayed higher risk of falls than pain-free controls. In this sense, it has been already reported that balance problems are considered as one of the top 10 most debilitating symptoms in FM [7]. Moreover, the observed values for risk of falls in the present study were similar to those previously reported in the elderly [259, 294] and in Parkinson patients [260]. Taking into account that around 30% of people over 65 may fall at least once a year [119, 295], one may speculate that the risk of falls in FM patients could represent an important limitation in their elderly life.

The analyses of body sway values during performance on the modified version of the Romberg’s balance test provided further support for the notion that FM may affect some subsystems responsible for postural control and balance. Body sways on the anterior-posterior and medial-lateral planes were significantly greater in FM patients than in pain-free controls. Furthermore, non-linear analyses of body sway time series showed significant group differences in the Hurst exponents (Figures 4.1 and 4.2 and Table 4.3). In the present study, we found that Hurst exponents were between .33 and .37 in pain-free controls and between .5 and .52 in FM patients. These data reveal that pain-free controls displayed an anti-persistent trend in body sway time series. This behavior implies that shifts in one direction are followed by shifts in the opposite direction, indicating that the motor control system searches to maintain a stable position over the time. By contrast, time series in FM patients were characterized by an uncorrelated pattern of body oscillations. This uncorrelated behavior shows a typical oscillation pattern where future movements do not depend on past movements; it is equivalent...
to random selecting a direction. Suggesting a serious disturbance in motor control system, leading to more unstable balance over the time and, possibly, to an increased risk of falls. Furthermore, the Hurst exponent values in pain-free controls were in concordance with previous findings [296], whereas values in FM patients were similar to those observed in patients with reduced mobility [297] [298].

Nevertheless, the present study has some limitations that should be taken into account for the interpretation of the results. Two-thirds of our FM patients were currently taking analgesic and antidepressant medication during data collection and, therefore, the possible side effects of these drugs on balance and gait cannot be completely discarded. In this sense, a recent study has shown that antidepressant use was one of the possible mediators for the association of obesity and falls in community living older persons [299]. It remains, however, unclear if similar effects could be observable in middle-age FM patients. Moreover, although our sample of FM patients displayed greater body-mass index than age-matched pain-free controls, they could not be considered as obese. Although prevalence of FM in men is significantly lower than in women, our sample only included women. Future studies should include representative samples of men, as well as medication-free and older participants to examine the mediator role of all these variables for the association of pain and balance in FM. Finally, it should be borne in mind that fatigue was assessed as a subjective symptom from the FIQ questionnaire. Further research is necessary to analyze if more objective and reliable measures of fatigue are also correlated with gait deficits in FM.

In conclusion, our results point towards significant impairments in balance in FM patients as compared with pain-free controls, as assessed by self-reports, standardized motor function tests and kinematic parameters extracted from participants’ video recordings. We found that pain intensity, fatigue (as measured by the FIQ questionnaire) and stiffness were the most relevant factors in explaining gait deficits in FM, rather than affective factors such as depression or anxiety. We have also found that the oscillation pattern for FM patients displays a temporal correlation without memory, which may be correlated with changes in the motor control system of patients. A higher Hurst exponent is also related with a bigger increase in oscillation amplitude in time. This abnormal behavior could explain higher risk of fall in this group. This lack
of anti-persistent correlation in FM group gives rise for new questions about the feedforward model in balance control for FM patients. All these findings highlight the relevant role of postural control and balance for daily activity functioning in FM. Thus, specific activities directed towards the modification of these altered gait and balance patterns may be included in regular physical intervention programs in FM. This represents a relevant contribution considering that most of previous research of balance in fibromyalgia was based on retrospective reports or on self-report measures rather than on objective measures of posture sway.

**Acknowledgments**

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Figures

**Figure 4.1:** Boxplot of Hurst exponents of antero-posterior body sway for FM patients (in red) and pain-free control subjects (in black).

**Figure 4.2:** Boxplot of Hurst exponents of medial-lateral body sway for FM patients (in red) and pain-free control subjects (in black).
### Table 4.1: Clinical characteristics of fibromyalgia patients and pain-free controls.

<table>
<thead>
<tr>
<th></th>
<th>Fibromyalgia patients (N = 26)</th>
<th>Pain-free controls (N = 16)</th>
<th>Cohen’s d</th>
<th>Effect-size r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>49.2 ± 8.0</td>
<td>43.5 ± 8.5</td>
<td>.69</td>
<td>.33</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>68.6 ± 10.9</td>
<td>64.2 ± 10.9</td>
<td>.40</td>
<td>.20</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.1 ± 6.4</td>
<td>163.3 ± 7.0</td>
<td>-.32</td>
<td>.16</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>26.5 ± 4.2</td>
<td>24.5 ± 4.1</td>
<td>.24</td>
<td>.12</td>
</tr>
<tr>
<td>FM History (yrs)</td>
<td>7.5 ± 5.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Medication (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Analgesic/relaxants/NSAID</td>
<td>69.23</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anxiolytics</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Fibromyalgia Impact Questionnaire (FIQ)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical impairment (0-3)</td>
<td>1.6 ± 0.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Feel good (0-7)</td>
<td>5.1 ± 1.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Work missed (0-7)</td>
<td>2.1 ± 2.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Do work (10 cm VAS)</td>
<td>8.3 ± 2.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pain (10 cm VAS)</td>
<td>8.2 ± 1.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fatigue (10 cm VAS)</td>
<td>8.9 ± 2.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rested (10 cm VAS)</td>
<td>8.1 ± 3.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stiffness (10 cm VAS)</td>
<td>7.6 ± 2.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anxiety (10 cm VAS)</td>
<td>7.6 ± 2.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Depression (10 cm VAS)</td>
<td>6.8 ± 3.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total FIQ score (0-100)</td>
<td>71.1 ± 16.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
**Table 4.2:** Mean and standard deviations of gait parameters during motor performance in fibromyalgia patients and pain-free controls.

<table>
<thead>
<tr>
<th></th>
<th>Fibromyalgia patients</th>
<th>Pain-free controls</th>
<th>Cohen’s $d$</th>
<th>Effect-size $r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standardized motor function tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berg scale for risk of falls (0–56)</td>
<td>44.7 ± 5.6</td>
<td>55.4 ± .6</td>
<td>-2.68</td>
<td>-.80</td>
</tr>
<tr>
<td>TUG (secs)</td>
<td>17.0 ± 5.2</td>
<td>8.2 ± 1.0</td>
<td>2.35</td>
<td>.76</td>
</tr>
<tr>
<td>Perceived effort after TUG (4–20)</td>
<td>12.3 ± 2.3</td>
<td>4.3 ± .5</td>
<td>4.80</td>
<td>.92</td>
</tr>
<tr>
<td>6MWT (m)</td>
<td>170.9 ± 46.9</td>
<td>330.1 ± 58.3</td>
<td>-3.0</td>
<td>-.83</td>
</tr>
<tr>
<td>Perceived effort after 6MWT (4–20)</td>
<td>14.1 ± 3.6</td>
<td>9.2 ± 3.1</td>
<td>1.45</td>
<td>.59</td>
</tr>
<tr>
<td>Gait parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gait velocity (cm/sec)</td>
<td>67.3 ± 17.7</td>
<td>112.0 ± 12.3</td>
<td>-2.97</td>
<td>.82</td>
</tr>
<tr>
<td>Gait duration (sec)</td>
<td>4.8 ± 1.4</td>
<td>2.7 ± .3</td>
<td>2.07</td>
<td>.72</td>
</tr>
<tr>
<td>Cadence (steps/min)</td>
<td>96.6 ± 19.4</td>
<td>115.5 ± 11.6</td>
<td>-1.18</td>
<td>.51</td>
</tr>
<tr>
<td>Stride Length (cm)</td>
<td>69.3 ± 14.3</td>
<td>99.2 ± 12.9</td>
<td>-2.19</td>
<td>.74</td>
</tr>
<tr>
<td>Step Length (cm)</td>
<td>58.6 ± 10.5</td>
<td>79.7 ± 8.6</td>
<td>-2.20</td>
<td>.74</td>
</tr>
<tr>
<td>Single support (%)</td>
<td>57.3 ± 7.0</td>
<td>66.1 ± 4.2</td>
<td>-1.52</td>
<td>.60</td>
</tr>
<tr>
<td>Swing phase (%)</td>
<td>29.3 ± 3.1</td>
<td>33.7 ± 3.0</td>
<td>-1.44</td>
<td>.58</td>
</tr>
</tbody>
</table>
**Table 4.3:** Mean and standard deviations of balance parameters in the anterior-posterior and the medial-lateral axes in fibromyalgia patients and pain-free controls.

<table>
<thead>
<tr>
<th></th>
<th>Fibromyalgia patients</th>
<th>Pain-free controls</th>
<th>Cohen’s $d$</th>
<th>Effect-size $r$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anterior-posterior axis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP Body sway (cm)</td>
<td>2.31 ± 0.99</td>
<td>1.07 ± 0.11</td>
<td>1.76</td>
<td>0.66</td>
</tr>
<tr>
<td>AP Hurst exponent</td>
<td>0.50 ± 0.10</td>
<td>0.37 ± 0.19</td>
<td>0.85</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Medial-lateral axis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ML Body sway (cm)</td>
<td>2.55 ± 0.92</td>
<td>1.08 ± 0.09</td>
<td>2.25</td>
<td>0.75</td>
</tr>
<tr>
<td>ML Hurst exponent</td>
<td>0.52 ± 0.10</td>
<td>0.31 ± 0.11</td>
<td>1.79</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Table 4.4: Pearson correlations of FIQ subscales with performance parameters on gait and balance tasks in fibromyalgia patient

<table>
<thead>
<tr>
<th>FIQ subscales</th>
<th>Pain</th>
<th>Physical Work Do</th>
<th>Rested Depression Anxiety Fatigue Stiffness impaired missed work</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standardized motor function tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berg scale (0–56)</td>
<td>.52*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TUG (secs)</td>
<td>.44*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Perceived effort after TUG (4–20)</td>
<td>–</td>
<td>.56**</td>
<td>–</td>
</tr>
<tr>
<td>6MWT (m)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Perceived effort after 6MWT (4–20)</td>
<td>.53*</td>
<td>–</td>
<td>.60*</td>
</tr>
<tr>
<td><strong>Gait parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gait velocity (cm/sec)</td>
<td>-.56*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gait duration (sec)</td>
<td>.54*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cadence (steps/min)</td>
<td>-.50*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stride Length (cm)</td>
<td>-.49*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Step Length (cm)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Single support (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Swing phase (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Balance parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP Body sway (cm)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ML Body sway (cm)</td>
<td>–</td>
<td>.68**</td>
<td>–</td>
</tr>
<tr>
<td>AP Hurst exponent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ML Hurst exponent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Asterisks indicate
* p < .05 and ** p < .01
4.2 Study 2: Effects of Catechol-O-methyltransferase haplotypes in the sleep patterns of patients with fibromyalgia

Abstract

In humans, sleep occupies a third of life so sleep disorders may seriously affect patients’ quality of life. Several studies have shown a close association between sleep disorders and pain sensitivity. Moreover, it is known that sleep disorders are highly prevalent in patients with fibromyalgia (FM). However, few studies have shown how genetic variants associated with pain intensity can affect sleep quality in FM patients. Objectives: The main objective of the present study was to examine differences in sleep architecture related to genetic variants (haplotypes) in the catechol-O-methyltransferase (COMT) gene associated with high and low sensitivity to pain in FM patients. Methods: This study included 26 female patients diagnosed with FM according to the criteria of the American College of Rheumatology presenting different COMT haplotypes. Two patients were excluded because their genetic material was lost. To test the effects of the COMT gene on clinical data and polysomnography measures, participants were divided in groups with low and high COMT activity. Patients underwent an overnight polysomnography and some standardized questionnaires on sleep, quality of life, anxiety and depression, and pain. Besides the classical study of sleep architecture and the frequency-band spectrum of EEG data from the polysomnographic record, we also applied a nonlinear analysis method to evaluate the dynamics of sleep stages in FM patients by performing a multiscale entropy analysis (MSE). Results: Patients with high and low COMT activity were comparable on age, medication, FM history and pain visual analogic scale (VAS). Only scores on the physical impairment subscale of the Fibromyalgia Impact Questionnaire (FIQ) ($p < .05$) and depression questionnaires (HAD) ($p < .05$) was statistically different between the groups. Regarding the sleep architecture, significant group differences were found in number of awakenings ($p < .05$). Analyses of entropy values revealed that patients with genetic haplotype associated with low COMT enzyme activity displayed increased entropy during REM and deep sleep over the right hemisphere than FM patients with genetic haplotype associated with high COMT enzyme ac-
Conclusion: Our findings suggest that specific genetic variations of the COMT gene might have different consequences for sleep architecture in FM patients.

Keywords: Fibromyalgia; Pain; Sleep; val158met; COMT; Power Spectrum; Multiscale Entropy

Introduction

In humans, sleep occupies a third of life so sleep disorders may seriously affect patients’ quality of life [300]. Several studies have shown a strong association between sleep disorders and pain sensitivity [52,141,156,172,301]. Affleck and collaborators [172] showed that a painful day is followed by a night of poor sleep, and conversely, a night of poor sleep is followed by a significantly more painful day among fibromyalgia patients. It is also known that many patients with chronic pain suffer from sleep disorders [302]. It has been estimated that 54-70% of chronic pain patients have difficulty to sleep, poor quality of sleep, no sleep, numerous night awakenings, daytime sleepiness and fatigue [153]. In the case of patients with fibromyalgia, musculoskeletal complaints were directly related to unrefreshing sleep [303]. Additionally, it has been suggested that changes in deep sleep stages (sleep stages III and IV) corresponding to the period of growth hormone (GHRH) release may influence the pain intensity experienced during the day. Moreover, there is increasing appreciation that suboptimal growth hormone secretion, leading to a state of adult growth hormone deficiency, may occur in the setting of chronic inflammatory disease, chronic corticosteroid use, and fibromyalgia [304].

It has been reported that pain sensitivity is related to some genetic haplotypes. Single-nucleotide polymorphisms (SNPs) in the gene encoding for the catecholamine-O-methyltransferase (COMT) enzyme have been extensively analyzed in association with pain perception and FM [29,71,73,74]. This enzyme is responsible for metabolizing catecholamines like dopamine, serotonin and noradrenaline, even as, contributes to interindividual differences in brain α oscillations, which are functionally related to executive performance such as counting tendency on a random number generation task in young adults [67]. Martínez-Jauand et al. [13] reported that COMT activity is associated with pain sensitivity in FM patients, with higher
activity of the COMT enzyme associated with lower pain sensitivity.

Genetic influences have also been involved in sleep disturbances [67, 78, 79]. Previous research with twins has highlighted the importance of genes to several subjectively defined aspects of sleep, such as sleep duration, and overall subjective sleep quality [78]. However, few studies have reported how different genetic haplotypes are associated with sleep disorders in FM patients. In this study, we perform a polysomnography (PSG) recording with 24 women with FM presenting 2 different haplotypes of the *val158met* polymorph of the *COMT* gene. Data were analyzed by using classical sleep architecture measures (NREM, REM, Latency, Efficiency, etc.), Power spectral analysis (PSA) and Multiscale Entropy (MSE) analysis of EEG signals in different sleep stages. Our aim was to examine differences in sleep architecture related to genetic variants (haplotypes) in the *COMT* gene associated with high and low sensitivity to pain in FM patients using linear and nonlinear analysis tools.

We hypothesized that these *COMT* haplotypes can be associated with an intensification of sleep disorders in FM patients, and, more specifically, that patients with higher activity of the COMT enzyme, have better sleep quality than those with low activity of COMT enzyme. We also hypothesized that the frequency analysis of the EEG, obtained at various sleep stages, would provide information about the pathophysiology underlying FM. Finally, considering that other studies have shown that sleep state EEG recordings present scale-free (fractal) properties [305], we conducted a MSE analysis to evaluate the complexity of sleep EEG recordings and to compare the outcomes of this non-linear assessment between the two genetic groups. The MSE provides multiple time scale information of a signal [201] and might reveal features that the architectural analysis and power spectra analysis are not able to. Considering that higher entropy is associated with healthier subjects [201], we believe that those patients with high COMT activity would present high complexity in EEG signals than low COMT activity ones.
Methods

Participants

Twenty-six women diagnosed with fibromyalgia participated in this study. FM patients were included if they fulfilled the 1990 classification criteria of the American College of Rheumatology [251] and had widespread pain as dominant symptom. Two patients were excluded because their genetic material was lost. Exclusion criteria were: pregnancy, neurological deficit not associated with pain, anxiety treatment, and sleep disorder prior to diagnosis of fibromyalgia. The subjects were recruited from different health centers and patients associations in Majorca (Spain) and presented comparable socio-demographic characteristics. This study was approved by the local Research Ethical Committee with reference number IB-1284/09.

The subjects of this study have participated in a previous project of genetic markers and haplotypes [13]. In that study have been analyzed val158met polymorphisms of COMT gene. They have identified 3 haplotypes of COMT val158met previously defined as low pain sensitivity (LPS), average pain sensitivity (APS) and high pain sensitivity (HPS). To test the effects of COMT val158met on clinical data and polysomnography measures, participants were divided in high COMT activity (carriers of at least one LPS haplotype) (n=13), and low COMT activity (carriers of only APS and/or HPS haplotypes) groups (n=11). In relation to genotype all participants in group low COMT activity are met homozygotes and group high COMT activity val carriers (val homozygotes and val/met heterozygotes). In the current work, we use the same group classification as Martínez-Jauand and collaborators [13].

