UAB
Universitat Autònoma de Barcelona

Doctoral Thesis

BIOLOGICAL TRAITS AND NEUROPHYSIOLOGICAL SUBSTRATES OF ANXIETY AND SOMATIC SYMPTOMS

Núria Mallorquí Bagué

Barcelona, 2014

Universitat Autònoma de Barcelona

Doctorate in Psychiatry and clinical Psychology.
Department of Psychiatry and Psychological Medicine. School of Medicine

Doctoral Thesis

BIOLOGICAL TRAITS AND NEUROPHYSIOLOGICAL SUBSTRATES OF ANXIETY AND SOMATIC SYMPTOMS

Ph.D. dissertation presented by Núria Mallorquí Bagué to obtain the grade of European Doctor by the Universitat Autònoma de Barcelona.

Directors:

Prof. Antonio Bulbena Vilarrasa & Dr. Guillem Pailhez Vindual.

Hospital del Mar-Parc de salut Mar and fundació IMIM; Department of Psychiatry and Psychological Medicine. School of Medicine, UAB.

In colaboration with: Prof. Hugo D. Critchley, Clinical Imaging Science Center. Brighton and Sussex Medical School, Brighton, UK. & Dr. Oscar Vilarroya Oliver, Neuroimaging Research Group. IMIM, Barcelona.

Barcelona, 2014

Prof. Antonio Bulbena and Dr. Guillem Pailhez certify that they have supervised and guided this doctoral thesis presented by Núria Mallorquí Bagué: “Biological traits and neurophysiological substrates of anxiety and somatic symptoms”. They hereby assert that this doctoral thesis fulfils the requirements to be defended.

Directors,

Prof. Antonio Bulbena Vilarrasa Dr. Guillem Pailhez Vindual

Núria Mallorquí Bagué

Barcelona, 2014.

Als meus pares,

i a l'Aniol.

Acknowledgements / Agraïments

During the process of this thesis I have been fortunate enough to be surrounded with some really inspiring and wonderful people that have helped me along the way. The completion of this thesis could not have been possible without their support, for which I am really thankful.

Als meus dos directors de tesis, per la vostra dedicació durant tots aquests anys, per brindar-me l'oportunitat d'aprendre al vostre costat i per fer possible aquest projecte: Antoni Bulbena, moltes gràcies per la teva inspiradora creativitat i experiència, per la complicitat i per ensenyar-me infinitat de coses. Guillem Pailhez, moltes gràcies per la teva proximitat i tot el coneixement transmés, també per ser tan pràctic i posar les coses fàcils, fins i tot en els moments més complicats.

Oscar Vilarroya, per acompanyar-me en els meus primers passos en el món de la neuroimatge, perquè sense tu aquest projecte no s'hauria fet possible. Moltes gràcies per la teva professionalitat i proximitat, com també per tots els consells i disponibilitat.

Hugo Critchley, thank you for mentoring me and letting me be part of your research team, for your generosity and for giving me endless opportunities in a relatively short period of time. Collaborating with you has been deeply nourishing.

Núria Roé, per ser tant pacient, comprensiva i per transmetrem tots els coneixements necessaris per entendre una mica més el món de la neuroimatge.

To all my colleagues and friends at CISC. Especially to Sarah Garfinkel, for working with me hand to hand and teaching me some certainly useful aspects of the experimental and neuroimaging research field; to Miriam

Engels, for being such a good research partner, for your friendship and for being the best proof-reader ever; to Jessica Eccles, for welcoming me and helping me make the most of my stage in Brighton; to Ella, Maud, Chiara, Arjun, Guisy and James, for all the amazing experiences shared.

A tots els companys de la URNC. Especialment al Jordi Fauquet per ajudar-me amb la complexitat de l'estadística; a l'Erika, la Susana i l'Elseline per compartir amb mi part d'aquest projecte; a la Marisol, per sempre col·laborar i procurar per tots; a la Chalia i a la Lorena, gràcies a les vostres dots organitzatives i alegria tot ha sigut millor; al Pepus, al Dani B. i a la Lili pels moments compartits.

Al departament de Psiquiatria i Medicina Legal de la Universitat Autònoma de Barcelona, especialment a l'Adolf Tobeña i a la Isabel Aledo per la seva ajuda.

Als meus companys de la Dexeus. Especialment al Josep Maria Farré, per creure en mi i donar-me suport per endinsar-me en l'aventura que ha culminat amb aquesta tesi; a la Gracia Lasheras, per la confiança i per les moltes converses sempre productives; a la Lidia H., al Broja, a la Cristina, a la Katty, a la Judit, al Conrad a la Rebeca i a la Lidia I., un gran equip!

A la Maria, la Fluvi, la Neus i la Nona que han viscut tot el procés de molt a prop. Per l'amistat que meu regalat (quantes coses que han passat en aquests anys, no sabria per on començar). Gràcies.

A les meves amigues de sempre, per una vida compartida.

A l'Aniol, per la teva manera de ser i d'estimar-me, per caminar al meu costat i compartir amb mi les subtileses del dia a dia; perquè junts tot sembla possible.

A la meva família, els meus referents. Als meus pares, per creure en mi i donar-me suport amb tot el que em proposo; per ser els meus pilars. Al meu germà Joaquim i a l'Esther, per guiar-me no només en la recerca...,per escoltar-me i tenir tanta paciència! A en Marc, el *pitiu* i l'alegria de la casa.

INDEX

LIST OF ACRONYMS

ACC	Anterior Cingulate Cortex
AD	Anxiety Disorder
BOLD	Blood oxygenation level dependent signal
CBT	Cognitive-Behavioural Therapy
DMPFC	Dorsomedial Prefrontal Cortex
DLPFC	Dorsolateral Prefrontal Cortex
fMRI	Functional magnetic resonance imaging
GAD	Generalized Anxiety Disorder
JHS	Joint Hypermobility Syndrome
JH	Joint Hypermobility
LSAS	Liebowitz social anxiety scale
MAIA	Multidimensional assessment of interoceptive awareness
MCC	Middle Cingulate Cortex
MINI	Mini international neuropsychiatric interview
MPFC	Medial Prefrontal Cortex
MRI	Magnetic resonance imaging
O-CPD	Obsessive-Compulsive Personality Disorder
OFC	Orbito Frontal Cortex
PA	Panic Attacks
PD	Panic Disorder
PFC	Prefrontal Cortex
POTS	Postural Orthostatic Tachycardia Syndrome
ROI	Regions of interest
SAD	Social Anxiety Disorder

SCID-I	Structured clinical interview for DSM axis-I disorders
SMH	Somatic marker hypothesis
sMRI	Structural magnetic resonance imaging
SPh	Specific Phobia
STAI	State/Trait anxiety inventory
VMPFC	Ventro Medial Prefrontal Cortex

ABSTRACT (1)

Anxiety is an emotion state that involves behavioural, psychological and physiological changes. Importantly at its excessive expression, anxiety is usually approached as a mind-body multisystem condition specifically characterized by feelings of tension, worried thoughts and physical changes that can lead to significant distress.

The aetiology of anxiety disorders (ADs) still remains unclear. However clinical and neurophysiological studies have described some important predisposing factors. Accordingly trait anxiety, body awareness and interoception have been widely used as vulnerability traits and potential markers for hypochondriasis, anxiety and somatization. In addition several brain regions have a key role in the development and maintenance of ADs, mostly those conferring the so called emotional brain (e.g.: amygdala, anterior cingulate cortex, insula and hippocampus). Besides Joint Hypermobility Syndrome (JHS), which is an inherited generalized collagen condition associated with abnormal autonomic reactivity, has shown to be a risk factor for developing ADs. Importantly, recent findings have point out a possible association between body awareness and JHS as well as specific brain regions that are likely to mediate the clinical expression of anxiety through JHS.

In order to better understand the aetiology of anxiety and somatic symptoms and thus help improving the treatment of ADs, we explore biological traits and neurophysiological substrates of anxiety and somatic symptoms in a general population with different ranges of joint

hypermobility (JH) as well as different ranges of non-clinical anxiety. Hence we conducted two different studies to: (1) explore the association between body awareness, sate anxiety and JH and (2) identify neural correlates of JH and trait/social anxiety. In the first study, all participants underwent a clinical examination for hypermobility, undertook tests of interoceptive sensitivity and completed questionnaire measures of state anxiety and body awareness. Also, some participants performed an emotional processing task during fMRI. In the second study, all participants were assessed with a structured clinical examination for hypermobility, completed validated questionnaire measures of anxiety tendency (i.e.: trait and social anxiety) and underwent a functional emotional paradigm using fMRI techniques.

Results showed that state anxiety negatively correlates with the attention regulation subscale and the trusting body sensations subscale of the body awareness self-reported measure. Also state anxiety positively correlated with higher accuracy of interoceptive sensitivity. JH was associated with higher state anxiety and demonstrated higher interoceptive accuracy than non-JH. Still, our mediation analysis revealed that interoceptive sensitivity mediated the association found between JH and state anxiety. Likewise, JH individuals were found to present a discrete but stronger neural reactivity to affective stimuli in brain areas known to be involved in emotional processing (particularly in anxiety) and interoception (i.e.: insula, brainstem), when compared to non-hypermobile participants. Results also show a significant association between trait anxiety and social anxiety, and between trait anxiety and JH. Our ROI analyses showed no associations with BOLD signals and the anxiety measures. However when

studying JH scores, ROI analyses showed a positive association between BOLD signals in the hippocampus as a response to crying faces compared to neutral faces. Additionally, the whole brain analysis showed that hypermobility scores were positively associated with an increased BOLD signal in some other key affective processing areas, such as the middle and anterior cingulate cortex, the fusiform gyros, the parahippocampal region, the orbitofrontal cortex and the cerebellum.

We present the first neuroimaging study of the relationship between anxiety tendencies (state-trait anxiety, social anxiety and hypermobility) that also examines interoceptive sensitivity in a non-clinical sample. Our findings have the potential to increase our understanding of the mechanisms through which vulnerability to anxiety disorders and somatic symptoms arises in people with trait anxiety, enhanced interoception and/or JH.

ABSTRACT (2)

L'ansietat és una emoció que involucra canvis fisiològics, psicològics i comportamentals. Quan l'ansietat és excessiva i per tant desadaptatitva, es caracteritza per sentiments de tensió, de preocupació i, s'acompanya de diferents sensacions corporals desagradables que produeixen gran malestar; minvant així l'autonomia personal del que la pateix.

La etiologia dels trastorns d'ansietat encara és poc clara. No obstant, estudis clínics i neurofisiològics han descrit alguns factors de predisposició ansiosa que resulten ser claus en el desenvolupament i manteniment d'aquests trastorns. Entre aquests factors de predisposició destaquen l'ansietat tret, la consciència corporal i la interocepció, que han estat àmpliament descrits com a trets de vulnerabilitat i marcadors potencials per a la hipocondria, l'ansietat i la somatització. A més a més vàries regions cerebrals hi tenen també un rol clau, especialment les que conformen l'anomenat cervell emocional (ex.: amígdala, còrtex cingulat anterior, insula i hipocamp). Per últim destacar també la Síndrome d'Hiperlaxitud Articular, una anomalia col·làgena generalitzada que està associada a irregularitats en la reactivitat autonòmica i descrita com a factor de risc pels trastorns d'ansietat. A més a més, troballes recents han destacat la possible associació entre la consciència corporal i la hiperlaxitud articular, com també determinades zones cerebrals que podrien mediar l'expressió clínica d'ansietat a través de la laxitud articular.

Per tal de comprendre millor la etiologia de l'ansietat i els símptomes somàtics i, així ajudar a millorar el tractament dels trastorns d'ansietat,

explorem trets biològics i neurofisiològics d'ansietat en una població que presenta diferents graus de laxitud articular i d'ansietat no clínica. Realitzem doncs dos estudis diferents: (1) explorar l'associació entre la consciència corporal, l'ansietat estat i la laxitud articular i, (2) identificar correlats neuronals d' hiperlaxitud articular, ansietat tret i ansietat social. En el primer estudi, tots els participants van ser examinats a través d'una exploració clínica d' hiperlaxitud articular, van realitzar tasques per mesurar la capacitat interoceptiva i van completar qüestionaris per mesurar les tendències ansioses (ansietat estat) i la consciència corporal. També, alguns participants van realitzar una tasca de processament emocional mitjançant tècniques de ressonància magnètica funcional. En el segon estudi, tots els participants van ser examinats a través de la mateixa exploració clínica per l'hiperlaxitud articular, van completar qüestionaris per mesurar tendències ansioses (ansietat tret i social) i van formar part d'un paradigma emocional mitjançant tècniques de ressonància magnètica funcional.

Els resultats obtinguts mostren que l'ansietat estat correlaciona positivament amb la capacitat interoceptiva (elevada percepció dels batecs del cor) i negativament amb dos de les subescales de mesura de consciència corporal: capacitat de regular l'atenció i confiança vers les sensacions corporals. Per altra banda, l' hiperlaxitud articular s'associa amb puntuacions més elevades en ansietat estat i s'observa que la capacitat interoceptiva fa de mediadora en l'associació obtinguda entre hiperlaxitud articular i ansietat estat. De la mateixa manera, els participants hiperlaxes presenten una reactivitat discretament més elevada en àrees involucrades

en el processament interoceptiu (insula, tronc encefàlic) durant un paradigma d'estimulació afectiva que els participants no hiperlaxes. Els resultats també mostren associacions significatives entre l'ansietat tret i l'ansietat social, i entre l'ansietat tret i la laxitud articular. Els anàlisis ROI no mostren cap associació entre la resposta BOLD i les mesures d'ansietat. No obstant, quan estudiem les puntuacions de laxitud articular en el contrast emocional (cares plorant vs. cares neutres), els anàlisis ROI mostren una associació positiva de la resposta BOLD a l'hipocamp com a resposta del paradigma emocional. A més a més, les anàlisis exploratòries no corregides mostren que les puntuacions en laxitud articular correlacionen positivament amb l'increment de la resposta BOLD en altres àrees importants pel processament afectiu: còrtex cingulat anterior i mitjà, girus fusiforme, àrea parahipocampal, còrtex orbito frontal i cerebel.

Presentem el primer estudi de neuroimatge sobre l'associació entre diferents tendències ansioses (ansietat estat-tret, ansietat social i laxitud articular) que també explora la interocepció en una població no clínica. Els nostres resultats tenen el potencial de millorar els coneixements dels mecanismes a través dels quals la vulnerabilitat pels trastorns d'ansietat i els símptomes somàtics apareixen en persones amb ansietat tret, capacitat interceptiva i hiperlaxitud articular.

1. INTRODUCTION

1.1 ANXIETY STATES

1.1.1 Anxiety as an emotion: from adaptive to excessive anxiety

Anxiety is an *emotion characterized by feelings of tension, worried thoughts and physical changes like increased blood pressure that interact* (American Psychological Association, 2014). Like all emotion states it can be experienced in varying degrees of intensity, frequency and duration. At a low range of expression, anxiety is adaptive and necessary for everyday life. However at an upper range (i.e.: when its expression is excessive in magnitude and/or duration and frequency) it can be overwhelming, hugely disruptive and evolve into different symptoms and into pathological anxiety.

Anxiety Disorders (ADs), which represent variant forms of pathological anxiety, are a group of mental disorders characterized by excessive and persisting feelings of fear, anxiety and related behavioural disturbances that interfere with a person's functioning and cause significant distress (American Psychiatry Association, 2013). Essentially, in its adaptive forms, fear is an emotion reaction to present events that protects us from current danger and anxiety is an emotion that prepares us to predict, control and avoid those danger events at the first place. This two states overlap, but they also differ: fear is more often associated with urges of autonomic arousal (necessary for fight or flight), thoughts of immediate danger and escape behaviours whereas anxiety is more often associated with muscle tension, cautious or avoidant behaviours and hypervigilance in preparation for future danger (American Psychiatry Association, 2013).

In ADs, biological, physiological, psychological, and social factors interact to create and maintain the described disruptive fear/anxiety feelings and its physical symptoms as well as behavioural maladaptive consequences (Barlow, 2002). There are various types of ADs, including Panic and Phobic Disorders, Generalized Anxiety Disorder (GAD), Separation Anxiety Disorder, Selective Mutism, Separation Anxiety Disorder and Anxiety Disorders induced by substance/medication or due to another medical condition (American Psychiatry Association, 2013). While each AD can be distinguished by its own characteristics, all of them include symptoms of anxiety. Thus, there are some common emotional (e.g.: uneasiness, fear, distress), cognitive (e.g.: negative and worry thoughts), behavioural (e.g.: avoidance, attachment undue) and physical (e.g.: tachycardia, muscle tension, dizziness) symptoms of anxiety and all them are crucial in ADs.

Somatic symptoms are usually key features of pathological and high state anxiety and are always linked to an acute activation of sympathetic axis of the Autonomic Nervous System. As for its nature, its manifestations cannot be considered in one dimension but in a multisystem approach, which includes musculoskeletal (e.g.: muscle tension, shakiness), respiratory (e.g.: dyspnoea), cardiovascular (e.g.: tachycardia), gastrointestinal (e.g.: diarrhoea), skin (e.g.: sweatiness), and genitourinary (e.g.: frequent urinary urgency) body systems. The perception and interpretation of these bodily states also play an important role in the pathogenesis of ADs and are extremely linked to the emotion state of the individual (Clark, 1986; Clark & Wells, 1995; Clark et al., 1997).

Epidemiological studies of adult community samples indicate that ADs are one of the most commonly occurring of the mental health disorders, affecting around 69.1 million individuals across Europe (Olesen, Gustavsson, Svensson, Wittchen & Jönsson, 2012; Sansone & Sansone, 2010). ADs typically have an early onset, usually last more than 6 months and frequently develop a chronic or relapsing course. As for its nature, they usually cause significantly prolonged distress and lifetime quality impairment (Rapaport, Clary, Fayyad & Endicott, 2005). There are different prevalence rates described for the disorders that comprise this heterogeneous condition, among them Social Anxiety Disorder (SAD, 10%) and Specific Phobia (SPh, 6-12%) have the highest lifetime prevalence. The remaining ADs, including Agoraphobia with and without Panic Disorder (PD) and GAD, have lifetime prevalence ratings of between 2 to 5% (Kessler, Ruscio, Shear & Wittchen, 2010). Moreover, evidence suggest that women are at considerable greater risk for suffering of anxiety and ADs than men; approximately a 2:1 ratio (American Psychiatry Association, 2013).

The aetiology of ADs is complex and still remains partly unknown. However it is widely accepted that there are multiple and inter-related factors involved in the development of pathological anxiety. These factors can be mainly categorized into biological vulnerabilities (e.g.: neural indices, genetic markers, trait anxiety, neuroticism, interoceptive sensitivity), psychological vulnerabilities (early learning experiences) and environmental stimuli and influences (life experiences). Once the biological and psychological vulnerabilities are in place, the environment can act as a trigger (Barlow, 2002). Each AD have different risk and prognosis factors,

however behavioural inhibition and neurotic disposition (negative affectivity and anxiety sensitivity) have shown to be relevant to most ADs (i.e.: phobic disorders, panic disorders, GAD) (See Table 1 for diagnostic features as well as risk of ADs not Substance/medication-induced, according to American Psychiatry Association, 2013: DSM-5 classification).

Table 1. Anxiety Disorders, not substance/medically-induced, classification and specific characteristics according to the Diagnostic and Statistical Manual of Mental Disorders, fifth Edition (DSM-5) (American Psychiatry Association, 2013).

