Hypermobility and Autonomic Hyperactivity: Relevance for the Expression of Psychiatric Symptoms

JESSICA ANNE ECCLES

A thesis submitted in partial fulfilment of the requirements of the University of Brighton and the University of Sussex for the degree of Doctor of Philosophy

February 2016
Abstract

Aims: The aim of this programme of work is to characterize the relevance of joint hypermobility and autonomic symptoms, particularly orthostatic intolerance, to clinical psychopathology. Joint hypermobility is a widespread, poorly recognized, connective tissue condition. Affected individuals are reportedly overrepresented among panic or anxiety disorders, irritable bowel syndrome, fibromyalgia, and chronic fatigue. Dysfunction or dysregulation of the autonomic nervous system, typically postural tachycardia syndrome is often found. Structural differences in amygdala have been reported in association with joint hypermobility. The relevance of hypermobility and autonomic dysfunction to general psychiatric conditions is currently poorly understood.

Methods: 400 adult patients attending psychiatric clinics were surveyed. Joint hypermobility was assessed using the Beighton scale and autonomic symptoms using the Autonomic Symptoms and Quality of Life Score. Additionally 70 participants (32 generalised anxiety disorder, 38 healthy controls), half of whom were classified as hypermobile, underwent functional magnetic resonance brain imaging, including emotional processing tasks, and specific tests of autonomic function.

Results: I demonstrate that rates of hypermobility are particularly high among adult patients attending psychiatric clinics ((OR, (95%CI) 2.38(1.95-2.90)), this observation validates and extends previous literature that has focused on the clinical expression of anxiety disorders. However I now show for the first time high rates of hypermobility in adults with diagnoses of Bipolar Disorder, Attention Deficit hyperactivity Disorder (ADHD) Autism Spectrum Conditions and Eating Disorders. The association of these conditions with joint hypermobility has not been previously recognised. I also show for the first time that the expression of symptoms of autonomic dysfunction, particularly high in this population, is both directly related to hypermobility status and also correlates with hypermobility score. I examine this further using autonomic function tests to show that anxious hypermobile participants exhibit increased sympathetic activation as demonstrated by sustained rise in heart rate in standing. In the clinical brain imaging sample, I used emotional processing and false physiological feedback tasks to provide new evidence that hypermobility, and interactions between hypermobility and anxiety, is associated with exaggerated reactivity within brain areas implicated in emotional processing and interoceptive control, including amygdala and insula cortex. Much of the heightening of neural responses within these brain regions correlated with, or was mediated by, measures of autonomic dysfunction.

Conclusions: Through new data contained within this thesis, I provide evidence to link joint hypermobility to a set of distinct psychopathological diagnoses, including the general expression of anxiety. Additionally, I provide experimental insight into a putative underlying mind-brain-body mechanism for this association, namely the aberrant engagement and control of autonomic nervous system. While the importance of joint hypermobility, and signs and symptoms of autonomic dysfunction, to the generation and maintenance of psychopathology has been poorly appreciated, this PhD, through a systematic set of studies goes some way toward a better characterisation of this relationship and its mediation by autonomic dysfunction. This has particular relevance for increasing clinical recognition of joint hypermobility itself across different medical disciplines and opens up new possibilities for personalised medicine.
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Acknowledgements

The work presented in this PhD would not have been possible without the generous support of the Medical Research Council who granted me a Clinical Research Training Fellowship to conduct this work. I also thank the Sussex Partnership NHS Foundation Trust Research and Development team for all their support and all of the patients and healthy volunteers who took part in this research. I am very much in debt to my inspirational supervisors Hugo Critchley and Neil Harrison who have given me so much encouragement, advice and support.

I must thank my colleagues at the Clinical Imaging Sciences Centre for all their invaluable assistance, particularly Sarah Garfinkel for helping me learn how to conduct functional MRI analyses and Cassandra Gould and David Watson for all their the help with programming. I am also grateful to the radiographers for all their assistance and Christina Lee for all her support.

I am indebted to the advice and encouragement of Professor Christopher Mathias and his team, particularly Andrew Owens, in teaching me about autonomic function tests and to the advice and encouragement of Professor Rodney Grahame and Professor Kevin Davies in teaching me about hypermobility, its importance, its extra-articular manifestations and, crucially its assessment.
Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to these or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed

Date
Definitions

ADHD  
Attention Deficit Hyperactivity Disorder

ALSPAC  
Avon Longitudinal Study of Parents and Children

ASD  
Autism Spectrum Disorder

ASI  
Anxiety Sensitivity Index

ASQoLS  
Autonomic Symptoms and Quality of Life Scale

BOLD  
Blood Oxygen Level Dependent

CI  
Confidence Interval

DSM  
Diagnostic and Statistical Manual

EDS  
Ehlers-Danlos Syndrome

EDS-HT  
Ehlers-Danlos Syndrome - Hypermobility Type

EPI  
Echo Planar Image

FEW  
Family Wise Error

FSS  
Fear Survey Schedule- Modified Wolpe Fear Scale

FWHM  
Full Width Half Maximum

GAD  
Generalized Anxiety Disorder

GHQ  
General Health Questionnaire

HAM-A  
Hamilton Anxiety Rating Scale

HAM-D  
Hamilton Rating Scale for Depression

ICD-10  
International Classification of Diseases-10th Edition

JH  
Joint Hypermobility

JHS  
Joint Hypermobility Syndrome
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>KDEF</td>
<td>Karolinska Directed Emotional Faces</td>
</tr>
<tr>
<td>LSAS</td>
<td>Liebowitz Social Anxiety Scale</td>
</tr>
<tr>
<td>MINI</td>
<td>International Neuropsychiatric Interview</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic Resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MVP</td>
<td>Mitral Valve Prolapse</td>
</tr>
<tr>
<td>NRES</td>
<td>National Research Ethics Service</td>
</tr>
<tr>
<td>OCD</td>
<td>Obsessive Compulsive Disorder</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PoTS</td>
<td>Postural Orthostatic Tachycardia Syndrome</td>
</tr>
<tr>
<td>RR</td>
<td>Relative Risk</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for DSM Disorders</td>
</tr>
<tr>
<td>SCL-90-R</td>
<td>Symptom Check List 90-R</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SMA</td>
<td>Supplementary Motor Area</td>
</tr>
<tr>
<td>SNRI</td>
<td>Serotonin–Norepinephrine Reuptake Inhibitors</td>
</tr>
<tr>
<td>SPIN</td>
<td>Social Phobia Inventory Polyvalent Psychiatric Interview</td>
</tr>
<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
</tr>
<tr>
<td>STAI</td>
<td>State-Trait Anxiety Inventory</td>
</tr>
<tr>
<td>TNXB</td>
<td>Tenascin</td>
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VVS  Vasovagal Syncope
Chapter 1: *Introduction*
1.1 Emotion, autonomic hyperactivity and psychiatric symptoms

Influential theories of emotion stress the role of bodily arousal states as the physiological basis of feeling states. This concept is embodied within psychological models of anxiety and anxiety disorders. Anxiety is a pervasive symptom across almost all psychiatric diagnoses, while anxiety disorders are themselves the most prevalent of mental illnesses. This PhD explores how common constitutional differences in bodily physiology (particularly Joint Hypermobility (JH) along with Postural Tachycardia syndrome (PoTS) and Vasovagal Syncope (VVS)) are linked to the clinical expression of psychiatric symptoms, notably anxiety. Joint hypermobility is a widespread, poorly recognized, connective tissue condition (Grahame, 2008). Estimates of its prevalence indicate joint hypermobility affects up to 25% of the general population (Remvig et al., 2007b, Clinch et al., 2011, Mulvey et al., 2013). Individuals with hypermobility are reported to be overrepresented among those with panic or anxiety disorders, for example Martin-Santos and colleagues found, in one of the first studies exploring the relationship between joint hypermobility and anxiety, very high rates of joint hypermobility in patients with anxiety (67.7%) compared to psychiatric and medical controls (10.1% and 12.5% respectively). They report that this translates to an adjusted odds ratio of developing anxiety given hypermobility of 16 (Martin-Santos et al., 1998). More recently, Smith and colleagues, in a large meta-analysis, find an odds ratio of 4.39 for suffering from anxiety if hypermobile (Smith et al., 2014). Hershenfeld and colleagues find psychiatric disorders present in 42.5% of patients suffering with Ehlers-Danlos syndromes, in which joint hypermobility is present (Hershenfeld et al., 2015). Hypermobility has also been linked to stress-sensitive psychosomatic disorders including irritable bowel syndrome (Zarate et al., 2010), fibromyalgia (Ofluoglu et al., 2006), and chronic fatigue (Nijs et al., 2006) and is associated with hypersensitivity to nociceptive stimuli (Grahame, 2008). Additionally, individuals with hypermobility often exhibit autonomic abnormalities (Gazit et al., 2003), typically postural tachycardia syndrome (Hakim and Grahame, 2004, Mathias et al., 2012), where there is an enhanced cardiovascular reactivity and a phenomenological overlap with
anxiety disorders (Mathias et al., 2012). Using structural magnetic resonance imaging of healthy hypermobile individuals, I have previously demonstrated volumetric differences in key structures including bilateral amygdala, whose integrity is necessary for normal emotion processing and where abnormalities have been linked to a variety of psychopathological disorders (Eccles et al., 2012). Thus direct and indirect evidence links hypermobility to psychiatric and stress-sensitive medical disorders.