Assessment of socio-demographic and clinical pain characteristics

All participants underwent an extensive medical and psychological assessment, including clinical pain characteristics through self-report questionnaires and a semi-structured clinical interview. The Fibromyalgia Impact Questionnaire (FIQ) [283], the West Haven–Yale Multidimensional Pain Inventory (WHYMPI) [258], the McGill Pain Questionnaire (MPQ) [257], Quality of life questionnaire (Espiditest) [254], Medical Outcome Study Short Form (SF-36) [256],
Hospital Anxiety and Depression Scale (HAD) [253] and two sleep questionnaires: the Pittsburgh Sleep Quality Index (PSQI) [272] and the Oviedo Sleep Questionnaire (OSQ) [273] was completed.

**Sleep polysomnography**

Polysomnographic recordings were obtained in all participants under similar and standardized conditions, in a sleep laboratory at the University of Balearic Islands (Spain). After one-hour of preparation, the recordings were initiated around 11pm and concluded around 7am of the next day, thus covering 8 hours of nocturnal recording in basal conditions. All the recordings were carried out with an infrared camera for nocturnal vision.

The EEG C3A2 and C4A1 monopolar channels were recorded according to the International 10-20 System [280, 281]. The settings for the recording were 0.5 Hz for the high-pass filter, 50 Hz for the low-pass filter, time constant of 0.4 seconds, and sensitivity of 50 µV/cm. The electrooculogram (EOG) electrodes were placed according Kales and Rechtschaffen manual [306], with low-pass filter at 15 Hz and high-pass filter 0.5 Hz was used. For the electromyogram (EMG), electrodes were placed on the muscles of the chin and fixed with gauze impregnated with collodion. EMG calibration was adjusted to achieve a deflection of 14 mm for a signal of 50 µV. We used a high-pass filter of 10 Hz and a low-pass filter of 70 Hz. For the electrocardiogram (EKG) were used three electrodes in the chest. Two electrodes were located in the intercostal spaces between the 3rd and the 4th rib: one at the right side and one at the left side of the chest. And a third electrode was located between the intercostal spaces of the 11th rib. We used high-pass filter of 0.5 Hz and low-pass filter of 50 Hz. The respiratory parameters were recorded by averaging the thoracic and abdominal movements for chest and abdomen elastic bands respectively.

**Frequency analysis of EEG**

The Power spectral analysis (PSA) seeks to describe the frequency content of a signal based on a finite set of data. The PSAs of EEG signals (C3A2, and C4A1) were carried out on PSG recordings with the classic frequency bands: delta (0.5 – 4 Hz), theta (4.5 – 8 Hz), alpha
(8.5 – 12 Hz) and beta (12.5 – 30 Hz) for each patient.

To do that, first we subdivided the EEG records into segments of 30 seconds each one. For each patient, we analyzed 15 of those segments: we took one segment of each of the sleep stages (I, II, III, IV, and REM) at three different moments of the PSG records (the beginning, the end, and the middle of the PSG record). We performed the frequencial analysis for each 5 seconds windows of those EEG segments, with an overlap of 2.5 seconds between subsequent windows.

**Multiscale Entropy analysis**

Multiscale Entropy (MSE) analysis measures the complexity of a time series by taking into account the entropy with respect to multiple temporal scales [307, 308]. It is a method for computing how a certain entropy measure varies as one successively coarsens a time series over multiple scales, by computing averages over non-overlapping intervals of fixed size (the scale).

The sample entropy in each coarse-graining step is evaluated as follows:

$$S_E(m, r, N) = -\ln \frac{U^{m+1}(r)}{U^m(r)}$$

The $U^m(r)$ and $U^{m+1}(r)$ factors represent the probability of two segments of length $m$ and $m + 1$, respectively, defined by the distance between two vectors $d[x^m(i), x^m(j)]$ [309, 310]. The distance is computed as:

$$d[x^m(i), x^m(j)] = \max ||x(i + k) - x(j + k)||k = 0, m - 1$$

The probability $U^m(r)$ is defined by the conditional probability of $d[x^m(i), x^m(j)] \leq r$ (following Heisz and McIntosh’s example analysis [311]).

We applied this to our 30 sec. artifact-free (visually removed) EEG data. We used the parameters $m = 2$, $r = 0.15$ (following Costa et al. [308]), $N = 20$ and the algorithm available at PhysioNet repository [312].

A study by Gow and coworkers [313] explains the choice of $m$ and $r$ is driven by two over-
arching factors: (1) maximizing the accuracy and confidence in the SampEn values obtained at each MSE scale and (2) optimizing the ability to distinguish any real, salient features in the dataset. In principle, the accuracy and confidence of the entropy estimate improve as the numbers of matches of length \( m \) and \( m + 1 \) increase. The number of matches can be increased by choosing small \( m \) (short templates) and large \( r \) (wide tolerance). Other studies explored the sensitivity of the MSE for different values of \( m \) and \( r \) \([314]-[316]\). They investigated as the result of changes MSE if using different values of \( m \) and \( r \) for the same data. The results found that although the absolute values of entropy changed to different values of \( m \) and \( r \), the relative changes were insignificant, i.e. the entropy values might change for different values of \( m \) and \( r \), but changed in the same way for all scales. The same authors found that the MSE values produces statistically reliable and more reproducible results when \( m = 2 \) and when \( r \) is between 10\% and 20\% \([313]\).

**Non-linear dynamics using surrogate data**

For the noisy and short time-series, standard chaotic dynamics algorithms can give spurious results, i.e. they can indicate the presence of the non-linear dynamics in completely random systems. To distinguish linearly correlated noise from chaotic systems, it is common to use surrogate data techniques \([317]\). The “surrogate” datasets are completely stochastic data sets that contain exactly the same linear correlations as that in the original time-series. In this study, surrogate data was obtained by shuffling the measures registered in the time series. Then, one can compute any nonlinear metric, using the same algorithm for both, original and surrogate data sets, in order to assess the effect of possible nonlinear interactions on the original time series. If the difference between the original and the surrogate metric is significantly larger than the standard deviation of the surrogate metric, then it is a strong indication of the non-linear structure in the investigated time-series.

We applied this comparison between MSE of the original sleep EEG data at REM stage, by shuffling the original EEG recording.
**Instrument and Data analyses**

Sleep recordings were performed using the device Respironics Alice 3 v 1.18, with a sampling rate of 100 Hz and resolution of 8 bits. The signals were amplified and digitized by a 5.0V analog-to-digital converter and a 0.5-50 Hz digital band-pass filter was applied.

For interpretation of the polysomnographic data, we followed the classification system of Kales and Rechtschaffen [306]. The diverse sleep stages were revised manually in 30-second epochs, and the successive NREM-REM cycles were recorded taking the REM periods into account. The percentages of the sleep stages relatives to total sleep time were determined.

Sleep efficiency was defined as the ratio between the total time in bed and the time in bed used for sleeping; sleep latency was defined by the time elapsed since the start of the study until the disappearance of alpha rhythm in the EEG (start of Stage II); time wakefulness after sleep on set was defined by the time of waking (active wakefulness more passive awake) uninterrupted less than 300 seconds (known as wake brief); and number of awakenings are defined as waking time (active wakefulness more passive awake) uninterrupted more than 300 seconds. NREM stages I and II were considered as light sleep, and NREM stages III and IV were considered as deep sleep.

Allele and genotype frequencies were estimated by gene counting and tested using chi-square test. To test the effects of the \textit{COMT} gene, participants were divided in low (carriers of only APS and/or HPS haplotypes) and high (carriers of at least one LPS haplotype) COMT activity. We then tested if there were differences in medication taken by groups with each polymorphism.

Socio-demographic information, sleep questionnaire data, sleep architecture and PSA results were analyzed by using analyses of Independent-Samples T Test considering as independent variables the genetic groups of each patient. For the PSA, we compared the relative power of the four different band frequencies (delta, theta, alpha, beta) for each sleep stage (stage I, stage II, stage III, stage IV, and REM).

For the MSE analysis, we used the Mann-Whitney U Test to compare the basic continuous variables between two polymorphism groups. To better observe these results was held one mul-
tivariate analyses of variance (MANOVAs) between the left (C3) and right (C4) hemispheres. The stages were grouped into REM, light sleep (stages I and II) and deep sleep (stages III and IV) stages. Although we used a multiscale entropy analysis corresponding to 1-20 time scale factor, for the MANOVA we used a smaller time scale factor of 5 points (1, 5, 10, 15 and 20) (according previous studies [318]). Statistical analyses were processed using IBM SPSS Statistics software (IBM SPSS Statistic, v22.0, Armonk,NY).

**Results**

From the total of Twenty-six patients include in this study, only twenty-four were successfully genotyped for the catechol-O-methyltransferase (COMT) val158met polymorphism (13 for group high COMT activity and 11 for group low COMT activity) and completed the Fibromyalgia Impact Questionnaire (FIQ), and sleep Questionnaires: the Pittsburgh Sleep Quality Index (PSQI) and the Oviedo Sleep Questionnaire (OSQ), as well as sleep polysomnography.

The general characteristics of the enrolled subjects are presented in Table 4.5. There was no statistically significant difference in age, medication, visual analogic scale of pain, or any questionnaire between the two genetic groups ($p > .05$). The mean disease duration of fibromyalgia was 9 years. FM patients with low COMT showed average total FIQ score of 75.44 ± 15.67 against an average of 66.8 ± 15.12 for high COMT activity patients. However, only physical impairment (FIQ) ($t(15) = −2.30, p = .03$) and depression (HAD) ($t(16) = −2.64, p = .02$) showed a statistically noticeable difference between genetic groups.

Regarding the medication we observed that 37.5% of patients take antidepressants; 50% take analgesic NSAID; 25% take muscle relaxants; 29.16% take anxiolytics; and 62.5% of patients take another type of medication. After performing a Chi-square test none of these drug groups showed significant differences.

---Table 4.5---

The figures 4.3a and 4.3b represent the different sleep stages diagram in patients with fibro-
myalgia with different haplotypes associated a COMT enzyme.

–Figures 4.3a and 4.3b–

EEG - Surrogate data

In order to analyze if non-linear features of sleep EEG data were due to random effects, surrogate and fake random data were obtained (Figure 4.5). It was observed that MSE values of real REM data were different from surrogate and random data starting from the scale factor 5.

–Figure 4.5–

EEG - Multiscale Entropy Analysis

We compared the MSE values for the same 2 groups of patients (high and low COMT activity subjects). The Mann-Whitney U test showed for data obtained while the participants were in sleep stage I, only one significant difference at C3A2 electrode (time scale = 16), and five significative differences for C4A1 (time scale = 4, 6, 7, 10 and 16). For sleep stage III, significant differences were observed in time scale 11 for C4A1. In REM sleep stage, significant differences were observed for C4A1 electrode (time scale = 14 and 18) (Figure 4.6).

– Figures 4.6 and 4.7 –

The MANOVAs revealed significant effects of sleep stage (light, deep sleep vs. REM) on MSE values at the following scales factors: sf 1 \[ F(2, 20) = 31.04; \ p < .000 \], sf 5 \[ F(2, 20) = 6.11; \ p < .008 \] and sf 20 \[ F(2, 20) = 3.94; \ p < .03 \]. In addition, an interaction between hemisphere, sleep stage and genetic group was found at sf10 \[ F(2, 20) = 7.58; \ p = .004 \] and between hemisphere and sleep stage at \[ F(2, 20) = 3.91; \ p = .04 \]. Post-hoc pairwise mean comparisons of the interaction effects revealed that FM patients with low COMT activity had greater entropy values during REM (sf14, sf18) and sleep stage III at C4 electrode, but lower entropy values during sleep stage I at C4 electrode, than FM patients with high COMT activity. No significant group differences were observed over the left hemisphere for any of the
sleep stages (Figures 4.6 and 4.7).

**Correlations**

In order to further assess if signal frequencies and entropy values of EEG were related to clinical symptoms in fibromyalgia, Pearson correlations were computed between values of PSA and MSE with scores of HAD and FIQ questionnaires (Tables 4.7 and 4.8). Results indicated that some subscales (“Work missed” and “Feel Good” of FIQ questionnaire and “Anxiety” as “Depression” of HAD and visual analogic scale of pain) were significantly correlated with at least with one parameter of entropy analysis. In relation to, the results showed significant correlations for the subscales “Do work”, “Pain” and “Rested” of FIQ questionnaire and “Anxiety” as “Depression” of HAD and visual analogic scale of pain. Thus, high pain scores were significantly correlated with the increase in the entropy of the EEG signal during REM, as well as to lower activity of delta band during deep sleep and increased activity of delta and theta band in light sleep.

**Discussion**

Although the multifactorial nature of fibromyalgia is well recognized, genetic factors are considered to be strong determinants of the disease, and numerous genes have been studied in connection with fibromyalgia [13, 58, 69, 70]. In the present study, we examined differences on sleep associated with the presence of the COMT gene. Previous studies have indicated that FM patients with low COMT activity had increased depression, affective distress and pain sensitivity than FM patients with high COMT activity [13]. In this work, we tested if these polymorphisms can be also correlated with an intensification of sleep disorders in FM patients. Besides the classical studies of sleep architecture and frequency analysis of EEG signal, a novel approach based on MSE analysis of EEG data was applied to characterize the sleep of these two subgroups of FM patients.

Regarding the self-report questionnaires, significant differences between genetic groups were observed only in physical impairment FIQ sub-scale and depression HAD sub-scale.
These values were in the same range of values showed for FM patients in previous studies [319,320]. Moreover, a meta-analysis study about the relationship between FM and COMT polymorphism demonstrated that the COMT val158met polymorphism may be associated with susceptibility to fibromyalgia, and that fibromyalgia patients carrying the met allele of the polymorphism may be correlated with higher FIQ scores as compared to those with the val allele [321]. It can occur because reduced COMT activity leads to elevated levels of catecholamines, such as epinephrine or norepinephrine, promoting pain production by stimulation of Beta2-adrenergic receptors in the peripheral and central nervous systems [68]. However, evidence for catecholamine levels increasing in fibromyalgia remains controversial [322]. Second, the COMT met-met genotype reduces the content of endogenous opioids-like peptides in the central nervous system, thus increasing the experience of pain [323]. Our results support this hypothesis and are in line with Lee, Ho and Song (2015) [321], as fibromyalgia patients presenting low COMT activity exhibited higher FIQ scores than those with high COMT activity.

Regarding the polysomnography, FM patients with high and low COMT activity differed in number of awakenings. Patients with high COMT activity show fewer awakenings than low COMT activity ones. The number of awakenings is usually correlated with the relative power of alpha band [67]. Patients with high COMT activity presented lower relative power of alpha band than patients with low COMT activity. According to Bodenmann et al. [67], mechanisms involving COMT activity contribute to interindividual differences in brain alpha oscillations, which are functionally related to executive functions in healthy individuals, and predict stable and frequency-specific interindividual variation in brain alpha oscillations in wakefulness, rapid-eye-movement (REM) sleep, and non-REM sleep [67, 301].

Patients with high and low COMT activity differed regarding the number of awakenings in sleep architecture, as well as to some questionnaires (FIQ and HAD). It is widely known [301] that antidepressants and anxiolytics have effects in the quality and architecture of sleep of waking sleep stage. Given that the use of antidepressants and anxiolytics was similar in patients with high and low COMT activity, it does not seem probable that group differences on quality of sleep and depression were due to medication.
Furthermore, it should be considered that frequency of temporal-parietal alpha band observed in FM subjects overlaps kappa band (7-11 Hz), a typical frequency band usually observed during wake-sleep stage [324] or during the deep sleep [325] in 11 to 30% of total sleep [326]. This kappa rhythm during wake-sleep stage has been related to reasoning and to anxiety [324]. Moreover, it has been found that kappa band activity in FM patients was more prominent than alpha rhythm during deep sleep [160]. Thus, it could be argued that feelings of unrefreshed sleep and fatigue during the day could be due to the presence of enhanced kappa activity during the sleep.

Some authors [201, 300] performed MSE analysis on EEG data aiming the identification of physiopathological disorders during sleep, especially given its robustness in the characterization of such patterns. We reproduced their method for a group of FM patients, in order to test if this type of analysis, can provide new information about disorders in fibromyalgia. The present findings indicated that FM patients with low COMT activity had greater entropy over the right hemisphere than FM patients with high COMT activity during deep sleep and REM, but not during light sleep. Moreover, it should be taken into account that increased alpha is related to micro-awakenings [67], and that FM patients with low COMT activity had 1.22 times more alpha events than FM patients with high COMT activity (Appendix C). Thus, it could be that higher entropy during REM stage in C4 could be related to the higher number of micro-awakenings. In any case, our findings may suggest that patients with the genetic haplotype high COMT activity displayed more complex brain functioning during sleep that patients low COMT activity.

Another point we should mention is the anatomic location of C3 and C4 electrodes. Both electrodes are located over four Brodman’s area: ba01, ba02, ba03 e ba04 [327]. Recordings from leads placed centrally (Cz) reflect EEG activity summed from both frontal and parietal regions and are considered the most sensitive for recording spindle activity [328, 329]. Conversely, spindle incidence is reduced in older adults [330], in various central nervous system diseases, including brain tumors, stroke, infection [331], depression [332], insomnia [333]; and with experimental auditory stimulation in NREM stage II [334].

Moreover, higher entropy in patients with high COMT activity could also be correlated
with more sadness, more movement (that can be inferred from the higher number of micro-
awakenings, and decreased deep stage), or a higher pain intensity (that can affect sleep quality).
Numbers of awakenings can increase the heterogeneity of signals increasing entropy. Results
from correlations with low predisposition to work and missed work days correlated with a
lower delta band activity in light sleep and increased entropy in the EEG signal during deep
sleep further provide support for this notion. Accordingly, Affleck and collaborators [172]
showed that a painful day is followed by a night of poor sleep, and conversely, a night of poor
sleep is followed by a significantly more painful day among FM patients. Our correlations
demonstrate that being anxious and depressive may lead to higher entropy of the EEG signal
during the light stage and deep sleep, for both groups of patients, regardless of antidepressants
and anxiolytics intake.