ANXIETY DISORDERS	DIAGNOSTIC FEATURES	RISK FACTORS
Specific Phobia	Persistent and out of proportion fear/anxiety about or avoidance of circumscribed objects or situations. None specific cognitive ideations.	*Temperamental*: negative affectivity, behavioural inhibition. *Environmental:* parental loss and separations or overprotectiveness, among others. *Genetic and physiological*: still being studied.
Social Anxiety Disorder (Social Phobia)	Fear, anxiety about or avoidance of social interactions and situations that involve the possibility of being scrutinized. The cognitive ideation is of being negatively evaluated or offending others.	*Temperamental*: Underlying traits like behavioural inhibition and fear of negative evaluation. *Environmental:* childhood maltreatment and adversity. *Genetic and physiological*: the described temperamental traits are genetically influenced.
Panic Attack (PA)	Abrupt surges of intense fear or intense discomfort that reaches a peak within minutes, accompanied by physical and/ or cognitive symptoms.	*Temperamental*: negative affectivity and anxiety sensitivity (for the onset). History of "fearful spells" for later PA. *Environmental:* smoking and identifiable individual stressors in the months before the first PA.

Panic Disorder (PD)	Recurrent unexpected PA and persistent concerns or worries about having more panic attacks or changes in his or her behaviour in maladaptive ways because of the PA.	*Temperamental*: the risk status for the diagnosis for PD is still unknown (see PA). *Environmental:* see PA. *Genetic and physiological*: multiple genes confer vulnerability to PD; however the exact genes remain unknown. Neural systems models for PD emphasize the amygdala and related structures.
Agoraphobia	Fear/anxiety about two or more of the following: using public transportation, being in open spaces or in enclosed places, standing in line or being in a crowd, being outside of the home alone. With thoughts that escape might be difficult or help might be unavailable if developing panic symptoms or other incapacitating or embarrassing symptoms.	*Temperamental*: Behavioural inhibition and neurotic disposition (negative affectivity and anxiety sensitivity). *Environmental:* Negative events in childhood and other stressful events (e.g.: being attacked or mugged). *Genetic and physiological*: Heritability for agoraphobia is 61% (the strongest and most specific association with the genetic factor that represents proneness to phobias).
Generalized Anxiety Disorder	Persistent and excessive anxiety/worry about various domains that the individuals find difficult to control. Also experiences of physical symptoms (e.g.: restlessness)	*Temperamental*: Behavioural inhibition, negative affectivity and harm avoidance. *Genetic and physiological:* 1/3 of its risk is genetic and overlaps with the neuroticism risk.

Selective mutism	Consistent failure to speak in social situations in which there is an expectation to speak even though the individual speaks in other situations.	*Environmental:* Social inhibition on the part of parents. *Genetic and physiological:* overlap with social phobia, may be shared genetic factors between these conditions.
Separation anxiety disorder	Fear /anxiety about separation from attachment figures to a degree that is developmentally inappropriate. Symptoms often develop in childhood but they can be expressed throughout adulthood as well.	*Environmental:* often develops after life stress, especially a loss. *Genetic and physiological:* heritability is estimated at 73% in a community of six-years-old twins. No references in adulthood.

1.1.2 Mind-body interaction models of emotion creation and anxiety

The mind and the body are intrinsically and dynamically coupled; perceptions, thoughts and feelings change and respond to the state of the body and vice-versa. Thus, to better understand the manifestations of pathological anxiety and its somatic symptoms a mind-body approach of emotion creation is required.

As mentioned above, emotions are commonly viewed as psychological states that involve behavioural, psychological and physiological changes and anxiety is usually approached as a mind and body multisystem condition. For many years, different empirical and scientific-based models have emerged in order to explain the creation of emotions in general and anxiety in particular in a mind-body context. Among them, of special relevance and influence are those of Clark and the cognitive-perceptual models' as well as the Damasio's "Somatic marker hypothesis".

Firstly, cognitive models (Clark, 1986; Salkovskis & Clark, 1993; Clark & Wells, 1995; Clark et al., 1997) emphasize the role of individual psychological processes in the perception and interpretation of one's bodily state and environment. The misinterpretations of body symptoms can lead to an amplification of these symptoms. Recent studies have shown how cognitions retain a central role in shaping the perception and interpretation of bodily sensations of arousal. The misattribution and misinterpretations of these feelings are recognised key factors in the development of anxiety states including panic and anxiety-related somatic symptoms (Herbert, Muth, Pollatos & Herbert, 2012). These findings have enlightened the

understanding of anxiety disorders and enabled treatment improvements (Clark & Beck, 2010; Hilchey, & Clark, 2014).

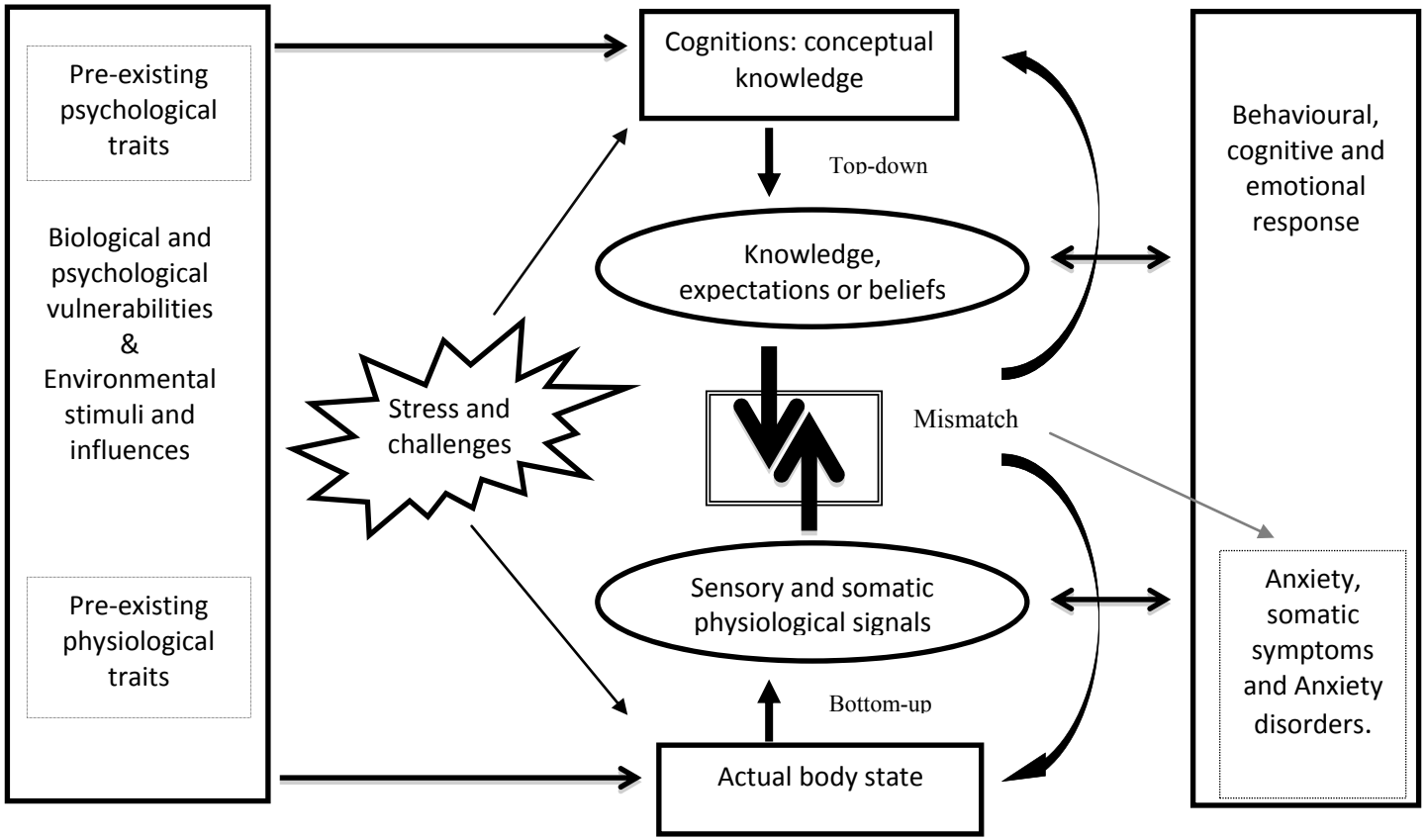
Secondly there is the notion of 'somatic markers', a theory that have provoked much discussion on the understanding of the subjective experience of emotion, where emphasis is given to visceral signals to emotional stimuli. Somatic marker hypothesis (SMH) is a modern successor and extension of the "James–Lange" theory of emotions (1884) proposed by Damasio (1996) and Craig (2004). According to James theory, emotions occur as a result of physiological reactions to events and interpretations of the afferent information from the body. The SMH was first proposed by Damasio (1996), and it postulates that bodily expression of emotion (e.g. autonomic arousal responses) can occur in the absence of awareness and bias behaviour and decision-making. For Damasio, emotion is the representation and regulation of homeostatic changes that occur in different levels of the body and brain in given situations. Thus, the brain makes predictions regarding the source of sensory information and adjusts these predictions against the information returning through the senses. In this framework, interoception processes play an important part underpinning processes such as preconscious homeostatic regulation, behavioural biases to the conscious experience of emotional feelings and the continuity of self-representation (Seth, Suzuki & Critchley, 2011).

Anxiety is coupled to both, the representation of the bodily arousal states and to the expectations and predictions about these bodily states (Critchley, Wiens, Rotshtein, Ohman & Dolan, 2004; Critchley & Harrison, 2013). The mismatch between actual and anticipated arousal states is of

utmost importance in the expression of anxiety. Furthermore, pre-existing states and traits are embedded within these processes and are described to be crucial vulnerability factors in the development of anxiety and somatic symptomatology (Paulus & Stein, 2006; Barlow, 2002) (See Figure 1 for this integrative model of anxiety and somatic symptoms).

The current understanding of mind-body interaction has allowed a better insight into the expression of adaptive and pathological emotions and cognitions as well as a better knowledge of the interactions that are presumed to fuel the development and maintenance of anxiety and somatic symptomatology. However, there is still a long way to go in improving the treatments given to these health impairments.

Figure 1. Mind-body interaction: underlying processes that presume to take place in developing and maintaining anxiety and somatic symptomatology.



1.2 BODY AWARENESS AND INTEROCEPTION

1.2.1 Body awareness definition

Body awareness is a construct that has emerged as a focus of scientific interest across a wide range of health topics. The term body awareness refers to both the attentional focus to the body senses and the capability to be aware of these sensations (Bekker, Croon, van Balkom & Vermee, 2008). This attention and capability to focus and detect bodily symptoms has usually been studied in anxiety and PD as a cognitive attitude characterized by an exaggerated focus on physical symptoms, rumination and magnification (‘‘somatosensory amplification’’). Thus, body awareness has been widely used as a vulnerability trait and a potential marker for hypochondriasis, anxiety and somatization (Kirmayer & Looper, 2006; Abramowitz, Schwartz & Whiteside, 2002; Cioffi, 1991). Accordingly, it has usually been conceived as a maladaptive cognitive attribution to the highly perceived bodily states.

Concerning body senses and in order to better understand its implications, it is also important to distinguish between these three concepts (Sherrington, 1906; Laskowski, 2000): “Proprioception”, which is the sense of the relative position of neighbouring parts of the body and strength of effort being employed in movement; “Exteroception” which is the sense of the outside world; “Interoception” which is the sense of the inner body (vide infra) and is highly relevant for the understanding of the underlying mechanisms of anxiety, thus highly relevant for the aims of this research project.

1.2.2 Interoception and its role in anxiety

Interoception is the ability to perceive changes in the physiological state of the body (Craig, 2003) and it is viewed as a constitutional trait of an individual which is stable over the lifespan. Furthermore, as previously mentioned, the perception of body states is considered to be essential to the generation of emotional feeling states (Damasio, 1996; Clark, 1986). The term interoception was first formulated by Sherrington (1906) to distinguish it from other types of body-sensation including proprioception which is nevertheless closely associated.

Interoception has recently been described at different levels and considered along three main dimensions (Garfinkel & Critchley, 2013) that describe the three different interoception constructs most commonly measured and investigated:

1) Interoceptive sensibility: dispositional tendency to be internally focused and hold self-referential beliefs about body tendencies (usually measured by questionnaires were individuals can express their subjective perception of their body tendencies).

2) Interoceptive sensitivity: objective accuracy in detection internal bodily sensations (largely measured by heartbeat detection or tracking tasks in laboratory premises).

3) Interoceptive awareness: metacognitive awareness of interoceptive sensitivity 'how well you know that you know" (e.g.: can be measured by confidence indexes when performing heartbeat tasks in laboratory premises or questionnaire measures). It is strongly influenced by mental

processes including attention, interpretation, appraisal, beliefs, memories, conditioning, attitudes and affect.

While strong emotional feelings are associated to stronger bodily responses, this effect is amplified in people with better representations of these changes. Individuals who present high levels of accuracy in judging their bodily signals (interoceptive sensitivity) experience emotions more intensely (Pollatos, Traut-Mattausch, Schroeder & Schandry, 2007; Wiens, Mezzacappa & Katkin, 2000). Besides, recent studies have suggested that interoceptive awareness modulates this enhanced intensity of the subjective emotion experience and affects individual traits related to emotion processing (Terasawa, Moriguchi, Tochizawa & Umeda, 2014).

Furthermore, behavioural studies consistently report a positive relationship between higher levels of anxiety and enhanced interoceptive sensitivity (measured using heartbeat detection tasks) in either ADs or general population samples (Mor & Winquist, 2002). Specifically, higher interoceptive sensitivity is found among PD individuals when compared to healthy participants (Domschke, Stevens, Pfleiderer & Gerlach, 2010) and it is reported to the same level in SAD, GAD and SPh (Tsakiris, Tajadura-Jiménez, Costantini, 2011). Interoceptive sensitivity is also positively related to anxiety measures in healthy subjects (Critchley et al., 2004; Willem Van der Does, Antony, Ehlers & Barsky, 2000). Additionally interoceptive awareness (metacognitive awareness of inner body states) has repeatedly been studied in anxiety and PD; high anxious individuals are conceived to present a maladaptive metacognitive attitude to body symptoms that lead to their magnification. There are generally two components of

metacognition: knowledge about cognition, and regulation of cognition (Schraw & Gregory, 1998; Beer & Moneta, 2012).

However, interoceptive awareness has also been proposed as a key component for the sense of self (Ehlers, Mayou, Sprigings & Birkhead, 2000). Thus, an alternative view of body awareness has emerged showing that the capacity to regulate affective arousal may be enhanced by heightened awareness of internal states (Flink, Nicholas, Boersma, Linton, 2009; Mehling et al., 2011). Likewise, 'mindfulness-based' therapeutic approaches have taken an alternative view of body awareness as promoting an adaptive, mindful and self-accepting approach to bodily attention (Kabat-Zinn, 1991; Kabat-Zinn, 1996). However, while mindfulness practice usually trains an enhanced awareness of bodily responses, performance accuracy on heartbeat perception tasks (i.e. interoceptive sensitivity) is generally not improved (Parkin et al., 2013). Interestingly, people's confidence in their heartbeat task accuracy (a metacognitive judgement of interoceptive awareness) does improve. These results reinforce the notion that interoceptive sensitivity is a trait that is not easily modified but that higher-level predictions about bodily state may be tuned to be more effective.

1.3 JOINT HYPEROMBILITY SYNDROME

1.3.1 Clinical and diagnostic features

Joint Hypermobility (JH) is an inherit collagen condition characterized by an increased joint mobility in passive and active movements. Collagen is a connective tissue fibrous protein component of bone, cartilage, tendon, blood vessels and other body constituents. This inherit qualitative collagen variation described in JH can cause a wide range of symptoms. When these symptoms occur, the condition is termed Joint Hypermobility Syndrome (JHS) and considered a systemic multisystem affection.

The estimated prevalence of JH in the general population (Western European) ranges between 10-15% and it is described to be higher in Chinese, Indians and Middle Eastern groups. It has a strong genetic component with autosomal dominant pattern; first degree relatives with the disorder can be identified in 50% of cases and it's more frequent in women than in men (3:1) (Ross & Grahame, 2011; Hakim, Cherkas, Grahame, Spector & MacGregor, 2004). JHS usually goes undiagnosed and its real prevalence in general population still reminds unknown. However some studies have shown a prevalence of 45% in reumathological clinical samples (Bravo & Wolff, 2006; Ross & Grahame, 2011; Beighton & Grahame, 2012)

There are a number of heritable related connective tissue disorders which present JH, such as Ehlers-Danlos Syndrome type 1 and 2, Marfan syndrome and JHS itself. As for their clinical manifestations, JHS (also referred as Ehlers-Danlos type 3) is described to have the lowest symptom

severity of all them. Still, it usually goes unnoticed and it can develop into a chronically disabling condition for particular individuals.

JHS presents multiple clinical features which are associated with the collagen abnormality and can be either articular or extra-articular: widespread musculoskeletal pain, multiple soft tissue lesions and fragility of supportive connective tissue and skin. In addition to the skin and musculoskeletal manifestations, JHS is associated with an increased incidence of mitral valve prolapse and high elasticity of the aortic wall (Grahame, Edwards, Pitcher, Gabell & Harvey, 1981; Yazici et al, 2004), hypotension, asthma (Morgan, Pearson, Davies, Gooi & Bird, 2007), severe Raynaud's phenomenon and gastro intestinal problems (reflux, irritable bowel syndrome, diverticulosis) (Fikree et al., 2014), as well as autonomic symptoms (Grahame & Hakim, 2008; Gazit, Nahir, Grahame & Jacob, 2003), anxiety disorders (particularly panic, agoraphobia and social phobia) (Bianchi, De Lima, Udina, Martin-Santos, Crippa, 2011; Martin-Santos et al., 1998) and some functional illnesses such as fibromyalgia, temporo mandibular joint disorder and chronic pain and fatigue (Keer & Grahame, 2003; Murray, Yashar, Uhlmann, Clauw & Petty, 2013).

In clinical practice as well as in research bases, the most frequently used scoring systems for JHS are the Beighton scale (Beighton, Grahame & Bird, 1989), the Brighton's (Grahame, Bird & Child, 2000) and Hospital del Mar's criteria (Bulbena et al., 1992). The Beighton score system is fast and easily conducted. It requires a physical examination consisting of five items (describing nine movements), that explores the joint mobility range of 5 body areas: wrists/thumb, knees, spine/hips, paired elbows and fifth

metacarpo-phalangeals. The maximum score is nine and the arbitrary cut-off point is set at 4 or more out of nine (see Table 1). Thus, scoring 4 or more is arbitrarily considered an indicative of the presence of generalized JH. This scoring system was originally set for epidemiological and research purposes and does not explore JH symptoms but only the mobility of the joints (JH) as an indicative of the collagen alteration.

Brighton criteria were published in order to improve the Beighton scores by adding some extra-articular features that could enable the diagnoses of the JHS and not only the presence of JH. Specifically, it consists of two major and eight minor criteria that endorse the revised Beighton system and, in addition, includes some other principal symptoms (e.g.: joint pain, dislocations, soft tissue lesions, uterine prolapse). Also, it proposes different cut-off points according to the age of the individual.