While vulnerability to anxiety disorders might plausibly originate in stereotyped patterns of autonomic response (as in postural tachycardia syndrome and vasovagal syncope (Kouakam et al., 2002, Beacher et al., 2009)), this association is clinically underappreciated. Thus, the number of patients with these autonomic conditions in a typical mental health setting is unknown. Moreover, brain substrates through which constitutional differences in bodily reactivity influence psychological processes are relatively poorly understood. My host laboratory (Critchley lab) is leading the delineation of these neural mechanisms in studies of selected dysautonomic, neurological and psychiatric patient groups and in healthy controls. Within this PhD, I extend this work to investigate the three physical phenotypes most strongly linked to enhanced vulnerability to anxiety disorder and neuropsychiatric symptoms in a broad clinical psychiatric population. My research will therefore characterize constitutional influences on psychiatric symptoms, particularly anxiety, and provide mechanistic insight into factors evoking and maintaining these symptoms. Ultimately this approach is relevant personalized psychological medicine, for example specific medicine targets, such as adrenoreceptors blockers, and specific cognitive therapy approaches that are based on physiological arousal, may be most appropriately tailored for individual patients.
1.2 Background and Literature Review

1.2.1 Physiological aspects of emotion
Influential theories of emotion acknowledge the expression of emotions along psychological, physiological and behavioural (action) dimensions, coupled to evolutionary drives for self-preservation and reproductive success. Emotional feelings provide a psychological link between sensory processing and motivational behaviour. These feeling states are proposed to arise from the embodiment of emotion and the mental representation of physical/physiological changes, generated by internal or external emotional triggers and accompanying behaviour. If a stimulus does not elicit a change in bodily state, then it is implausible that it will have a subjective or objective emotional impact. Such a view has a long history: Over 100 years ago, Carl Lange and William James argued that the difference between emotion and non-emotion is the body’s reaction (Lange and James, 1967, James, 1894, Lange, 1885). Even Descartes’s writing acknowledges the dependence of emotional experience on bodily arousal. Criticism of this argument (Cannon, 1927) typically focuses on the relative lack of specificity of the bodily (arousal) response, which in turn can be countered by ‘constructionist’ proposals such as the two-stage model of emotion of Schachter and Singer (Schachter and Singer, 1962). This model (backed up by some experimental evidence) suggests that arousal triggers and intensifies emotional feelings, yet the cognitive appraisal of what triggered the bodily response determines which emotion is experienced (Schachter and Singer, 1962). Barrett proposes an extension of this model of emotion whereby emotional experience is a confluence of two dimensions, valence (positive or negative) and arousal (high or low) (Barrett et al., 2007). In anxiety states in particular, the occurrence of physiological responses can come to the perceptual foreground and their appraisal, and/or misinterpretation is a recognised factor in the genesis of pathological anxiety and anxiety disorders (Barlow, 1988, Clark, 1986). Moreover, anxiety is a pervasive symptom across almost all psychological disorders, while anxiety disorders represent the most frequent psychiatric illness, with a lifetime prevalence of 16.6% noted in
a large pooled study (Somers et al., 2006), c.f. 6.7% noted in major depressive disorder (Waraich et al., 2004).

Perceptions, cognitions and emotions interact with the control of bodily state in specific ways at different levels of the neuraxis (Critchley et al., 2013). Correspondingly, emotional processes influence the internal state of physiological arousal; first automatically through autonomic nerves (where there is sympathetic and parasympathetic antagonism); second indirectly though changes in respiration, skeletomotor activity or posture under partial or full volitional control, or third; as consequences of enacting motivated behaviours. In parallel, the internal physiological state of the body influences mental processes, such that; the ‘interoceptive’ representation of internal bodily state can grab attention, compete for representational or cognitive resources and interrupt on-going thoughts and feelings; interoceptive information can be fully integrated with other perceptual representations e.g. where the bodily sensations have primary reinforcing (e.g. rewarding) properties or where physiological arousal enhances the encoding of information into memory; The interoceptive state can serve as a changeable context (e.g. ‘occasion setter’ (Bouton et al., 2001, Bouton, 1993)) for emotional and cognitive processes, as exemplified when information learned in low arousal is best recalled in low arousal. Together interaction between the generation and representation of (autonomically-mediated) changes in internal bodily state may be critical to emotion. In particular, the degree to which a change in bodily state is predictable or explicable by the context or behaviour is relevant: It is proposed that Emotional feelings arise from mismatch (prediction error – see below) between observed and expected autonomic state and a need to interpret and account for this difference. More specifically, inaccurate or imprecise predictions about the internal state of the body, relative to afferent representation, may give rise to feelings of anxiety or threat (e.g. (Paulus and Stein, 2006, Simmons et al., 2008, Gray et al., 2007), particularly when confronted by ambiguous signals, driving interpretative processes (Critchley et al., 2013, Critchley, 2005).
1.2.1.1 Predictive coding and prediction error

Clark, drawing on a large and diverse literature, argues that brains are essentially prediction machines: They are bundles of cells that support perception and action by constantly attempting to match incoming sensory inputs with top-down expectations or predictions (Clark, 2013).

As Seth (Seth, 2013) writes, the view that prediction and error correction provide fundamental principles for understanding brain operation is gaining increasing traction with cognitive science, e.g. (Clark, 2013, Friston et al., 2006). In the guise of ‘predictive coding’ perceptual content is seen as resulting from probabilistic, knowledge-driven inference on the external causes of sensory signals. In order to support adaptive responses, the brain must discover information about the likely causes of sensory signals (i.e., perception) without direct access to these causes, using only information in the flux of sensory signals themselves. Simply put ‘predictive coding’ is a data processing strategy whereby signals are presented by generative models, with its origins in the insights of Von Helmoltz and reaching recent prominence in the ‘Bayesian brain’ hypothesis. Predictive coding is typically implemented by functional architectures in which top-down signals convey predictions and bottom-up signals convey prediction error (Seth, 2013), see figure below. Early work focused on the role of predictive coding in vision (Rao and Ballard, 1999) and computational approaches to motor control (Wolpert, 1997)
Figure 1.1: Functional architecture of predictive coding, adapted from Seth (Seth, 2013) and Seth et al (Seth et al., 2011). A: A schematic of hierarchical predictive coding across three cortical regions, the ‘lowest’ on left (R1) and the ‘highest’ on the right (R3). Bottom up projections (red) originate from error units (light orange) in superficial cortical layers and terminate on ‘state units’ (light blue) in the deep layers of their targets, whereas top-down projections (dark blue) that convey predictions originate in deep layers and project to superficial layers of their targets. Prediction errors are associated with precisions (inverse variances), which determine the relative influence of the bottom-up and top-down signal flow. Top-down precision weighting (dashed lines) regulates the post-synaptic gain of prediction error projection neurons possibly by neuromodulation. Triangles represent pyramidal (projection) neurons; circles represent inhibitory interneurons. Solid black lines depict local circuit interactions wherein descending predictions are resolved with ascending prediction errors. B: The influence of precisions on Bayesian inference and predictive coding.
The curves represent probability distributions over the value of a sensory signal (x-axis). On the left, high precision-weighting of sensory signals (red) enhances their influence on the posterior (green) and expectation (dotted lines) as compared to the prior (blue). In the right, low precision-weighting of sensory signals has the opposite effect on posteriors and expectations.
Clark argues that this predictive coding is achieved using a hierarchical generative model that ultimately aims to minimize this prediction error within a bidirectional cascade of cortical processing. I.e. such errors look to be corrected within a cascade of cortical processing events in which higher-level systems attempt to predict the inputs to lower-level ones on the basis of their own emerging models of the causal structure of the world (i.e. the signal source). Errors in predicting lower level inputs cause the higher-level models to adapt so as to reduce the discrepancy (Clark, 2013)