According to a study published in 2010 by Sitges et al. [318], chronic pain patients dis-
played increased MSE and fractal dimensionality values over the right parietal region, sug-
gest ing that non-painful stimulation of the left hand elicits an enhanced alertness-like state and
a greater activation of neuronal cell assemblies over the contralateral somatosensory cortex,
when compared with healthy subjects. Additionally, the pattern of EEG frequency band over
sensorimotor and temporal regions in these patients suggests an abnormal engagement of brain
networks related to emotional processing. According to the authors, the higher entropy found
in the right-sided parietal EEG activity could reveal this system’s mode of brain operation in
chronic pain. On the contrary, repetitive stimulation (like the one delivered to the right hand)
can be easily predicted by the system, which therefore does not need to lead to a flexible,
alertness-like state in chronic pain.

The val158met polymorphism of COMT appears to influence pain sensitivity through cen-
tral mechanisms such as reduced responses in µ-opioid endogenous system to sustained pain,
higher receptor binding and enhanced brain processing of sensory and affective pain compo-
nents in met carriers [69, 335]. Martínez-Jauand and coworkers [13] demonstrated that the
frequency of genetic variations associated with low COMT enzyme activity was higher in FM
patients than in healthy controls, and that FM patients who possess those genetic combinations
displayed an increased sensitivity to experimental pain.
It is already known that chronic pain patients present a higher complexity in the EEG signal than healthy subjects [318]. Goldberger et al. [336], suggest that the MSE analysis of EEG is coherent with the theory of complexity loss in disease [336], which relates disease to less complex biomedical signals. Our results suggest that the MSE analysis showed statistically lower entropy for low COMT activity patients than for high COMT activity ones at certain time scales.

Conclusions

In summary, it was found that patients with different genetic haplotype correlated with low COMT enzyme activity displayed increased MSE in their EEG during REM sleep in C4, at relatively high time scale frequency. Previous studies have reported altered complexity of MSE for sleep EEG signals in health people [199], in patients with Parkinson [201], depression [337], and EEG signals Alzheimer disease [338,339], of biological signals (EKG, EEG, balance-related center of pressure dynamics - COP) [308,313,314]. Therefore, the nonlinear analysis for example the MSE approach combined by a wide range of time scale analysis of EEG may be regarded as a powerful complementary tool for assessing the characteristics of patients with FM. Hence, this work represents a relevant contribution considering that most of previous research of sleep in fibromyalgia was based on retrospective reports or on self-report measures and sleep architecture conventional methods, using PSG.

The present study has some limitations that should be considered for the interpretation of the results. Firstly, the sample of the study was very small and, therefore, our conclusions should be taken as preliminary. Furthermore, the lack of control pain-free subjects did not allow us to analyze if the effects of the COMT polymorphism in FM patients were modulated in a different way under the presence of chronic pain. We subdivided the EEG records into subsets of 30 seconds each one. For our analysis we used only three subsets for each sleep stage along 3 different moments of sleep recording (at the beginning, at the end, and at the middle of the sleep recording). Also, we only used two central electrodes for EEG recordings; the values of MSE from multiple electrodes in EEG may give more information about brain
dynamics. For future research it would be interesting to use more electrodes as well as to make a multiscale analysis of the entire sleep recording. It should be also noted that many patients with chronic pain in the present study were taking antidepressants and anxiolytics during data collection and, therefore, the possible side effects of these drugs on sleep could not be completely discarded. Nevertheless, it should be noted that after excluding those patients who were taking such medication, used such medicines, the same differences appeared regarding sleep architecture and questionnaires were observed between the two groups of patients (high and low COMT activity). This probably suggests that differences in number of awakenings were not influenced by medication. However, it should be tested with a higher number of patients to explore the possible interaction of medication and COMT haplotypes. Furthermore, our finding were obtained on female adults and, therefore, no information about modulatory effect of gender on the association between sleep patterns and the COMT haplotypes on the val158met polymorphism in fibromyalgia patients compared with healthy controls could be explored.

Our findings revealed that patients, with low COMT activity were more affected by fibromyalgia than high COMT activity ones. The importance of genetic conditions in the definition of a healthy sleep and sleep disorders is increasingly recognized today. Val158met is considered to be one of the most relevant functional polymorphism of the COMT gene involved in fibromyalgia [186, 340, 341]. Nevertheless, the COMT gene is probably not a gene for a particular disease but might have some critical effects on pre-frontal cognitive performance, behavioral profile, sleep architecture, sleep EEG, vulnerability to sleep loss, and response to stimulant treatment [186]. Over the years, it has became obvious that FM is not a homogeneous condition and that different subtypes of patients might exist [13, 342, 344]. In this sense, our findings suggest that specific genetic variations of the COMT gene might have different consequences for sleep quality for FM patients.

**Acknowledgments**

We thank the Fibromyalgia Associations of Inca and Felanitx (Majorca, Spain) for their support by patients recruitment. We also thank to Mercedes Martínez-Jauand for performing analysis of
genetic haplotypes in the subjects of this study. Thank you so much to Priscila Aquino, Serena Carollo and colleagues from the lab: Anna Zamorano, Carolina Sitges, Ana Gonzales, Ana Mantecón, Xisca Roselló and Juan Gea, for their help in the last polysomnography recordings; and to Professor Mourad Akaarir for teaching me very patiently to correct and analyze the polysomnography. This work was supported by fellowships from the Universitat de les Illes Balears (Majorca, Spain) and the Brazilian National Council of Research and Development (CNPQ, Brazil) (201499/2012-6) to IC, as well as by grants from the Spanish Ministry of Economy and Competitiveness and European Regional Development Funds (♯PSI2010-19372) and the Spanish Ministry of Education and Culture (♯SAF2007-66878-C02-02) and JGVM was supported by a Brazilian grant (CNPq, process 306571/2011-0).
Figures
Figure 4.3: Hypnograms of patients with different COMT activities: (a) Hypnogram of a FM patient with high COMT activity. (b) Hypnogram of a FM patient with low COMT activity. Sleep stages are represented in the Y-axis by the symbols (I-IV, stages of sleep; REM, Rapid eye movement; Movement, refers to the movement out of awakening period.
Figure 4.4: Average of EEG power spectra of sleep record for high and low COMT activity patients. Relative power values for each frequency band and standard errors (bars) of PSA of electroencephalography (EEG) according sleep stage of patients with FM associated with sensitivity catechol-O-methyltransferase (COMT). The color dark grey represents high COMT activity and light grey is a low COMT activity patients. \(\delta\) = delta, \(\theta\) = theta, \(\alpha\) = alpha and \(\beta\) = beta bands.
Figure 4.5: Surrogate analysis of EEG sleep data. Red line represents the entropy of REM stage EEG data at 20 different scales (1-20). Yellow and Blue lines represent the MSE for a surrogate version of the EEG data and a fake random series at the same 20 scales, respectively. The surrogate outcome is quite similar to the random data and totally different from the REM segment. It proves that “shuffling” the EEG time series will always break its time correlation.
Figure 4.6: MSE analysis of EEG data of sleep record. Multiscale entropy (MSE) analysis of electroencephalography (EEG) according to sleep stage of patients with FM associated with sensitivity catechol-O-methyltransferase (COMT). The color blue represents high COMT activity group and the color red represents low COMT activity group, and with “x” representing the significant differences of MSE at certain time scale factor. The S1 represents stage I, S2 stage II, S3 stage III and S4 stage IV for sleep.
Figure 4.7: Mean multiscale entropy (MSE) values of EEG data of sleep record for time scale factors 1, 5, 10, 15, 20 at C3 and C4 electrodes associated with high and low COMT activity. Mean scores and standard errors (bars) of MSE analysis of electroencephalography (EEG) according sleep stage of patients with FM associated with sensitivity catechol-O-methyltransferase (COMT). The color dark grey represents high COMT activity and light grey is a low COMT activity patients.
Tables
Table 4.5: Clinical characteristics of fibromyalgia patients associated with catechol-O-methyltransferase (COMT) val158met polymorphism.

<table>
<thead>
<tr>
<th></th>
<th>High COMT</th>
<th>Low COMT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N = 13</strong></td>
<td><strong>N = 11</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>51.08 ± 8.03</td>
<td>52.5 ± 7.07</td>
</tr>
<tr>
<td><strong>FM History (yrs)</strong></td>
<td>9.08 ± 8.08</td>
<td>9.30 ± 5.05</td>
</tr>
<tr>
<td><strong>Medication(%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
<td>46.15</td>
<td>23.07</td>
</tr>
<tr>
<td>Analgesics/NSAID</td>
<td>53.84</td>
<td>34.46</td>
</tr>
<tr>
<td>Muscle relaxants</td>
<td>34.46</td>
<td>7.69</td>
</tr>
<tr>
<td>Anxiolytics</td>
<td>34.46</td>
<td>7.69</td>
</tr>
<tr>
<td>Other drugs</td>
<td>69.23</td>
<td>46.15</td>
</tr>
<tr>
<td><strong>Visual Analogic Scale (0-10)</strong></td>
<td>6.00 ± 2.4</td>
<td>5.50 ± 2.17</td>
</tr>
<tr>
<td><strong>Fibromyalgia Impact Questionnaire (FIQ)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical impairment (0-3)*</td>
<td>1.19 ± .83</td>
<td>2.04 ± .63</td>
</tr>
<tr>
<td>Feel good (0-7)</td>
<td>1.40 ± 1.07</td>
<td>.57 ± .97</td>
</tr>
<tr>
<td>Work missed (0-7)</td>
<td>2.70 ± 2.16</td>
<td>4.12 ± 2.69</td>
</tr>
<tr>
<td>Do work (10 cm VAS)</td>
<td>7.55 ± 2.84</td>
<td>9.2 ± .95</td>
</tr>
<tr>
<td>Pain (10 cm VAS)</td>
<td>8.10 ± 1.24</td>
<td>8.92 ± .93</td>
</tr>
<tr>
<td>Fatigue (10 cm VAS)</td>
<td>8.88 ± 1.27</td>
<td>9.21 ± .76</td>
</tr>
<tr>
<td>Rested (10 cm VAS)</td>
<td>8.0 ± 2.92</td>
<td>7.64 ± 3.36</td>
</tr>
<tr>
<td>Stiffness (10 cm VAS)</td>
<td>7.5 ± 2.95</td>
<td>6.86 ± 3.14</td>
</tr>
<tr>
<td>Anxiety (10 cm VAS)</td>
<td>7.95 ± 1.78</td>
<td>7.29 ± 3.25</td>
</tr>
<tr>
<td>Depression (10 cm VAS)</td>
<td>5.75 ± 3.27</td>
<td>5.4 ± 3.47</td>
</tr>
<tr>
<td><strong>Total FIQ score (0-100)</strong></td>
<td>66.80 ± 15.12</td>
<td>75.44 ± 15.67</td>
</tr>
</tbody>
</table>

**Hospital Anxiety and Depression Scale (HADS)**

<table>
<thead>
<tr>
<th></th>
<th>High COMT</th>
<th>Low COMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety</td>
<td>11.27 ± 3.35</td>
<td>9.89 ± 6.09</td>
</tr>
<tr>
<td>Depression*</td>
<td>9.18 ± 1.25</td>
<td>11.43 ± 2.37</td>
</tr>
</tbody>
</table>

*p < .05

* Chi-square test was used

NSAID = nonsteroidal anti-inflammatory drugs

High COMT activity = Haplotype LPS carries; Low COMT activity= HPS-APS carries
Table 4.6: Scores obtained in the polysomnographic variables and sleep questionnaires associated with catechol-O-methyltransferase (COMT) val158met polymorphism.

<table>
<thead>
<tr>
<th>Polysomnographic variables</th>
<th>High COMT N = 13</th>
<th>Low COMT N = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sleep time (min)</td>
<td>350.11 ± 84.91</td>
<td>368.18 ± 66.14</td>
</tr>
<tr>
<td>Time in bed (min)</td>
<td>459.38 ± 27.91</td>
<td>484.54 ± 15.65</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>76.04 ± 17.85</td>
<td>75.86 ± 12.66</td>
</tr>
<tr>
<td>Time of wakefulness after sleep onset (min)</td>
<td>43.11 ± 49.57</td>
<td>34.64 ± 31.42</td>
</tr>
<tr>
<td>Number of awakenings*</td>
<td>41.15 ± 30.06</td>
<td>64.68 ± 23.13</td>
</tr>
<tr>
<td>Micro-waking REM</td>
<td>22.31 ± 16.73</td>
<td>38.79 ± 26.34</td>
</tr>
<tr>
<td>Micro-waking NREM</td>
<td>22.32 ± 19.11</td>
<td>23.38 ± 13.57</td>
</tr>
<tr>
<td>Micro-waking Total</td>
<td>22.89 ± 13.08</td>
<td>27.30 ± 13.26</td>
</tr>
<tr>
<td>Stage I (%)</td>
<td>30.01 ± 18.34</td>
<td>24.74 ± 8.07</td>
</tr>
<tr>
<td>Stage II (%)</td>
<td>39.10 ± 9.57</td>
<td>33.20 ± 9.0</td>
</tr>
<tr>
<td>Stage III (%)</td>
<td>11.03 ± 7.29</td>
<td>9.29 ± 5.45</td>
</tr>
<tr>
<td>Stage IV (%)</td>
<td>4.0 ± 4.5</td>
<td>6.0 ± 5.9</td>
</tr>
<tr>
<td>REM Stage (%)</td>
<td>10.56 ± 30.0</td>
<td>10.43 ± 56.7</td>
</tr>
<tr>
<td>REM latency (minutes)</td>
<td>165.15 ± 95.81</td>
<td>140.27 ± 116.06</td>
</tr>
<tr>
<td>Sleep latency (min)</td>
<td>22.31 ± 17.82</td>
<td>34.14 ± 27.09</td>
</tr>
</tbody>
</table>

Sleep Questionnaires

| OSQ Insomnia                                | 27.33 ± 6.85     | 28.43 ± 4.65    |
| OSQ Hyperinsomnia                          | 9.0 ± 4.33       | 8.57 ± 4.43     |
| PSQI Total                                 | 10.70 ± 3.97     | 10.57 ± 3.31    |

*<p> < .05
REM = Rapid Eye Movement; OSQ = Oviedo Sleep Questionnaire; PSQI = Pittsburgh Sleep Quality Index
High COMT activity = Haplotype LPS carries; Low COMT activity = HPS-APS carries
**Table 4.7:** Significant correlations between some questionnaires and frequencies bands of PSA values of sleep stages in fibromyalgia patients.

<table>
<thead>
<tr>
<th></th>
<th>FIQ</th>
<th>HAD Anxiety</th>
<th>VAS Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Light sleep stage at C3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta band</td>
<td>-.44*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theta band</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha band</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Light sleep stage at C4</strong></td>
<td>-.44*</td>
<td></td>
<td>.42*</td>
</tr>
<tr>
<td>Delta band</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theta band</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha band</td>
<td></td>
<td>-.49*</td>
<td></td>
</tr>
<tr>
<td><strong>Deep sleep stage at C3</strong></td>
<td>-.45*</td>
<td>-.57**</td>
<td></td>
</tr>
<tr>
<td>Delta band</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>REM sleep stage at C3</strong></td>
<td>-.57*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* indicates $p < .05$

** indicates $p < .01$

FIQ = Fibromyalgia Impact Questionnaires
HAD = Hospital Anxiety and Depression scale
VAS = Visual Analogic Scale

---

**Table 4.8:** Significant correlations between some questionnaires and entropy values of sleep stages in fibromyalgia patients.

<table>
<thead>
<tr>
<th></th>
<th>FIQ</th>
<th>HAD Anxiety</th>
<th>VAS Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Light sleep stage at C3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entropy at Scale 1</td>
<td></td>
<td>.57*</td>
<td></td>
</tr>
<tr>
<td><strong>Light sleep stage at C4</strong></td>
<td></td>
<td>.58*</td>
<td></td>
</tr>
<tr>
<td>Entropy at Scale 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entropy at Scale 10</td>
<td>.62*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entropy at Scale 15</td>
<td>.53*</td>
<td></td>
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</tr>
<tr>
<td>Entropy at Scale 20</td>
<td>.60*</td>
<td></td>
<td>.54*</td>
</tr>
<tr>
<td><strong>Deep sleep stage at C4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entropy at Scale 05</td>
<td>.58*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entropy at Scale 10</td>
<td>.56*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entropy at Scale 15</td>
<td>.65**</td>
<td></td>
<td>.49*</td>
</tr>
<tr>
<td>Entropy at Scale 20</td>
<td>.56*</td>
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<td></td>
</tr>
<tr>
<td><strong>REM sleep stage at C4</strong></td>
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<tr>
<td>Entropy at Scale 1</td>
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</tr>
</tbody>
</table>

* indicates $p < .05$

** indicates $p < .01$

FIQ = Fibromyalgia Impact Questionnaires
HAD = Hospital Anxiety and Depression scale
VAS = Visual Analogic Scale

---

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4.3 Study 3: Impact of genetic variation in COMT (val158met) on emotion processing among patients with fibromyalgia

Abstract

Fibromyalgia is a chronic pain condition characterized by negative affective mood state and central sensitization. Although the etiology of fibromyalgia is still unclear, abnormal brain processing of affective-related body information and enhanced pain sensitivity have been discussed as relevant hallmarks of this syndrome. Recently, it has been also emphasized that the val158met polymorphism (substitution of valine by methionine at codon 158) of the catechol-O-methyltransferase (COMT) gene could be associated with pain sensitivity and mood in fibromyalgia patients. In particular, met homozygotes are characterized by low activity of the COMT enzyme, together with increased pain sensitivity and negative mood as compared with val carriers (val homozygotes and val/met heterozygotes). The aim of the present study was to assess if these two subgroups of patients with fibromyalgia were different on brain processing of affective startle modulation. For this purpose, amplitudes of event-related potentials, power densities at several EEG band frequencies and multiscale entropy of EEG time-series elicited by affective stimuli were analyzed. Twenty fibromyalgia patients were genotyped and classified into low vs. high COMT activity subgroups. The task consisted on the presentation of pleasant, unpleasant and neutral pictures followed by an acoustic startle probe in some trials. Results indicated that FM patients with low COMT activity showed more depression and physical impairment, as well as more reduced N100 amplitudes and higher entropy values than FM patients with high COMT activity. These findings provide preliminary evidence for the modulatory role of genetic haplotypes involved in the regulation of catecholamines on brain processing of affective information in patients with fibromyalgia.