The Hospital del Mar criteria were obtained after a multivariate analysis of margins from the original Carter & Wilkinson's, the Beighton's and the Rotés' scoring systems. This new scale showed consistent indicators of reliability, internal consistency and predictive validity and provided evidence for using different scores for each gender. It consists of ten items (measuring articular and extra-articular features) and similarly to the Beighton's method one point is given for each criterion up to a maximum score of 10; a high score may be an indicative of JHS. Authors propose a cut-off point of 3/4 for men and 4/5 for women. Correlation between these systems is high but the scope is slightly different.

Despite diagnoses and clinical protocols have changed since JHS was first described, its multifaceted nature often leads to confusion in making its

diagnosis and it often remains unrecognized. Hence the scientific community highlights the necessity to intensify the attention given to this condition as well as further specify and unify the cut-off points and gold standards of the JHS validated criteria (Remvig, Flycht, Christensen & Juul-Kristensen, 2014; Ross & Grahame, 2011; Remvig et al., 2011) which still remains debated.

Table 1.Nine-point Beighton score for joint hypermobility syndrome

One point is gained for each side of the body for the first four movements listed below, such that the hypermobility score is a maximum of 9 if all are positive.

1. Passive apposition of the thumbs to the flexor aspects of the forearm (one point for each thumb).

2. Hyperextension of the knee beyond 10º (one point for each knee).

3. Passive dorsiflexion of the little fingers beyond 90º (one point for each hand).

4. Hyperextension of the elbows beyond 10º (one point for each elbow).

5. Forward flexion of the trunk with knees fully extended so that the palms of the hands rest flat on the floor (one point).

Table 2. Brighton criteria for classification of joint hypermobility syndrome (1998).

JHS is diagnosed in the presence of two major criteria, one major criterion plus two minor criteria or four minor criteria. Two minor criteria will suffice where there is an unequivocally affected first degree relative. The syndrome is excluded by the presence of Marfan's or Ehlers-Danlos syndromes (other than the hypermobility type of Ehlers-Danlos syndrome).

Major criteria

- Beighton score of ≥4 (either currently or previously)
- Arthralgia for longer than three months in four or more joints

Minor criteria

- Beighton score of 1, 2, or 3 (0, 1, 2, or 3 if aged >50 years)
- Arthralgia in one to three joints or back pain or spondylosis, spondylolysis and/or spondylolisthesis
- Dislocation in more than one joint or in one joint on more than one occasion
- Three or more soft tissue lesions (e.g., epicondylitis, tenosynovitis, bursitis)
- Marfanoid habitus (tall, slim, ratio of span to height greater than 1.03 and/or ratio of upper segment to lower segment less than 0.89, arachnodactyly)
- Abnormal skin: hyperextensibility, thin skin, papyraceous scarring
- Eye signs: drooping eyelids, myopia, or antimongoloid slant
- Varicose veins, hernia, or uterine or rectal prolapse

Table 3. Hospital del Mar criteria (Bulbena et al., 1992) for JHS.

Male patients scoring 4 or more are considered cases; female patients are considered cases with scores 5 or over.

Upper extremities

- Passive apposition of the thumb to the flexor aspect of the fore arm at a distance oflessthan21mm.
- The passive dorsiflexion of the fifth finger is 90º or more.
- The active hyperextension of the elbow is 10º or more.
- External rotation of the shoulder up to more than 85º.

Lower extremities. Supine position

- The passive hip abduction can be taken to an angle of 85º or more.
- Hypermobility of the rotula.
- Hypermobility of the ankle and foot.
- Dorsal flexion of the toe of 90º or more.

Lower extremities. Prone position

- Hyperflexion of the knee.
- Ecchymoses.

1.3.2 Anxiety and the role of collagen tissue

The association between anxiety and JHS was first observed in clinical practice when clinicians noticed that a wide array of the anxiety patients seeking for treatment did also present JHS. The first study reporting this association dates of 1988 (Bulbena, Duró, Mateo, Porta & Vallejo, 1988) and it was later replicated in 1993 (Bulbena et al. 1993); both case-control studies conducted within rheumatologic outpatient population. Results revealed that 67.7% of the JHS patients did also present anxiety and confirmed the statistical significance of this association when compared to a non-hypermobile sample. Specifically, JHS patients presented higher prevalence of PD, SPh and SAD when compared to the control group. No associations were found between JHS and GAD.

The first evidence of this association in a clinical anxiety sample was reported in a case-control study conducted for Martín-Santos et al. (1998). Results showed a 70% prevalence of JHS within the PD group, which was significantly higher than the prevalence observed in the control groups (i.e.: psychiatric disorders control group, medical with non-anxious history control group). These was then further tested in general population samples, showing that non-clinical JH participants presented higher probabilities to suffer from PD, SAD and/or Agoraphobia (Bulbena et al., 2004a) and also presented higher trait anxiety when measured by means of STAI-trait (Bulbena et al., 2004b). Besides, in a longitudinal study JH was found to be a risk factor for developing anxiety disorders (Bulbena et al., 2011).

The robust and already widely acknowledged association between JHS and ADs in general has specifically proven to be even stronger within PD and SAD (Garcia-Campayo, Asso, Alda, 2011; Garcia-Campayo, Asso, Alda, Andres, & Sobradiel, 2010). Additionally, recent studies have reported that JHS individuals also experience significantly greater perceptions of fear, somatic symptoms and functional illnesses and have a higher probability of demonstrating depression than those without JHS (Smith, et al., 2014; Garcia-Campavo et al, 2011). Finally, a new contribution has recently been made on this matter; an unexpected association between JHS and obsessive-compulsive personality disorder (O-CPD) has emerged. JHS patients showed a high rate of personality disorders (21%) and an observed prevalence rate of 10.6% for O-CPD (Pasquini et al., 2013), a personality disorder extremely linked to anxiety. Replications of these results are still needed.

Despite the described clinical associations are widely studied and corroborated, their underpinning mechanisms still remain unclear. There is evidence that PD and JHS individuals could share a common genetic aetiology characterised by a DUP25 mutation (Gratacos et al., 2001; Collier, 2002; Tabiner, et al., 2003; Weiland, Kraus & Speicher, 2003). However, further studies will help to disentangle and better understand this major genetic susceptibility factor for panic/phobic ADs and JHS.

1.3.3 Dysautonomia (Autonomic Dysfunctions)

A further factor may underlie the association of JHS and anxiety in the form of autonomic conditions. Autonomic Dysfunctions (or Dysautonomia)

are any disease or malfunctioning of the autonomic nervous system which cause a large variety of symptoms (e.g.: heart rate and blood pressure alterations) that can greatly alter a person's quality of life.

Autonomic dysfunctions symptoms have been reported to occur in JHS including fatigue, syncope, palpitations, heat intolerance, nausea, chest discomfort, etc. Still, JHS has not only been associated to these symptoms but also to the following Autonomic Conditions: Postural Orthostatic Tachycardia Syndrome (POTS), Orthostatic Hypotension and uncategorized orthostatic hypotension. According to a relatively recent study, Autonomic conditions' prevalence in JHS rates up to 78% whereas in non-JHS is of 10% (Gazit et al., 2003).

Furthermore, this increasingly recognized association between JHS and autonomic conditions has been supported by different recent studies conducted with different sample populations. Accordingly, in a recent study 50% of POTS patients express JHS (Mathias, et al, 2011). One account attributes the postural tachycardia to a deficit in normal vascular response (vasoconstriction) consequent upon abnormal vascular collagen, suggesting the link between JHS and anxiety may in part originate from constitutional differences in cardiovascular responsiveness.

1.4 EMOTIONAL BRAIN AND NEUROIMAGING

1.4.1 Emotion and anxiety: neuroimaging features

Numerous neuroimaging studies have been conducted so far to determine structural and functional neuronal pathways of emotion and pathological anxiety. To do so, studies have been focused in both non-clinical and clinical samples. Notably the study of individuals suffering from pathological anxiety has not only explored different brain representations as compared to non-clinical individuals but also specific characteristics that differ among the ADs. Still human models of anxiety in healthy individuals help to reveal neural pathways underlying the development of ADs. In its non-pathological form anxiety can be divided into two categories: state anxiety which is a measure of the immediate level of anxiety and trait anxiety which reflects the long-term tendency of an individual to show an increased anxiety response.

The study of human ADs has benefited greatly from these neuroimaging techniques that have promoted the identification of structural and functional characteristics underlying anxiety emotional response and widely contributed to the diagnosis and treatment of these mental disorders (Paulus, 2008). Thus neuroimaging data raises the question of the neurobiological cause of ADs, opening up new reflections not only on treatments but also on the nosology of these disorders.

Structural Magnetic Resonance Imaging (sMRI) studies

Structural magnetic resonance imaging (sMRI) has enabled mapping of the anxious human brain and has contributed substantially to the understanding of anxiety. The consulted structural neuroimaging studies conducted with healthy subjects in anxiety indicate that left amygdala volumes predict anxiety with decreased amygdala volumes associated with higher anxiety on both state and trait anxiety measures (Blackmon K, 2011). Furthermore, studies implicate reduced thickness in medial orbitofrontal cortex and volume increase in nucleus accumbens with trait anxiety (Kühn, Schubert & Gallinat, 2011; Spampinato, Wood, De Simone & Grafman, 2009) with also some rising results that correlated trait anxiety with volumetric differences in the temporal lobe (e.g.: hippocampus) (Montag, Reuter, Jurkiewicz, Markett & Panksepp, 2013). Thus, the volumetric variability on these regions may be associated with the development of trait anxiety and be implicated in the pathogenesis of ADs.

Likewise, the studies conducted with clinical samples are of utmost importance. The most consistent finding in quantitative neuroimaging studies of PD and Agoraphobia show a reduced volume of Amygdala, Hippocampus (particularly the left parahippocampal gyrus, which is a region that surrounds the hippocampus) and also a volume reduction in some cortical areas such as anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) as well as temporal and frontal cortices, as compared to control individuals. Furthermore, they can also show increased volume (grey matter increase) in the brainstem nuclei (involved in the autonomic arousal circuitry and specifically shown to be involved in the generation of panic

attacks), the insula, superior temporal gyrus (Uchida et al., 2008; Del Casale et al., 2013; Protopopescu, et al. 2006; Lai & Wu, 2012; Dresler, et al., 2013). Moreover, phobic avoidance may be related to abnormalities at the temporal lobe, prefrontal cortical areas, and brain stem and, anticipatory anxiety may be related to abnormalities of limbic structures (Freitas, Busatto, McGuire & Crippa, 2008). Few studies using sMRI in GAD have been conducted so far; however there is a possible association with this disorder and larger amygdala and dorsomedial prefrontal cortex (DMPFC) volumes (Schienle, Ebner & Schäfer, 2011; Ferrari, Busatto, McGuire & Crippa, 2008). Finally, brain structure studies on anatomical differences in SAD report mixed and partially contradictory findings. Although some studies report no differences in whole brain analyses and in ROIs analyses (amygdala, putamen, caudate and thalamus) when comparing SAD individuals to healthy controls (Argyropoulos, Bell & Nutt, 2001; Freitas et al., 2008), some recent studies bring up indicators of structural differences such as cortical thickness increased in left insula, right ACC and right temporal pole as well as in right dorsolateral prefrontal cortex (DLPFC) and right parietal cortex when comparing SAD individuals to control ones (Brühl et al., 2013).

In conclusion, studies of anatomical differences in anxiety suggest a structural anxiety network and raise the possibility that structural abnormalities may result in a greater vulnerability to anxiety or conversely that elevated anxiety symptoms may result in structural changes. However, the exact brain circuitry and the mechanisms underlying AD pathophysiology still remain in debate.

Functional Magnetic Resonance Imaging (fMRI) studies

Functional magnetic resonance imaging (fMRI) has enabled mapping the activity of the emotion related brain areas associated to anxiety and has contributed substantially to the understanding of pathological anxiety. Importantly, the majority of functional neuroimaging studies investigating emotional responses and pathological anxiety are designed according to symptom provocation paradigms (e.g.: face paradigm with angry faces presented to individuals with different ranges of social anxiety scores) in order to elicit specific emotions (Holzschneider & Mulert, 2011).

There is substantial evidence from neuroimaging studies that in humans, the extent to which a stimulus is identified as emotive and linked to the production of an affective state appears to depend upon the level of activity within two neural systems (Phillips, Drevets, Rauch & Lane, 2003):

(1) A ventral neural system including the amygdala, insula, ventral striatum, ventral ACC and PFC that supports the identification of the emotional significance of a stimulus, production of an affective state and the automatic regulation of emotional responses.

(2) A dorsal neural system including the hippocampus, dorsal ACC and PFC that supports the regulation of affective states and subsequent behaviour.

The interplay of these systems is indeed involved in the expression of fear and anxiety. Specifically hyperactivity of the Amygdala during symptom provocation is related to the experienced symptoms of anxiety (Damsa,

Kosel & Moussally, 2009) and hyperactivity of regions referred as “the fear network” are involved in both the development and maintenance of anxiety conditions (Phan, Wager, Taylor & Liberzon, 2002; Etkin, 2010; Nitschke, Sarinopoulos, Mackiewicz, Schaefer & Davidson, 2006).

“The fear network regions” entail ACC that is involved in approach, avoidance and fear learning (Onoda, et al. 2008; Milad et al., 2007), Insula that is a central structure for emotion processing, subjective feelings and interoceptive awareness (Critchley et al., 2004) and the Amygdala itself (Bremner, 2004; Kent & Rauch, 2003) that has a critical role in threat assessment. However there are more brain structures that presume to be involved in anxiety and are described to present enhanced activity during symptom provocation, such as the orbitofrontal cortex (OFC) that is relevant for computing and in the service of decision making, the ventromedial prefrontal cortex (VMPFC) and the hippocampus, both displaying a top-down governance over the amygdala and capable of inhibiting fear responding. Furthermore hippocampus is also involved in the mediation of contextual conditioning (Craske et al., 2009; Brooks et al., 2012; Bach et al., 2014). Finally, subcortical centres such as hypothalamus and periaqueductal grey matter also contribute to anxiety through more proximal coupling of bodily states to motivational feelings, including fear (Feinstein et al., 2013). Markedly, amygdala and insula connectivity during emotion processing has also been observed in anxiety-prone individuals (i.e.: individuals with increased trait anxiety rates) (Stein et al., 2007) and in negative affectivity (Vizueta, Patrick, Jiang, Thomas & He, 2012).

These data suggest that ADs may be usefully conceptualized as differentially affecting emotional reactivity and regulatory processes that involve different neurobiological mechanisms. A growing body of data have reported the implication of the already mentioned brain regions in all the ADs (including amygdala, insula and hippocampal areas) yet no differential and specifically sustainable data in fMRI is found for any of the ADs individually (Damsa et al, 2009; Holzschneider & Mulert, 2011). For example, according to face processing paradigms SAD is described as a dysfunction of five main cerebral areas: Amygdala, MPFC, Insula, Hippocampus and the DLPFC (Pietrini, 2010; Blair, 2011; Pejic, Hermann, Vaitl, & Stark, 2013) and PD with Agoraphobia revealed enhanced activations in areas associated with the fear circuit including amygdala, insula and hippocampal areas in response to emotional valence paradigms (Wittmann, et al., 2011).

In conclusion, ADs are associated with higher activity of the “fear network” during symptom provocation (hyperactivated amygdala, cingulate cortex and insula) and insufficient top-down regulation through frontal brain region (Holzschneider & Mulert, 2011). Notably amygdala, insula and ACC hyperactivity during symptom provocation is consistent with the role of these regions in the generation and representation of visceral arousal states linked to emotional feelings (Critchley & Harrison, 2013.

1.4.2 Interoception in emotion and anxiety: brain representation

Human neuroimaging studies identify a set of brain regions as neural substrates for emotional feelings, affective responses and the

representation of internal bodily signals. Insular cortex is particularly linked to the representation of internal bodily responses and it has therefore been proposed as interoceptive cortex (Critchley et al., 2004). However amygdala, hypothalamus, OFC and especially ACC also share the property of bidirectional functional connections to the autonomic nervous system and support behaviours and/or cognitions associated with mood and motivation (Craig, 2009).

During performance of heartbeat perception tasks (a paradigm designed for measuring interoceptive sensitivity) in healthy individuals, right and anterior insula and ACC activity predicts actual interoceptive sensitivity (task performance accuracy). Also enhanced activities in insula, somatomotor and cingulate cortices are associated to good performance in heart perception tasks (Pollatos, et al., 2007).

Brain activation during emotional and/or interoceptive judgements overlap in anterior insula and adjacent inferior frontal operculum (Zaki, Davis & Ochsner, 2012) and the engagement of anterior insular cortex is shown to mediate the relationship between interoceptive sensibility and social anxiety. Still, functional analyses link right insula to individual experiences of anxiety, consistent with this region as the substrate through which interoceptive representations emerge as consciously accessible emotional feeling states (Critchley et al, 2004). Furthermore right anterior insula and VMPFC are activated when evaluating self-referential statements relating to both bodily states and emotional feelings (Terasawa, Fukushima & Umeda, 2013). Importantly, these studies suggest that the role of these

brain regions in ADs and fear/anxiety circuitry may be grounded on interoceptive processing (Damsa et al., 2009; Nagai Kishi, & Kato, 2007).

1.4.3 Joint hypermobility: brain structural differentiation

Up until now no studies have been conducted with functional neuroimaging techniques in hypermobile samples and only one with structural neuroimaging techniques. In this relatively recent and small study, non-clinically anxious individuals with JH (cut-off point set at scoring more than one at the Beighton criteria) from a general population sample were shown to manifest structural differences in emotional-processing brain regions, notably having larger amygdala volume bilaterally compared to participants without hypermobility (Eccles et al., 2012).

Interestingly this same study (Eccles, et al., 2012) shows that JH participants score higher on tests of interoceptive sensibility (subjective awareness of internal body measured by means of "Porges body perception questionnaire" (PBPQ)) and present a trend towards higher anxiety (assessed with the beck anxiety inventory) than the ones with no JH.

1.5 AIMS AND HYPOTHESES

1.5.1 Experimental approach and global aim

For many years clinical researches have been studying anxiety and somatic symptoms in order to better understand their aetiology and thus, improve their treatments. In particular, some traits such as interoception or trait anxiety have been described to be high risk factors for developing ADs and somatic conditions.

Additionally, JHS is a multisystem collagen condition that has not only been strongly linked to anxiety and somatic symptoms but has also been described as a high risk factor for developing ADs (specially the panic/phobic ones). However, little is yet known about the biological and neurophysiological mechanisms underlying this clinical association.

The global aim of this research project is to explore biological traits and neurophysiological substrates of anxiety and somatic symptoms in general population with different ranges of hypermobility as well as different ranges of non-clinical anxiety.

We divided this project in two main aims that lead to two different studies. The main and specific aims of each of them, as well as the hypotheses are describe below.

1.5.2 Main aims

- To clarify the association among anxiety, body awareness and JH in a non-clinical sample.

- To identify neuronal correlates of JH and anxiety tendencies (i.e.: trait anxiety and social anxiety) in a non-clinical sample.

1.5.3 Specific aims

Study 1

- To study the association between state anxiety and body awareness.
- To elucidate whether state anxiety is associated with higher accuracy in interoceptive sensitivity.
- To establish whether Joint Hypermobility individuals present higher state anxiety as compared to non-Joint Hypermobility individuals.
- To study the association between Joint Hypermobility and body awareness.
- To explore the association between Joint Hypermobility and interoceptive sensitivity.
- To explore specific brain reactivity in structures associated with interoceptive sensitivity (i.e.: insular cortex and anterior cingulate cortex) during emotional processing in Joint Hypermobility.