Analysing the work of Sokolov, Bridgeman writes the cortex codes not stimulus properties but stimulus information, i.e. the difference between signal and expectation (Bridgeman, 2013). Prediction error reports the “surprise” induced by a mismatch between the sensory signals encountered and those predicted (Clark, 2013)

Edwards et al, drawing on the free-energy principle (Friston et al., 2006, Feldman and Friston, 2010), assert that any adaptive change made by a biological system must attempt to minimise long-term average surprise, which means unpredicted sensations, stating that organisms can minimize this sensory surprise by constructing a hierarchical model of how sensations (exteroceptive, interoceptive and proprioceptive) are caused. Sensory surprise can be minimised by reducing prediction errors, based on the predictions of the model, either by changing sensory samples through actions or by changing the predictions through perception. In this framework, perception corresponds to optimising the model by changing synaptic activity and connection strength to minimise prediction error – predictive coding. In predictive coding this surprise – or free energy - is minimised at each level of the cortical hierarchy by changing levels of activity in neural populations encoding predictions and prediction errors, namely prediction units and prediction error units (Edwards et al., 2012).

Seth et al state that predictive coding is a powerful framework for conceiving of the neural mechanisms that underlie perception, cognition and action, positing that predictive coding models describe counter flowing top-down prediction/expectation signals and bottom-up prediction error signals.
Successful perception, cognition and action are associated with successful suppression (“explaining away”) of prediction error. They argue that very sense of “presence” is the result of successful suppression by top-down predictions of informative interoceptive signals evoked (directly) by autonomic control signals and (indirectly) by bodily responses to afferent sensory signals (Seth et al., 2011).

1.2.1.2 Relevance to psychiatric symptoms

Seth and Critchley argue that emotion itself is constituted by continually updated predictions of the causes of interoceptive input, arguing these predictions are shaped by generative models informed by “efference copies” of visceral, autonomic and motor control signals, generated, compared and updated within a salience network anchored on the anterior insular and anterior cingulate cortices that engage brainstem regions as targets for visceromotor control and relays of afferent interoceptive signals (Seth and Critchley, 2013), see Figure 1.2 below. In their model of Embodied Predictive Interoception Coding Barret and Simmons, integrating an anatomical model of cortico-cortical connections with Bayesian active inference principles, propose that agranular visceromotor cortices contribute to interoception by issuing interoceptive predictions. They state that disruption to interoceptive prediction could function as a common vulnerability for mental and physical illness (Barrett and Simmons, 2015). Similarly Farb et al posit that the tension between expected and felt bodily sensation implicates interoception in a variety of affective and psychosomatic disorders (Farb et al., 2015).
Figure 1.2: Interoceptive predictive coding model of Seth and Critchley according to which subjective feeling states are constituted by continually updated predictions of the causes of interoceptive input, adapted from Seth and Critchley (Seth and Critchley, 2013)
Mismatch and misattribution of interoceptive cues appears key in disorders of the self (Critchley et al., 2013). In a “comparator model” of schizophrenia, it is proposed that disturbances of self (e.g., delusions of control) arise as a consequence of problems in predictive coding, reflecting confusion between evaluation of changes in sensations caused by the self and changes associated with external causes: Sensory effects of self are attributed instead to external forces (Frith, 2012). A similar prediction error is implicated as a central mechanism in generation of anxiety. Specifically, Paulus and Stein (Paulus and Stein, 2006) argue that an altered signal of impeding aversive body state provides the basic link between altered interoception and anxiety. They argue that individuals who are prone to anxiety show an altered interoceptive prediction signal, i.e. they experience an augmented signalling of the difference between observed and expected body state. They state two possibilities of altered prediction signalling. Firstly, anxiety-prone individuals may experience an attenuated baseline interoceptive state and a normal interoceptive expectation resulting in a larger error signal. Alternatively anxiety-prone individuals may have a normal baseline interoceptive state but an exaggerated expected body state resulting in a larger error signal. They suggest evidence is in favour of the second hypothesis, citing Critchley et al (Critchley et al., 2004) and suggest the anterior insula as a substrate for this prediction signal. They suggest that the enhanced difference signal drives the continue engagement of cognitive resources to “solve the problem,” which manifests as aimless, non-goal directed cognitive activity that is experienced as generalized or anticipatory anxiety or worry (Paulus and Stein, 2006).

This is similar to Edwards’ “Bayesian account of ‘hysteria’” which posits functional motor and sensory symptoms arise as a consequence of overly precise priors which may lead to the overweighting of bottom-up inputs that
accord with those priors, mediated by attentional processes (Edwards et al., 2012).

1.2.2 Autonomic control and the central nervous system
The autonomic nervous system is critical to the internalised physiological expression of emotion, and by extension to associated emotional feelings. Autonomic regulation supports homeostasis through the coordination of the activity across bodily organs, glands and blood vessels. The term ‘autonomic’ derives from the fact that autonomic regulation is largely beyond conscious control, supported by both simple and complex reflexes which orchestrate bodily states to match (meet and anticipate) the behavioural context, but which ultimately prioritises vegetative processes necessary for survival. The autonomic nervous system is subdivided into two branches; the sympathetic nervous system and the parasympathetic nervous system. These axes, for the most part, act antagonistically and have distinct anatomical organization, neurochemistry and activation dynamics. The sympathetic nervous system, in which spinal preganglionic fibres relay at (cholinergic nicotinic) ganglia within the paravertebral sympathetic chain, acts on visceral organ mainly (not exclusively) via release of the catecholamine noradrenaline from postganglionic fibres at the effector synapse. The parasympathetic nervous system, in which ganglia lie close to effector organs act via acetylcholine on muscarinic receptors on the effector organ or, in some instances on pre-synaptically to inhibit sympathetic nerves. Sympathetic activation and parasympathetic withdrawal change the internal physiology of the body from prioritising vegetative functions (rest digest evaluate) toward the facilitation of action including vigilance and responses to potential threat (flight and fight) (Brading, 1999).

1.2.3 Interoception
Interoception refers to sensitivity to stimuli originating from within the body and in its original definition by Sherrington is distinguished from the sensing of stimulation from outside the body (exteroception) and of the body’s
position in space (proprioception) (Sherrington, 1948). As Garfinkel and Critchley write, while interoception encompasses both skeletomuscular and circulating (humoral) signals, more emphasis is generally given to afferent information from visceral organs and vascular system (Garfinkel and Critchley, 2013). The viscerosensory nerves that carry this information typically also form the afferent limb of autonomic nervous reflexes and higher levels of autonomic regulation. Correspondingly interoception is linked to low-level homeostatic control processes that to a large extent are managed pre-consciously by peripheral, brain stem and subcortical structures. The notion that emotional feelings arise from internal bodily sensations is influential and continues to drive an interest in interoception. Peripheral theories of emotion, e.g. that formulated in the 19th century by William James and Carl Lange (Lange et al., 1967), have empirical support ((Cannon, 1927, Lazarus, 1991, Schachter and Singer, 1962)). It is broadly accepted that visceral bodily states (of arousal) at very least can contribute to (and intensify) emotional feelings.

Wiens, gathering behavioural and neuroimaging evidence argues, centrally integrated (physiological) feedback plays a role in emotional experience and proposes a two-level model regarding the relationship between interoception and emotional experiences (both phenomenology and awareness). In this model (see Figure 1.3 below) he divides interoception into three parts (central representation of feedback from whole body, the perception of actual physiological changes and the perception of illusory physiological changes) and argues that whereas first level emotional experience (phenomenology) is affected only by the central representation of feedback from the whole body, second-level experience (awareness) is also affected by the perception of actual and illusory physiological changes, and this perception triggers an attribution process leading to self-reported emotional experience. He argues that interoceptive accuracy affects this process by allowing better discrimination between actual and illusory changes (Wiens, 2005).
Figure 1.3: Wiens’ hypothetical model of effects of interoception on emotional experience, adapted from Wiens (Wiens 2005)
Patients with increased anxiety sensitivity as well as panic disorder and other anxiety disorders, such as social anxiety disorder or generalized anxiety disorder, generally report hypervigilance for somatic sensations (interoceptive sensibility), for a review see (Domschke et al., 2010). There is a substantial body of work that demonstrates that increased interoception is positively associated with greater intensity of emotional experience, e.g. (Barrett et al., 2004, Herbert et al., 2007, Pollatos et al., 2007a, Pollatos et al., 2007b) and conversely interoceptive accuracy is negatively associated with alexithymia (Herbert et al., 2011). For example, people who are better at ‘heartbeat detection’ tasks rate emotional film clips as more intense compared to those who don’t across emotional valences (Wiens et al., 2000).