Keywords: Fibromyalgia; Pain; Startle; Emotion
Introduction

Fibromyalgia (FM) is a chronic syndrome characterized by widespread pain, fatigue and unre-
refreshed awaking problems, as well as affective disturbances, cognitive deficits and somatic
symptoms [5]. FM has a around 2% to prevalence in the population and appears to affect
women more frequently than men [249,345,346], with a peak age of onset in the range 30-
50 years [249,347]. Patients with fibromyalgia have 2-3 times higher healthcare costs [348,
349] and report poorer well-being compared to healthy persons [350]. Although its etiology is
still unclear, FM has been associated with abnormal neuroendocrine function [351] and brain
activity [219,220,352,353], as well as with genetic factors [13,354]. Additionally, it has been
emphasized the role of emotions in the origin and maintenance of FM, suggesting that patients
might be particularly vulnerable to the effects of negative mood [50,51,221]. In this sense,
it has been suggested that clinical symptoms could be at least partially related to an altered
functioning of central nervous system (central sensitization or hyperexcitability) during the
processing of somatosensory and affective information [227] which, in turn, could be linked to
a malfunctioning of the monoamine system [236,237].

The val158met polymorphism in the gene encoding catechol-O-methyltransferase (COMT)
leading to a substitution of valine (val) by methionine (met) at codon 158 has been associ-
ated with a three- to four-fold reduction in the enzymatic degradation activity in the synaptic
cleft [227]. Thus, it has been shown that val allele homozygotes exhibited higher COMT
activity and lower dopaminergic signaling in prefrontal cortex [64], hippocampus [355], and
corticolimbic structures [356] than met allele homozygotes (low COMT activity). Moreover,
there is evidence for a role of genetically induced changes in catecholamine metabolism in
modulating the generation of alpha EEG oscillations during wakefulness and sleep in healthy
individuals. For instance, it has been found that val homozygotes compared with met homozy-
gotes displayed slower α peak frequency in waking, as well as reduced power activity in the
11–13 Hz EEG band, independently of vigilance/sleep state or experimental interventions such
as sleep deprivation or administration of stimulants [67]. Empirical evidence has further shown
that genetic differences on COMT enzymatic activity could be also associated with interindi-
vidual differences in cognitive and emotional processing. Thus, carriers of the met allele of COMT were previously found to more efficiently avoid perseverative errors than carriers of the val allele \[357, 358\], while carriers of the val allele displayed higher levels of emotional regulation \[65, 66\].

Previous neuroimaging studies have shown that the COMT val158met genotype may also affect functional activation and connectivity of brain circuits involved in affective arousal and regulation in healthy individuals. COMT genotype was related to brain responses to unpleasant stimuli in the limbic system and the connected prefrontal areas, with higher reactivity to unpleasant stimuli \[359, 360\] and more enhanced functional connectivity \[361\] in carriers of met alleles than in carriers of val alleles. Furthermore, variations in the gene coding for COMT appear to be linked to variability in pain sensitivity \[13, 29, 70, 71, 323, 362, 363\]. Fibromyalgia patients with met/met alleles (low COMT activity) showed higher sensitivity to thermal and pressure pain stimuli than patients carrying val alleles (high COMT activity) \[13\], and healthy met homozygotes showed higher pain levels following a single opioid dose \[363\] and lower capacity to activate $\mu$-opioid neurotransmission in thalamus, basal ganglia, limbic and paralimbic areas in response to a sustained pain challenge \[323\]. Nevertheless, physiological mechanisms that explain these differences on COMT activity and pain sensitivity are far to be clear. Thus, for instance, it has been suggested \[341\] that met carriers may have a 20-fold reduction in COMT activity mainly due to stable local stem-loop structures in the val158 region and leading to enhanced pain sensitivity. By contrast, val/met heterozygotes may have a 3-fold reduction in COMT activity mainly due to reduced stability of the enzyme at normal physiologic temperatures and leading to moderate pain sensitivity.

In the present study, the affective modulation of the startle response was used to characterize the impact of genetic variation in COMT on emotion processing among patients with fibromyalgia. For this purpose, a psychophysiological paradigm which allows the recording of involuntary responses (peripheral or central) to a sudden, intense stimulus, during the presentation of affective stimuli (slides depicting scenes of different emotional valence) has been frequently used (see Grillon Baas, 2003, for review) \[224\]. The affective modulation of the startle reflex refers to a well-known and replicated phenomenon whereby the amplitude of the
startle reflex is potentiated or inhibited by the presentation of negative or positive stimuli, respectively [364–366]. According to Lang’s priming motivational hypothesis, aversive pictures would induce an emotional state that acts to prime this defensive system, thus enhancing the startle reflex [367, 368]. There is huge evidence showing that affective startle reflex modulation may be helpful to address individual differences on emotional processing in patients with affective disorders (anxiety, depression, phobia) [224]. Nevertheless, research on the affective modulation of protective reflexes in fibromyalgia is still scarce and contradictory results have been found. Two studies found that patients with FM displayed similar affective modulation than healthy individuals when startle eyeblink was elicited by noise probes [206] or pressure pain [16]. By contrast, two recent studies observed a lack of affective modulation of startle reflex in FM patients [218, 225].

In the present study, we examined the impact of the COMT val158met polymorphism on brain correlates of affective startle reflex modulation among patients with fibromyalgia. A recent study found that COMT genotype significantly affected startle reflex modulation by aversive stimuli, with met homozygotes exhibiting a markedly potentiated startle reflex compared with val carriers [369]. Given prior findings linking the met allele to enhanced pain sensitivity and affective symptoms, we hypothesized that FM patients with the met/met genotype (low COMT activity) would show greater brain activity elicited by unpleasant stimuli than FM patients carrying a val allele (val/met and val/val genotypes) (high COMT activity).

**Material and Methods**

**Participants**

The same sample of 26 women diagnosed with fibromyalgia (FM) (mean age 50.55 ± 8.26) from Study 2 also participated in the present study. Genetic information about the val158 polymorphism of the COMT gene was obtained from their participation in a previous study of our laboratory [13]. However, two patients were excluded because their genetic material was lost, and four patients were excluded from the analysis due to poor data quality in the electroencephalography (EEG) recording. To test the effects of the val158met polymorphism of the
COMT gene on clinical data and emotional processing, participants were divided according with the number of \textit{met} alleles into low COMT activity (\textit{met} homozygotes) (n=10) and high COMT activity (\textit{val} homozygotes and \textit{val/met} genotype) (n=10). This classification was according with previous literature \cite{13,29,70,71,323,362,363}. Participants were given a detailed explanation of the experimental procedure and they signed a written informed consent prior to participate in the study. The experimental procedure was approved by the local ethics committee.

\textit{Self-report measures}

All patients underwent an assessment of clinical pain characteristics through self-report questionnaires. The Spanish versions of the Fibromyalgia Impact Questionnaire (FIQ) \cite{283}, the West Haven–Yale Multidimensional Pain Inventory (WHYMPI) \cite{258}, the McGill Pain Questionnaire (MPQ) \cite{257}, Quality of life questionnaireEspiditest \cite{254}, Medical Outcome Study Short Form (SF-36) \cite{256}, Hospital Anxiety and Depression Scale (HAD) \cite{253}, as well as two sleep Questionnaires: the Pittsburgh Sleep Quality Index (PSQI) \cite{272} and the Oviedo Sleep Questionnaire (OSQ) \cite{273} were fulfilled.

At the end of the experiment patients also completed the Self-Assessment Manikin (SAM) \cite{274} to rate pleasantness and arousal elicited by affective pictures. This instrument consists of two sets of humanoid figures representing the dimensions of pleasantness and arousal. Each rating scale includes nine levels of intensity, ranging from a smiling to a frowning figure for pleasantness and from an apparently agitated to a sleepy-looking figure for arousal \cite{275}. Participants were instructed to assess how they felt while viewing each pictures by using these scales.

\textit{Emotion-induction task}

Stimuli were selected from the International Affective Picture System (IAPS) \cite{206,276,277} grouped into three categories: pleasant, neutral and unpleasant. The IAPS constitutes a standardized and exhaustively investigated set of color pictures containing more than 900 items for studying emotion and attention \cite{55}. The content of the pictures ranges from everyday ob-
jects and scenes (e.gr., household furniture and landscapes) to extremely rare (e.gr., mutilated bodies) or exciting scenes (e.gr., erotic nudes, extreme sports). The IAPS also contents a detailed list of average ratings of valence and arousal elicted by each picture separated by gender and age. In the present study, sixty-six pictures were selected according with their average valence and arousal ratings in the Spanish version of the IAPS [370,371]. The stimulus contents were chosen such that there was no overlap in IAPS normative affective valence ratings, i.e. the three stimulus contents were distinct and representative of each affect category (pleasant, neutral and unpleasant). Mean and standard deviation of valence and arousal ratings for the selected stimulus categories from normative data were as following:

**Table 4.9:** Mean and standard deviations of the ratings from the selected pictures in the three affective categories used in the present study and obtained from standardized data published by Lang et al.(2008) [372].

<table>
<thead>
<tr>
<th></th>
<th>Pleasant</th>
<th>Neutral</th>
<th>Unpleasant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Valence</strong></td>
<td>7.54 ± 1.54</td>
<td>5.06 ± 1.34</td>
<td>2.22 ± 1.38</td>
</tr>
<tr>
<td><strong>Arousal</strong></td>
<td>6.43 ± 2.26</td>
<td>3.45 ± 2.01</td>
<td>6.52 ± 2.11</td>
</tr>
</tbody>
</table>

Each trial lasted 16 seconds and began with a white fixation cross on a black screen during 8 seconds followed by the affective picture presentation during 6 seconds and a period of 2 seconds for valence and arousal rating. The order of stimulus presentation was randomized for each participant. A startle probe was also pseudo randomly presented either during the fixation cross (in 12 trials, as described in Figure 4.8-A) or during the stimulus presentation (in 54 trials, as described in Figure 4.8-B). The startle probe consisted of an intense tone (white noise, intensity 105 db, duration 50 ms).

*The IAPS slide numbers were as follows: pleasant 4510, 4531, 4533, 4607, 4608, 4651, 4652, 4653, 4659, 5621, 5623, 5626, 5629, 8080, 8161, 8170, 8210, 8370, 8380, 8420, 8470, 8490; neutral 2190, 2200, 5500, 7000, 7010, 7025, 7030, 7040, 7050, 7060, 7080, 7090, 7100, 7130, 7150, 7170, 7217, 7490, 7500, 7550, 7700; unpleasant 1090, 1290, 2120, 2691, 2710, 2750, 3160, 3230, 6200, 6831, 6940, 9181, 9404, 9420, 9421, 9440, 9490, 9520, 9560, 9611, 9920, 9921
Figure 4.8: Trial design - Trials always started with a white fixation cross on a black screen (8 seconds) followed by the affective picture presentation (6 seconds) and a period for valence and arousal ratings (2 seconds). In addition, an acoustic startle was presented either during the last 2 seconds of blank screen presentation (A) or during the last second of picture presentation (B).

**EEG recording and analysis data**

Electroencephalography was recorded from 32 scalp electrodes placed following the international 10/20 system with reference electrodes at ear mastoids. An electrooculogram channel was obtained by placing one electrode above and one electrode below the left eye. Electrode impedance was kept below 10 KΩ. The signals were registered by a BrainAmp MR amplifier at a sampling rate of 1000 Hz, with high and low pass filter settings at 0.01 Hz and 70 Hz, respectively. A 50 Hz notch filter was also applied.

**Procedure**

Participant’s fulfilled the questionnaires after arrival to the laboratory, immediately after informed consent was obtained. Participants were seated in a dimly lit, sound-attenuated room in front of a computer screen at a viewing distance of 110 cm. They were instructed to view the picture stimuli and to rate their valence and arousal after each trial by using a Self-Assessment Manikin (SAM) \[354]\). The experiment lasted around 15 minutes.

**EEG Data preprocessing**

EEG waveforms were initially segmented in epochs of 1000 ms duration (-100 ms to 900 ms relative to stimuli onset), filtered (40 Hz low pass and 0.05 Hz high pass), and baseline
corrected (from -100 ms to 0 ms). Then, eye movement artifacts were corrected by using Gratton & Coles algorithm \cite{373}. Finally, an artifact rejection protocol with following criteria was applied to obtain artifact-free time-series: maximal allowed voltage step/sampling point 75 $\mu$V, minimum allowed amplitude -75 $\mu$V, maximal allowed amplitude 75 $\mu$V, and maximum allowed absolute difference 75 $\mu$V.

After removing artifacts, EEG data elicited by affective picture stimuli were analyzed by computing linear (evoked potential amplitudes at different latencies, and power density at different band frequencies), and non-linear parameters (multiscale entropy) from the time-series at each electrode location and each affective condition. For the analyses of visual evoked potentials (VEPs), artifact-free EEG time-series (or wave-forms) were averaged separately for each affective images (pleasant, neutral and unpleasant). Amplitudes of VEP components were obtained by computing the local maxima at Cz in the latency range of 100 to 200 ms (N100) and in the latency range of 200 to 300 ms (P200) after stimulus onset, as well as at Pz in the latency range of 300 to 450 ms (P300).

For the analyses of brain oscillations, fast-Fourier transformation was applied to artifact-free time-series and power density was computed for the following frequency bands: \textit{delta} (2 – 3.9 Hz), \textit{theta$_1$} (4 – 5.9 Hz), \textit{theta$_2$} (6 – 7.9 Hz), \textit{alpha$_1$} (8 – 9.9 Hz), \textit{alpha$_2$} (10 – 11.9 Hz), \textit{beta$_1$} (12 – 17.9 Hz) and \textit{beta$_2$} (18 – 22 Hz).

For the analyses of multiscale entropy (MSE), artifact-free time-series were first down sampled to generate multiple time series of varying time scales and sample entropy (irregularity pattern of the time-series) was computed by using the following algorithm (available at \url{http://www.physionet.org/physiotools/mse}):

\[
\text{Sample entropy (m, r, N)} = -\ln \frac{A_m (r, N)}{B_m (r, N)},
\]

where \( B_m \) is the probability that two patterns will match for \( m \) points, \( A_m \) is the probability that two patterns will match for \( m + 1 \) points, and \( N \) is the time scale \cite{318}. For calculation purposes, the parameter \( r \) of the algorithm was set to 15% of the standard deviation and the parameter \( m \) was set to 2 \cite{308, 374}. Thus, the complexity of time series was measured by taking into account the entropy with respect to multiple temporal scales \cite{375}. This is a method
for computing how a certain entropy measure varies as one successively coarsens a time-series over multiple scales, by computing averages over non-overlapping intervals of fixed size (the scale). In the simple way, the multiscale entropy is the calculation of the sample entropy for multiple scales of the original time series, generating an entropy pattern for different scales, hence making a better characterization of the system complexity. In the present study, the algorithm was applied to artifact-free EEG signals by using 5 scales factors (1, 5, 10, 15, 20) [219].

**Statistical analyses**

For statistical analyses, parameters obtained at each electrode location (VEP amplitudes, power densities and multiscale entropies) were grouped into following brain regions: F7, F3, Fz, F4, F8, FT7, FC3, FCz, FC4, FT8 (frontal); T7, C3, Cz, C4, T8, TP7, CP3, CPz, CP4, TP8 (central); P7, P3, Pz, P4, P8, O1, Oz, O2 (parieto-occipital). To further test topographical effects, EEG electrode locations were also grouped into left and right hemisphere (frontal: F7, F3, FC3 vs. F8, F4, FC4; central: T7, C3, CP3 vs. T8, C4, CP4; parieto-occipital: P7, P3, O1 vs. P8, P4, O2).

EEG parameters were statistically analyzed by using multivariate analyses of variance (MANOVA) for repeated measures with genetic group (high and low COMT activity) as between-subjects factor, and stimuli (pleasant, neutral vs. unpleasant) and brain location (frontal, central, parieto-occipital) as within-subject factors. Subjective ratings during the picture viewing task (valence and arousal) were statistically analyzed by using MANOVAs for repeated measures with genetic group (high and low COMT activity) as between-subjects factor, and stimuli (pleasant, neutral vs. unpleasant) as within-subject factor. In case of violation of the sphericity assumption, degrees of freedom were corrected by using Greenhouse-Geisser epsilons.

To test significant differences between groups of patients on sociodemographic and questionnaire data, t-tests for independent samples were used. Correlations were tested by using Pearson correlation coefficients. Significant results were set at $p < 0.05$.  

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Results

Twenty patients were genotyped for the catechol-O-methyltransferase (COMT) \textit{val158met} polymorphism. As result, 10 patients were classified as \textit{met} homozygotes (\textit{high} COMT activity), and 10 patients were classified as \textit{val} carriers (\textit{low} COMT activity).

Sociodemographic and clinical characteristics

Table 4.10 displays the sociodemographic and clinical characteristics of the two subgroups of patients. No significant differences were observed between the subgroups with \textit{high} and \textit{low} COMT activity on age, years since FM diagnosis, medication, current pain intensity, and self-report questionnaires (all \textit{ps} > .05). Nevertheless, there were significant group differences on physical impairment scores from the FIQ [\textit{t}(13) = -2.69, \textit{p} = .02] and Depression scores from the HADS [\textit{t}(14) = -2.44, \textit{p} = .03] (Table 4.10), showing that patients with \textit{low} COMT activity were more depressed and more physically impaired than patients with \textit{high} COMT activity.