Study 2

- To study the association among Joint Hypermobility, trait anxiety and social anxiety.
- To identify whether brain reactivity of emotional regions to the presentations of visual stimuli with emotional valence is associated with a non-clinical range of trait anxiety and social anxiety.

- To explore whether brain reactivity of emotional regions to the presentations of visual stimuli with emotional valence is associated with joint hypermobility.

1.5.4 Hypotheses

Study 1

1. State anxiety will be associated with body awareness measures: it will positively correlate with perception of body sensations but negatively correlate with adaptive cognitive tendencies towards body sensations (e.g.: tendency not to worry about body sensations).
2. State anxiety will be positively correlated with higher interoceptive sensitivity accuracy.
3. Joint Hypermobility individuals will be associated with higher state anxiety.
4. Joint Hypermobility individuals will present higher perception of body sensations and lower adaptive cognitive tendencies towards body sensations (e.g.: tendency not to worry about body sensations).
5. Joint Hypermobility individuals will present higher interoceptive sensitivity accuracy.
6. The underlying neural substrates of interoception (i.e.: insular cortex and anterior cingulate cortex) will be associated to hypermobility through an enhanced affective reactivity.

Study 2

7. Joint Hypermobility and anxiety scores (i.e.: trait and social anxiety) will all be positively correlated.
8. Trait and social anxiety will positively correlate with higher BOLD signal in anxiety described regions of interest (i.e.: amygdala, cingulate cortex, insula, hippocampus and orbitofrontal cortex) in response to stimuli with emotional contents.
9. Joint Hypermobility will positively correlate with higher BOLD signal in anxiety described regions of interest (i.e.: amygdala, cingulate cortex, insula, hippocampus and orbitofrontal cortex) in response to stimuli with emotional contents.

2. METHODS

2.1 STUDY 1: Joint hypermobility, body awareness and anxiety.

2.1.1 Participants

The final sample consisted of thirty-six healthy volunteers (16 male and 20 female) that participated in the study after informed consent and eligibility screening (were hypermobility was considered and neurological or psychiatric disorders were excluded). All participants underwent a structured clinical examination for hypermobility, undertook tests of interoceptive sensitivity and completed validated questionnaire measures of state anxiety and body awareness tendency. Twenty right handed participants randomly selected from this sample also performed an emotional processing task during fMRI, lasting approximately 20 minutes that were divided into two functional runs of 10 minutes each. One participant was removed from the fMRI analyses due to non-compliance with experimental procedures and excessive movement in the scanner, resulting in 19 (9 male and 10 female) right handed participants.

This study was approved by the local Research Ethics and Governance Committee. Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 16 subjects were necessary in each group to recognize as statistically significant a difference greater than or equal to 0.5 units in interoceptive sensitivity. The common standard deviation is assumed to be 0.5. For correlation analyses 37 participants were estimated necessary, with a correlation coefficient of 0.45.

2.1.2 Measures

Ratings of state anxiety were acquired using the State Trait Anxiety

Inventory (STAI), state sub-scale (Spielberger, Gorsuch & Lushene, 1970); it consists on 40 items in a Likert scale of 3 points that evaluate state anxiety. Hypermobility symptoms were quantified using the Beighton clinical exploration of JH (Beighton et al., 1989) which was conducted by a trained and validated clinician (according to the basis of the clinical rheumatologists' standards, kappa inter-examiners reliability ranged from 0, 8 to 1). The Beighton scoring system consists of five items that describe nine movements for exploring the mobility grade on some of the patient joints; the highest score is nine and an accepted cut-off point among the scientific and clinical community is ≥5 for women and ≥4 for men. Interoceptive sensitivity was measured by objective means using adaptations of two different heartbeat detection tasks (mental tracking and heartbeat perception; Schandry, 1981; Katkin, Wiens & Ohman, 2001). Participants' body sensibility (i.e. their subjectively assessed body sensations) and the mental processes and cognitive disposition towards body sensations (body and interoceptive awareness) (Garfinkel & Critchley, 2013) was also inferred from answers on the Multidimensional Assessment of Interoceptive Awareness (MAIA) questionnaire (Mehling et al., 2012) which is composed of the following sub-scales: Noticing (awareness of uncomfortable, comfortable, and neutral body sensations), Not-Distracting (tendency not to ignore or distract oneself from sensations of pain or discomfort), Not-Worrying (tendency not to worry or experience emotional distress with sensations of pain or discomfort), Attention Regulation (ability to sustain and control attention to body sensations), Emotional Awareness (awareness of the connection between body sensations and emotional states), Self-Regulation (ability to regulate distress by attention to body

sensations), Body Listening (active listening to the body for insight) and Trusting (experience of one's body as safe and trustworthy).

Heartbeat detection tasks

Interoceptive sensitivity was assessed using two tasks: these were modified versions of the heartbeat perception task (Katkin et al., 2001) and the mental tracking task (Schandry, 1981), run using in-house Matlab software (Mathworks Inc. Sherborn, M.A.). In the heartbeat perception task, each participant was asked to judge whether a tone was or was not synchronised with his/her heartbeat across 15 different blocks. Each block consisted of 10 tones presented at 440 Hz and having 100ms duration, triggered by the participant's heartbeat. Presentation of the tones was timed to coincide with systole (i.e. the cardiac ejection period, when heartbeats are typically felt) or to occur later (i.e. delayed relative to the heartbeat). Tasks were run using a pulse-oximetry signal from the left index finger. Assuming an average delay of 250 ms between the R-wave and the arrival of the pulse wave (e.g., Payne, Symeonides, Webb & Maxwell, 2006), this task setup delivered tones at around 250 ms or 550 ms after the ECG R-wave, corresponding respectively to the maximum and minimum synchronicity judgements reported in systematic studies of heartbeat detection (Wiens & Palmer, 2001). In the mental tracking task, the participant was asked to count silently each heartbeat felt (without manually checking) over cued intervals; i.e. from the time he/she heard "start" to when he/she heard "stop". This task procedure was repeated over six intervals, using time-windows of 25, 30, 35, 40, 45 and 50s, presented in randomised order. Following each block of both tasks

described, the participant responded with a judgment (synchronous/asynchronous or number of heartbeats).

2.1.3 Functional neuroimaging paradigm

During fMRI acquisition each participant was presented with images projected on a screen depicting neutral, angry or sad scenes in successive counterbalanced blocks. Images were derived from the International Affective Pictures System (IAPS; Lang, Greenwald, Bradley & Hamm, 1993) and supplemented with similar valence-matched images. Participants were trained on the paradigm before scanning. The functional imaging study was split into two different runs: each run started with a fixation cross that lasted 10 seconds. Images were blocked in fours by emotion-type and each image was displayed for 5 seconds, with 10 seconds separating each block. Each participant completed 40 randomised blocks, viewing 72 neutral pictures, 32 angry scenes, 32 anger eliciting and 32 sad pictures. During the task the participant performed an incidental task deciding whether pictures depicted animate or inanimate scenes.

2.1.4 Image acquisition parameters

Neuroimaging data were acquired using a Siemens Avanto 1.5 Tesla MRI scanner (Siemens, Erlangen, Germany) with 32 channel headcoil and upgraded gradients. Functional imaging involved the acquisition of echo-planar datasets sensitive to BOLD (Blood Oxygen Level Dependent) contrast from axial slices (anterioposterior phase encode direction) tilted 30 degrees from intercommissural plane to minimize T2* signal dropout from orbitofrontal and anterior temporal regions. Thirty-five 3 mm slices with a

0.75 mm interslice gap provided full brain coverage with an in-plane resolution of 3 x 3 mm (TE 42 ms, volume TR 2.620 ms). Following acquisition of the functional dataset, full brain T1-weighted structural scans were acquired from each participant (MPRAGE, 0.9 mm3 voxels, 192 slices, 1,160/4.24 ms TR/TE, 300 ms inversion time, 230 x 230 mm2 FOV).

2.1.5 Image processing

Images were pre-processed within SPM8 (http:// www.fil.ion.ucl.ac.uk/spm/) implemented in MATLAB 7.14 (Mathworks Inc. Sherborn, M.A.). The initial four functional volumes were discarded to allow for equilibration of net magnetization. Images were then spatially realigned and unwrapped, and spatially normalized to standard MNI space (Montreal Neurologic Institute) and movement regressors added. Normalized functional scans were smoothed with an 8 mm Gaussian smoothing kernel using SPM8.

2.1.6 Statistical neuroimaging analysis

At the first level of analysis, neural activity inferred from voxel-wise changes in BOLD response across conditions were assessed for each participant according to the general linear model. The regressors-of-interest were convolved with the canonical hemodynamic response function implemented in SPM and optimal parameter estimates were computed using a least squares function. The models at the first level were constructed coding for emotion type: angers scenes versus neutral scenes, sad versus neutral scenes (corresponding to an increased neural response for the sad or anger scenes, as compared to neutral scenes) was applied to

estimate the effect size for each participant and generate the associated statistical parametric map.

Second level analyses isolated brain activity pertaining to these emotional contrasts as a function of the variable of interest (hypermobility). Functional voxel-wise *t-test* group comparison analysis for hypermobile group (n=9; mean age ± SD: 23, 75 ± 3, 19 years) versus non hypermobile group (n=10; mean age ± SD: 25, 27 ± 6, 1 years) was conducted. The analysis was performed within preselected regions of interest (ROIs): insular cortex and anterior cingulate cortex. These were chosen on the basis of being described as important processing regions for interceptive sensitivity. Finally, to complement ROI analyses, whole brain analyses (*p*-value threshold set at 0.001, uncorrected) were also performed at the established contrasts for exploratory purposes.

2.1.7 Statistical analysis of clinical variables and questionnaire measures

SPSS 20.0 was implemented to analyse descriptive data, and measures of anxiety, JH and interoception task performance. A Pearson Chi-square analysis for testing sex and age sample homogeneity was performed. Correlation tests were executed among the studied variables (Pearson correlation index and Point-Biserial according to the test application criteria). Based on all previous studies and literature, when studying the positive association between JH and state anxiety as well as state anxiety and interoception sensitivity (i.e.: heart beat detection tasks) one-tailed analyses were performed in the required analyses. We also tested whether the associations found on the whole sample (n=36) were extensive to the sub-group of the sample that underwent the emotional paradigm through

fMRI techniques (n=19).

Additionally, we conducted mediation analysis to infer causality regarding the relationship between JH, interoceptive ability and state anxiety. Specifically, we tested pathways linking JH (as predictor) to interoceptive accuracy (as mediator) and state anxiety (as dependent variable). Mediation first requires that the predictor is significantly and independently related to all mediators and to the dependent variable (Baron & Kenny, 1986). JH was then entered in a multiple regression model along with interoceptive accuracy and state anxiety to test for diminished significance when entering the mediator.

2.2 STUDY 2: The neural signature of emotional processing in anxiety and Joint hypermobility

2.2.1 Participants

The final sample consisted of fifty-one participants free from any Axis I disorder, psychotropic medication or any other pathology that could interfere in the fMRI paradigm brain response and/or in the JH assessment. All participants were assessed by the Mini International Neuropsychiatric Interview (MINI) (Sheehan, 1998) and the Structured clinical interview for DSM-Axis I disorders (SCID-I) (First, Spitzer, Gibbon & Williams, 1999) as well as evaluated through a medical and psychiatric history. Participants were also assessed with a structured clinical examination for JH, completed validated questionnaire measures of anxiety and underwent a functional emotional paradigm using fMRI techniques. A given complete description of the fMRI and clinical examination before written informed consent was obtained, and all of them voluntarily agreed to take part in the study.

Parc de Salut Mar Barcelona Clinical Research Ethical Committee approval was obtained. Sample size was estimated at a minimum of 53 participants, accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test with a correlation coefficient of 0.45. It was anticipated a drop-out rate of 0.3.

2.2.2 Measures

All participants were assessed with the Beighton exploration for JH (Beighton, 1988) in order to quantify their grade of joint mobility. This exploration was conducted by a trained and validated clinician (according to

the basis of the clinical rheumatologists' standards, kappa inter-examiners reliability ranged from 0, 8 to 1). The Beighton scoring system consists of five items that describe nine movements for exploring the mobility grade on some of the patient joints; the highest score is nine. According to the aims of this study we explore the whole spectrum of hypermobility, however Beighton scoring system also have a scientific and clinically accepted cut-off point (≥5 for women and ≥4 for men).

Ratings of trait anxiety and social anxiety were acquired using the two following self-reported questionnaires: (1) State Trait Anxiety Inventory (STAI), trait subscale (Spielberger et al., 1982). It consists on 40 items in a Likert scale of 3 points that evaluate trait anxiety. Manual refers Cronbach's alpha coefficient of= 0.87. (2) Liebowitz Social Anxiety Scale (LSAS) (Liebowitz, 1987) in its Spanish version adapted by (Bobes et al., 1999). It consists of 24 items in a Likert scale form 0-3 points that evaluate social phobia and the role that it takes into the subject life.

2.2.3 Functional neuroimaging paradigm

During the acquisition subjects participated in an event-related paradigm of emotional facial stimuli in which two groups of images were presented: 14 images of crying faces and 14 images of neutral faces. Images were derived from the Gur et al., (2002) series and supplemented with similar valence-matched images. The stimuli were displayed for 1500 ms, followed by an interstimulus interval of between 750 to 1300 ms, with mean trial duration of 2500 ms. Participants were trained on the task before the beginning of the fMRI session. The order of picture presentation

was randomized for each participant and the presentation counterbalanced across subjects.

2.2.4 Image acquisition parameters

Images were acquired in a Philips Achieva 3T scanner. T1-weighted images were obtained using a FSPGR sequence (TR: 8.2 ms, TE: 3.7 ms, FA: 8º, matrix size: 256x256x180, voxel size: 0.94 mm x 0.94 mm x 1.00 mm, gap: 0 mm). An EPI-T2* sequence allowed obtaining the functional volumes, each comprising 30 three mm-thick slices (TR 3000 ms, TE: 35 ms, FA: 90º, in-plane voxel size 1.80 mm x 1.80 mm, Slice thickness 3.0 mm, gap = 1.0 mm, matrix size: 128x128, 30 slices).

2.2.5 Image processing

Images were pre-processed within SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) implemented in MATLAB 7.14 (Mathworks Inc. Sherborn, M.A.). The first three volumes of each session were discarded to remove non steady-state effects. Head motion correction was carried out by means of spatial interpolation, employing parameters derived from a six-parameter rigid body transformation with a least squares algorithm. Functional images were realigned to the first volume, normalized to the MNI EPI template and smoothed with a 12 mm FWHM kernel using SPM8. The linear contrasts cry > neutral was applied to estimate the effect sizes for each participant.

2.2.6 Statistical neuroimaging analysis

At the first level of analysis, voxel-wise changes in blood oxygen level dependence (BOLD) response across conditions were analysed according to the general linear model and assessed for each subject. The regressors of interest were convolved with the canonical hemodynamic response function implemented in SPM and optimal parameter estimations were computed using a least squares function. A linear contrast crying face > neutral face (corresponding to an increased BOLD response for the crying faces, as compared to neutral faces) was applied to estimate the effect for each participant and generate the associated statistical parametric map.

Second level analyses were performed with the main studied variables (i.e.: JH, trait anxiety and social anxiety scores) and the established crying face > neutral face contrast. Correlation analysis (p-value threshold was set at 0.05, FWE-corrected) was carried out to check for the dependence of the contrast values on the JH, trait anxiety and social anxiety scale values. The parameter estimated represented the magnitude of correlation between task-specific activations and the subjects-specific measure of JH or anxiety. We used JH as a continuous variable provided that we wanted to study the whole spectrum of joint mobility and not only the ones that accomplish the pathological threshold. We also used a continuous variable for the anxiety scores provided that we did not have clinical participants and wanted to study the whole spectrum of non-pathological punctuations. The analysis was performed within each of a set of preselected regions of interest (ROIs): amygdala, hippocampus, insular cortex, and anterior cingulate cortex. These were chosen on the basis of being described as important

affective processing regions (Brooks et al., 2012; Smith, Stephan, Rugg & Dolan, 2006; Stein et al., 2007). ROI masks were obtained from the WFU pickatlas (Maldjian, Laurienti, Burdette & Kraft, 2003; Maldjian, Laurienti & Burdette, 2004) brain maps and *p*-value threshold was set at 0.05, FEW-corrected. Significant activation regions were described and individual contrast *beta* values (without the studied variables as a covariate) were plotted against JH values and correlation analysis was performed to obtain scatterplot graphic. Clinimetric variable measurements (JH as well as trait and social anxiety) and correlations with ROI contrast values extracted from peak voxels in SPM were carried out using SPSS 20.0 and controlled for gender. Finally, to complement ROI analysis, whole brain analysis (*p*-value threshold set at 0.001, uncorrected) was also performed at the established crying face > neutral face contrast for exploratory purposes.

2.2.7 Statistical analysis of clinical variables and questionnaires

SPSS 20.0 was implemented to analyse descriptive data and measures of anxiety and hypermobility. A Pearson Chi-square analysis for testing sex and age sample homogeneity was performed. Pearson correlation analyses were executed with the studied variables (i.e.: social anxiety, trait anxiety and JH scores) in order to establish the associations among them.

3. RESULTS

3.1 STUDY 1: Joint Hypermobility, body awareness and anxiety.

3.1.1 Sociodemographic and clinical variables data.

Thirty-six non-clinical volunteers (16 male and 20 female) participated in the study (Table 1). There were no significant differences in anxiety or body awareness scores between male and female participants. According to the suggested cut-off point for JH (≥5 for women and ≥4 for men), fourteen (38.9%) of the sample participants had JH. Nineteen (9 male and 10 female) participants did also perform the emotional processing task during fMRI. According to the suggested cut-off point, nine (42.1%) of the sample participants met criteria for JH. Sample homogeneity was granted by no significant difference of age or sex.

Table 1. Descriptive data for anxiety, body awareness and Joint Hypermobility measures (*n*=36)

	Min	Max	Mean	SD
Age	20.00	42.00	24.83	5.04
Joint Hypermobility* (0-9)	0.00	9.00	3.40	2.69
STAI-State (20-80)	20.00	54.00	33.86	10.72
MAIA noticing (0-5)	1.00	4.25	3.03	0.85
MAIA not-distracting (0-5)	0.00	3.67	2.04	0.86
MAIA not-worrying (0-5)	1.33	4.66	3.06	0.83
MAIA attention regulation (0-5)	0.71	5.00	3.02	1.05
MAIA emotional awareness (0-5)	1.60	4.80	3.09	0.83
MAIA Self-regulation (0-5)	0.00	5.00	2.74	1.09
MAIA Body listening (0-5)	0.00	4.00	1.77	1.02
MAIA Trusting (0-5)	0.33	5.00	3.49	1.14

STAI-State, state trait anxiety inventory-state subscale; MAIA, Multidimensional Assessment of Interoceptive Awareness; *Beighton Joint Hypermobility assessment.

3.1.2 State anxiety

Body Awareness

Across participants, the MAIA attention regulation (i.e. ability to control the attention given to body sensations) and the trusting body sensations subscales, negatively correlated with state anxiety scores (r=-0.370, p<=0.031; r=-0.340, p=0.049, consecutively). No further associations were found between the body awareness subscales and state anxiety.