Garfinkel argues that interoception should be divided into three dimensions – sensibility, accuracy and awareness (Garfinkel et al., 2015). Sensibility is a dispositional tendency to be internally focused and includes self-reported beliefs about body tendencies. Accuracy refers to objective accuracy in the detection of internal bodily sensations. This is typically measured by heartbeat detection tasks: heartbeat perception task (Katkin et al., 2001) and the mental tracking task (Schandry, 1981). Awareness is metacognitive awareness of interoceptive accuracy, e.g. confidence-accuracy correspondence.

Interoception has long been implicated in the expression and pathophysiology of anxiety disorders, with heightened anxiety a consequence of augmented detection of a difference between observed and expected bodily states. Insula cortex is proposed to mediate anxiety via (mis)match of interoceptive signals; subjective anxiety is associated with enhanced interoceptive prediction error signals (Paulus and Stein, 2006).

1.2.4 Neural correlates

As noted, anxiety may well be a consequence of augmented detection of differences between observed and expected bodily states (Critchley et al., 2013, Paulus and Stein, 2006). Regions of insula cortex are implicated as a substrate for anxiety through is role in representing interoceptive signals and
interoceptive prediction error signals (Paulus and Stein, 2006). Highly anxious individuals show increased anterior insula cortex activity during emotion processing. Moreover, the experimental induction of mismatch between predicted and actual interoceptive signals via false physiological feedback engages both insula cortex and amygdala (a region implicated in threat responses) (Garfinkel et al., 2014) in a manner that predicts changes in the attributed emotionality of ambiguous stimuli (Gray et al., 2007).

The interoceptive role of insula, and its association with emotional feelings, is part of a broader network of regions sensitive to both (external) behavioural salience and (internal) emotional arousal. Insula, found bilaterally beneath the temporal and frontal lobes, enjoys widespread connectivity to parietal, frontal and limbic regions, and is typically divided into function sub-regions, see Craig (Craig, 2015). Broadly speaking the posterior insula is site of interoceptive representation of internal bodily state, mid insula incorporates polymodal integration and anterior insula the subjective feeling of emotional states, see Figure below.
Figure 1.4: Functional anatomy of insula sub-regions, adapted from Craig (Craig, 2015) and Palvuluri and May (Pavuluri and May, 2015) showing posterior-to-mid-to-anterior integration. Integration of salience in the middle insula is built upon the interoceptive representation in the posterior insula. It culminates in the anterior insula in the complete representation of ongoing feeling (Craig, 2015).
Across many studies of emotion, there is co-activation of anterior cingulate and bilateral insular cortices, e.g. see Medford and Critchley (Medford and Critchley, 2010). This pattern of activity is very similar to that evoked by behavioural (cognitive and physical) effort (Paus et al., 1998), when people are given pain stimuli, and when anxiety is provoked in anxious patients (Nitschke et al., 2009). Engagement of this arousal matrix is also observed across different tasks during states of cardiovascular or sympathetic arousal (Critchley 2005) and in many cases is accompanied responses within amygdala and dorsal brainstem centres that are more proximate regulators of efferent autonomic drive and modulation of associated reflexes. The activity of ventromedial prefrontal / orbitofrontal cortices and adjacent subgenual anterior cingulate cortex typically shows a negative correlation with states of autonomic arousal (i.e. shifts from sympathetic to parasympathetic dominance (Wager et al., 2009, Critchley et al., 2013). These regions are also closely linked to emotional processes, notably the subgenual cingulate is dysfunctional in major depression, and even a target for surgical treatment of depression with deep brain stimulation (Mayberg et al., 2005). The experiential feeling of anxiety, a conscious experience, associated with uneasiness, painfulness, or disturbing suspense, is often associated with rumination or perseverative cognition. Anxiety disorders are common, very disabling and have high personal and social costs; they typically include Panic Disorder, Obsessive Compulsive Disorder, Social Anxiety Disorder, Post Traumatic Stress Disorder and Generalized Anxiety disorder. Core symptoms include both cognitive and physiological ones. Core cognitive preoccupations are regarding harm, escape, avoidance, and worry. Associated physiological symptoms include hypervigilance, blushing, dizziness, dyspnoea, palpitations and sweating – all symptoms of activation of the sympathetic nervous system (Nutt et al., 2008). Bouton argues that panic disorder itself indeed might develop because exposure to panic attacks cases the conditioning of anxiety to exteroceptive and interoceptive (i.e. physiological) cues (Bouton et al., 2001). The experience of worry (perseverative cognition) is itself associated with abnormal autonomic profiles (Brosschot et al., 2006, Ottaviani et al., 2015).
Where there is mismatch between intended and actual autonomic state, corrective efferent reactions are accompanied by interpretative processes: The unconscious operation of autonomic nervous system can be interrupted by deviations from expected state, i.e. we become aware of our autonomic bodily state when we experience changes in internal state that are ‘unpredicted’ by control centres (Critchley et al., 2013, Seth et al., 2011). Interestingly, healthy individuals with hypermobility report increased subjective sensitivity to autonomic bodily changes, a psychological trait that has been associated with an increased predisposition to anxiety disorder (Eccles et al., 2012).

1.2.5 Historical descriptions of joint hypermobility
The first clinical description of articular hypermobility is attributed to Hippocrates, who, in the fourth century B.C., described the Scythians, a race of Iranian horse-riding nomads inhabiting the region that now forms the Ukraine, as having humidity, flabbiness and atony such that they were unable to use their weapons. Their main problem in warfare was that hyperlaxity of the elbow and shoulder joints prevented them from drawing their bows effectively (Beighton et al., 1999).

Clinical features suggestive of hypermobility syndrome are also illustrated in a painting "The Three Graces" (1638-1640) by Peter Paul Rubens, Prado, Madrid (Figure 1.5). Manifest hypermobility of the hand has also been found in two other ancient paintings: "Saint Cyriaque" in the Heller Retable by Mathias Grünewald (1450-1528), Frankfurt, and "The wounded man" by Gaspare Traversi, Venice (1732-1769) (Dequeker, 2001).
Figure 1.5: “The Three Graces” by Peter Paul Rubens (1577–1640). The grace in the middle has scoliosis and a positive Trendelenburg sign. The grace on the left shows hyperextension of the finger and flat feet (Dequeker, 2001).
1.2.6 Recognition of joint hypermobility syndrome

Over the last 50 years, there has been increasing recognition of the phenomenon of joint hypermobility and its relevance to orthopaedic and rheumatological symptoms. Sutro demonstrated rheumatological symptoms (knee effusions) in patients with hypermobility (Sutro, 1947). Carter and Wilkinson described the association between joint laxity and congenital dislocation of the hip (Carter and Wilkinson, 1964). Kirk in 1967 coined the term ‘hypermobility syndrome’ to describe musculoskeletal complaints associated with joint hypermobility (Kirk et al., 1967).