Subjective Ratings elicited by viewing affective stimuli

The MANOVA yielded significant effects due to stimuli on arousal (\textit{F}[2, 38] = 25.49, \textit{p} = .000) and valence ratings (\textit{F}[2, 38] = 204.69, \textit{p} = .000), indicating that unpleasant pictures were rated as the most arousing and negative, followed by the pleasant and neutral pictures (Figures 4.9 and 4.10). No significant differences due to group were observed either on valence or arousal ratings.
Amplitudes of event-related potentials

Within the first 450ms interval after stimuli onset, visual-evoked potentials (VEP) elicited by affective stimuli were characterized by a negative peak (N100) followed by a positive peak (P200), and a second positive peak (P300). The statistical analyses of the amplitudes at these components revealed following results:

- N100 (100-200ms after stimulus onset). Statistical analyses revealed significant effects of brain location ($F[2, 36] = 18.17, p = .000$), and brain location × genetic group ($F[2, 36] = 5.98, p = .02$). Post-hoc mean comparisons of the interaction effect showed that patients with high COMT activity (val carriers) displayed greater N100 amplitudes than patients with low COMT activity (met homozygotes) at parieto-occipital electrodes ($p = .016$), whereas no group differences were observed at frontal or central electrodes. Furthermore, post-hoc pairwise mean comparisons indicated that patients with high COMT activity (val carriers) displayed greater N100 amplitudes at frontal ($p = .001$) and central ($p < .001$) than at parieto-occipital electrodes, whereas no effects due to brain location was observed in patients with low COMT activity (met homozygotes).

- Figure 4.12
- Figure 4.13

- P200 (200-300ms after stimulus onset). The statistical analyses revealed significant effects of brain location ($F[2, 36] = 12.33, p = .001$), indicating that P200 amplitudes were higher at parieto-occipital electrodes than at frontal ($p = .02$) or central electrodes ($p = .02$). No effects due to genetic group or its interaction were significant.

- Figure 4.14

- P300 (300-450ms after stimulus onset). The statistical analyses revealed significant effects of brain location ($F[2, 36] = 22.55, p = .000$) and brain location × stimuli ($F[4, 72] = 3.86, p = .03$). Post-hoc pairwise mean comparisons of the brain location × stimuli interaction effect indicated that unpleasant stimuli elicited greater P300
amplitudes than neutral stimuli at parieto-occipital electrodes \((p = .032)\), whereas no further differences appeared at other brain locations. No effects due to genetic group or its interaction were significant.

– Figure 4.15 –

**EEG power density**

MANOVAs on power density were computed for each frequency band by grouping the electrodes at frontal (F7, F3, Fz, F4, F8, FT7, FC3, FCz, FC4, FT8), central (T7, C3, Cz, C4, T8, TP7, CP3, CPz, CP4, TP8), and parieto-occipital locations (P7, P3, Pz, P4, P8, O1, Oz, O2). No group differences were found in any of the band frequencies. Regarding the effects of brain location and stimuli, following results were obtained:

- **Delta** \((2−3.9\, \text{Hz})\). The MANOVA revealed a significant main effect due to brain location (frontal, central and parieto-occipital), \((F[2, 36] = 56.76, p = .000)\), showing that delta power displayed higher density at frontal than at central region \((p = .000)\) and greater at parieto-occipital than at central \((p = .000)\). No effects due to stimuli were observed.

- **Theta** \(_1\) \((4−5.9\, \text{Hz})\) and **theta** \(_2\) \((6−7.9\, \text{Hz})\). The multivariate test all suggest the presence of main effect for brain location on power density of theta\(_1\), \((F[2, 36] = 45.31, p = .000)\). Regarding power density of theta\(_2\), the MANOVA revealed a main effect for brain location \((F[2, 36] = 24.69, p = .000)\), indicating that theta\(_1\) power displayed higher density at frontal than at central region \((p = .000)\) and greater at parieto-occipital than at central \((p = .000)\). In theta\(_2\) power was greater at frontal than at central \((p = .02)\) and greater at parieto-occipital than at central \((p = .001)\). No effects due to stimuli were observed.

- **Alpha** \(_1\) \((8 − 9.9\, \text{Hz})\) and **alpha** \(_2\) \((10 − 11.9\, \text{Hz})\). Statistical analyses further revealed significant effects of brain location brain location \((F[2, 36] = 15.99, p = .000)\) indicating that alpha\(_1\) power was greater at parieto-occipital than at frontal \((p = .008)\) and greater at central than at parieto-occipital region \((p = .01)\). No effects due to stimuli were observed.
In *Alpha*$_2$: A Significant main effects in *brain location* ($F[2, 36] = 7.10, p = .003$) and *brain location $\times$ stimuli* ($F[4, 72] = 2.57, p = .04$), showing that *alpha$_2$* power was higher at parieto-occipital than frontal ($p = .08$) or central region ($p = .06$). The post-hoc pairwise mean comparisons of the *brain location $\times$ stimuli* did not give any result.

- *Beta$_1$* ($12 - 17.9$Hz) and *beta$_2$* ($18 - 22$Hz). Statistical analyses further revealed significant effects of *brain location* for *beta$_1$* analysis, ($F[2, 36] = 6.49, p = .004$), and *beta$_2$* ($F[2, 36] = 9.33, p = .001$), indicating that *beta$_1$* power displayed higher density at parieto-occipital than at central region ($p = .03$). In *beta$_2$* power was greater at frontal than at central ($p = .01$ or parieto-occipital ($p = .04$). No effects due to stimuli were observed.

- *Table 4.11, 4.12 and 4.13* –

**EEG - Multiscale Entropy Analysis**

The MANOVAs on entropy values revealed significant effects due to factors *stimuli* in the scale 10 ($F[2, 36] = 3.47, p = .05$), 15 ($F[2, 36] = 4.20, p = .02$), and 20 ($F[2, 36] = 4.05, p = .03$), indicating that the neutral stimuli elicited the highest values of entropy followed by the pleasant and unpleasant stimuli.

Significant differences due to the factor *hemisphere* was found in the scale 10 ($F[1, 18] = 5.37, p = .04$) and 15 ($F[1, 18] = 4.92, p = .04$), indicating that MSE values were greater at the left *hemisphere*.

Significant differences due to *brain location* were observed in the scale 1 ($F[2, 36] = 9.49, p = .001$), 5 ($F[2, 36] = 12.05, p = .000$), 10 ($F[2, 36] = 8.76, p = .001$), 15 ($F[2, 36] = 11.64, p = .001$) and 20 ($F[2, 36] = 22.67, p = .000$), showing that MSE values were greater at central than at parieto-occipital electrode locations.

A significant *hemisphere $\times$ genetic group* interaction effect was observed in the scale 5 ($F[1, 18] = 4.56, p = .05$) and a non-significant trend in scale 10 ($F[1, 18] = .15, p = .057$),
indicating that FM patients with high COMT activity displayed lower MSE values than patients with low COMT activity at the left hemisphere. In addition, it was found that MSE values were higher over the left hemisphere than over the right hemisphere in FM patients with low COMT activity, but not in patients with high COMT activity.

– Figure 4.11 –

Correlations

In order to further assess if power density, entropy and ERP amplitudes were related to clinical symptoms in fibromyalgia, Pearson correlations were computed between scores of self-report questionnaires (HAD and FIQ) and values of the different brain activity parameters (ERP amplitudes, power density and MSE values) (4.14 - 4.20).

– Tables 4.14 - 4.20 –

Results indicated that several subscales (“Do Work”, “Fatigue”, “Pain” and “Rested” of FIQ questionnaire and “Anxiety” as “Depression” of HAD) yielded significant correlations at least with one parameter of ERP amplitudes. It was observed that increased anxiety and depression were correlated with attenuated amplitudes of N100, P200 and P300. In relation to power density, significant correlations were obtained in the subscales subscales “Pain”, “Fatigue”, “Rested”, “Depression” and “Total FIQ” of FIQ questionnaire. Enhanced anxiety and depression were related to increased power density at delta, alpha and beta frequency bands. Regarding the multiscale entropy values, significant correlations were obtained in the subscales “Fatigue”, “Rested”, “Depression” of subscales FIQ questionnaire. Increased entropy values on the scales 1, 5 and 10 were correlated with increased anxiety and depression.

Discussion

Previous studies had demonstrated that patients with fibromyalgia displayed an abnormal brain processing of affective stimuli in comparison with healthy, pain-free controls [16,50,218,220,224,227,376]. Although its etiology is still unclear, fibromyalgia has been linked to central hyperexcitability, leading to enhanced pain sensitivity and, probably, to some plastic changes.
in emotional brain processing [12,15,50,227]. In addition, there is some evidence showing the val158met polymorphism of the catechol-O-methyltransferase (COMT) gene plays a relevant role in exacerbated pain sensitivity and affective disorders in patients with fibromyalgia [13,29,71,323]. Thus, for instance, it has been observed that percentage of met homozygotes is greater in patients with fibromyalgia than in the healthy population [13,14,323]. Moreover, it has been found that individuals with met alleles in this functional polymorphism have lower activity of the COMT enzyme and are more pain sensitive than individuals carrying a val allele [13,67,71,323,362]. According to these previous findings, the main objective of the present study was to assess if two genotypes of the val158met polymorphism of the catechol-O-methyltransferase (COMT) gene could differentiate brain responses to affective stimuli in patients with fibromyalgia. Basically, the present study revealed that FM patients with high and low COMT activity differed on self-reports of depression and physical impairment, as well as on N100 amplitudes of the visual ERPs and on entropy values of the EEG. FM patients with low COMT activity were more depressed and more physically impaired, and displayed more reduced N100 amplitudes and higher entropy values over the left hemisphere than FM patients with high COMT activity.

In general, scores from self-report questionnaires were in the same range as values reported in FM patients in previous works [319,320]. However, although there were no significant group differences on pain intensity, patients with low COMT activity (met homozygotes) were more depressed and more physically impaired than patients with high COMT activity (val carriers). In this sense, our findings were in agreement with previous studies, demonstrating that the COMT val158met polymorphism was associated with susceptibility to fibromyalgia, and that met homozygotes displayed higher sensitivity to pain [323], as well as higher anxiety and depression scores [377,380] than val carriers. Moreover, the fact that there were no significant differences between the two subgroups of FM patients on the number and type of pain drugs for alleviating their symptoms, further indicated that differences between patients with high and low COMT was affecting affective symptoms rather than pain intensity. These findings are in disagreement with those observed by previous studies in fibromyalgia [377] and cancer [381], indicating that subgroups of patients based on their COMT genotype exhibited
different physical and psychological outcomes, as well as different responses to pain medica-
tion. Nevertheless, this may be due to the small percentage (20% for both groups) of patients
taking antidepressants. In a recent study of our lab, it was reported that 80% of patients with
fibromyalgia were using antidepressants and that only 50% of these patients had a diagnosis of
depression [218].

In this line, the analyses of visual evoked brain amplitudes elicited by the viewing of affect-
tive stimuli revealed significant differences on N100 amplitudes due to patients’ classification
based on val158met polymorphism of the COMT gene. Basically, patients with high COMT
activity (val carriers) displayed greater N100 amplitudes to all affective stimuli over parieto-
occipital electrodes than patients with low COMT activity (met homozygotes). According with
previous research [318, 352, 376, 382, 383], this enhancement of N100 amplitudes over parieto-
occipital regions may be signaling the strong involvement of an attentional brain network dur-
ding the affective viewing task. Taking into account that picture presentations were frequently
followed by acoustic startles (regardless of emotional content), this attentional brain network
could be responsible for the activation of a generalized arousal and alert mechanism elicited by
external information (in this case, paired presentation of affective pictures and acoustic startle
 probes). By contrast, the reduced involvement of this attentional brain network in FM patients
with low COMT activity could be associated with a more reduced ability to be distracted with
exteroceptive cues during pain processing.

Nevertheless, no differences due to subgroups of patients based on the val158met polymor-
phism were observed on subjective ratings of affective stimuli. A recent study proposed that
the val158met polymorphism of the COMT gene could play a key role in phenotypic expres-
sion of pain in fibromyalgia, given that FM patients carrying the met/met genotype had more
severe psychological and functional impact scores than val carriers [384]. Moreover, it seems
that COMT variants moderate not only pain but also maladaptive coping processes in patients
with FM [385]. Thus, for instance, it has been demonstrated that met homozygotes experienced
more pain on those days when pain catastrophizing and pain attention scores were elevated, as
well as stronger reduction of positive affect on days when pain was elevated [73, 386]. All
these findings support a key role of the COMT gene in pain-related affective reactivity and
pain-related cognitions in patients with FM [385]. Nevertheless, the fact that both subgroups of patients had the same level of clinical pain may have led to inconclusive results regarding the val158met polymorphism of the COMT gene in this study.

In spite of the lack of group differences on subjective ratings elicited by the presentation of affective pictures, the present findings revealed that stimuli were appropriate for examining affective processing. As it was already observed in previous studies [218, 387], the three categories of affective stimuli clearly differed on valence ratings, and pleasant and unpleasant pictures were different from neutral ones on arousal ratings. Contrary to our expectations, we observed that although patients with low COMT activity were more depressed (but with similar pain intensity) than patients with high COMT activity, there were no significant group differences on valence and arousal ratings of affective stimuli. Our findings are partially in agreement with those observed in a previous study of our lab, showing that patients with FM patients rated the three categories of affective stimuli as less negative, but more arousing than healthy controls [218]. Furthermore, the present results seem to be in line with the notion that abnormal processing of affective stimuli in FM could be related to the presence of chronic pain rather than to affective symptoms such as depression. Previous reports have already shown that patients with FM were characterized by an abnormal affective processing including a significant bias towards the processing of negative information, impaired affect regulation, as well as increased emotional avoidance, catastrophizing, and alexithymia [50, 218, 219, 387–389] or generalized hypervigilance towards affective stimuli [390, 391]. Thus, it appears that patients with FM display a generalized deficit in the processing and self-regulation of all types of affective stimuli (regardless of their emotional contents) and, particularly, when affective information was arising from bodily signals together with somatic painful sensations. In any case, the ERP findings of the present study suggest that the role of the functional polymorphisms of the COMT gene in the cognitive and affective symptoms of chronic pain syndrome such as fibromyalgia should be further examined.

Regarding power density of brain oscillations elicited by affective stimuli, we observed that FM patients with low and high COMT activity displayed similar values of power density at several EEG band frequencies. Some studies have previously shown that patients with chronic
pain displayed significant reductions in these frequency bands in during resting state [392] and task-related conditions [393] as compared to healthy controls. It has been argued that reductions in alpha activity over posterior brain regions could be part of a general orienting and attentional response towards any kind of stimulation [394], or reflect a central and specific processing of the attention system during sustained painful stimulation [395]. Our findings do not provide evidence for a differential effect based on the genetic profile of patients with fibromyalgia.

Regarding multiscale entropy, patients with low COMT activity have higher entropy values than patient’s high COMT activity over the left hemisphere. These findings are partially in agreement with those observed by Sitges et al. 2010 [318], indicating that chronic pain patients displayed higher entropy and fractal dimension over the right than over the left parietal hemisphere, whereas no differences appeared in healthy controls. In the last years, the theory of nonlinear dynamical systems has been applied to electroencephalographic (EEG) and magnetoencephalographic (MEG) data in order to capture the macroscopic spatial and temporal dynamics of brain activity [396–400], and has provided suitable methods to understand how the brain works in healthy and pathological states [400]. For instance, nonlinear measures such as fractal dimension and entropy have proved successful in quantifying the complexity [401] and the regularity of EEG time series (i.e., the predictability of future amplitude values of the EEG based on one or two previous amplitude values), providing information about the flexibility that the brain needs to respond to cognitive demands [307, 374, 402]. Thus, low EEG entropy (reflecting enhanced EEG regularity and low brain complexity) has been associated with several physiological and pathological states including sleep, anesthesia, schizophrenia, Parkinson’s and Alzheimer’s diseases [201, 300, 400, 402] whereas high entropy has been observed in patients with chronic pain [318].

The present study has some limitations that should be taken into account for interpreting the findings. Firstly, the study sample was very small, and conclusions should be considered preliminary. The lack of a pain-free, control group does not allow to test if differences due to the COMT polymorphism were specific for FM patients or are present in individuals without chronic pain and FM symptoms. Moreover, many patients were treated with analgesics and antidepressants, which may have an effect on the EEG signals.
In summary, the present study revealed that subgroups of FM patients based on genetic haplotypes associated with COMT enzyme activity displayed significant differences on subjective self-reports of depression and physical impairment, as well as on N100 amplitudes and entropy values. The fact that patients with low COMT activity showed more depression, physical impairment, reduced N100 amplitudes and higher entropy values suggest that patients with this haplotype were more impaired than FM patients with high COMT activity. Moreover, these results support the notion that genetic polymorphisms may play a significant role for understanding of cognitive, affective and somatic symptoms in fibromyalgia.

Acknowledgments

We thank the Fibromyalgia Associations of Inca and Felanitx (Majorca, Spain) for their support during patient recruitment. We also thank to Mercedes Martínez-Jauand for performing analysis of genetic haplotypes. Thank you so much to Priscila Aquino, Serena Carollo, for the help in the EEG recordings. This work was supported by fellowships from the Universitat de les Illes Balears (Majorca, Spain) and the Brazilian National Council of Research and Development (CNPQ, Brazil) (201499/2012-6) to IC, as well as by grants from the Spanish Ministry of Economy and Competitiveness and European Regional Development Funds (PSI2010-19372) and the Spanish Ministry of Education and Culture (SAF2007-66878-C02-2) and JGVM was supported by a Brazilian grant (CNPq, process 306571/2011-0).
**Figures**

**Figure 4.9:** Arousal ratings. Mean scores and standard errors of the mean for arousal ratings during the presentation images in patients with high and low COMT activity according SAM. The color light grey represents low COMT activity, and dark grey represents high COMT activity group. SAM = Self-Assessment Manikin.
Figure 4.10: Valence ratings. Mean scores and standard errors of the mean for arousal ratings during the presentation images in patients with high and low COMT activity according SAM. The color light grey represents low COMT activity, and dark grey represents high COMT activity group. SAM = Self-Assessment Manikin.
Figure 4.11: MSE analysis for EEG data of startle record. Barplot of mean multiscale entropy (MSE) values for scale factors 1, 5, 10, 15, 20 for groups high and low COMT activity in FM patients. The color light grey represents low COMT activity, and dark grey represents high COMT activity group.
Figure 4.12: Boxplot of N100 ERP analysis of EEG data of startle record at electrode Cz. Event-related potentials elicited by frequent (at Cz) and topographical distribution on the N100 amplitudes for both groups high and low COMT activity.