Interoceptive sensitivity

State anxiety positively correlated with a better performance in the mental tracking interoceptive sensitivity task (r=0.284; p=0.046). This association was also observed in the higher range of state anxiety individuals (Q3 of the sample; n=9), which did also present a higher accuracy in the mental tracking interoceptive task (r_{pb}=0.385; p=0.010) than the non-higher state anxiety individuals. No further associations were found between heartbeat detection tasks performance and state anxiety.

3.1.3 Joint Hypermobility

Body awareness

No differences were found on the MAIA body awareness subscales when comparing hypermobile individuals to non-hypermobile.

Interoceptive sensitivity

Hypermobile individuals presented a positive association with the accuracy in the mental tracking task as compared with non-hypermobile

individuals (r_{pb} =0.387; p=0.020). No further associations were found between the heart beat detection tasks and JH.

Anxiety

Hypermobile individuals presented significantly higher punctuations in state anxiety than non-hypermobile individuals *(*r_{pb} = 0.318; *p*= 0.029).

3.1.4 Mediation analysis of interoception in hypermobility and anxiety

When exploring the relationship between interoceptive sensitivity (by means of heartbeat mental tracking task) and the different grades of state anxiety and JH, interoception was observed to mediate the association between JH and state anxiety (Figure1).

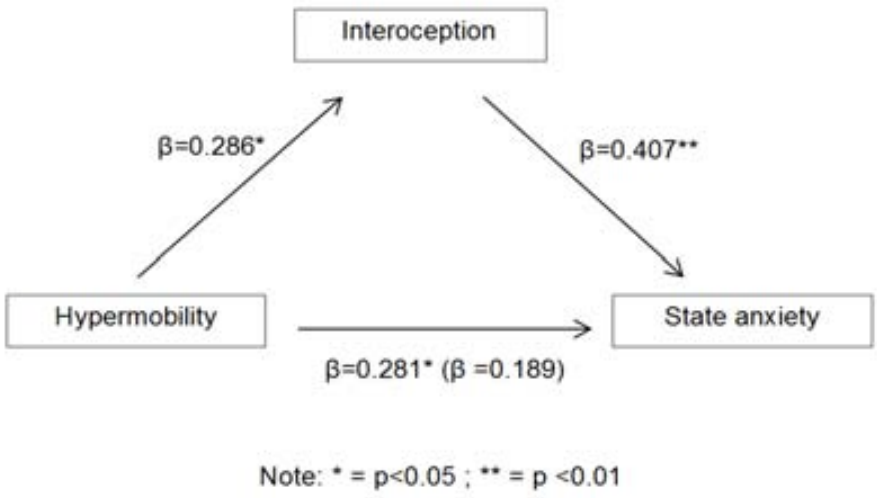


Figure 1. Graphic showing the regression coefficients, with the coefficients (*β*) for the effect of hypermobility on state anxiety with the latter (when entering interoception into the model) in parentheses.

3.1.5 Functional imaging data

Hypermobile participants of the sub-group that underwent the functional paradigm (n=19) did show significantly higher punctuations in state anxiety (r_{pb}=0.387; p=0.050) and higher accuracy in the mental tracking interoceptive task (r_{pb}=0.438; p=0.030) than non-hypermobile participants. Thus the association found in the whole sample is extensive to this sub-group of the sample.

ROI analysis

When comparing hypermobile as to non-hypermobile individuals, no significant associations were found for the studied interoception and emotion involved regions of interest (i.e.: insular cortex and anterior cingulated cortex).

Whole brain analysis

During the processing of sad versus neutral imagery a discrete set of brain regions associated with emotion and anxiety manifested greater responses within the hypermobile group. These included insular cortex, brainstem, parietal and sensorimotor cortices, inferolateral prefrontal cortex and temporal cortices (Table 2, Figure 2). During the processing of anger versus neutral imagery a discrete set of brain regions also demonstrated enhanced activity within the hypermobile group including cerebellum, temporal cortices and thalamus (Table 3).

Table 2. Activity seen in sad versus neutral images when comparing hypermobility participants (high Beighton score) to non-hypermobility participants (low Beighton score) (cluster size >10; p = 0.001, uncorrected).

Brain Area	**Side**	**Cluster Size**	**Coordinates**			***t*-Value**
Insula	R	63	42	2	4	5.76
Rolandic Operculum	R	118	64	8	14	5.34
Rolandic Operculum	R	118	46	4	14	4.37
Rolandic Operculum	R	63	46	-4	8	4.96
Frontal Inferior Operculum	R	118	52	10	14	3.95
Triangular part of Frontal Inferior Gyrus	R	41	58	30	2	5.92
Brainstem		15	-16	-20	-26	4.67
Cerebellum (Crus1)	L	84	-46	-68	-26	4.89
Cerebellum (Crus1)	L	12	-20	-74	-30	3.86
Cerebellum		59	-24	44	-4	4.17
Cerebellum (8)	R	27	30	-42	46	4.32
Supra Marginal Gyrus	R	45	64	-26	30	5.04
Postcentral Gyrus	R	19	60	-10	-30	3.91
Postcentral Gyrus	R	16	58	-10	32	4.41
Middle Temporal Gyrus	R	19	64	-8	-22	3.82
Inferior Temporal Gyrus	L	21	-46	-6	-34	4.61
Inferior Temporal Gyrus	L	21	-54	-4	-32	3.86

Table 3. Activity seen in anger versus neutral images when comparing hypermobility participants (high Beighton score) to non-hypermobility participants (low Beighton score) (cluster size >10; p = 0.001, uncorrected).

Brain Area	**Side**	**Cluster Size**	**Coordinates**			***t*-Value**
Cerebellum (crus1)	L	49	-46	-60	-34	4.89
Middle Temporal Gyrus	L	31	-66	-18	-20	4.69
Inferior Temporal Gyrus	L	31	-54	-18	-26	4.41
Middle Temporal Pole	L	14	36	2	-32	4.32
Thalamus	R	15	10	-8	6	4.22

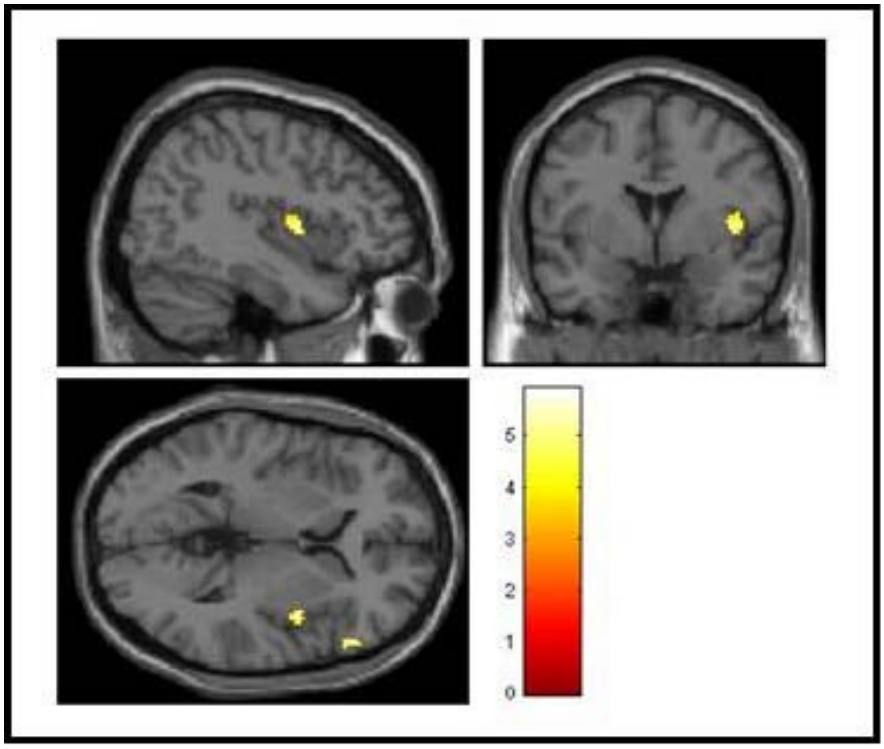


Figure 2. Enhanced right insular cortex activation in sad versus neutral contrast in hypermobility individuals compared to non-hypermobility individuals (cluster size >10; p = 0.001, uncorrected).

3.2 STUDY 2: The neural signature of emotional processing in anxiety and Joint Hypermobility

3.2.1 Sociodemographic and clinical variables data

Fifty one non-clinical volunteers (33 females and 18 males) participated in the study. The mean age of the sample was of 33.94 (SD=4.70). The self-reported measures of anxiety—trait anxiety and social anxiety—were significantly inter-correlated across the 51 participants. In addition JH did significantly correlate with trait anxiety scores (Table 4). Sample homogeneity was granted by no significant difference of age or sex.

Table 4. Self-reported anxiety measures and Joint Hypermobility scores (*n*=51)

	Min	**Max**	**Mean**	**SD**	***r*- Pearson correlation index (*p* value)**	
					Trait anxiety	Social Anxiety
Trait anxiety (20-80) *	22	33	16.41	7.03	---	0.417(<0.01)
Social Anxiety (0-144)**	0	68	28.98	17.33	0.417(<0.01)	---
Hypermobility (0-9) ***	0	9	2.94	2.64	0.303(0.031)	0.109(0.446)

*Measured by means of STAI-T, Spielberger State Trait Anxiety Inventory-trait subscale;
** Measured by means of LSAS, Liebowitz Social Anxiety Scale;
*** Beighton hypermobility assessment.

3.2.2 Functional imaging data

ROI analysis

When performing ROI analysis for the described emotional regions of interest, JH scores showed a positive correlation with the BOLD signal in the left hippocampus for the crying over neutral face contrast (t = 4.08; cluster p (FEW-cor) = 0.019; cluster size= 14; r=0.286) (Figure 3). No significant association on JH was found in any of the other studied regions of interest and no significant associations were found for the described emotional regions of interest on the non-clinical range of trait anxiety and social anxiety.

Whole brain analysis

During the processing of crying faces images, a discrete set of brain regions showed greater responses with increasing JH score. These included: right anterior and mid cingulum, right and left fusiform gyrus, left parahippocampus, and left hippocampus as well as right cerebellum (Table 5). All these results were uncorrected with a p-threshold of 0.001.

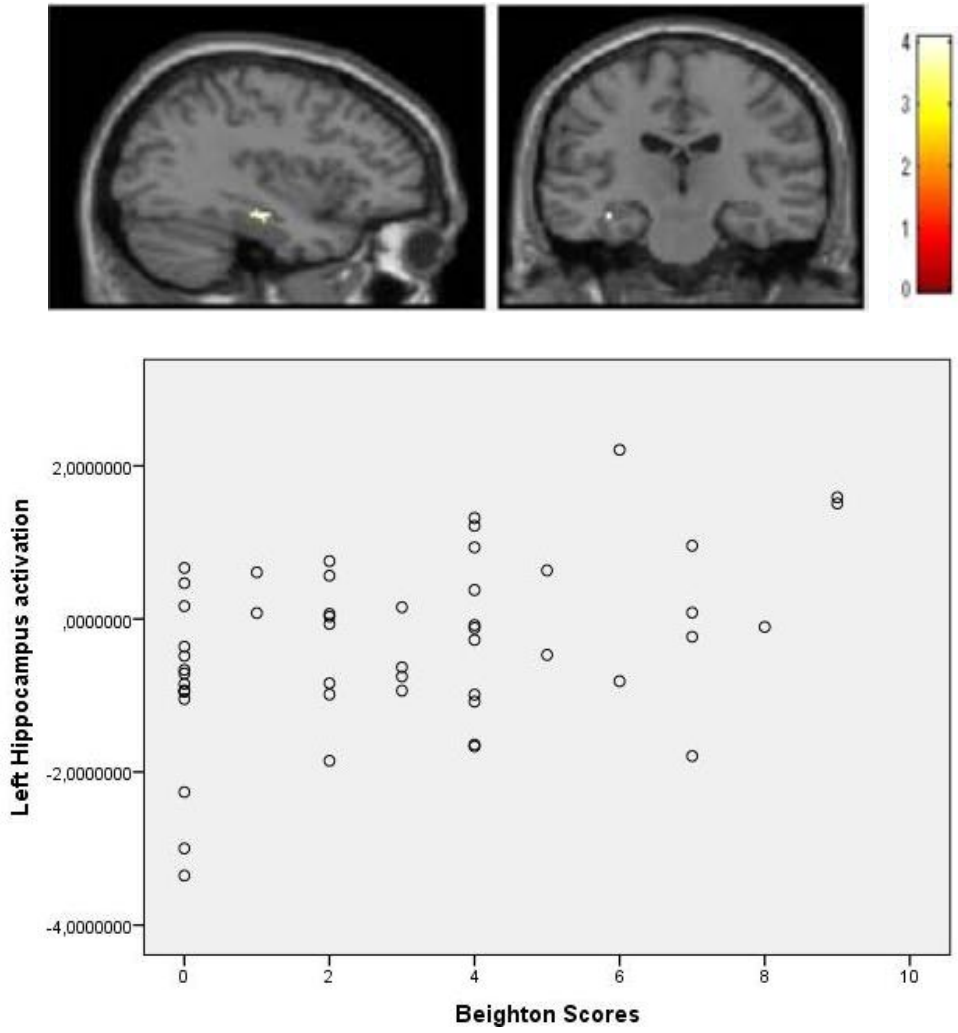


Figure 3. Activation map obtained for left Hippocampus ROI's analysis of a correlation test with Beighton hypermobility scores of cry face > neutral face contrast ($p<0.05$ FWE-Corrected). At the top of the figure, the brain mapping images show all the activated clusters. At the lower part of the figure, the activation response and individual Beighton Joint Hypermobility scores in the peak voxel [-38 -24 -14] of the cluster is displayed.

Table 5. Whole-brain analysis of the activity seen in crying face versus neutral face images with correlation analysis with hypermobility scores (cluster size>10; p = 0.001, uncorrected).

Brain Area	**Side**	**Cluster Size**	**Coordinates**			***t*-Value**
Middle Cingulate Cortex	L	240	-16	-30	46	4.90
Middle Cingulate Cortex	R	80	14	-18	48	3.47
Anterior Cingulate Cortex	R	117	16	36	24	3.77
Anterior Cingulate Cortex	R	14	14	50	18	3.48
Hippocampus	L	79	-40	-22	-18	4.19
Parahippocampal Gyrus	L	62	-20	-30	-16	3.98
Fusiform Gyrus	L	62	-26	-36	-16	3.75
Fusiform Gyrus	R	16	30	-4	-38	3.72
Fusiform Gyrus	R	16	36	-10	-38	3.39
Supplementary Motor Area	R	80	6	-16	50	3.41
Medial Orbital Frontal	R	57	14	62	-4	3.81
Sub Orbital Frontal	R	57	16	54	-8	3.56
Postcentral Gyrus	L	33	-40	-22	54	3.74
Cerebellum_4_5	R	26	28	-36	-32	3.62

4. DISCUSSION

4.1 GENERAL DISCUSSION

The present study explored biological traits and neurophysiological substrates of anxiety and somatic symptoms in a general population with different ranges of hypermobility as well as different ranges of non-clinical anxiety. We aimed at clarifying the underling association among anxiety, body awareness and JH as well as identifying neuronal correlates of anxiety tendencies (state, trait and social anxiety) and JH. For this purpose we first explored in a non-clinical sample the association among state anxiety, body awareness and sensibility and, JH through questionnaire measures, interoception accuracy controlled tasks and also through an emotional paradigm with fMRI techniques (focusing the analysis on critical affective processing regions involved in interoception). Secondly, in another non-clinical sample we examined the association between trait anxiety, social anxiety and JH. Furthermore we explored the neural signatures of emotion processing associated with every each of these three variables (focusing the analysis on critical affective processing regions associated with anxiety).

State anxiety negatively correlated with the attention regulation subscale (i.e. ability to control attention to body sensations) and the trusting body sensations subscale of the body awareness questionnaire. Furthermore state anxiety did also positively correlate with higher accuracy of interoceptive sensitivity (i.e.: mental tracking interoceptive task). Hence state anxiety is not only found to be associated with enhanced interoception but also with some considered less adaptive mental processes and cognition tendencies towards body sensations. These results were extended to the higher-range of state anxiety individuals of our

sample as compared to the rest of the individuals. These observations are noteworthy; interoceptive sensitivity as a trait is associated with increased risk of anxiety and developing ADs (Domschke et al., 2010). Our results support these previous studies and thus the primary hypothesis that increased interoceptive sensitivity is associated with increased anxiety also in a non-clinical population. However, this observation is further extended to support those cognitive models of anxiety (e.g.: Clark, 1986) that suggest the capacity to accurately predict, interpret and control bodily changes is compromised in people with anxiety. Thus the concurrence of increased perceptual sensitivity to physiological arousal signals (in this paradigm, heart-beat detection through mental tracking task) and diminished confidence in the interpretation and ability to control their attention to bodily sensations (in this study MAIA subscales measurements) is characteristic of high-state-anxiety individuals (Paulus & Stein, 2006). Importantly, there is growing interest and increasing empirical support for the therapeutic use of intervention approaches that include an enhanced awareness of bodily processes (Parkin et al., 2013). Future studies may clarify whether such individuals could benefit from a psychotherapy approach (e.g.: mindfulness, CBT) that would let them take advantage of their interoceptive accuracy trait in terms of having a more adaptive and quick response to bodily signals.

Hypermobile participants were found to experience significantly higher state anxiety than non-hypermobile participants. JH is widely acknowledged to be strongly linked to the clinical expression of anxiety (Bianchi et al., 2012; Garcia-Campayo et al., 2011; Bulbena et al., 2011). Additionally,

similarly to the results found with state anxiety, hypermobile individuals demonstrated higher interoceptive accuracy when performing the mental tracking task of the heart beat detection task. Still, our mediation analysis revealed that interoceptive sensitivity mediated the association found between JH and state anxiety. These findings validate an earlier observation of increased interoceptive sensibility in individuals with hypermobile features (Eccles et al., 2012). No more studies have been conducted so far with interoceptive sensitivity measures and hypermobility. However, an autonomic 'vascular' account provides one potential mechanism for this. Collagen is a connective tissue fibrous protein component of bone, cartilage, tendon, blood vessels and other body constituents; people with the previously described variant of collagen (i.e.: JHS), may show relative problems with orthostatic vasoconstriction, in extreme cases linked to a form of dysautonomia known as postural orthostatic tachycardia syndrome (POTS, commonly associated with JH), where increased heart rate compensates to maintain blood pressure (Freeman et al., 2011). This increased cardiac response may condition increased interoceptive sensitivity (Pollatos et al., 2007) with affective consequences. Nevertheless, this account emphasizes heart rate changes and it is known that the interoceptive sensitivity in hypermobile individuals (and even the dysautonomia associated with POTS) extends beyond the cardiovascular system (Mathias et al., 2011).