The syndrome (rather than joint hypermobility itself) is now commonly defined by the Brighton Criteria (Grahame et al., 2000) which stipulate that for a diagnosis of joint hypermobility syndrome presence of joint hypermobility is required plus musculoskeletal or connective tissue symptoms (e.g. prolapse, easy bruising, dislocations). It is clear that joint hypermobility is common to the hereditary disorders of connective tissue (e.g. Marfan Syndrome, Osteogenesis Imperfecta, Ehlers-Danlos Syndromes) and many argue that joint hypermobility syndrome is synonymous with Ehlers-Danlos III (also known as hypermobility type EDS) (Tinkle et al., 2009). Interestingly the criteria for each vary, see discussion below. Over recent years there has been growing recognition of the extra-articular features of joint hypermobility: these span almost every system of the body, unsurprising as connective tissue is not confined to joints (see Table 1.1).
<table>
<thead>
<tr>
<th><strong>System</strong></th>
<th><strong>Condition</strong></th>
<th><strong>Reference</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiratory</strong></td>
<td>Asthma</td>
<td>(Morgan et al., 2007)</td>
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<tr>
<td><strong>Neurological</strong></td>
<td>Chiari malformation type I</td>
<td>(Milhorat et al., 2007)</td>
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<td></td>
<td>Postural tachycardia syndrome</td>
<td>(Mathias et al., 2012)</td>
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<td></td>
<td>Carpal tunnel syndrome</td>
<td>(Aktas et al., 2008)</td>
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<td></td>
<td>Developmental co-ordination disorder</td>
<td>(Kirby and Davies, 2007)</td>
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<td></td>
<td>Headache attributed to spontaneous</td>
<td>(Schievink et al., 2004)</td>
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<td></td>
<td>cerebrospinal fluid leakage</td>
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<td></td>
<td>Migraine</td>
<td>(Bendik et al., 2011)</td>
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<td></td>
<td>New daily persistent headache</td>
<td>(Rozen et al., 2006)</td>
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<tr>
<td></td>
<td>Somatosensory amplification</td>
<td>(Baeza-Velasco et al., 2011)</td>
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<tr>
<td><strong>Gastro-intestinal</strong></td>
<td>Chronic constipation</td>
<td>(de Kort et al., 2003)</td>
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<tr>
<td></td>
<td>Crohn’s disease</td>
<td>(Vounotrypidis et al., 2009)</td>
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<td></td>
<td>Faecal incontinence</td>
<td>(Arunkalaivanan et al., 2009)</td>
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<tr>
<td></td>
<td>Functional gastrointestinal disorder</td>
<td>(Zarate et al., 2010)</td>
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<tr>
<td></td>
<td>Hiatus hernia</td>
<td>(Al-Rawi et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Rectal evacuatory dysfunction</td>
<td>(Mohammed et al., 2010)</td>
</tr>
<tr>
<td><strong>Genito-urinary</strong></td>
<td>Pelvic organ prolapse</td>
<td>(Lammers et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Urinary stress incontinence</td>
<td>(Karan et al., 2004)</td>
</tr>
<tr>
<td><strong>Psychiatric</strong></td>
<td>Attention deficit hyperactivity disorder</td>
<td>(Koldas Dogan et al., 2011)</td>
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<tr>
<td></td>
<td>Anxiety</td>
<td>See later review, e.g. (Martin-Santos et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>Psychological distress</td>
<td>(Baeza-Velasco et al., 2011)</td>
</tr>
<tr>
<td><strong>Cardio-vascular</strong></td>
<td>Mitral valve prolapse (MVP)</td>
<td>(Yazici et al., 2004)</td>
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</table>
**Pain disorders and stress-sensitive medical disorders**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic fatigue syndrome</td>
<td>(Nijs et al., 2006)</td>
</tr>
<tr>
<td>Chronic regional pain syndrome</td>
<td>(Stoler and Oaklander, 2006)</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>(Ofluoglu et al., 2006)</td>
</tr>
</tbody>
</table>

**Table 1.1: Example extra-articular disorders associated with joint hypermobility with associated references.**
A discussion of all literature relating to joint hypermobility is beyond the scope of this thesis. Instead I will focus my review on several key areas relevant to the objectives of my doctoral project:

- pathophysiology and aetiology of hypermobility
- epidemiology and measurement of hypermobility
- relationship between hypermobility and autonomic dysfunction, including postural tachycardia syndrome
- relevance of hypermobility to psychiatric disorders
- neuroimaging of joint hypermobility and postural tachycardia syndrome

1.2.7 Pathophysiology and aetiology of joint hypermobility

It has been argued that joint hypermobility is a consequence of Gaussian normality and not of particular pathological significance, e.g. (Leone et al., 2009). Indeed it is both an asset and a liability for many dancers and sportsman (Grahame and Jenkins, 1972). Others would argue that it is a source of considerable morbidity and frequently overlooked and misunderstood by doctors (Grahame, 2008, Grahame and Bird, 2001). Hypermobility syndrome (also known as Ehlers Danlos – Hypermobility type) is purported to be a hereditary connective tissue disorder (Grahame, 1999) – defined by association of joint hypermobility, widespread pain and skin features (Beighton et al., 1998).

1.2.7.1 Genetics of joint hypermobility

Clinicians have observed that joint laxity runs in families and joint hypermobility appears to be an autosomal dominant trait. However, there is a marked preponderance among females and it is posited that it is an autosomal dominant trait with incomplete penetrance, variable expressivity, and influenced by sex (Castori, 2012). Unlike the other heritable disorders of connective tissue, few genes have been reliably and consistently demonstrated to be implicated in joint hypermobility.

Most EDS subtypes, in which joint hypermobility is a frequent feature, are caused by mutations in genes encoding collagen chains or proteins involved
in their biogenesis, and there are several established mutations, eg COL5A1, COL5A2, COL3A1, COL1A1, COL1A2, see Malfait et al and Castori (Malfait et al., 2006, Castori, 2012) for an overview, however in contrast to other variants, the genetic basis of EDS-HT remains unclear. Tenascin-X is a large extracellular matrix glycoprotein. 151 patients with EDS screened for tenascin deficiencies (Schalkwijk et al., 2001) and these were found in 5 patients and 3 siblings. Haploinsufficiency of tenascin-X was found in 6 of 80 patients with EDS-HT (Zweers et al., 2004a). Abnormalities of tenascin-x have been associated with reduced collagen density in skin (Zweers et al., 2004b) consistent with mice models in which tenascin-x deficiency mimics Ehlers-Danlos syndrome in mice through alteration of collagen deposition (Mao et al., 2002).

1.2.7.2 Research linking hypermobility to aberrant connective tissue
It is hypothesised that qualitative or quantitative variations of the normal extracellular matrix architecture lead to increased laxity (Zarate et al., 2010), likely collagens, elastin, fibrillins, etc (Grahame, 1999). Joint hypermobility is frequently reported to be a collagen disorder in the literature (Bulbena et al., 2006, Krapf and Goldstein, 2013) however the evidence base for this assumption is small, and often indirect, see below:

Handler et al find in small samples patients with HMS ratio type III collagen to type III+ type I collagen is increased (Handler et al., 1985). Additionally in 22 hypermobile females compared to 42 age matched controls, abnormal (increased) ratio of type III collagen to type III + I collagen observed in skin samples (Child, 1986). Normally ratio is 18:21%, in HMS 28%:46%, as reported by Russek (Russek, 1999). Electron microscopy of skin samples revealed marked decreased proportion of thick collagen fibres and increased fine collagen fibres compared to age matched controls. (Child, 1986). In 10 women with joint hypermobility and temporomandibular joint dysfunction skin biopsies demonstrated reduced levels of total collagen and greater ratio of type III collagen to type III + I collagen (Westling et al., 1992). In a small study of newborns, the umbilical cords of babies with confirmed congenital dislocation of the hip (known to be associated with joint hypermobility
(Carter and Wilkinson, 1964)) were assayed and higher collagen III/I ratio was found in these newborns compared to controls (Jensen et al., 1986).

In a study of 43 women with pelvic organ prolapse, joint hypermobility was found to be present in 35% of patients and associated with increased concentration of type I procollagen in urine, and in hypermobile patients with recurrent prolapses, significantly higher concentrations of type III procollagen were found (Knuuti et al., 2011). In a study of 85 patients with varying forms of EDS, groups including EDS-HT, abnormal collagen ultrastructure was observed including collagen bundles that regularly contained numerous composite collagen fibrils with enlarged flower-like cross sections (Hausser and Anton-Lamprecht, 1994). More recently Iodice and colleagues found a reduction in collagen IV fibres in basement membranes and blood vessels in the skin in a study of 20 patients who had both postural tachycardia syndrome and joint hypermobility syndrome (Iodice et al., 2015)