Figure 4.13: N100 ERP analysis of EEG data of startle record at electrode Cz. Event-related potentials elicited by frequent (at Cz) and topographical distribution on the N100 amplitudes for both groups high and low COMT activity.
Figure 4.14: P200 ERP analysis of EEG data of startle record at electrode Cz. Event-related potentials elicited by frequent (at Cz) and topographical distribution on the P200 amplitudes for both groups high and low COMT activity.

Figure 4.15: P300 ERP analysis of EEG data of startle record at electrode Cz. Event-related potentials elicited by frequent (at Pz) and topographical distribution on the P300 amplitudes for both groups high and low COMT activity.
Tables
Table 4.10: Clinical characteristics of fibromyalgia patients associated with catechol-O-methyltransferase (COMT) val158met polymorphism.

<table>
<thead>
<tr>
<th></th>
<th>High COMT N = 10</th>
<th>Low COMT N = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>50.4 ± 8.7</td>
<td>50.7 ± 8.3</td>
</tr>
<tr>
<td>FM History (yrs)</td>
<td>10.1 ± 8.7</td>
<td>8.5 ± 5.4</td>
</tr>
<tr>
<td>Medication(%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Analgesic/NSAID</td>
<td>25</td>
<td>30</td>
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<tr>
<td>Muscle relaxants</td>
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<td>0</td>
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<tr>
<td>Anxiolytics</td>
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<td>15</td>
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<tr>
<td>Other drugs</td>
<td>35</td>
<td>25</td>
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<tr>
<td>Visual Analogic Scale (0-10)</td>
<td>5.5 ± 1.8</td>
<td>5.0 ± 2.2</td>
</tr>
<tr>
<td>Fibromyalgia Impact Questionnaire (FIQ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical impairment (0-3)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.14 ± .71</td>
<td>2.03 ± .57</td>
</tr>
<tr>
<td>Feel good (0-7)</td>
<td>1.43 ± .50</td>
<td>1.13 ± .92</td>
</tr>
<tr>
<td>Work missed (0-7)</td>
<td>3.29 ± 1.97</td>
<td>4.11 ± 2.48</td>
</tr>
<tr>
<td>Do work (10 cm VAS)</td>
<td>7.9 ± 1.88</td>
<td>9.06 ± .97</td>
</tr>
<tr>
<td>Pain (10 cm VAS)</td>
<td>7.93 ± 1.20</td>
<td>8.75 ± 1.0</td>
</tr>
<tr>
<td>Fatigue (10 cm VAS)</td>
<td>8.76 ± 1.4</td>
<td>8.63 ± 1.8</td>
</tr>
<tr>
<td>Rested (10 cm VAS)</td>
<td>8.5 ± 1.82</td>
<td>6.81 ± 3.90</td>
</tr>
<tr>
<td>Stiffness (10 cm VAS)</td>
<td>8.5 ± 1.82</td>
<td>6.81 ± 1.8</td>
</tr>
<tr>
<td>Anxiety (10 cm VAS)</td>
<td>8.5 ± .91</td>
<td>7.5 ± 3.07</td>
</tr>
<tr>
<td>Depression (10 cm VAS)</td>
<td>6.36 ± 3.1</td>
<td>5.7 ± 3.3</td>
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<tr>
<td>Total FIQ score (0-100)</td>
<td>68.9 ± 14.73</td>
<td>74.12 ± 14.98</td>
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<tr>
<td>Hospital Anxiety and Depression Scale (HADS)</td>
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<tr>
<td>Anxiety</td>
<td>10.50 ± 3.46</td>
<td>9.75 ± 5.65</td>
</tr>
<tr>
<td>Depression*</td>
<td>9.0 ± 1.31</td>
<td>11.25 ± .97</td>
</tr>
</tbody>
</table>

<sup>p</sup> < .05
<sup>a</sup> Chi-square test was used
NSAID = nonsteroidal anti-inflammatory drugs
High COMT activity = Haplotype LPS carries;
Low COMT activity= HPS-APS carries
Table 4.11: Average of EEG relative power spectra in *frontal electrodes* in patients with haplotypes for gene COMT

<table>
<thead>
<tr>
<th></th>
<th>Pleasant Mean(SD)</th>
<th>Neutral Mean(SD)</th>
<th>Unpleasant Mean(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Delta (2 – 3.9 Hz)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High COMT</td>
<td>29.08 (2.09)</td>
<td>29.25 (1.84)</td>
<td>29.00 (1.77)</td>
</tr>
<tr>
<td>Low COMT</td>
<td>27.06 (2.09)</td>
<td>27.11 (2.21)</td>
<td>27.08 (1.81)</td>
</tr>
<tr>
<td><strong>Theta1 (4 – 5.9 Hz)</strong></td>
<td></td>
<td></td>
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<tr>
<td>High COMT</td>
<td>25.91 (2.64)</td>
<td>25.96 (2.80)</td>
<td>26.07 (2.91)</td>
</tr>
<tr>
<td>Low COMT</td>
<td>24.92 (2.61)</td>
<td>24.91 (2.92)</td>
<td>25.22 (2.99)</td>
</tr>
<tr>
<td><strong>Theta2 (6 – 7.9 Hz)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>High COMT</td>
<td>24.20 (2.57)</td>
<td>24.39 (2.77)</td>
<td>24.28 (2.41)</td>
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<tr>
<td>Low COMT</td>
<td>22.95 (3.01)</td>
<td>23.19 (2.89)</td>
<td>22.92 (2.99)</td>
</tr>
<tr>
<td><strong>Alpha1 (8 – 9.9 Hz)</strong></td>
<td></td>
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<tr>
<td>High COMT</td>
<td>22.09 (3.32)</td>
<td>22.12 (2.95)</td>
<td>21.94 (3.05)</td>
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<tr>
<td>Low COMT</td>
<td>28.21 (1.85)</td>
<td>28.03 (1.47)</td>
<td>28.20 (2.02)</td>
</tr>
<tr>
<td><strong>Alpha2 (10 – 11.9 Hz)</strong></td>
<td></td>
<td></td>
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<tr>
<td>High COMT</td>
<td>26.02 (1.78)</td>
<td>25.76 (1.62)</td>
<td>26.17 (1.67)</td>
</tr>
<tr>
<td>Low COMT</td>
<td>25.12 (1.79)</td>
<td>25.03 (1.97)</td>
<td>25.17 (1.97)</td>
</tr>
<tr>
<td><strong>Beta1 (12 – 17.9 Hz)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>High COMT</td>
<td>24.64 (2.03)</td>
<td>24.83 (2.03)</td>
<td>24.72 (1.92)</td>
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<td>Low COMT</td>
<td>23.62 (1.72)</td>
<td>23.92 (1.66)</td>
<td>23.73 (1.78)</td>
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<tr>
<td><strong>Beta2 (18 – 22 Hz)</strong></td>
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<td></td>
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<tr>
<td>High COMT</td>
<td>22.42 (2.24)</td>
<td>22.49 (2.44)</td>
<td>22.44 (2.38)</td>
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<tr>
<td>Low COMT</td>
<td>21.73 (2.86)</td>
<td>21.92 (2.89)</td>
<td>21.73 (3.24)</td>
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Table 4.12: Average of EEG relative power spectra in central electrodes in patients with haplotypes for COMT gene

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<th>Pleasant Mean(SD)</th>
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<tr>
<td><strong>Delta (2 – 3.9 Hz)</strong></td>
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<tr>
<td>High COMT</td>
<td>27.46 (1.57)</td>
<td>27.46 (1.41)</td>
<td>27.15 (1.44)</td>
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<td>Low COMT</td>
<td>26.88 (1.97)</td>
<td>28.77 (1.82)</td>
<td>26.92 (2.07)</td>
</tr>
<tr>
<td><strong>Theta1 (4 – 5.9 Hz)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>High COMT</td>
<td>25.54 (1.76)</td>
<td>25.39 (2.19)</td>
<td>25.65 (1.67)</td>
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<tr>
<td>Low COMT</td>
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<td>25.08 (1.85)</td>
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<td><strong>Theta2 (6 – 7.9 Hz)</strong></td>
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<td></td>
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<tr>
<td>High COMT</td>
<td>24.92 (3.02)</td>
<td>25.04 (3.33)</td>
<td>25.25 (3.36)</td>
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<tr>
<td>Low COMT</td>
<td>24.62 (2.28)</td>
<td>24.44 (2.16)</td>
<td>24.62 (2.23)</td>
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<tr>
<td><strong>Alpha1 (8 – 9.9 Hz)</strong></td>
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<tr>
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<td>24.79 (3.19)</td>
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<td>Low COMT</td>
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<td><strong>Alpha2 (10 – 11.9 Hz)</strong></td>
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<tr>
<td>High COMT</td>
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<td><strong>Beta1 (12 – 17.9 Hz)</strong></td>
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<tr>
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<td>22.33 (3.31)</td>
<td>22.58 (3.43)</td>
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<td>Low COMT</td>
<td>22.06 (2.46)</td>
<td>21.13 (3.26)</td>
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<tr>
<td><strong>Beta2 (18 – 22 Hz)</strong></td>
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<tr>
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<td>21.01 (3.54)</td>
<td>22.37 (3.23)</td>
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<td>Low COMT</td>
<td>21.10 (2.73)</td>
<td>22.07 (2.74)</td>
<td>21.05 (3.07)</td>
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Table 4.13: Average of EEG relative power spectra in *parieto-occipital electrodes* in patients with haplotypes for gene COMT

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<tr>
<th>Frequency</th>
<th>Pleasant Mean(SD)</th>
<th>Neutral Mean(SD)</th>
<th>Unpleasant Mean(SD)</th>
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</thead>
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<tr>
<td><strong>Delta (2 – 3.9 Hz)</strong></td>
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<td></td>
</tr>
<tr>
<td>High COMT</td>
<td>28.71 (1.58)</td>
<td>28.41 (1.63)</td>
<td>28.65 (1.65)</td>
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<td>Low COMT</td>
<td>27.17 (1.75)</td>
<td>26.85 (1.78)</td>
<td>27.16 (1.47)</td>
</tr>
<tr>
<td><strong>Theta1 (4 – 5.9 Hz)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High COMT</td>
<td>26.26 (2.52)</td>
<td>26.18 (2.66)</td>
<td>26.43 (2.75)</td>
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<tr>
<td>Low COMT</td>
<td>25.86 (2.60)</td>
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<td>25.78 (2.94)</td>
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<td><strong>Theta2 (6 – 7.9 Hz)</strong></td>
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<tr>
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<td>25.03 (2.62)</td>
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<td>24.77 (2.58)</td>
</tr>
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<td>Low COMT</td>
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<td>23.21 (3.26)</td>
<td>22.78 (3.16)</td>
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<tr>
<td><strong>Alpha1 (8 – 9.9 Hz)</strong></td>
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<tr>
<td>High COMT</td>
<td>25.36 (2.25)</td>
<td>25.52 (2.03)</td>
<td>25.37 (2.07)</td>
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Table 4.14: Significant correlations between questionnaires and amplitudes of ERP components elicited by affective pictures in FM patients

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<th>HAD</th>
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<tr>
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<td>Do work</td>
<td>Fatigue</td>
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<tr>
<td>Pleasant images</td>
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<tr>
<td>N100 Central</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Neutral images</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P200 PO</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unpleasant images</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N100 Central</td>
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<td>–</td>
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<td>P200 Frontal</td>
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<td>P200 PO</td>
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<td>P300 Central</td>
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</table>

* indicates $p < .05$
** indicates $p < .01$

FIQ = Fibromyalgia Impact Questionnaires
HAD = Hospital Anxiety and Depression scale
PO = parieto occipital electrodes
Table 4.15: Significant correlations between questionnaires and power density values at different EEG frequency bands elicited by pleasant pictures in fibromyalgia patients.

<table>
<thead>
<tr>
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* indicates $p < .05$

** indicates $p < .01$

FIQ = Fibromyalgia Impact Questionnaires

PO = Parieto-Occipital
Table 4.16: Significant correlations between some questionnaires and frequencies same as before waves of PSA values of neutral emotion in fibromyalgia patients.

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* indicates $p < .05$

** indicates $p < .01$

FIQ = Fibromyalgia Impact Questionnaires

PO = Parieto-Occipital
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* indicates $p < .05$

** indicates $p < .01$

FIQ = Fibromyalgia Impact Questionnaires
HAD = Hospital Anxiety and Depression scale
PO = Parieto-Occipital
Table 4.18: Significant correlations between questionnaires and entropy values elicited by pleasant pictures in fibromyalgia patients.

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* indicates p < .05
** indicates p < .01
FIQ = Fibromyalgia Impact Questionnaires
PO = Parieto-Ociptal
Table 4.19: Significant correlations between questionnaires and entropy values elicited by neutral pictures in fibromyalgia patients.

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* indicates $p < .05$

** indicates $p < .01$

FIQ = Fibromyalgia Impact Questionnaires

PO = Parieto-Occiptal
Table 4.20: Significant correlations between questionnaires and entropy values elicited by unpleasant pictures in fibromyalgia patients.

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* indicates $p < .05$

** indicates $p < .01$

FIQ = Fibromyalgia Impact Questionnaires
PO = Parieto-Occiptal
General discussion and future perspectives

In this section, we summarize the main conclusions of this thesis, integrating the different findings and linking them to the existing literature.

5.1 General discussion

In recent years, central mechanisms responsible for enhanced pain sensitivity in chronic pain syndromes such as hyperexcitability of the central nervous system or deficits in inhibitory pain mechanisms, have been widely recognized [12, 14, 15, 403]. However, the pathophysiology associated with other relevant symptoms, such as reduced physical activity, sleep disturbances or affective distress are still unknown. In the present work, we focused on fibromyalgia (FM) syndrome, a chronic pain condition in which central sensitization is accompanied by motor, sleep and affective symptoms associated with chronic pain. Basically, the present work examines the effects of chronic pain on gait and balance (study 1), sleep quality (study 2) and response to affective pictures (study 3) by comparing fibromyalgia patients and healthy controls (study 1), as well as two subgroups of fibromyalgia patients based on genetic characteristics (studies 2 and 3).

The previous literature revised in the first section of this work revealed that although FM patients seem to display relevant deficits on gait and balance, most of the evidence comes from
retrospective data and self-report measures. Therefore, the main objective of the first study was to compare FM patients and pain-free controls in several objective measures of motor function by using standardized methods and video recordings. In a similar way, previous studies have shown that genetic factors could substantially contribute to the development and maintenance of enhanced pain sensitivity in several somatic syndromes such as fibromyalgia [61, 62].

In this sense, functional polymorphisms of genes encoding catechol-O-methyltransferase, dopamine type 4 receptors, serotonin 5-hydroxytryptamine 2A receptors and serotonin transporters have been significantly involved in pain regulation [33]. Based on previous literature showing that the val158met polymorphism of the COMT gene was associated with differences on pain sensitivity in FM patients, two studies were carried in the present thesis to test the influence of this functional polymorphism on sleep (study 2) and affective processing (study 3). The val158met polymorphism of the COMT gene was selected to classify a sample of FM patients into met homozygotes (displaying high sensitivity to pain stimuli) and val carriers (with low pain sensitivity) [13, 70, 71, 323, 325, 362].

In summary, the present work was based on three major hypotheses: 1) FM patients would display significant gait and balance deficits as compared with pain-free, healthy controls; 2) FM patients with low COMT enzyme activity (met homozygotes) would have a poorer quality of sleep than patients with high activity of COMT enzyme (val carriers); and 3) FM patients with low COMT enzyme activity (met homozygotes) would display more altered brain processing of affective information than patients with high COMT (val carriers). To test these hypotheses, balance and gait tasks (study 1), a polysomnography recording (study 2), and recording of event-related brain activity triggered by emotional pictures (study 3) were used as experimental tasks.

The findings from these studies have highlighted the influence of biomechanical abnormalities and genetic factors in patients with fibromyalgia. In particular, study 1 revealed that FM patients displayed significant reductions in speed, stride length and cycle frequency. Moreover, FM patients showed greater body sways on the anterior-posterior and medial-lateral planes than pain-free controls, suggesting a relevant alteration of subsystems responsible for postural control and balance which could lead to increase the risk of falls and thus to limit daily functioning.
Our findings are consistent with previous studies \cite{112,114,264} and emphasize the need to take into account motor deficits together with the rest of somatic, affective and cognitive symptoms during the clinical assessment and intervention in fibromyalgia.

Results from study 2 further indicated that although sleep disturbances are frequent in patients with chronic pain, those patients with low COMT activity had poorer quality of sleep (higher number of awakenings during the night), together with more depression and higher impact of pain than FM patients with high COMT activity. Thus, although there were no significant group differences neither in sleep architecture nor in power density of EEG frequency bands, our findings suggest that this functional polymorphism of the COMT gene could be also modulating sleep disturbances in FM patients. Furthermore, the small interhemispheric differences on entropy values in patients with low COMT activity also point towards the existence of relevant differences on brain activity during sleep that could be associated with this polymorphism. Nevertheless, the small size of our study sample precludes any significant and relevant conclusion from our results and requires further research in future studies.