With regards to the functional neuroimaging paradigm, no significant activation response was found when conducting ROI analysis on critical affective processing regions involved in interoception (i.e.: insular cortex

and ACC) for hypermobile participants. However in the whole brain exploratory analysis, hypermobile individuals were found to present a discrete but stronger neural reactivity to affective stimulation in brain areas known to be involved in emotional processing, particularly in anxiety (i.e.: insula, brainstem, thalamus), when compared to non-hypermobile participants. Specifically, hypermobile participants presented higher activation to sad scenes in areas implicated in interoceptive representation, feeling states, self-representation and pain processing (i.e.: insular cortex, parietal and sensoriomotor cortices); in areas associated with the autonomic arousal circuitry and specifically shown to be involved in the generation of panic attacks (i.e.: brainstem); in areas implicated in executive control processes (i.e.: inferolateral prefrontal cortex); as well as in areas involved in encoding socially salient visual information (i.e.: temporal cortices) and executive control processes (i.e. inferolateral prefrontal cortex). Hypermobile participants also revealed enhanced activity to anger scenes within thalamus and inferotemproal cortex. In social interaction, insula is involved in emotional processing and empathy (Lamm & Singer, 2010) but also in homeostasis; it maps and controls autonomic functions regulation of the sympathetic and parasympathetic systems (Critchley & Harrison, 2013; Gianaros, Onyewuenyi, Sheu, Christie & Critchley, 2012). Furthermore, right anterior insula aids interoceptive awareness of body states, such as the ability to detect the timing of one's own heartbeat (Simmons et al., 2012). Thus, the enhanced activity in the insular cortex likely supports the association between hypermobility and interoceptive sensitivity (high accuracy in heartbeat detection) and, by extension, its association to anxiety. These findings show a tendency to

greater affective reactivity among the hypermobile participants within emotion-related brain areas. Thus hypermobile individuals do not only have greater interoceptive accuracy but also higher emotional reactivity to visual stimuli with affective salience. Similar higher affective reactivity has also been described behaviourally in anxiety disorders, particularly SAD, where patients also display hyper-reactivity within limbic brain regions (Goldin, Manber, Hakimi, Canli & Gross, 2009).

When examining the association among trait anxiety, social anxiety and JH in a non-clinical sample we explored both the association of the anxiety and JH measures, as well as brain responses to facial visual stimuli with emotional cues using fMRI techniques. We focused the analysis on critical affective processing regions (i.e.: amygdala, hippocampus, insular cortex, and anterior cingulated cortex). Our ROI analyses showed no associations with BOLD signals and the anxiety measures. Although the studied regions of interested are key areas of emotion processing and are involved in anxiety in both clinical (Damsa et al., 2009; Etkin, 2010; Onoda et al., 2008; Holzschneider & Mulert, 2011) and non-clinical samples where trait anxiety was studied (Murray, Stein, Simmons, Feinstein & Paulus, 2007), the non-clinical range of anxiety scores of this sample may have not got enough consistency to report individual brain reactivity differences. However, our results may also second the obtained in other studies, were the brain biases with SAD could not be unequivocally extended to subclinical social anxiety (Abraham et al., 2013).

However when studying hypermobility scores, the ROI analysis showed that there was a positive association between BOLD signals in the

hippocampus as a response to crying faces compared to neutral faces. This is noteworthy, previous studies have not only proven that hippocampus plays a key role in memory, learning and spatial coding (Smith & Mizumori, 2006) but also have recently suggested it is remarkably implicated in the emotional behaviour as well as in the pathophysiology of ADs (Shin & Liberzon, 2010). Moreover neuroimaging studies have also revealed that hippocampus is vulnerable to stress and also implicated in pain perception and Fibromyalgia (Aoki, Inokuchi & Suwa, 2013). Likewise, a recent functional magnetic resonance imaging study investigates how hippocampus individual reaction differences in pain modulation induced by anxiety relate to somatization. Results show that daily physical symptoms cause a chronic condition that weakens the ability of the hippocampus to distinguish anxiety states. The dysfunction of the anxiety network (including hippocampus) known to be associated with pain modulation, probably underpins the manifestation of somatizations (Gondo et al., 2012). Consequently, in our study, the higher activation response of the hippocampus associated with higher hypermobility scores give support towards a differential neural processing of emotional cues in hypermobility that can underlie the manifestation of anxiety and somatic symptoms through this collagen condition.

Additionally, the exploratory whole brain analysis showed that hypermobility scores were positively associated with an increased BOLD signal in some other key affective processing areas, such as the middle and anterior cingulate cortex, the fusiform gyrus, the parahipocampal region, the orbitofrontal cortex and the cerebellum. Despite being uncorrected

results, the fact that all of them are key emotional processing areas recaps interest. In this sense, neuroimaging studies have described the relevance of the middle and anterior cingulate cortex in avoidance and fear learning, emotion and apathy (Holzschneider & Mulert, 2011) and also the contribution of the orbitofrontal cortex in emotion regulation and reward in decision making (Golkar, 2012). Additionally, the fusiform gyrus has been found to be critical in face processing and the parahippocampal region in direct attention to emotional information (Ziaei, Peira & Persson, 2013; Pujol, et al., 2009). The cerebellum, in its part, has been involved in regulating fear and pleasure responses (Wolf, Rapoport & Schweizer, 2009).

Interestingly, the areas we have found to be associated with hypermobility scores are crucial in the emotional brain response of subjects suffering from clinical anxiety, especially the cingulate cortex and fusiform gyrus (Holzschneider & Mulert, 2011). We consider that this association might well be an expression of the overrepresentation of ADs in hypermobility. Accordingly, in our sample, trait anxiety was indeed strongly inter-correlated with social anxiety and JH. These results are consistent with reports from previous studies. High trait anxiety has repeatedly been found to be linked to increased responsiveness to stressors and leads to higher vulnerability to state anxiety and to ADs (Endler & Kocovski, 2001; Wilken, Smith, Tola & Mann, 2000). Likewise, a previous study conducted in a non-clinical population revealed the association between hypermobility and trait anxiety (Bulbena et al., 2004b). Our results replicate previous ones and highlight the value existence of “anxious vulnerability”, in the sense that

high trait anxious participants may develop anxiety particularly when they suffer from this heritable collagen anomaly.

To conclude, we present the first neurophysiological study that explores the association of non-clinical ranges of different anxiety tendencies (i.e.: state, trait and social anxiety) and hypermobility that also examines interoceptive sensitivity (heartbeat detection) in a non-clinical sample. The interactions observed among anxiety, hypermobility and interoception, as well as the enhanced activity found in specific emotional brain regions provide a starting point for future researches. Our findings have the potential to inform about innovation in therapeutic approaches and increase our understanding of the mechanisms through which vulnerability to anxiety disorders and somatic symptoms arises in people with specific anxiety tendencies, enhanced interoception and a heritable variant of collagen.

4.2 LIMITATIONS

There are different limitations of this research project. Firstly, when exploring the association of body awareness, anxiety and JH, a relatively constrained number of participants underwent these whole detailed assessments. However, the sample size was estimated to be sufficient. Secondly, when studying neuronal correlates of anxiety and hypermobility the sample consisted of a larger number of women participants compared to men participants. However, the sample was proven to be homogeneous and the analyses were controlled for the sex variable. Another possible limitation is the use of a general population sample free from psychological

or neurological disorders. This allowed us to study the emotional processing associated with anxiety, interoception and hypermobility in a non-clinical sample. However, due to the high association between the measured anxiety variables (i.e.: state anxiety, trait anxiety, social anxiety and JH) with ADs, a non-clinical sample of the general population may hinder the identification of their neural underpinnings. Future studies should nevertheless replicate our findings in clinical patient groups. Lastly, while we used an accepted cut-off point of hypermobility among the scientific and clinical community, this still remains a point of discussion.

4.3 IMPLICATIONS FOR RESEARCH AND FUTURE DIRECTIONS

This research project sheds light on the biological traits and neurophysiological substrates of anxiety and somatic symptoms that arise in general population with different ranges of non-clinical anxiety and/or JH. A special emphasis is given into the role that interoception as a trait and cognitive processes involved in body perception play in these anxiety and somatic symptoms.

Body awareness and interoception is a construct that has emerged as a subject of scientific and clinical interest across a wide range of health topics but especially among the anxiety related ones. Enhanced body awareness has shown to be a vulnerability factor for anxiety related disorders but some therapeutic approaches have demonstrated that it can also reduce anxiety symptoms. Our results display substantial data and replicate previous studies showing that interoception as a trait and difficulties controlling attention towards bodily sensations coupled with not trusting

bodily signals is linked to higher state anxiety in a non-clinical sample population.

Additionally, to date little is known about the precise mechanisms underpinning the association between anxiety and hypermobility. However, our findings provide insight to better understand their pathogenesis. The results link the presence of state anxiety in JH to enhanced interoceptive sensitivity and a discrete but heightened reactivity notably in insular cortex.

These findings highlight the dependence of emotional state on bodily context, and increase our understanding of the mechanisms through which vulnerability to ADs arises in people bearing specific anxiety tendencies as well as a heritable variant of collagen. Future studies will usefully replicate and extend these findings to clarify whether enhanced interoception patients, observed in either JH or other anxiety related sample populations, might benefit from a psychotherapeutic approach that enables a more adaptive attitude towards body signals.

When studying the neuronal correlates of trait, social anxiety and JH. No results were found for any of the studied anxiety measures, future studies should replicate these results to help clarifying whether this is a generalizable finding or it is constrained to the analysed sample. When studying JH neuronal correlates, results show an enhanced activation in hippocampus during processing of visual stimuli with affective valence. Thus results show there might be a specific neuronal signature of hypermobility. However, future studies should further study and replicate these findings in non-clinical samples as well as explore the neural underpinnings of JH and its links to the expression of ADs in clinical

samples. Clinical practice could probably benefit from these findings that can help to infer functional brain singularities associated to Anxiety in JHS patients. Finally, as for the revealed results regarding hippocampus, future studies should explore whether hypermobile individuals present to some extent, specific attention and memory processes.

5. CONCLUSIONS

This thesis aimed at studying biological traits and neurophysiological substrates of anxiety and somatic symptoms in general populations with different ranges of hypermobility as well as different ranges of non-clinical anxiety. The main conclusions of the thesis derived from the objectives and the significance of the results are:

1. State anxiety is associated with body awareness measures. Specifically it negatively correlates with two of the body awareness sub-scales (i.e.: trusting body sensations and attention regulation to body sensations). Thus the higher the state anxiety, the lower the ability to control the attention given to body sensations and the trust on these sensations.
2. State anxiety is positively correlated with interoceptive sensitivity accuracy. Hence the higher the state anxiety, the higher the accuracy in interoceptive sensitivity.
3. Joint Hypermobile individuals present higher state anxiety than non-hypermobile individuals
4. Joint Hypermobile individuals do not present differences on body awareness measures when compared to non-hypermobile individuals.
5. Joint Hypermobile individuals present higher interoceptive sensitivity accuracy than non-hypermobile individuals.
6. The underlying neural substrates of interoception (particularly enhanced insular cortex activity in response to stimuli with emotional valences) are discretely associated to hypermobility as compared to non-hypermobility.

7. Trait anxiety is positively correlated with social anxiety and with the grade of hypermobility. Thus the higher the trait anxiety, the higher the social anxiety and the greater the grade of Joint Hypermobility.

8. No patterns of neural activation in response to stimuli with emotional contents are associated to trait and social anxiety.

9. The grade of Joint Hypermobility is associated with enhanced hippocampus activation and a discrete set of emotion described regions (particularly cingulate cortex and orbitofrontal cortex) in response to stimuli with emotional contents.

6. REFERENCES

Abraham, A., Kaufmann, C., Redlich, R., Hermann, A., Stark, R., Stevens, S., Hermann, C. (2013). Self-referential and anxiety-relevant information processing in subclinical social anxiety: an fMRI study. B*rainImagingBehav*, 7, (1), 35-48. doi 10.1007/s11682-012-9188-x.

Abramowitz, J.S., Schwartz, S.A & Whiteside, S.P. (2002). A contemporary conceptual model of hypochondriasis. *Mayo Clin Proc,* 77, 1323–1330. doi:10.1016/S0025-6196(11)62432-4.

Anxiety (n.d.). Retrieved from Marc 18th, 2014, from American Pyschiological Association website, http://www.apa.org/topics/anxiety.

American Psychiatry Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders, fifth Edition (DSM-5)*. Washington DC: American Psychiatric Association.

Aoki, Y., Inokuchi, R. & Suwa, H. (2013). Reduced N-acetylaspartate in the hippocampus in patients with fibromyalgia: a meta-analysis. *Psychiatry Res*, 30, 213(3), 242-8. doi: 10.1016/j.pscychresns.2013.03.008.

Argyropoulos, S.V., Bell, C.J. & Nutt, D.J. (2001). Brain function in social anxiety disorder. *Psychiatr Clin North Am,* 24(4), 707-22.

Bach, D.R., Guitart-Masip, M., Packard, P.A., Miró, J., Falip, M., Fuentemilla, L. & Dolan, R.J. (2014). Human hippocampus arbitrates approach-avoidance conflict. *Curr Biol,* 3, 24(5), 541-7. doi: 10.1016/j.cub.2014.01.046.

Barlow, D. H. (2002). *Anxiety and its Disorders: The Nature and Treatment of Anxiety and Panic* (2nd ed.). New York: Guilford Press.

Baron, R. M. & Kenny, D. A. (1986) "The Moderator-Mediator Variable Distinction in Social Psychological Research – Conceptual, Strategic, and Statistical Considerations". *Journal of Personality and Social Psychology*, 51(6), 1173– 1182.doi:10.1037/0022-3514.51.6.1173

Beighton, P.H. (1988). Hypermobility scoring. *Br J Rheumatol*, 27, 163.

Beighton, P.H., Grahame, R. & Bird, H.A. (2012). *Hypermobility of joints* (4th ed). Berlin: Springer.

Beer, N., & Moneta, G. B. (2012). Coping and perceived stress as a function of positive metacognitions and positive meta-emotions. *Individual Differences Research*, 10(2), 105–116.

Bekker, M.H., Croon, M.A., Van Balkom, E.G. & Vermee, J.B. (2008). Predicting individual differences in autonomy-connectedness: the role of body awareness, alexithymia, and assertiveness. *J ClinPsychol*, 64: 747–765. doi: 10.1002/jclp.20486.

Bianchi Sanches, S., De Lima Osório, F., Udina, M., Martin-Santos, R. & Crippa, J.A. (2012). Anxiety and joint hypermobility association: a systematic review. *RBP*, 34(Suppl), 53-68. doi: 10.1590/S1516-44462012000500005.

Blackmon, K., Barr, W.B., Carlson, C., Devinsky, O., Dubois, J., Pogash, D., ...Thesen, T. (2011). Structural evidence for involvement of a left amygdala-orbitofrontal network in subclinical anxiety. *Psychiatry Res,* 194(3), 296-303. doi: 10.1016/j.pscychresns.2011.05.007.

Blair, K.S., Geraci, M., Korelitz, K., Otero, M., Towbin, K., Ernst, M., ...Pine, D.S. (2011). The pathology of social phobia is independent of developmental changes in face processing. *Am J Psychiatry*, 168(11), 1202-9. doi: 10.1176/appi.ajp.2011.10121740.

Bobes, J., Badía, X., Luque, A., García, M., González, M.P. & Dal-Ré, R. (1999). Validación de las versiones en español de los cuestionarios Liebowitz Social Anxiety Scale, Social Anxiety and Distres Scale y Sheehan Disability Inventory para la evaluación de la fobia social. *Med Clin (Barc)*, 112(14), 530-8.

Bravo, J.F. & Wolff, C. (2006). Clinical study of hereditary disorders of connective tissues in a Chilean population: joint hypermobility syndrome and vascular Ehlers-Danlos syndrome. *Arthritis Rheum*, 54(2), 515-23. doi: 10.1002/art.21557

Bremner, J.D. (2004). Brain imaging in anxiety disorders. *Exp Rev Neurother*, 4, 275–284.

Brooks, S.J., Savov, V., Allzén, E., Benedict, C., Fredriksson, R. & Schiöth, H.B. (2012). Exposure to subliminal arousing stimuli induces robust activation in the amygdala, hippocampus, anterior cingulate, insular còrtex and primary visual cortex: A systematic meta-analysis of fMRIstudies. *Neuro Image,* 59(3), 2962–2973. doi:10.1016/j.neuroimage.2011.09.077.

Brühl, A.B., Hänggi, J., Baur, V., Rufer, M., Delsignore, A., Weidt, S., ...Herwig, U. (2013). Increased cortical thickness in a frontoparietal network in social anxiety disorder. *Hum. Brain Mapp*. Advance online publication. doi: 10.1002/hbm.22378

Bulbena, A., Duró, J.C., Porta, M., Faus, S., Vallescar, R. & Martín-Santos, R. (1992). Clinical assessment of Hypermobility of joints: Assembling criteria. *J Rheumatol*, 19, 115-22.

Bulbena, A., Duró, JC., Porta, M., Martín-Santos, R., Mateo, A., Molina, L., ...Vallejo, J. (1993). Anxiety disorder in the joint hypermobility syndrome. *Psychiatric Res*, 43, 59-68.

Bulbena, A., Duró, JC., Mateo, A., Porta, M., & Vallejo, J. (1988). Joint hypermobility síndrome and anxiety disorders. *Lancet*, 2, 694-670.

Bulbena, A., Gago, J., Martín-Santos, R., Porta, M., Dasquens, J., & Berrios, GE. (2004a). Anxiety disorder & joint laxity a definitive link. *Neurology, Psychiatry and Brain Research,* 11, 137-40.

Bulbena, A., Agullo, A., Pailhez, G., Martin-Santos, R., Porta, M., Guitart, J., & Gago, J. (2004b). Is joint hypermobility related to anxiety in a nonclinical population also? *Psychosomatics*, 45, 432-7. doi: 10.1176/appi.psy.45.5.432.

Bulbena, A., Gago, J., Pailhez, G., Sperry, L., Fullana, M. & Vilarroya, O. (2011). Joint hypermobility syndrome is a risk factor trait for anxiety disorders: a 15-year follow-up cohort study. *Gen Hosp Psychiatry*, 33(4), 363-70. doi: 10.1016/j.genhosppsych.2011.03.004.

Cioffi, D. (1991). Beyond attentional strategies: cognitive-perceptual model of somatic interpretation. *Psychol Bull*, 109, 25–41. doi: 10.1037/0033-2909.109.1.25

Clark, D.M. & Wells, A. (1995). *A cognitive model of social phobia*. New York: Guildford Press.

Clark, D.M. (1986). A cognitive approach to panic. *Behaviour Research and Therapy*, 24, 461-470.

Clark, D.M., Salkovskis, P.M., Ost, L.G., Breitholtz, E., Koehler, K.A., Westiling, B.E., ...Gelder, M. (1997). Misinterpretations of body sensations in panic disorder. *Journal of Consulting and Clinical Psychology*, 65, 203-213. doi: 10.1037/0022-006X.65.2.203

Clark, D.A. & Beck, A. T. (2010). *Cognitive therapy of anxiety disorders: Science and practice*. New York, US: Guilford Press.

Collier, D.A. (2002). FISH, flexible joints and panic: are anxiety disorders really expressions of instability in the human genome? *Br J Psychiatry*, 181, 457-9. doi: 10.1192/bjp.181.6.457

Craig, A.D. (2003). Interoception: the sense of the physiological condition of the body. *Curr Opin Neurobiol*, 13(4), 500-5. doi: 10.1016/S0959-4388(03)00090-4

Craig, A.D. (2004). Human feelings: why are some more aware than others?. *Trends Cogn Sci*, 8(6), 239-41. doi:10.1016/j.tics.2004.04.004Craig, A.D. (2009). How do you feel-now? The anterior insula and human awareness. *Nature Reviews Neuroscience,* 10, 59-70. doi:10.1038/nrn2555

Craske, M.G., Rauch, S.L., Ursano, R., Prenoveau, J., Pine, D.S. & Zinbarg, R.E. (2009). What is an anxiety disorder? *Depress Anxiety*, 26(12), 1066-85. doi: 10.1002/da.20633.