1.2.8 Epidemiology and measurement of hypermobility

Internationally, there is no agreement on the measurement and definition of joint hypermobility (Remvig et al., 2011, Remvig et al., 2007b, Remvig et al., 2007a, Grahame, 2001). Joint hypermobility is most frequently measured by the Beighton Scale (Beighton et al., 1973) (see Figure 1.6). However a number of other methods exist including Carter Wilkinson, Rotes-Querol and Hospital del Mar methods (Beighton et al., 2012). Bulbena and colleagues found that correlation was good between all the above scales (Bulbena et al., 1992). Experts in hypermobility such as Rodney Grahame assert that any available measurement, including the Beighton scale may easily miss clinically significant hypermobility in joints not surveyed such as the shoulder or fingers. Available prevalence literature uses different cut-offs of the Beighton scale to define joint hypermobility, ranging from greater than or equal to 3 points to greater than equal to 6. Most define joint hypermobility as present at a cut off of 4 (e.g. (Clinch et al., 2011)), and indeed a Beighton score of 4 is a major criteria for the diagnosis of joint hypermobility syndrome (Brighton Criteria) (Grahame et al., 2000) (see Table 1.2 for diagnostic
criteria). However, in the presence of several other criteria the syndrome can be diagnosed with a Beighton score as low as 1. The Villefranche diagnostic criteria for hypermobility EDS stipulate a score of 5 or more (Beighton et al., 1998). Remvig and colleagues reviewed the literature on the diagnostic criteria and found that reproducibility of the tests was generally good (Remvig et al., 2007a) but agreed that the categorisation of joint hypermobility was up for debate. This renders interpretation and comparison of many of the studies exploring associations with joint hypermobility difficult. Grahame and Hakim have designed a 5 point self-report questionnaire, for detecting joint hypermobility, which correctly identifies 84% of all cases and controls (Hakim and Grahame, 2003).
Figure 1.6: Beighton scale, reproduced from Hypermobility Syndromes Association. 1. Hyperextension of trunk demonstrated by placing hands flat on floor without bending the knee. 2. Hyperextension of the elbow to > 10°. 3. Hyperextension of the knee to > 10°. 4. Opposition of thumb to volar aspect of ipsilateral forearm. 5. Passive dorsiflexion of fifth metacarpophalangeal joint to 90 °. For manoeuvre 1 score 1 point, for 2-5 score 1 point for each side of the body. Maximum score is 9. The scale is assessed by a clinician.
<table>
<thead>
<tr>
<th>Major Criteria</th>
<th>Brighton Criteria</th>
<th>Villefranche Criteria for EDS-HT</th>
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<tbody>
<tr>
<td>Beighton score of 4/9 or greater (either currently or historically).</td>
<td>Beighton score of 5/9 or greater.</td>
<td></td>
</tr>
<tr>
<td>Arthralgia for longer than 3 months in 4 or more joints.</td>
<td>Hyperextensible and/or smooth velvety skin</td>
<td></td>
</tr>
<tr>
<td>Minor Criteria</td>
<td>Beighton score of 1, 2 or 3/9 (0, 1, 2 or 3 if aged 50+).</td>
<td>Recurrent dislocations.</td>
</tr>
<tr>
<td>Arthralgia (&gt; 3 months) in one to three joints or back pain (&gt; 3 months), spondylosis, spondylosis/spondylolisthesis.</td>
<td>Chronic joint/limb pain</td>
<td></td>
</tr>
<tr>
<td>Dislocation/subluxation in more than one joint, or in one joint on more than one occasion.</td>
<td>Positive family history</td>
<td></td>
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<tr>
<td>Soft tissue rheumatism. &gt; 3 lesions (e.g. epicondylitis, tenosynovitis, bursitis).</td>
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<tr>
<td>Marfanoid habitus (tall, slim, span/height ratio &gt;1.03, upper: lower segment ratio less than 0.89, arachnodactyly [positive Steinberg/wrist signs].</td>
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<tr>
<td>Abnormal skin: striae, hyperextensibility, thin skin, papyraceous scarring.</td>
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<tr>
<td>Eye signs: drooping eyelids or myopia or anti-mongoloid slant.</td>
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Varicose veins or hernia or uterine/rectal prolapse.

Table 1.2: Diagnostic criteria. Revised Brighton Criteria for the diagnosis of joint hypermobility syndrome (left). Joint hypermobility syndrome is diagnosed in the presence two major criteria, or one major and two minor criteria, or four minor criteria. Two minor criteria will suffice where there is an unequivocally affected first-degree relative. Joint Hypermobility Syndrome is excluded by presence of Marfan or Ehlers-Danlos syndromes other than the hypermobility type of Ehlers-Danlos syndrome as defined by the Ghent 1996 and Villefranche 1998 criteria respectively. Criteria Major 1 and Minor 1 are mutually exclusive as are Major 2 and Minor 2 (Grahame et al., 2000). Villefranche Criteria for Ehlers-Danlos Syndrome (Hypermobility Type) (right). No clear indication on precise numbers of major and minor criteria for Ehlers-Danlos syndrome (hypermobility type) is specified (Beighton et al., 1998). However the presence of at least 1 major and 1 minor criteria is usually necessary for proceeding in molecular confirmation for the other Ehlers-Danlos syndrome subtypes with a known, prevalent molecular cause. The presence of at least two major criteria is strongly indicative for a definite diagnosis of the specific Ehlers-Danlos syndrome subtype (Castori, 2012).
Epidemiological studies of joint hypermobility have largely been performed in selected groups, making it difficult to generalise prevalence estimates or draw definitive conclusions. Many papers cite prevalence of joint hypermobility between 10 and 20% of the general population, e.g. (Krapf and Goldstein, 2013). Beighton and colleagues demonstrated that hypermobility appears to be more common in females than males and age related (Beighton et al., 1973). However, this was in an African population. Ethnic background appears to influence hypermobility with higher rates amongst Iraqis and Yoruba Africans and low rates amongst native New Zealanders (Remvig et al., 2007b). Rates also appear to be higher in musicians (Grahame, 1993), ballet dancers (Briggs et al., 2009) and gymnasts. The largest study to date of clinician-assessed joint hypermobility prevalence in a British population comes from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort (6,022 participants), which finds 27.5% of 14 year old females have a Beighton score of 4 or more, and 10.6% of males, (Clinch et al., 2011). A large epidemiological survey (12,853 participants) of adults, using a self-report measure, finds a very similar prevalence rate of 18.3% (Mulvey et al., 2013)

1.2.9 Hypermobility and the autonomic nervous system
There is growing clinical and laboratory evidence of autonomic nervous system dysfunction in joint hypermobility. Hakim and Grahame found increased symptoms suggestive of autonomic dysfunction in hypermobility syndrome patients compared to controls, including symptoms pre-syncope, palpitations and gastrointestinal disturbances (Hakim and Grahame, 2004). Formal laboratory abnormalities include orthostatic hypotension, postural orthostatic tachycardia syndrome, and uncategorized orthostatic intolerance, which are found in over three-quarters of hypermobile patients compared to one tenth of controls (Gazit et al., 2003). Patients with joint hypermobility syndrome have a greater mean drop in systolic blood pressure during hyperventilation, and a greater increase in systolic blood pressure after a cold pressor test, than controls, indicating sympathetic over-activity. Again implicating sympathetic over-activity, they also have evidence of heightened
vasoconstriction mediated by alpha-adrenergic and beta-adrenergic hyper-responsiveness (as assessed by administration of phenylephrine and as assessed by administration of isoproterenol) (Gazit et al., 2003).

Wendele and colleagues find measures of heart rate reactivity and reactions to the Valsalva maneuver, during autonomic functional testing, also indicate autonomic dysregulation in joint hypermobility syndrome patients compared to controls (De Wandele et al., 2013)

In Postural tachycardia syndrome, characterized by a marked rise in heart rate of 30 beats-per-minute or greater occurring within 10 min of head-up tilt or standing from supine, or a heart rate while upright of >120 beats per minute, there is a strong phenomenological overlap with anxiety disorders: Patients report symptoms similar to panic, including dizziness, palpitations and gastrointestinal disturbance (Mathias et al., 2012). Postural tachycardia syndrome, in the UK at least, is very frequently associated with joint hypermobility syndrome: ninety-six per cent of postural tachycardia syndrome patients attending a UK tertiary autonomic disorders service have been reported to have joint hypermobility (Owens et al., in preparation). In an examination of 114 individuals with joint hypermobility syndrome, over 40% fulfilled criteria for postural tachycardia syndrome (Hakim et al., 2009).

A large survey of Brazilian students suggests that symptoms of autonomic dysfunction associated with hypermobility may be more frequent in females than males (Sanches et al., 2014). In postural tachycardia syndrome, in which there is a clear association with joint hypermobility, a study by Kanjwal and colleagues found that patients were highly symptomatic, reporting frequent clinical symptoms that were suggestive of autonomic dysfunction, principally orthostatic intolerance. Recurrent pre-syncope, syncope, orthostatic palpitations, exercise intolerance, and fatigue were the most frequent symptoms reported (Kanjwal et al., 2011).
1.2.10 Relationship between hypermobility and anxiety

Bulbena and colleagues were the first to report the association between joint hypermobility and anxiety in rheumatology patients in a case-control study (Bulbena et al., 1993). The over-representation of hypermobility in anxiety disorder patients was subsequently confirmed in later studies (e.g. (Bulbena et al., 1996, Martin-Santos et al., 1998)). Further studies have also consistently demonstrated this association in non-clinical populations (e.g. (Bulbena et al., 2004a, Bulbena et al., 2004b)) and in a large general population cohort (Bulbena et al., 2011).