A further evidence for the modulatory role of the functional val158met polymorphism of the COMT gene in fibromyalgia comes from results of study 3. Here, it was found that FM patients with low COMT activity displayed more reduced N100 amplitudes and higher entropy values to all types of affective stimuli (pleasant, unpleasant, and neutral) than FM patients with high COMT activity. Considering that FM patients generally showed reduced amplitudes in the early ERP components to different types of affective information \cite{50,318}, the present findings would suggest that low COMT activity as expressed by one of the val158met genotypes (met homozygotes) could be associated with a worsening of affective symptoms in fibromyalgia. Again, this would also emphasize the role of genetic information for the designing of specific treatments or interventions according with the current notion of personalized medicine.

The experimental techniques used in this thesis were basically in accordance with the multidimensional nature of fibromyalgia. Thus, apart from the use of objectives measures of gait and balance in study 1, the thesis focused on the modulatory role of genetic factors in sleep and affective disturbances in fibromyalgia. According with a biopsychosocial model of chronic diseases such as fibromyalgia, the experience of health should emerge from the interaction
between genetic factors and physiological mechanisms (biology), emotion and cognition (psychology), and sociocultural environment (social world). In this sense, our study has contributed to a better understanding of pain-related physical and psychological symptoms in fibromyalgia, as well as to underline the need of a multidisciplinary approach for the assessment of patients with fibromyalgia. Furthermore, bearing in mind that biopsychosocial models have demonstrated a high effectiveness for rehabilitation of patients with chronic pain in several studies [404, 405] and meta-analyses [406, 407], our findings emphasize the notion that genetic factors could be also helpful for the development of personalized assessment and rehabilitation in fibromyalgia. The results presented in this thesis could also help to develop new diagnosis as well as new strategies for treatment of fibromyalgia patients. The complex nature of fibromyalgia urges the development of new diagnostic methods able to integrate all the clinical information [407]. One example of such new integrative methods is the so-called expert systems [407, 408]. An expert system is an interactive knowledge-based computational software that embodies wise and experience employed by leading experts in a specific field. Our results could represent an important contribution to the development of such a platform within the field of pain management, as it could integrate self-report, behavioral, neurophysiological and genetic data. An integrated evaluation system could also allow the development of better treatment strategies. For example, the development of new multidisciplinary and interdisciplinary health intervention addressed to different components of the patients (e.g. biological, psychological, and social), like an intervention based on the biopsychosocial model of health and illness. In addition, the results from the present work emphasize the need for a new approach that allows the examination of genetic and epigenetic interactions with motor deficits, sleep and psychosocial symptoms to provide a better quality of life for patients living with fibromyalgia.

### 5.2 Limitations

The following limitations and shortcomings should be taken into account when interpreting the findings from the present work:
1. Low spatial resolution of EEG during sleep recordings. Only 2 EEG channels were used during the polysomnography study (study 2). The electrodes were located at C3 and C4 scalp regions.

2. Medication in patients with fibromyalgia. Several patients with FM were currently taking painkillers and antidepressants during data collection. Therefore, potential side effects of these drugs on the motor performance, sleep and cognitive processing tasks could not be completely discarded.

3. Lack of a male sample. Although the prevalence of FM is significantly lower among men than among women [409], it is important to take into account that our results could be only referred to female patients because of the absence of men in our samples.

4. Although one the initial goal of the thesis was to analyze if the two haplotypes of the val158met polymorphism of the COMT gene were different in motor function, sleep and affective processing in FM patients, study 1 could not include an analysis of the two subgroups of FM patients based on this polymorphism due to technical difficulties.

5. Studies 2 and 3 were applied only to fibromyalgia patients with different genetic haplotypes without a control group of pain-free subjects.

6. No data was collected about the startle eyeblink reflex in the study 3. Given that the startle was presented when viewing the affective pictures, it would be also interesting to analyze the modulatory effect of emotion on startle reflex as a further measure of affective processing. Nevertheless, previous results from our lab [218] already indicated that affective modulation of startle reflex was significantly reduced in fibromyalgia patients. It would be necessary to test in future studies if this reduction of affective modulation could be due to the presence of patients with low COMT activity.

5.3 Future perspectives

Regarding future research, the results described in this work should lead to the planning of new studies to examine the role of genetic factors on movement alterations among patients
with fibromyalgia. In this sense, it should be mentioned that the initial goal of the present thesis was to examine the influence of the val158met polymorphism of the COMT gene on motor function. This should be completed in the future for a better understanding of the role of this genetic marker on motor function, sleep and affect in fibromyalgia. It would be also interesting to test new intervention protocols for improvements of quality of sleep, gait and balance parameters, and quality of life in these patients. Currently, new physiotherapy approaches have been developed integrating a multidisciplinary approach, as the use of games in rehabilitation. Gold and collaborators recently hypothesized that Virtual Reality (VR) can induce pain distraction through attention on multi modal sensory stimuli and diminish of pain related emotions [410]. Studies, which have used VR for pain distraction, have shown positive effect on burn patients, cancer patients and people with regional pain syndrome [411–413], and fibromyalgia patients [414]. Thus, future studies on rehabilitation programs in FM should take into account the idea that different subgroups of patients based on genetic haplotypes could differentially benefit from physical and psychological rehabilitation.

In relation to sleep study, future studies should examine if FM patients could benefit from those programs focusing on light therapy, and test again if genetic factors may play a relevant role for improvement of quality of sleep. This therapy – also referred to have heliotherapy, bright light therapy, or phototherapy – involves the use of exposure to artificial light as a means to treat various conditions. It is frequently used to treat seasonal affective disorder, a type of depression that occurs at the same time each year, generally in the fall or winter when there are fewer hours of daylight [415]. It can also be used to treat other types of depression, mood disorders [416] as well as various sleep disorders [417]. The aim of such a study would be to observe whether after the light therapy, patients who have low COMT enzyme activity would show an improvement in their quality of sleep.

Regarding the study of emotions, it could be very useful to analyze the patterns of brain connectivity in emotions as suggested by Rosário et al [418]. This would characterize the functional connectivity of the brain network generated from our EEG data obtained in patients with different genetic haplotype in the emotional image viewing (pleasant, neutral and unpleasant pictures).
Fibromyalgia is a chronic pain syndrome mostly characterized by enhanced pain sensitivity, sleep and affective disturbances, leading to physical limitations and to impairments in daily functioning. Although its etiology is still unknown, it has been shown that some functional polymorphisms like the val158met of the COMT gene may play an important role in the exacerbation of these symptoms. The present thesis has mainly focused on the examination of physical impairment in patients with fibromyalgia by using different approaches: motor performance tasks (gait and balance), polysomnographic recordings, and recording of event-related brain activity triggered by emotional pictures. For this purpose, a group of FM patients and a group of pain-free controls were compared in study 1, and two subgroups of FM patients based on a genetic polymorphism responsible of the level of COMT activity were compared in the study 2 and 3.

The main findings of this work were the following:

1. FM patients displayed significant deficits on balance parameters compared with healthy controls. Pain intensity, stiffness and depression are associated to reductions in speed, stride length and cycle frequency in patients with FM. Body sway data reveal that pain-free controls displayed a non-persistent trend in body sway time series leading to the maintenance of a stable position over the time. By contrast, body sway time series in FM patients were characterized by an uncorrelated pattern of body oscillations, suggesting
a relevant alteration of subsystems responsible for postural control and balance which could lead to increase the risk of falls and thus to limit daily functioning.

2. FM patients presenting low COMT activity haplotype had poorer quality of sleep, together with more depression and higher impact of pain than FM patients with high COMT activity. Although not statistically significant, there are evidences in the MSE analysis that suggest that patients with low COMT activity present small interhemispheric differences on entropy values. Hence, our findings suggest that this functional polymorphism of the COMT gene could be also modulating sleep disturbances in FM patients.

3. FM patients with low COMT activity display more reduced evoked potentials N100 and higher entropy values for all types of affective stimuli (pleasant, unpleasant, and neutral) than FM patients with high COMT activity. This reduction in N100 could be associated to a worsening of affective symptoms in fibromyalgia and to increased anxiety and depression of the patients.

Although fibromyalgia patients show similar clinical profiles, our findings indicate that the group of patients that present lower COMT activity could have a slightly higher incidence of emotional and physical problems. Patients with low COMT enzyme activity may live in a higher state of alert, under higher levels of stress and depression. It represents a challenge for health professionals of different specialities. Our work could emphasize the role of genetic information for the development of personalized assessment and rehabilitation in fibromyalgia. A better treatment is a key step to enhance the self-esteem of the patients, providing optimal conditions for the development of individual skills, and thus improving their quality of life.
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Appendices
Appendix A - The electrode montage used in the emotion study for PSA and ERP analysis.

Figure 7.1: The electrode montage used in the emotion study. In light grey is a 32 electrodes used in EEG record. The 28 electrodes selected for PSA and ERP analysis are indicated by the circles with colored borders. The color orange, represents frontal electrodes; the color green, represents central electrodes and the color blue, represents parieto-occipital electrodes.
Appendix B - The electrode montage used in the emotion study for MSE analysis.

Figure 7.2: The electrode montage used in the emotion study. In light grey is a 32 electrodes used in EEG record. The 18 electrodes selected for MSE analysis are indicated by the circles with colored borders. The color orange, represents frontal electrodes; the color green, represents central electrodes and the color blue, represents parieto-occipital electrodes.
Appendix C - Scores obtained in the polysomnographic variables

Table 7.1: Scores obtained in the polysomnographic variables associated with catechol-O-methyltransferase (COMT) val158met polymorphism.

<table>
<thead>
<tr>
<th>Polysomnographic variables</th>
<th>High COMT</th>
<th>Low COMT</th>
<th>F</th>
<th>T</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 13</td>
<td>N = 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Sleep time (min)</td>
<td>350.11 ± 84.91</td>
<td>368.18 ± 66.14</td>
<td>-.57</td>
<td>.21</td>
<td>.57</td>
</tr>
<tr>
<td>Time in bed (min)</td>
<td>459.38 ± 27.91</td>
<td>484.54 ± 15.65</td>
<td>-2.65</td>
<td>4.79</td>
<td>.01</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>76.04 ± 17.85</td>
<td>75.86 ± 12.66</td>
<td>.03</td>
<td>.49</td>
<td>.98</td>
</tr>
<tr>
<td>Time of wakefulness after sleep onset (min)</td>
<td>43.11 ± 49.57</td>
<td>34.64 ± 31.42</td>
<td>.49</td>
<td>.59</td>
<td>.63</td>
</tr>
<tr>
<td>Number of awakenings*</td>
<td>41.15 ± 30.06</td>
<td>64.68 ± 23.13</td>
<td>-2.12</td>
<td>.65</td>
<td>.04</td>
</tr>
<tr>
<td>Micro-waking REM</td>
<td>22.31 ± 16.73</td>
<td>38.79 ± 26.34</td>
<td>4.66</td>
<td>-1.86</td>
<td>.07</td>
</tr>
<tr>
<td>Micro-waking NREM</td>
<td>22.32 ± 19.11</td>
<td>23.38 ± 13.57</td>
<td>.58</td>
<td>-.15</td>
<td>.88</td>
</tr>
<tr>
<td>Micro-waking Total</td>
<td>22.89 ± 13.08</td>
<td>27.30 ± 13.26</td>
<td>.009</td>
<td>-.82</td>
<td>.42</td>
</tr>
<tr>
<td>Phase I (%)</td>
<td>30.01 ± 18.34</td>
<td>24.74 ± 8.07</td>
<td>.88</td>
<td>.38</td>
<td>.38</td>
</tr>
<tr>
<td>Phase II (%)</td>
<td>39.10 ± 9.57</td>
<td>33.20 ± 9.0</td>
<td>1.54</td>
<td>.37</td>
<td>.14</td>
</tr>
<tr>
<td>Phase III (%)</td>
<td>11.03 ± 7.29</td>
<td>9.29 ± 5.45</td>
<td>.65</td>
<td>.58</td>
<td>.52</td>
</tr>
<tr>
<td>Phase IV (%)</td>
<td>4.0 ± 4.5</td>
<td>6.0 ± 5.9</td>
<td>-1.04</td>
<td>1.39</td>
<td>.31</td>
</tr>
<tr>
<td>REM phase (%)</td>
<td>10.56 ± 30.0</td>
<td>10.43 ± 56.7</td>
<td>.70</td>
<td>4.9</td>
<td>.94</td>
</tr>
<tr>
<td>REM latency (minutes)</td>
<td>165.15 ± 95.81</td>
<td>140.27 ± 116.06</td>
<td>.57</td>
<td>.27</td>
<td>.57</td>
</tr>
<tr>
<td>Sleep latency (min)</td>
<td>22.31 ± 17.82</td>
<td>34.14 ± 27.09</td>
<td>.57</td>
<td>2.3</td>
<td>.21</td>
</tr>
</tbody>
</table>

Sleep events, /h

<p>| | | | | | |</p>
<table>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha - Waking</td>
<td>401.3 ± 576.6</td>
<td>382.5 ± 289.6</td>
<td>.24</td>
<td>-.82</td>
<td>.42</td>
</tr>
<tr>
<td>Alpha - S1</td>
<td>182.5 ± 144.1</td>
<td>224.2 ± 230.4</td>
<td>.62</td>
<td>-.54</td>
<td>.59</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th></th>
<th>Mean ± Standard Deviation</th>
<th>Mean ± Standard Deviation</th>
<th>t-value</th>
<th>p-value1</th>
<th>p-value2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha - S2</td>
<td>273.8 ± 173.7</td>
<td>313.3 ± 280.6</td>
<td>2.78</td>
<td>-.42</td>
<td>.68</td>
</tr>
<tr>
<td>Alpha - S3</td>
<td>61.7 ± 71.5</td>
<td>60.7 ± 87.5</td>
<td>.15</td>
<td>.03</td>
<td>.98</td>
</tr>
<tr>
<td>Alpha - S4</td>
<td>17.2 ± 26.9</td>
<td>35.82 ± 86.8</td>
<td>2.08</td>
<td>-.73</td>
<td>.47</td>
</tr>
<tr>
<td>Alpha - REM</td>
<td>16.92 ± 18.9</td>
<td>24.9 ± 44.1</td>
<td>2.46</td>
<td>-.76</td>
<td>.45</td>
</tr>
<tr>
<td>Delta - Waking</td>
<td>389.15 ± 306.2</td>
<td>536.1 ± 405.7</td>
<td>.74</td>
<td>-1.01</td>
<td>.32</td>
</tr>
<tr>
<td>Delta - S1</td>
<td>311.6 ± 193.2</td>
<td>406.4 ± 260.6</td>
<td>.01</td>
<td>-1.02</td>
<td>.32</td>
</tr>
<tr>
<td>Delta - S2</td>
<td>717.7 ± 469.2</td>
<td>983.2 ± 612.8</td>
<td>.05</td>
<td>-1.20</td>
<td>.24</td>
</tr>
<tr>
<td>Delta - S3</td>
<td>614.3 ± 604.6</td>
<td>691.6 ± 608.0</td>
<td>.09</td>
<td>-.31</td>
<td>.76</td>
</tr>
<tr>
<td>Delta - S4</td>
<td>383.2 ± 661.1</td>
<td>551.2 ± 738.9</td>
<td>.20</td>
<td>-.59</td>
<td>.56</td>
</tr>
<tr>
<td>Delta - REM</td>
<td>37.3 ± 39.2</td>
<td>51.5 ± 49.65</td>
<td>2.34</td>
<td>-.75</td>
<td>.44</td>
</tr>
<tr>
<td>Spindles - Waking</td>
<td>41.3 ± 49.5</td>
<td>22.7 ± 30.0</td>
<td>3.7</td>
<td>1.08</td>
<td>.29</td>
</tr>
<tr>
<td>Spindles - S1</td>
<td>184.1 ± 232.7</td>
<td>100.3 ± 168.9</td>
<td>1.01</td>
<td>.99</td>
<td>.33</td>
</tr>
<tr>
<td>Spindles - S2</td>
<td>612.6 ± 1014.0</td>
<td>208.6 ± 312.2</td>
<td>2.5</td>
<td>1.27</td>
<td>.22</td>
</tr>
<tr>
<td>Spindles - S3</td>
<td>45.9 ± 57.0</td>
<td>16.6 ± 28.8</td>
<td>5.1</td>
<td>1.54</td>
<td>.14</td>
</tr>
<tr>
<td>Spindles - S4</td>
<td>8.08 ± 13.45</td>
<td>6.36 ± 12.5</td>
<td>.09</td>
<td>.32</td>
<td>.75</td>
</tr>
<tr>
<td>Spindles - REM</td>
<td>3.46 ± 6.4</td>
<td>2.4 ± 6.0</td>
<td>.24</td>
<td>.39</td>
<td>.70</td>
</tr>
</tbody>
</table>

*ps < .05

Degree of freedom = 22 FM = fibromyalgia; REM = Rapid Eye Movement; OSQ = Oviedo Sleep Questionnaire

High COMT activity = Haplotype LPS carries; Low COMT activity = HPS-APS carries
Annex A - Document of agreement between the co-authors of articles
Altered functional performance in patients with fibromyalgia

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12 Aug 2016

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www.frontiersin.org

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London, 20/9/2016
Annex B - List of drugs taken by the patients

The following description of the drugs was obtained in Vademecum and Drugs.com website [419], [420].

Antidepressants

Tricyclic (TCAs)/ Non-selective monoamine reuptake inhibitors

Amitriptyline

- Comercial Name in Spain: Deprelio/ Tryptizol
- Objective: Inhibits the reuptake of serotonin and noradrenaline and has sedative action
- Indication: major depression, manic depression, anxiety states associated with depression, neuralgic pain and severe chronic pain
- Effects: increase in the quantity of sleep potentiation of analgesic action of endorphins and muscle relaxation.
- Side Effects: Daytime somnolence, sedation, dry mouth, blurred vision, weight gain, fluid retention, dizziness, constipation, sexual dysfunction, palpitations, worsening of Restless Legs Syndrome, hypotension, etc.
- Sleep Side Effects: Increased latency of REM sleep, decreased total sleep time and REM variable effect on sleep efficiency.