Critchley, H.D., Wiens, S., Rotshtein, P., Ohman, A. & Dolan, R.J. (2004). Neural systems supporting interoceptive awareness. *Nat Neurosci,* 7:189–195. doi:10.1038/nn1176

Critchley, H., & Harrison, N. (2013). Visceral influences on brain and behavior. *Neuron*, 77(4), 624-38. doi: 10.1016/j.neuron.2013.02.008

Damasio, A.R. (1996). The somatic marker hypothesis and the possible functions of prefrontal cortex. Philosophical Transactions of the Royal Society of London – Series B: Biological Sciences, 351, 1413–1420. doi: 10.1098/rstb.1996.0125.

Damsa, C., Kosel, M. & Moussally, J. (2009). Current status of brain imaging in anxiety disorders. *Curr Opin Psychiatry*, 22, 96–110. doi: 10.1097/YCO.0b013e328319bd10.

Del Casale, A., Serata, D., Rapinesi, C., Kotzalidis, G.D., Angeletti, G., Tatarelli, R., ...Girardi, P. (2013). Structural neuroimaging in patients with panic disorder: findings and limitations of recent studies. *PsychiatrDanub*, 25(2), 108- 14.

Domschke, K., Stevens, S., Pfleiderer, B. & Geralch, A.L. (2010). Interoceptive sensitivity in anxiety and anxiety disorders: an overview and integration f neurobiological findings. *Clin Psychol Rev*, 30(1), 1-11. doi: 10.1016/j.cpr.2009.08.008

Dresler, T., Guhn, A., Tupak, S.V., Ehlis, A.C., Herrmann, M.J., Fallgatter, A.J., ...Domschke, K. (2013). Revise the revised? New dimensions of the neuroanatomical hypothesis of panic disorder. *J Neural Transm,* 120(1), 3-29. doi: 10.1007/s00702-012-0811-1.

Eccles, J.A., Beacher, F.D., Gray, M.A., Jones, C.L., Minati, L., Harrison, N.A. & Critchley, H.D. (2012). Brain structure and joint hypermobility: relevance to the expression of psychiatric symptoms. *Br J Psychiatry*, 200(6), 508-9. doi: 10.1192/bjp.bp.111.092460.

Ehlers, A., Mayou, R.A., Sprigings, D.C. & Birkhead, J.. (2000). Psychological and perceptual factors associated with arrhythmias and benign palpitations. *Psychosom Med*, 62(5), 693-702.

Endler, N.S. & Kocovski, N.L. (2001). State and trait anxiety revisited. *J Anxiety Disord*, 15(3), 231-45. doi: 10.1016/S0887-6185(01)00060-3

Etkin, A. (2010). Functional neuroanatomy of anxiety: a neural circuit perspective. *Curr Top Behav Neurosci*, 2, 251-77.

Feinstein, J.S., Buzza, C., Hurlemann, R., Follmer, R.L., Dahdaleh, N.S., Coryell, W.H., ...Wemmie, J.A. (2013). Fear and panic in humans with bilateral amígdala damage. *Nat Neurosci,* 16(3), 270-2. doi:10.1038/nn.3323.

Ferrari, M.C., Busatto, G.F., McGuire, P.K. & Crippa, J.A. (2008). Structural magnetic resonance imaging in anxiety disorders: an update of research findings. *Rev Bras Psiquiatr*, 30(3), 251-64. doi: 10.1590/S1516-44462008000300013.

Fikree, A., Grahame, R., Aktar, R., Farmer, A.D., Hakim, A.J., Morris, J.K., ...Aziz, Q. (2014). A Prospective Evaluation of Undiagnosed Joint Hypermobility Syndrome in Patients With Gastrointestinal Symptoms. *Clinical Gastroenterology and Hepatology*.Advance online publication. doi:10.1016/j.cgh.2014.01.014

First, M.B., Spitzer, R.L., Gibbon, M. & Williams, J.B.W. (1999). *Entrevista Clínica Estructurada para los Trastornos del Eje I del DSM-IV. Versión Clínica (SCID- I)*. Barcelona: Masson, S.A.

Flink, I.K., Nicholas, M.K., Boersma, K. & Linton, S.J. (2009). Reducing the threat value of chronic pain: A preliminary replicated single-case study of interoceptive exposure versus distraction in six individuals with chronic back pain. *Behav Res Ther* 47, 721–728. doi: 10.1016/j.brat.2009.05.003.

Freeman, R., Wieling, W., Axelrod, F.B., Benditt, D.G., Benarroch, E., Biaggioni, I., ...van Dijk, J.G. (2011). Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Clin Auton Res*, 21(2), 69-72. doi: 10.1016/j.autneu.2011.02.004.

Freitas, M.C., Busatto, G.F., McGuire, F.K. & Crippa J.A. (2008). Structural magnetic ressonance imaging in anxiety disorders: an update of research findings. *Rev. Bras. Psiquiatr*, 30 (3), 251-264. doi: 10.1590/S1516-44462008000300013.

Garcia-Campayo, J., Asso, E. & Alda, M. (2011). Joint hypermobility and anxiety: the state of the art. *Curr Psychiatry Rep*, 13(1), 18-25. doi: 10.1007/s11920-010-0164-0.

Garcìa-Campavo J., Asso, E., Alda, M., Andres, E.M. & Sobradiel, N. (2010). Association between joint hypermobility syndrome and panic disorder: a case–control study. *Psychosomatics*, 51 (1), 55–61. doi: 10.1016/S0033-3182(10)70659-9.

Garfinkel, S.N. & Critchley, H.D. (2013). Interoception, emotion and brain: new insights link internal physiology to social behaviour. Commentary on: "Anterior insular cortex mediates bodily sensibility and social anxiety" by Terasawa et al. (2012). *Soc Cogn Affect Neurosci*, 8(3), 231-4. doi: 10.1093/scan/nss140.

Gazit, Y., Nahir, A.M., Grahame, R. & Jacob, G. (2003). Dysautonomia in the joint hypermobility syndrome. *Am J Med*, 115(1), 33-40. doi:10.1016/S0002-9343(03)00235-3

Gianaros, P.J., Onyewuenyi, I.C., Sheu, L.K., Christie, I. & Critchley, H.D. (2012). Brain systems for baroreflex suppression during stress in humans. *Human Brain Mapping*, 33(7), 1700-16. doi: 10.1002/hbm.21315.

Goldin, P.R., Manber, T., Hakimi, S., Canli, T. & Gross, J.J. (2009). Neural bases of social anxiety disorder: emotional reactivity and cognitive regulation during social and physical threat. *Arch Gen Psychiatry*, 66(2), 170-80. doi: 10.1001/archgenpsychiatry.2008.525.

Golkar, A., Lonsdorf, T.B., Olsson, A., Lindstrom, K.M., Berrebi, J., Fransson, P.,...Öhman, A. (2012). Distinct contributions of the dorsolateral prefrontal and orbitofrontal cortex during emotion regulation. *PLoS One*, 7(11), e48107. doi: 10.1371/journal.pone.0048107.

Gondo, M., Moriguchi, Y., Kodama, N., Sato, N., Sudo, N., Kubo, C. & Komaki, G. (2012). Daily physical complaints and hippocampal function: an fMRI study of pain modulation by anxiety. *Neuroimage*, 15, 63(3), 1011-9. doi: 10.1016/j.neuroimage..07.025.

Grahame, R., Edwards, J.C., Pitcher, D., Gabell, A. & Harvey, W. (1981). A clinical and echocardiographic study of patients with the hypermobility syndrome. *Ann Rheum Dis*, 40, 541-6.

Grahame, R. & Hakim, A.J. (2008). Hypermobility. *Curr Opin Rheumatol*, 20(1), 106-10. doi: 10.1097/BOR.0b013e3282f31790.

Grahame, R., Bird, H.A, Child, A. (2000). The revised (Brighton 1998) criteria for the diagnosis of benign joint hypermobility syndrome. *J Rheumatol*, 27(7), 1777-79.

Gratacos, M., Nadal, M., Martín-Santos, R., Pujana, M., Gago, J., Peral, B., ...Estivill, X. (2001). A polymorphic genomic duplication on human chromosome 15 is a susceptibility factor for panic and phobic disorders. *Cell*, 106(3), 367-79. doi: 10.1016/S0092-8674(01)00447-0

Gur, R.C., Sara, R., Hagendoorn, M., Marom, O., Hughett, P., Macy, L., ...Gur, R.E. (2002). A method for obtaining 3-dimensional facial expressions and its standardization for use in neurocognitive studies.*J.Neurosci. Methods*, 115(2), 137-43. doi: 10.1016/S0165-0270(02)00006-7

Hakim, A.J, Cherkas, L.F., Grahame, R., Spector, T.D., MacGregor, A.J. (2004). The genetic epidemiology of joint hypermobility: a population study of female twins. *Arthritis Rheum,* 50(8), 2640–2644. doi: 10.1002/art.20376

Herbert, B.M., Muth, E.R., Pollatos, O. & Herbert, C. (2012). Interoception across modalities: on the relationship between cardiac awareness and the sensitivity for gastric functions. *PLoS One,* 7(5), e36646. doi: 10.1371/journal.pone.0036646

Hilchey, C.A. & Clark, D.A. (2014). Context in Anxiety Sensitivity: The Role of Expectancy, Catastrophic Misinterpretations and Diminished Reappraisal in Response to Hypothetical Physical Arousal Scenarios. *Cognitive Therapy and Research*. Advance online publication. doi 10.1007/s10608-013-9594-4.

Holzschneider, K. & Mulert, C. (2011). Neuroimaging in anxiety disorders. *Dialogues Clin Neurosci*, 13(4), 453-461.

Kabat-Zinn, J. (1991). *Full catastrophe living: using the wisdom of your body and mind to face stress, pain, and illness, DC*: Delta Trade Paperbacks.

Kabat-Zinn, J. (1996). *Full catastrophe living: how to cope with stress, pain and illness using mindfulness meditation*. DC: Piatkus.

Katkin, E., Wiens, S. & Ohman, A. (2001). Nonconsious fear condition, visceral perception, and the development of gut feelings. *Psychol Sci*, 12(5): 366-370. doi: 10.1111/1467-9280.00368

Keer, R. & Grahame, R. (2003). *Hypermobility syndrome*. New York: Butterworth Heinemann.

Kent, J.M. & Rauch, S.L. (2003). Neurocircuitry of anxiety disorders. *Curr Psychiatry Rep*, 5(4), 266–273. doi: 10.1007/s11920-003-0055-8

Kessler, R.C., Ruscio, A.M., Shear, K. & Wittchen, H.U. (2010). Epidemiology of anxiety disorders. *Curr Top Behav Neurosci,* 2, 21-35.

Kirmayer, L.J. & Looper, K.J. (2006). Abnormal illness behaviour: physiological, psychological and social dimensions of coping with distress. *Curr Opin Psychiatry.* 19(1), 54–60.

Kühn, S., Schubert, F. & Gallinat, J. (2011). Structural correlates of trait anxiety: reduced thickness in medial orbitofrontal cortex accompanied by volume increase in nucleus accumbens*. J Affect Disord,* 134, 315-9. doi: 10.1016/j.jad.2011.06.003.

Lai, C.H. & Wu, Y.T. (2012). Fronto-temporo-insula gray matter alterations of first-episode, drug-naïve and very late-onset panic disorder patients. *J Affect Disord*, 140(3), 285-91. doi: 10.1016/j.jad.2012.01.049.

Lamm, C. & Singer, T. (2010). The role of anterior insular cortex in social emotions. *Brain Struct Funct*, 214 (5-6), 579-91. doi: 10.1007/s00429-010-0251-3.

Lang, P.J., Greenwald, M.K., Bradley, M.M. & Hamm, A.O. (1993). Looking at pictures: Affctive, facial, visceral, and behavioral reactions. *Psychophysiology*, 30, 261–273.

Laskowski, E.R. (2000). Proprioception. *Scient Princ Sports Rehab* 11, 323–340.

Liebowitz, M.R. (1987). Social Phobia. *Mod Probl Pharmacopsychiatry*, 22, 141-173.

Maldjian, J.A., Laurienti, P.J., Burdette, J.B. & Kraft, R.A. (2003). An Automated Method for Neuroanatomic and Cytoarchitectonic Atlas-based Interrogation of fMRI Data Sets. *NeuroImage*, 19 (3), 1233-1239. doi: 10.1016/S1053-8119(03)00169-1

Maldjian, J.A., Laurienti, P.J. & Burdette, J.H. (2004). Precentral Gyrus Discrepancy in Electronic Versions of the Talairach Atlas. *Neuroimage*,21(1),450-455.doi:10.1016/j.neuroimage.2003.09.032.

Martin-Santos, R., Bulbena, A., Porta, M., Gago, J., Molina, L. & Duro, J.C. (1998). Association between the joint hypermobility syndrome and panic disorder. *Am J Psychiatry*, 155(11), 1578–83.

Mathias, C.J., Low, D.A., Iodice, V., Owens, A.P., Kirbis, M. & Grahame, R. (2011). Postural tachycardia syndrome--current experience and concepts. *Nat Rev Neurol*, 8(1), 22-34. doi: 10.1038/nrneurol.2011.187

Maussa, I., Wilhelma, F. & Grossa, J. (2004). Is there less to social anxiety than meets the eye? Emotion experience, expression, and bodily responding. Cognition & Emotion, Volume 18, 5, 631-642. doi:10.1080/02699930341000112

Mehling, W.E., Price, C., Daubenmier, J.J., Acree, M., Bartmess, E., Stewart, A. (2012). The Multidimensional Assessment of Interoceptive Awareness (MAIA). *PLoS ONE* 7, 11, e48230. doi: 10.1371/journal.pone.0048230.

Mehling, W.E., Wrubel, J., Daubenmier, J.J., Price, C.J., Kerr, C.E., Silow, T., ...Stewart, A. (2011). Body Awareness: a phenomenological inquiry into the common ground of mind- body therapies. *Philos Ethics Humanit Med* 6, 6. Advance online publication. doi:10.1186/1747-5341-6-6.

Milad, M.R., Quirk, G.J., Pitman, R.K., Orr, S.P., Fischl, B., Rauch S.L. (2007). A role for the human dorsal anterior cingulate cortex in fear expression. *Biol Psychiatry* 15, 62 (10), 1191–1194. doi:10.1016/j.biopsych.2007.04.032.

Montag, C., Reuter, M., Jurkiewicz, M., Markett, S. & Panksepp, J. (2013). Imaging the structure of the human anxious brain: a review of findings from neuroscientific personality psychology. *Rev Neurosci*, 24(2), 167-90. doi: 10.1515/revneuro-2012-0085.

Mor, N., & Winquist, J. (2002). Self-focused attention and negative affect: a meta-analysis. *Psychol Bull*, 128 (4), 638-62. doi: 10.1037/0033-2909.128.4.638.

Morgan, A.W., Pearson, S.B., Davies, S., Gooi, H.C. & Bird, H.A. (2007). Asthma and airways collapse in two heritable disorders of connective tissue. *Ann Rheum Dis,* 66(10), 1369–73.

Murray, B., Stein., M.D., Simmons, A.N., Feinstein, J.S. & Paulus, M.P. (2007). Increased Amygdala and Insula Activation During Emotion Processing in Anxiety-Prone Subjects. *Am J Psychiatry*, 164, 318-327. doi:10.1176/appi.ajp.164.2.318

Murray, B., Yashar, B.M., Uhlmann, W.R., Clauw, D.J. & Petty, E.M. (2013). Ehlers- Danlos syndrome, hypermobility type: A characterization of the patients' lived experience. *Am J Med Genet A*, 161A (12), 2981-8. doi: 10.1002/ajmg.a.36293.

Nagai, M., Kishi, K. & Kato, S. (2007). Insular cortex and neuropsychiatric disorders: a review of recent literature. *Eur Psychiatry*, 22(6), 387-94. doi:10.1016/j.eurpsy.2007.02.006

Nitschke, J.B., Sarinopoulos, I., Mackiewicz, K.L., Schaefer, H.S. & Davidson, R.J. (2006). Functional neuroanatomy of aversion and its anticipation.*Neuroimage,*29(1),106–116. doi:10.1016/j.neuroimage. 2005.06.068

Olesen, J., Gustavsson, A., Svensson, M., Wittchen, H.U. & Jönsson, B. (2012). On behalf of the CDBE2010 study group and the European Brain Council: The economic cost of brain disorders in Europe. *European Journal of Neurology*, 19, 155-162. doi:10.1111/j.1468-1331.2011.03590.x

Onoda, K., Okamoto, Y., Toki, S., Ueda, K., Shishida, K., Kinoshita A, ...Yamawaki S. (2008). Anterior cingulate cortex modulates preparatory activation during certain anticipation of negative picture. *Neuropsychologia.* 15, 46(1), 102-10. doi: 10.1016/j.neuropsychologia.2007.08.006

Pasquini, M., Celletti, C., Berardelli, I., Roselli, V., Mastroeni, S., Castori, M., ...Camerota, F. (2013). Unexpected association between joint hypermobility syndrome/Ehlers-Danlos syndrome hypermobility type and obsessive-compulsive personality disorder. *Rheumatol Int*. Advance online publication. doi: 10.1007/s00296-013-2901-2.

Parkin, L., Morgan, R., Rosselli, A., Howard, M., Sheppard, A., Evans, D., ...Dunn, B. (2013). Exploring the Relationship Between Mindfulness and Cardiac Perception. *Mindfulne,* Advance online publication. doi 10.1007/s12671-012-0181-7.

Paulus, M.P. (2008). The role of neuroimaging for the diagnosis and treatment of anxiety disorders. *Depress Anxiety*, 25(4), 348–356. doi: 10.1002/da.20499.

Paulus, M.P & Stein, M.B. (2006). An insular view of anxiety. *Biol Psychiatry*, 60(4), 383-7. doi:10.1016/j.biopsych.2006.03.042

Payne, R.A., Symeonides, C.N., Webb, D.J. & Maxwell, S.R. (2006). Pulse transit time measured from the ECG: an unreliable marker of beat-to-beat blood pressure. *J. Appl. Physiol*, 100(1), 136–141. doi: 10. 1152/japplphysiol.00657.

Pejic, T., Hermann, A., Vaitl, D. & Stark, R. (2013). Social anxiety modulates amygdala activation during social conditioning. *Soc Cogn Affect Neurosci*. 8(3), 267-76. doi: 10.1093/scan/nsr095.

Phan, K.L., Wager, T., Taylor, SF. & Liberzon, I. (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage*, 16(2), 331–348. doi: 10.1006/nimg.2002.1087

Phillips, M.L., Drevets, W.C., Rauch, S.L. & Lane, R. (2003). Neurobiology of emotion perception I: The neural basis of normal emotion perception. *Biol Psychiatry,* 54(5), 504–514. doi:10.1016/S0006-3223(03)00168-9

Pietrini, F., Godini, L., Lazzeretti, L., Benni, L., Pracucci, C., Talamba, G.A. & Faravelli C. (2010). Neuroimaging and neurobiology of social anxiety. *Riv Psichiatr*, 45(6), 349-60.