The bulk of the literature exploring the relationship between joint hypermobility and psychiatric problems focuses on anxiety. The methodologies, research participants, nature of controls and classification of joint hypermobility in the existing literature all show considerable heterogeneity, which is a limitation. The specific association with anxiety is the subject of two fairly comprehensive recent reviews (Sanches et al., 2012, Garcia-Campayo et al., 2011). Please see Table 1.3 for a review of all literature exploring relationship between joint hypermobility and anxiety.

<table>
<thead>
<tr>
<th>Author</th>
<th>Design</th>
<th>Setting and sample</th>
<th>Diagnostic tools</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association with anxiety in rheumatology patients</td>
<td>Case-control</td>
<td>Rheumatology outpatients 114 JH 59 controls (rheumatology patients)</td>
<td>Beighton&gt;5 SCID III</td>
<td>In JH Any anxiety OR 10.7 (4.8-23.8) Panic disorder +/-agoraphobia OR 7 (2.3-20.1) Simple phobia OR 5.8 (2.0-16.2) Generalized anxiety disorder OR 2.5 (0.6-9.4)</td>
</tr>
<tr>
<td>(Bulbena et al., 1993), Spain</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Study Details</td>
<td>Study Type</td>
<td>Participant Details</td>
<td>Measurement Details</td>
<td>Results</td>
</tr>
<tr>
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</tr>
</tbody>
</table>
| Lumley et al., 1994, USA | Case-control | EDS research clinic  
21 EDS III/JH  
20 controls (other EDS) | Villefranche criteria  
SCL | EDS/JH greater score anxiety, depression subscales of SCL |
| Gulsun et al., 2007, Turkey | Case-control | General medical outpatients  
52 Thorax deformity (21 with JH, 31 no JH)  
40 healthy controls | SCID  
HAM-A  
Beighton score >5 | JH >score than cases without  
All cases > anxiety disorders than controls |
| Ercolani et al., 2008, Italy | Case-control | General medical outpatients  
30 JH  
25 healthy controls  
30 fibromyalgia | SCL  
Beighton ≥5 | JH group> significant psychological distress and increased frequency/intensity of somatic symptoms |
| Pasquini et al., 2014, Italy | Case-control | 47 JHS/EDS III  
45 healthy controls | Brighton criteria  
Villefranche criteria | JHS/EDS higher scores on HAM-A and HAM-D.  
Overall OR 4.3 |
### Association with anxiety in anxiety patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Participants</th>
<th>Diagnostic Criteria</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Martin-Santos et al., 1998), Spain</td>
<td>Case-control</td>
<td>Psychiatric outpatients 99 Panic disorder patients 99 Psychiatric controls (no anxiety) 64 Medical controls (no anxiety)</td>
<td>SCID III Beighton ≥5</td>
<td>JH and Panic disorder+/- agoraphobia association vs psychiatric controls OR 18.6 (8-6 – 40.5) vs medical controls OR 14.7 Prevalence of JH amongst patients with anxiety 67.7%</td>
</tr>
<tr>
<td>(Bulbena et al., 1996), Spain</td>
<td>Case-control</td>
<td>Psychiatric outpatients 99 Panic disorder patients 99 Psychiatric controls (no anxiety) 64 Medical controls (no anxiety)</td>
<td>BMI</td>
<td>Asthenic somatotype 33.3 % in panic disorder+/- agoraphobia, 19.2% psychiatric controls, 18.7% medical controls</td>
</tr>
<tr>
<td>(Benjamin et al., 2001), Israel</td>
<td>Case-control</td>
<td>Anxiety disorder</td>
<td>SCID Beighton ≥5</td>
<td>JH 13% in panic+/-</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Study Details</td>
<td>Methods</td>
<td>Findings</td>
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<tr>
<td>(Gulpek et al., 2004), Turkey</td>
<td>Case-control</td>
<td>Psychiatric clinics 101 panic disorder+/- agoraphobia 39 controls (undergraduates)</td>
<td>SCID Beighton ≥5 Echocardiogram</td>
<td>agoraphobia JH 15% in controls No difference between groups</td>
</tr>
<tr>
<td>(Garcia Campayo et al., 2010), Spain</td>
<td>Case-control</td>
<td>Psychiatric clinics 42 panic disorder+/- agoraphobia +MVP 35 panic disorder+/- agoraphobia – MVP 38 controls - MVP</td>
<td>SPPI Beighton ≥5</td>
<td>JH and panic disorder – non-significant MVP may affect prevalence of JH in panic patients</td>
</tr>
</tbody>
</table>

**Association with anxiety in other populations**

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Study Details</th>
<th>Methods</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bulbena et al., 2014), Spain</td>
<td>Case-control</td>
<td>Psychiatric clinics 55 Panic disorder+/- agoraphobia 55 controls (psychiatric controls – no anxiety disorder) 55 controls (fibromyalgia) 55 controls (healthy)</td>
<td>SPPI Beighton ≥5</td>
<td>JH &amp; Panic disorder+/- agoraphobia vs psychiatric controls OR 13.2 (5-47) vs fibromyalgia OR 4.7 4.7(2-10) vs healthy controls OR 20.6(5-36)</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Population Description</td>
<td>Tool(s)</td>
<td>Findings</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------</td>
<td>--------------------------------------------</td>
<td>------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Bulbena et al., 2006, Spain</td>
<td>Control</td>
<td>2004b</td>
<td>SCID</td>
<td>Increased OR for agoraphobia OR 8.19 (3.4-19.7); agoraphobia OR 5.89 (3-11.7); social phobia OR 7.79 (2.4-24.9) No increased OR for simple phobia, OCD, GAD, dysthymic disorder or depression</td>
</tr>
<tr>
<td>(Bulbena et al., 2004a), Spain</td>
<td>Cross-sectional</td>
<td>Medical department of a company 526 subjects</td>
<td>STAI Hospital del Mar</td>
<td>JH &gt; trait anxiety</td>
</tr>
<tr>
<td>Baeza-Velasco and Bulbena, 2009, France</td>
<td>Cross-sectional</td>
<td>Internet survey of 158 tall people</td>
<td>LSAS Beighton &gt;4</td>
<td>High rate of JH and social phobia in tall people</td>
</tr>
<tr>
<td>Baeza-Velasco et al., 2011, Chile</td>
<td>Case-control</td>
<td>University students 50 JH 50 controls</td>
<td>Beighton ≥5 HADS SCID</td>
<td>JH: greater use of antidepressants and anxiolytics, anxiety background, anxiety symptoms and ‘psychosomatic’ diseases</td>
</tr>
<tr>
<td>Pailhez et al., 2011, Spain</td>
<td>Cross-sectional</td>
<td>150 High school students</td>
<td>FSS Chocolate consumption rate</td>
<td>Mean fear severity score higher in JH Higher use of chocolate</td>
</tr>
<tr>
<td>Study (Year, Country)</td>
<td>Study Design</td>
<td>Sample Size</td>
<td>Mental Health Measures</td>
<td>JH Patients RR</td>
</tr>
<tr>
<td>-------------------------------</td>
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</tr>
<tr>
<td>Bulbena et al., 2011, Spain</td>
<td>Cohort Study</td>
<td>137 General Population</td>
<td>SCID, STAI, Beighton ≥5</td>
<td>Hospital del Mar</td>
</tr>
<tr>
<td>Scheper et al., 2013, Netherlands</td>
<td>Case-Control</td>
<td>36 Dancers, 36 Controls</td>
<td>Beighton ≥4</td>
<td>HADS</td>
</tr>
<tr>
<td>Pasquini et al., 2014, Italy</td>
<td>Case-Control</td>
<td>301 Female Psychology Students</td>
<td>Hakim &amp; Grahame score ≥2</td>
<td>STAI</td>
</tr>
<tr>
<td>Baeza-Velasco et al., 2014, France</td>
<td>Cross-Sectional</td>
<td>2600 Brazilian Students</td>
<td>Hakim &amp; Grahame score ≥2</td>
<td>No association with BAI in whole sample</td>
</tr>
</tbody>
</table>
In JH women, JH correlates BAI and SPIN, and strongly with autonomic subscale of BAI, but not in men.