Imipramine

- Comercial Name in Spain: Imipramina
- Objective: Inhibits the reuptake of norepinephrine and serotonin approximately the same extent.
- **Indication:** It is suitable for all forms of depression, depression associated with personality disorders or chronic alcoholism, anxiety, panic, night terrors and severe chronic pain. In fibromyalgia is widely used for pain control.

- **Side Effects:** Dry mouth, blurred vision, headache, drowsiness, dizziness, constipation, nausea, vomiting, loss of appetite, diarrhea, stomach cramps, weight gain/loss, and increased sweating may occur.

- **Sleep Side Effects:** Increasing REM sleep time and the time of non-REM sleep stage 2, sleepiness; insomnia.

**Doxepin**

- **Commercial Name in Spain:** Anafranil

- **Objective:** Blocks the reuptake of neurotransmitters from neuronal membrane.

- **Indication:** Psychoneurotic disorders where anxiety and/or depression are prominent symptoms: anxiety neurosis with or without somatic symptoms, reactive depression, anxious.

- **Effects:** It is also used to control sleep, due to its antihistamine capacity.

- **Side Effects:** Dry mouth, blurred vision, constipation. Withdrawal symptoms: nausea, headache, malaise.

- **Sleep Side Effects:** Sleepiness.

**Clomipramine**

- **Commercial Name in Spain:** Clomipramina

- **Objective:** Inhibits the reuptake of norepinephrine and serotonin and is antihistaminínicas properties.

- **Indication:** Suitable for depression, phobias, panic attacks, premature ejaculation, anorexia nervosa, obsessive-compulsive syndrome, chronic somatic diseases and chronic pain.
Sleep Side Effects: increasing REM sleep time and the time of non-REM sleep stage 2.

**Selective Serotonin Reuptake Inhibitors (SSRI)**

**Citalopram**

- **Commercial Name in Spain**: Citalopram
- **Objective**: More selective serotonin reuptake inhibitor. Devoid of effect on the reuptake of norepinephrine, dopamine and GABA.
- **Indication**: Promote increased amount of serotonin between neurons and reduce fatigue, improve reasoning and the spirit of the patient. As with other antidepressants, recommended doses for the treatment of pain and sleep are much smaller than those required for antidepressant action. But, even at low doses have anxiolytic activity.
- **Side Effects**: agitation, nervousness, decreased libido, anxiety, confusional state, disturbance in attention, lethargy, headache, tremor, dizziness, paresthesia, asthenia, fatigue
- **Sleep Side Effects**: Increased latency of REM sleep, reduction of total REM sleep time and decreased sleep efficiency.

**Fluoxetine**

- **Commercial Name in Spain**: Fluoxetina
- **Objective**: Fluoxetine (Prozac) appears to be the weakest of the SSRIs. Due to its very long half-life and relatively weak potency as a serotonin inhibitor fluoxetine has less prominent discontinuation symptoms than many other antidepressants.
- **Indication**: Suitable for depressive disorders and obsessive-compulsive disorder (OCD)
- **Side Effects**: anxiety, nervousness, restlessness, tension, decreased libido, impaired attention, dizziness, dysgeusia, lethargy, tremors, etc.
- **Sleep Side Effects**: increase wakefulness during sleep.
Escitalopram

- Comercial Name in Spain: Escitalopram/Esertia

- Objective: work by increasing serotonin, are the serotonin reuptake inhibitors (SSRIs).

- Indication: Treatment of major depressive episodes; Panic disorder with or without agoraphobia; Social anxiety disorder (social phobia); Generalized anxiety disorder; Obsessive-compulsive disorder (OCD)

- Side Effects: dry mouth; weight gain; dizziness, paraesthesia, tremor; sinusitis; increased sweating; arthralgia, myalgia; decreased appetite, increased appetite; fatigue, pyrexia; ejaculation disorder, impotence; anxiety, restlessness, abnormal dreams, decreased libido, etc.

- Sleep Side Effects: insomnia, drowsiness, yawning.

Duloxetine

- Comercial Name in Spain: Duloxetina/Cymbalta

- Objective: Reuptake inhibitor of serotonin and norepinephrine. It weakly inhibits dopamine reuptake with no significant affinity for histaminergic, dopaminergic, cholinergic and adrenergic receptors.

- Indication: Its main purpose is to treat all forms of depression, stress urinary incontinence, peripheral pain of diabetic neuropathy and migraine.

- Side Effects: Headache, dizziness, lethargy, tremors, paresthesia; decreased appetite; agitation, decreased libido, anxiety, abnormal orgasm; nausea, dry mouth, constipation, diarrhea, abdominal pain, vomiting; musculoskeletal pain, muscle spasm; dysuria; erectile dysfunction, ejaculation disorder, delayed ejaculation; falls, fatigue; etc.

- Sleep Side Effects: sleepiness, insomnia, abnormal dreams.
Velafaxine

- Comercial Name in Spain: Velafanixa/Zarelis Retar

- Objective: It is related to the enhancement of monoaminergic activity in the CNS. In pre-clinical studies have shown that venlafaxine and its major metabolite, O-desmethylvenlafaxine, are potent inhibitors of the serotonin and noradrenaline. Weakly inhibits dopamine reuptake.

- Indication: Used to treat varying degrees of depression, obsessive-compulsive disorders and reduction of fatigue.

- Side Effects: Asthenia, chills, fatigue; hypertension, vasodilation, palpitations; decreased appetite, constipation, nausea, vomiting; decreased libido, dizziness, dry mouth, headache, hypertension, nervousness, paresthesia, sedation, drowsiness, tremor, confusion, depersonalization; yawns; sweating; anomalies in accommodation, mydriasis, visual disturbances; tinnitus; ejaculation / abnormal orgasm (male), anorgasmia, erectile dysfunction, impaired urination, menstrual disorders, dysuria, frequency.

- Sleep Side Effects: sleepiness, insomnia, abnormal dreams.

**Antagonists of alpha-2 adrenoceptors:**

**Mitarzapine**

- Comercial Name in Spain: Mitarzepina/Afloyan

- Objective: Tetracyclic antidepressants are different from the others because it acts in blocking the neuronal uptake of amines and therefore induces an increase of noradrenaline and serotonin.

- Indication: major depression, manic depression, anxiety states associated with depression, neuralgic pain and severe chronic pain.

- Side Effects: Weight gain; sedation, headache, lethargy, dizziness, tremor; dry mouth,
nausea, diarrhea, vomiting; rash; arthralgia, myalgia, back pain; increased appetite; orthostatic hypotension; peripheral edema, fatigue; abnormal dreams, confusion, anxiety.

- Sleep Side Effects: sleepiness, insomnia, abnormal dreams.

**Serotonin reuptake inhibitors and alpha-2 antagonist (IRSA)**

**Trazodone**

- Comercial Name in Spain: Trazodona/Deprax

- Objective: Also blocks histamine receptor (responsible for strong sedative effect) and alpha-1 and is believed to be able to increase the GABA neurotransmitter activity.

- Indication: suitable for deep depression, for the treatment of bulimia, kleptomania, migraine prophylaxis, relieving the symptoms of alcohol withdrawal, to control aggressive behavior in patients with autism and mental retardation, to diabetic neuropathy, to anxiety and the sleep disturbances

- Effects: Contributes to reduce the number of intermittent awakenings during sleep and increases the amount of deep sleep during the night,

- Side Effects: dizziness, vertigo, tremor, memory impairment, paresthesia, dystonia, fatigue.

- Sleep Side Effects: insomnia, nightmares, drowsiness.

**Analgesics**

**Selective agonists of serotonin receptors**

**Tramadol**

- Comercial Name in Spain: Dolpar/Pazital/Pontalsic/Zaldiar

- Objective: Structurally tramadol is not an opiate, but it exhibits some opioid properties. Tramadol shows a selective interaction with mu receptors, which are responsi-
ble for nociception, and has weak pharmacodynamic activity on other opioid receptors. At the same time, it acts synergistically on neuroamine transmission by inhibiting synaptic noradrenaline (norepinephrine) reuptake and inducing intrasynaptic serotonin (5-hydroxytryptamine; 5-HT) release.

- Indication: used to treat moderate to severe pain.
- Side Effects: alteration of mood, diarrhea, abdominal pain, dyspepsia, flatulence.
- Sleep Side Effects: sleep disturbances.

**Almotriptan**

- Comercial Name in Spain: Almogran
- Objective: Treating acute migraine headaches with or without aura (eg, dark spots, flashing lights, wavy lines).
- Indication: used to treat moderate to severe migraine.
- Side Effects: Dizziness; drowsiness; dry mouth; headache; nausea; vomiting.
- Sleep Side Effects: sleepiness.

**Paracetamol**

- Comercial Name in Spain: Paracetamol
- Objective: is a pain reliever and a fever reducer.
- Indication: s used to treat many conditions such as headache, muscle aches, arthritis, backache, toothaches, colds, and fevers. It relieves pain in mild arthritis.
- Side Effects: Rare: Malaise, increased levels of transaminases, hypotension, hepatotoxicity, rash, blood disorders, hypoglycemia, sterile pyuria. The paracetamol with codeine, codeine can because it may be diminished attention span, so it must be taken into account when driving, operating machinery or performing other tasks that may entail some danger.
• Sleep Side Effects: -

**Anxiolytics**

**Benzodiazepines**

**Diazepam**

- Comercial Name in Spain: Valium/Diazepan
- Objective: It facilitates the binding of GABA to its receptor and increases its activity. It acts on the limbic system, thalamus and hypothalamus. Locking action produces peripheral SNA or extrapyramidal side effects. Prolonged action.
- Indication: is used to treat anxiety disorders, alcohol withdrawal symptoms, or muscle spasms.
- Side Effects: Plunted affect, reduced alertness, confusion, fatigue, headache, dizziness, muscle weakness, ataxia or double vision, amnesia, depression, psychiatric and paradoxical reactions; respiratory depression.
- Sleep Side Effects: sleepiness.

**Lorazepam**

- Comercial Name in Spain: Lorazepam
- Objective: It affects chemicals in the brain that may become unbalanced and cause anxiety.
- Indication: is used to treat anxiety disorders.
- Side Effects: Sedation, blunted affect, reduced alertness, fatigue, headache, shortness of breath, ataxia, diplopia, confusion, depression, unmasking of depression, dizziness, fatigue, muscular weakness, psychiatric and paradoxical reactions.
- Sleep Side Effects: sleepiness.
Alprazolam

- Commercial Name in Spain: Trankimazim
- Objective: It affects chemicals in the brain that may become unbalanced and cause anxiety
- Indication: used to treat anxiety disorders, panic disorders, and anxiety caused by depression.
- Side Effects: Decreased appetite; confusional state, depression, disorientation, decreased libido; sedation, ataxia, impaired balance, coordination abnormal, memory impairment, dysarthria, disturbance in attention, lethargy, dizziness, headache; blurry vision; constipation, dry mouth, nausea; fatigue, irritability.
- Sleep Side Effects: sleepiness, hypersomnia.

Hypnotics

Melatonin receptor agonists

Melatonina

- Commercial Name in Spain: Melatonina
- Objective: It is believed that the activity of melatonin at the MT1, MT2 and MT3 receptors contributes to its stimulant properties of sleep, as these receptors (mainly MT1 and MT2) are involved in the regulation of circadian rhythms and sleep.
- Indication: Short-term treatment of primary insomnia.
- Side Effects: Headaches, Dizziness, abdominal discomfort, mild anxiety, irritability, confusion and short-lasting feelings of depression.
- Sleep Side Effects: sleepiness.
Nonsteroidal anti-inflammatory

Oral selective inhibitor of cyclooxygenase-2 (COX-2).

Etoricoxib

- Comercial Name in Spain: Arcoxia
- Objective: is a nonsteroidal anti-inflammatory drug (NSAID).
- Indication: helps to reduce the pain and swelling (inflammation) in the joints; and muscles of people with osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and gout.
- Side Effects: Edema / fluid retention; dizziness, headache; palpitations, arrhythmia; HTA; bronchospasm; abdominal pain, constipation, flatulence, gastritis, heartburn / acid reflux, diarrhea, dyspepsia, epigastric discomfort, nausea, vomiting, esophagitis, oral ulcer; bruising; increased AST and ALT; alveolar osteitis.
- Sleep Side Effects: -

Celecoxibe

- Comercial Name in Spain: Artilog/Celebreix
- Objective: is a nonsteroidal anti-inflammatory drug (NSAID).
- Indication: is used to treat Ankylosing Spondylitis, Familial Adenomatous Polyposis, Fibromatosis, Juvenile Rheumatoid Arthritis, Osteoarthritis, Pain, Period Pain, Rheumatoid Arthritis and Spondyloarthritis.
- Side Effects: More common: Cough; fever; skin rash; sneezing; sore throat; swelling on the face, fingers, feet, or lower legs.
- Sleep Side Effects: -
Dexketoprofen

- Comercial Name in Spain: Enantyum
- Objective: is a nonsteroidal anti-inflammatory drug (NSAID).
- Indication: Decreases prostaglandin synthesis by inhibiting cyclooxygenase.
- Side Effects: Nausea, vomiting, abdominal pain, dyspepsia, diarrhea, pain at the site of injection.
- Sleep Side Effects: -

Ibuprofen

- Comercial Name in Spain: Ibuprofeno/ Espidifen
- Objective: is a nonsteroidal anti-inflammatory drug (NSAID).
- Indication: It works by reducing hormones that cause inflammation and pain in the body. Is used to reduce fever and treat pain or inflammation caused by many conditions such as headache, toothache, back pain, arthritis, menstrual cramps.
- Side Effects: hemorrhage, vomiting, decreased hemoglobin, hypertension, eosinophilia, and anemia. Other side effects include: upper gastrointestinal hemorrhage, upper gastrointestinal tract ulcer, dizziness, and dyspepsia.
- Sleep Side Effects: -

Naproxen

- Comercial Name in Spain: Naproxeno
- Objective: is a nonsteroidal anti-inflammatory drug (NSAID).
- Indication: It works by reducing hormones that cause inflammation and pain in the body. Is used to treat pain or inflammation caused by conditions such as arthritis, ankylosing spondylitis, tendinitis, bursitis, gout, or menstrual cramps.
• Side Effects: More common: Belching, bruising, difficult or labored breathing, feeling of indigestion, headache, itching skin, large, flat, blue, or purplish patches in the skin, pain in the chest below, the breastbone shortness of breath, skin eruptions, stomach pain, etc

• Sleep Side Effects: -

**Piroxicam**

• Comercial Name in Spain: Vitaxicam

• Objective: is a nonsteroidal anti-inflammatory drug (NSAID).

• Indication: This medicine works by reducing substances in the body that cause pain and inflammation.

• Side Effects: More common: Bloating, bloody or black, tarry stools, burning upper abdominal or stomach pain, burning upper abdominal or stomach pain, cloudy urine, severe abdominal or stomach pain, cramping, or burning, severe abdominal or stomach pain, cramping or burning, etc

• Sleep Side Effects: -

**Muscle relaxant**

**Benzoadizepines**

**Tetrazepam**

• Comercial Name in Spain: Myolastan

• Objective: Centrally acting muscle relaxant.

• Indication: Painful contractures; Muscle spasm; Chronic low back pain; crick; sciatica.

• Side Effects: Paradoxical reactions with anxiety, agitation, aggression, confusion of ideas, hallucinations; anterograde amnesia.
• Sleep Side Effects: -

**Antiepileptics**

**Gabapetin**

- Comercial Name in Spain: Gabapentina

- Objective: It reduces the release of monoamine neurotransmitters and increases GABA turnover in several brain areas.

- Indication: Peripheral neuropathic pain; Epilepsy.

- Side Effects: Viral infection, pneumonia, respiratory infection, urinary tract infection, infection, otitis media; leucopenia; anorexia, increased appetite; hostility, confusion and emotional lability, depression, anxiety, nervousness, abnormal thinking; dizziness, ataxia, convulsions, hyperkinesias, dysarthria, amnesia, tremor, insomnia, headache, sensations such as paresthesia, hypoesthesia, coordination abnormal, nystagmus, increase / decrease / absence of reflexes; visual disturbances such as amblyopia, diplopia; dizziness; etc.

- Sleep Side Effects: sleep disturbances

**Pregabalin**

- Comercial Name in Spain: Pregabalina

- Objective: GABA analog. It binds to an auxiliary subunit channel voltage-dependent Ca CNS, potentially displacing [3H] -gabapentin.


- Side Effects: Dizziness, ataxia, impaired concentration, coordination abnormal, memory impairment, tremor, dysarthria, paraesthesia; increased appetite; euphoria, confusion,
decreased libido, irritability; blurred vision, diplopia; dizziness; dry mouth, constipation, vomiting, flatulence; erectile dysfunction; fatigue, peripheral edema, edema, abnormal gait; weight gain.

- Sleep Side Effects: sleepiness

**Sleep inducers**

**Zolpidem**

- Comercial Name in Spain: Zolpidem

- Objective: Specific agonist of central receptors belonging to the GABA receptor complex macromolecular omega-modulating the ion channel openers chlorine.

- Indication: is used to treat insomnia.

- Side Effects: Blunted affect, reduced alertness, confusion, fatigue, headache, dizziness, amnesia, hallucinations, agitation, ataxia, dizziness, double vision, depression, back pain, muscle weakness, diarrhea, nausea, vomiting, abdominal pain, urinary tract infection upper and lower respiratory, skin reactions.

- Sleep Side Effects: Daytime sleepiness, worsening of insomnia, nightmares.
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