Pollatos, O., Herbert, B.M., Matthias, E. & Schandry, R. (2007). Heart rate response after emotional picture presentation is modulated by interoceptive awareness. *Int J Psychophysiol*, 63(1), 117-24. doi: 10.1016/j.ijpsycho.2006.09.003

Pollatos, O., Traut-Mattausch, E., Schroeder, H. & Schandry, R. (2007). Interoceptive awareness mediates the relationship between anxiety and the intensity of unpleasant feelings. *Journal of Anxiety Disorders*, 21(7), 931-943. doi: 10.1016/j.janxdis.2006.12.004

Protopopescu, X., Pan, H., Tuescher, O., Cloitre, M., Goldstein, M., Engelien, A., ...Silbersweig, D. (2006). Increased brainstem volume in panic disorder: a voxel-based morphometric study. *Neuroreport,* 17(4), 361-3. doi: 10.1097/01.wnr.0000203354.80438.1

Pujol, J., Harrison, B.J., Ortiz, H., Deus, J., Soriano-Mas, C., López-Solà, M., Yücel, M., ...Cardoner, N. (2009). Influence of the fusiform gyrus on amígdala response to emotional faces in the non-clinical range of social anxiety. *Psychol Med*, 39(7), 1177-87. doi: 10.1017/S003329170800500X.

Rapaport, M.H., Clary, C., Fayyad, R. & Endicott, J. (2005). Quality-of-life impairment in depressive and anxiety disorders. *Am J Psychiatry*, 162(6), 1171-8. doi:10.1176/appi.ajp.162.6.1171

Remvig L., Engelbert, R.H., Berglund, B., Bulbena, A., Byers, P.H., Grahame, R., ...Wekre, L.L. (2011). Need for a consensus on the methods by which to measure joint mobility and the definition of norms for hypermobility that reflect age, gender and ethnic-dependent variation: is revision of criteria for joint hypermobility syndrome and Ehlers_Danlos syndrome hypermobility type indicated? Rheumatology, 50, 1169-1171. doi:10.1093/rheumatology/ker140.

Remvig, L., Flycht, L., Christensen, K.B. & Juul-Kristensen B. (2014). Lack of consensus on tests and criteria for generalized joint hypermobility, Ehlers–Danlos syndrome: Hypermobile type and joint hypermobility syndrome.*Am J Med Genet A,* 164(3), 591-6. doi: 10.1002/ajmg.a.36402.

Ross, J. & Grahame, R. (2011). Easily mised? Joint hypermobility syndrome. *BMJ*, 342, c7167. doi: http://dx.doi.org/10.1136/bmj.c7167

Salkovskis, P.M., & Clark, D.M. (1993). Panic disorder and hypochondriasis. *Advances in Behaviour Research and Therapy*, 15, 23–48. doi: 10.1016/0146-6402(93)90002-J

Sansone, R.A. & Sansone, L.A. (2010). Psychiatric Disorders: a global look at facts and figures. *Psychiatry (Edgmont)*, 7(12), 16–19.

Schandry, R., (1981). Heart beat perception and emotional experience. *Psychophysiology,* 18(4), 483–488.

Schienle, A., Ebner, F. & Schäfer, A. (2011). Localized gray matter volume abnormalities in generalized anxiety disorder. *Eur Arch Psychiatry Clin Neurosci*, 261(4):303-7. doi: 10.1007/s00406-010-0147-5.

Schraw, G. (1998). "Promoting general metacognitive awareness". *Instructional Science,* 26, 113–125. doi:10.1023/A:1003044231033.

Seth, A.K., Suzuki, K. & Critchley, H.D. (2011). An interoceptive predictive coding model of conscious presence. *Front Psychol*, 2, 395.

Sheehan, D.V, Lecrubier, Y., Harnett-Sheehan, K., Janavs, J., Weiller, E., Bonora, L.I., ...Dunbar, G.C. (1998). "The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10". *J Clin Psychiatry*, 59 Suppl 20. 22–33. In its Spanish 5.0.0 version validated in our country by Ferrando, L., Bobes, J., Gibert, J. Soto, M., Soto, O., Instituto IAP. 1995.

Sherrington, C.S. (1906). The integrative action of the nervous system. New Haven, CT: Yale University Press.

Shin, L.M. & Liberzon, I. (2010). The Neurocircuitry of Fear, Stress, and Anxiety Disorders. *Neuropsychopharmacology*, 35(1), 169–191. doi: 10.1038/npp.2009.83.

Simmons, W.K., Avery, J.A., Barcalow, J.C., Bodurka, J., Drevets, W.C. & Bellgowan, P. (2012). Keeping the body in mind: Insula functional organization and functional connectivity integrate interoceptive, exteroceptive, and emotional awareness. *Hum Brain Mapp*, 34(11), 2944-58. doi: 10.1002/hbm.22113.

Slotnick, S. D. (2004). Visual memory and visual perception recruit common neural substrates. *Behavioral and Cognitive Neuroscience Reviews*, 3(4), 207–221. doi: 10.1177/1534582304274070

Smith, A.P.R, Stephan, K.E., Rugg, M.D. & Dolan, R.J. (2006). Task and content modulate amygdala-hippocampal connectivity in emotional retrieval. *Neuron,* 49 (4), 631-638. Doi:10.1016/j.neuron.2005.12.02

Smith, D.M. & Mizumori, S.J. (2006). Hippocampal place cells, context, and episodic memory. Hippocampus, 16(9), 716-29. doi: 10.1002/hipo.20208

Smith, T.O., Easton, V., Bacon, H., Jerman, E., Armon, K., Poland, F., Macgregor, A.J. (2014). The relationship between benign joint hypermobility syndrome and psychological distress: a systematic review and meta-analysis. Rheumatology (Oxford), 53(1), 114-22. doi: 10.1093/rheumatology/ket317.

Stein, J.L., Wiedholz, L.M., Bassett, D.S., Weinberger, D.R., Zink, C.F., Mattay, V.S. & Meyer-Lindenberg, A. (2007). A validated network of effective amygdala connectivity. *NeuroImage*, 36(3), 736-745. doi: 10.1016/j.neuroimage.2007.03.022

Spampinato, M.V., Wood, J.N., De Simone, V. & Grafman, J. (2009). Neural correlates of anxiety in healthy volunteers: a voxel-based morphometry study. *J Neuropsychiatry Clin Neurosci*, 21 (2), 199-205. doi: 10.1176/appi.neuropsych.21.2.199.

Spielberger, C.D., Gorusch, R.L. & Lushene, R. (1982). STAI Cuestionario de Ansiedad Estado/Rasgo (1st ed). Madrid: Tecnicos Especialistas Asociados (TEA).

Spielberger, C.D, Gorsuch, R.L, & Lushene, R. (1970). *STAI Manual for the State-Trait.. Anxiety Inventory*. Palo alto: California: Consuling Psychologist Press.

Tabiner, M., Youings, S., Dennis, N., Baldwin, D., Buis, C., Mayers, A., ...Crolla, J.A. (2003). Failure to Find DUP25 in Patients with Anxiety Disorders, in Control Individuals, or in Previously Reported Positive Control Cell Lines. *Am J Hum Genet*, 72(3), 535–538. doi:10.1086/367777.

Terasawa, Y., Moriguchi, Y., Tochizawa, S. & Umeda, S. (2014). Interoceptive sensitivity predicts sensitivity to the emotions of others. *Cognition & Emotion,* published online 21 Feb 2014. doi:10.1080/02699931.2014.888988.

Terasawa, Y., Fukushima, H. & Umeda, S. (2013). How does interoceptive awareness interact with the subjective experience of emotion? An fMRI study. *Hum Brain Mapp*, 34(3), 598-612. doi: 10.1002/hbm.21458.

Tsakiris, M., Tajadura-Jiménez, A. & Costantini, M. (2011). Just a heartbeat away from one's body: interoceptive sensitivity predicts malleability of body-representations. *Proc Biol* Sci, 278(1717), 2470-6. doi: 10.1098/rspb.2010.2547.

Uchida, R.R, Del-Ben, C.M., Araújo, D., Busatto-Filho, G., Duran, F.L., Crippa, J.A. & Graeff, F.G. (2008). Correlation between voxel based morphometry and manual volumetry in magnetic resonance images of the human brain. *An Acad Bras Cienc*, 80(1), 149-56. doi: 10.1590/S0001-37652008000100010.

Vizueta, N., Patrick, C.J., Jiang, Y., Thomas, K.M. & He, S. (2012). Dispositional fear, negative affectivity, and neuroimaging response to visually suppressed emotional faces. *Neuroimage,* 59, 761–771. doi:10.1016/j.neuroimage.2011.07.015.

Weiland, Y., Kraus, J. & Speicher, M.R. (2003). A Multicolor FISH Assay Does Not Detect DUP25 in Control Individuals or in Reported Positive Control Cells. *Am J Hum Genet*, 72(5), 1349–1352. doi:10.1086/375168.

Wittmann, A., Schlagenhauf, F., John, T., Guhn, A., Rehbein, H., Siegmund, A., ... Ströhle, A. (2011). A new paradigm (Westphal-Paradigm) to study the neural correlates of panic disorder with agoraphobia. *Eur Arch Psychiatry Clin Neurosci*, 261(3)185-94. doi: 10.1007/s00406-010-0167-1.

Wiens, S., Mezzacappa, E.S. & Katkin, E.S. (2000). Heartbeat detection and the experience of emotions. *Cognition and Emotion*, 14(3), 417-427. doi: 10.1080/026999300378905.

Wiens, S., & Palmer S. N. (2001). Quadratic trend analysis and heartbeat detection. *Biological Psychology*, 58(2), 159-175. Doi: 10.1016/S0301-0511(01)00110-7.

Wilken, J.A., Smith, B.D., Tola, K. & Mann, M. (2000). Trait anxiety and prior exposure to non-stressful stimuli: effects on psychophysiological arousal and anxiety. *Int J Psychophysiol*, 37(3), 233-42. doi: 10.1016/S0167-8760(00)00082-9.

Willem Van der Does A.J., Antony, M.M., Ehlers, A. & Barsky, A.J. (2000). Heartbeat perception in panic disorder: a reanalysis. *Behav Res Ther*, 38(1), 47-62. doi: 10.1016/S0005-7967(98)00184-3.

Wolf, U., Rapoport, M.J. & Schweizer, T.A. (2009). Evaluating the affective component of the cerebellar cognitive affective syndrome. *J. Neuropsychiatry Clin. Neurosci*, 21 (3), 245–53. doi: 10.1176/appi.neuropsych.21.3.245.

Yazici, M., Ataoglu, S., Makarc, S. Sari, I., Erbilen, E., Albayrak, S., ... Uyan, C. (2004). The relationship between echocardiographic features of mitral valve and elastic properties of aortic wall and Beighton hypermobility score in patients with mitral valve prolapse. *Jpn Heart J*, 45(3), 447-60. doi: 10.1536/jhj.45.447

Zaki, J., Davis, J.I. & Ochsner, K.N. (2012). Overlapping activity in anterior insula during interoception and emotional experience. *Neurimage*, 62 (1), 493-9. doi: 10.1016/j.neuroimage.2012.05.012.

Ziaei, M., Peira, N. & Persson, J. (2013). Brain systems underlying attentional control and emotional distraction during working memory encoding. *Neuroimage,* 87, 276–286. doi:10.1016/j.neuroimage.2013.10.048.

APPENDICES: Studies submitted for publication

Article submitted for publication to: *Psychosomatic Medicne*, 18.02.2014

Running head: Hypermobility interoception and anxiety

A systemic collagen condition (joint hypermobility) is related to enhanced interoception, anxiety and affective reactivity: an fMRI and psychophysiological investigation.

Mallorquí-Bagué N, MSc[1,2,3], Garfinkel SN, PhD[1,4,5], Engels M, MSc[1,8], Eccles JA, MB BCh'[1,4,] Pailhez G, MD, PhD[2,6,7],Bulbena A, MD, MSc(Cantab)[2,6,7], Critchley HD, MBChB, DPhil.[1,4,5]

[1]Department of Psychiatry, Brighton and Sussex Medical School, University of Sussex, Falmer, UK.
[2]Doctorate program in Psychiatry. Department of Psychiatry and Forensic Medicine. School of Medicine. Universitat Autonoma de Barcelona (UAB). Spain.
[3]Psychiatry, Psychology and Psychosomatics department. Institut Universitari Quirón Dexeus. Barcelona. Spain.
[4] Mood and Anxiety Research Sussex (MARS), Suseex Partnership NHS Foundation Trust, UK.
[5]Sackler Centre for Consciousness Science, University of Sussex, Falmer, UK.
[6]Anxiety Unit. Institute of Neuropsychiatry and Addictions (INAD). Hospital del Mar. Universitat Autònoma de Barcelona. Barcelona. Spain.
[7]IMIM (Hospital del Mar Medical Research Institute), Barcelona. Spain.
[8]Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, The Netherlands.

Abstract

Objective*:** States of physiological arousal contribute to the expression of anxiety. Interoceptive sensitivity describes an individual's disposition to detect internal bodily signals and is a recognised stable trait associated with anxiety. Increasingly a common variation in the connective tissue protein *collagen,* manifest as joint hypermobility, is recognised as a risk factor to anxiety and related disorders. The aim of this study was to explore the link between anxiety, interoceptive sensitivity and hypermobility in a sub-clinical population. ***Methods: Thirty-six healthy volunteers undertook interoceptive sensitivity tests, a clinical examination for hypermobility and completed validated questionnaire measures of state, trait anxiety and body awareness tendency. Nineteen participants also performed an emotional processing paradigm during functional neuroimaging. ***Results:*** We confirmed a significant relationship between state anxiety score and joint hypermobility. Interoceptive accuracy was associated with both state anxiety and hypermobility and was formally shown to mediate the relationship between these two conditions. Hypermobile, when compared to non-hypermobile, participants displayed heightened neural reactivity to sad and angry scenes within brain regions implicated in anxious feeling states, notably insular cortex. ***Conclusions:*** These findings highlight the dependence of emotional state on bodily context, and increase our understanding of the mechanisms through which vulnerability to anxiety disorders arises in people bearing a heritable variant of collagen.

Key words: Anxiety, Functional Magnetic Resonance Imaging (fMRI), Interoception, emotion, Joint hypermobility/psychology

Article submitted for publication to: *The British Journal of Psychiatry*, 07.04.2014

Running head: Emotional processing in the Joint Hypermobility Syndrome

The neural bases for anxiety and related somatic symptoms in the Joint Hypermobility Syndrome: An event-related fMRI study

Mallorquí-Bagué N, MSc[1,2,3] Bulbena A, MD, MSc(Cantab)[1,4,5] , Roé-Vellvé N. PhD[3], Hoekzema E, PhD[3,6], Carmona S, PhD[3,7], Barba-Müller E, MSc[1,3], Fauquet J, PhD [3,8], Pailhez G, MD, PhD[1,4,5], Vilarroya O, MD, PhD[1,3]

[1]Doctorate program in Psychiatry. Department of Psychiatry and Forensic Medicine. School of Medicine. Campus de la Universitat Autonoma de Barcelona (UAB). Spain.
[2]Psychiatry, Psychology and Psychosomatics department. Institut Universitari Quirón Dexeus. Barcelona. Spain.
[3]Neuroimaging Research Group.Fundació IMIM. Doctor Aiguader, 88. 08003 Barcelona, Spain.
[4]Fundació IMIM (Hospital del Mar Medical Research Institute), Barcelona. Spain.
[5]Anxiety Unit. Institute of Neuropsychiatry and Addictions (INAD). Hospital del Mar. Universitat Autònoma de Barcelona. Barcelona. Spain.
[6] Netherlands Institute for Neuroscience, Meibergdreef 47, 1105 BA Amsterdam, the Netherlands
[7]Centro de Investigación Biomédica en Red de Salud mental, Spain (SC, MD); Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain; Departamento de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, Madrid, Spain
[8] Dept. Psicobiologia i Metodologia de les Ciències de la Salut. Campus de la UAB. 08193 Bellaterra (Cerdanyola del Vallés), Barcelona, Spain

Abstract

Background: Joint Hypermobility Syndrome (JHS) has been repeatedly found associated to anxiety, and also to Fibromyalgia, Irritable Bowell S. and TemporoMandibular S. However the neural underpinnings of this association still remain unclear. The aim of this study is to explore brain responses to facial visual stimuli with emotional cues using fMRI techniques in general population with different ranges of hypermobility. **Methods:** Sample included 51 participants (18 males, 33 females) free from any psychological or physical pathology that could interfere in the studied variables. All them fulfilled the State-Trait Anxiety Inventory, were assessed with the Beighton system for hypermobility and underwent an emotional face processing fMRI paradigm. **Results:** Trait anxiety scores were significantly correlated with state anxiety and hypermobility scores. Region of Interest (ROI) analyses showed a positive correlation between BOLD signals in the hippocampus and hypermobility scores for the crying faces versus neutral faces contrast. No results were found for any of the other studied ROIs. Additionally, whole brain analysis showed that hypermobility scores were positively associated with several other key affective processing areas (i.e. the middle and anterior cingulate gyrus, fusiform gyrus, parahippocampal region, orbitofrontal cortex, and cerebellum). **Conclusions:** Our results show that hypermobility scores are associated with trait anxiety and stronger brain responses to emotional faces in emotion processing brain areas (including hippocampus) described to be linked to anxiety and somatic symptoms. These findings increase our understanding of the emotional processing in people bearing this heritable

variant of collagen and the mechanisms through which vulnerability to anxiety and somatic symptoms arises in this population**. Key words**: Funcional Magnetic Resonance Imaging (fMRI), emotion, Joint Hypermobility/ Anxiety, Psychosomatics *

Article submitted for publication to: *Harvard Review of Psychiatry*, 02.04.2014

Running head: Mind-body interactions

Mind-body interactions in anxiety and related conditions.

Núria Mallorquí-Bagué, MSc[1,2,3], Antoni Bulbena, MD, MSc(Cantab)[2,6,7], Guillem Pailhez, MD, PhD[2,6,7], Sarah N Garfinkel, PhD[1,4,5], Hugo D Critchley, MBChB, DPhil.[1,4,5]

[1]Department of Psychiatry, Brighton and Sussex Medical School, University of Sussex, Falmer, UK.
[2]Doctorate program in Psychiatry. Department of Psychiatry and Forensic Medicine. School of Medicine. Universitat Autonoma de Barcelona (UAB). Spain.
[3]Psychiatry, Psychology and Psychosomatics department. Institut Universitari Quirón Dexeus. Barcelona. Spain.
[4] Mood and Anxiety Research Sussex (MARS), Suseex Partnership NHS Foundation Trust, UK.
[5]Sackler Centre for Consciousness Science, University of Sussex, Falmer, UK.
[6]Anxiety Unit. Institute of Neuropsychiatry and Addictions (INAD). Hospital del Mar. Universitat Autònoma de Barcelona. Barcelona. Spain.
[7]IMIM (Hospital del Mar Medical Research Institute), Barcelona. Spain.

Abstract

Anxiety and somatic disorders have a high prevalence in the general population. A mechanistic understanding of how different factors contribute to the development and maintenance of these heterogeneous set of mental disorders is crucial to optimize treatments. In this article, we present a redefined model of body-mind interaction in anxiety and somatic conditions, with an emphasis on interaction between bottom-up and top-down processes. Consideration is given to the role played in this interaction by pre-disposing physiological and psychological traits (e.g. interoception, anxiety sensitivity and trait anxiety) and, the levels at which mindfulness approaches may exert therapeutic benefit. The proposed model of body-mind interaction in anxiety and somatic conditions is appraised in the context of Joint Hypermobility Syndrome, a constitutional variant associated with autonomic abnormalities and vulnerability to anxiety disorders.

Keywords: Anxiety Disorders/aetiology, Somatic Disorders/aetiology, Models/Psychological, interoception, emotion/cognition, Joint hypermobility/ psychology.