(Mallorqui-Bague et al., 2014), UK

Cross-sectional

36 healthy volunteers

Beighton>4 (men)
Beighton>5 (women)
STAI

JH higher state anxiety

Table 1.3: Table reviewing literature exploring association between joint hypermobility and anxiety (both disorder and traits). Adapted from (Sanches et al., 2012). ASI: Anxiety Sensitivity Index; BAI: Beck Anxiety Inventory; DSM: Diagnostic and Statistical Manual; EDS: Ehlers-Danlos Syndrome; FSS: Fear Survey Schedule- Modified Wolpe Fear Scale; GAD: Generalized Anxiety Disorder; GHQ: General Health Questionnaire; HAM-A: Hamilton Anxiety Rating Scale; HAM-D: Hamilton Rating Scale for Depression; JH: Joint Hypermobility; JHS: Joint Hypermobility Syndrome; LSAS: Liebowitz Social Anxiety Scale; MVP: Mitral Valve Prolapse; OCD: Obsessive Compulsive Disorder; OR: Odds Ratio; RR: Relative Risk; SCID: Structured Clinical Interview for DSM Disorders; SPIN : Social Phobia Inventory Polyvalent Psychiatric Interview; STAI: State-Trait Anxiety Inventory; SCL-90- R: Symptom Check List 90-R;
This table summarises 22 different studies across a variety of population types (e.g. rheumatology outpatients, anxiety outpatients, tall people). It incorporates a variety of different methodologies, different instruments and, crucially different criteria and definitions of joint hypermobility. However, in spite of this it consistently shows an association between joint hypermobility and anxiety and related disorders and behaviours.

Despite methodological heterogeneity a recent meta-analysis of 14 published case-control and cohort, (not all of those available in literature) pooling 3957 participants, solidly demonstrates and further confirms that hypermobile people are significantly overrepresented in people experiencing a variety of manifestations of anxiety. In addition to significantly increased rates of anxiety disorder (OR 4.39, CI 1.92-10.4) and panic disorder (OR 6.72, 95% CI 2.22, 20.35) compared to non-hypermobile individuals (p<0.005), hypermobile people experience more intense fear (p<0.05) and agoraphobia (p<0.05) (Smith et al., 2014).

In addition, the literature would suggest that hypermobile individuals are overrepresented in disorders in which anxiety is frequently co-morbid e.g. obsessive compulsive personality disorder (Pasquini et al., 2014) and neurodevelopmental disorders, such as Attention Deficit Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) (e.g. (Koldas Dogan et al., 2011, Tantam et al., 1990). However, the only studies of ADHD are in children and all papers reporting an association with ASD are case-studies.

In addition to overrepresentation in anxiety disorder, hypermobile individuals display a range of heightened scores on anxiety scales (e.g. (Bulbena et al., 2004a) and psychological distress and behaviours that the authors claim may attempt to mitigate anxiety including chocolate (Pailhez et al., 2011), tobacco and alcohol consumption (Baeza-Velasco et al., 2014). On the premise of literature regarding the anxiolytic effects of chocolate (e.g. (Dallard et al., 2001)) Pailhez and colleagues found that hypermobile participants were more likely to say yes to the question 'If you feel anxious, do you sometimes take chocolate to calm down?'. Hypermobile individuals
experienced higher somatosensory pain amplification (Baeza-Velasco et al., 2011).

The studies reporting the association between anxiety and hypermobility have been heterogeneous and this may cause limitations in the interpretation of the data. These limitations are described below.

Both anxiety disorder and anxiety levels have been studied and have ranged over a number of years in which diagnostic criteria will have changed. Some studies have used SCID III (e.g. (Bulbena et al., 2004b)), others SCID IV, some DSM IV to define anxiety disorder (e.g. (Ercolani et al., 2008)). A variety of rating scales have been used including STAI, HAM-A, ASI, FSS.

As discussed previously internationally, there is no agreement on the measurement and definition of joint hypermobility (Remvig et al., 2011, Remvig et al., 2007b, Remvig et al., 2007a, Grahame, 2001). Joint hypermobility is most frequently measured by the Beighton Score (Beighton et al., 1973). Available prevalence literature uses different cut-offs of the Beighton scale to define joint hypermobility, ranging from greater than or equal to 3 points to greater than equal to 6. Most studies of hypermobility define joint hypermobility as present at a cut off of 4 and indeed a Beighton score of 4 is a major criteria for the diagnosis of joint hypermobility syndrome (Brighton Criteria) (Grahame et al., 2000), however in the presence of several other criteria JHS can be diagnosed with a Beighton score as low as 1. Many of the studies in the anxiety literature have conflated hypermobility as defined by the Beighton scale as joint hypermobility syndrome.

1.2.11 Neuroimaging of hypermobility and dysautonomia: relevance to anxiety and emotional processing

Two recent papers have attempted to investigate neural correlates of hypermobility in healthy volunteers. In a voxel-based-morphometry study of 72 healthy (i.e. non-clinically anxious) participants, structural differences in key emotion processing brain regions, notably affecting the amygdala bilaterally, were observed. The hypermobile group (as assessed by Beighton
scale) as a whole also displayed decreased anterior cingulate and left parietal cortical volume while the degree of hypermobility correlated negatively with both superior temporal and inferior parietal volume (Eccles et al., 2012).

In the first, albeit small, functional neuroimaging study of 19 healthy volunteers, hypermobile (n=9) participants showed differences in emotional processing compared to non-hypermobile. Hypermobility was assessed using the Beighton Scale. The aim of this study was to test the brain basis of hypermobility, specifically using emotional tasks to probe emotional circuitry in the brain. All participants were shown, in a block design, either neutral, angry or sad scenes from the International Affective Pictures System (IAPS). During the task participants made an incidental judgement on whether pictures depicted animate or inanimate scenes. Hypermobile participants demonstrated significantly higher state anxiety scores and interceptive accuracy than non-hypermobile participants. When looking at neural activity in the sad vs neutral contrast, hypermobile participants compared to non-hypermobile participants showed greater activity in areas including insular cortex, brain stem, parietal and sensorimotor cortices, inferolateral prefrontal cortex, temporal cortices and thalamus. In the anger versus neutral contrast hypermobile participants compared to non-hypermobile participants demonstrated increased activity in insula, temporal gyri, cerebellum and thalamus (Mallorqui-Bague et al., 2014).

The authors of the study argue that hypermobile individuals manifest stronger neural reactive to affective stimulation within brain regions known to be involved in emotional processing, particularly anxiety (i.e., insula, brainstem, thalamus) and specifically in areas implicated in interoceptive representation, feeling states and self-representation. They also argue that enhanced activity within insular cortex likely supports their finding of association between hypermobility and interoceptive accuracy, and by extension its association to anxiety (Mallorqui-Bague et al., 2014), see Figure below.
Figure 1.7: Neuroimaging of hypermobility. A: Right and left insula activation in sad vs. neutral condition in hypermobile individuals compared to controls in a functional neuroimaging experiment. B: Left insula activation in anger vs. neutral condition in hypermobile individuals compared to controls in a functional neuroimaging experiment (Mallorqui-Bague et al., 2014). C: Bilateral amygdala enlargement in hypermobile individuals compared to controls in a structural neuroimaging experiment (Eccles et al., 2012).
Together, these neuroimaging data suggest that specific brain regions mediate the interaction between psychological processes and the physiological state of the body, in a manner ultimately crucial to the generation of anxiety and related symptoms in the joint hypermobility phenotype. The amygdala is a key region supporting motivational and behaviours and emotional memory; it is implicated in threat processing, generation of bodily arousal reactions and the expression of mood symptoms. These neuroimaging data implicate the amygdala and insular cortices as the most likely neural substrate underlying the association between of hypermobility and clinical anxiety and psychosomatic disorders. Speculatively, potential mechanisms that may further account for the mediating role of amygdala between anxiety and hypermobility include heightened susceptibility to the threat of pain and a perturbation of autonomic afferent feedback (Nicotra et al., 2006). Hypermobility syndrome is associated with pain syndrome such as fibromyalgia, irritable bowel syndrome and chronic regional pain syndrome. Differences in amygdala reactivity are also reported for these (Tracey and Bushnell, 2009, Eccles et al., 2012).

Additionally insight into the neural correlates of dysautonomia can be gained from the neuroimaging of postural tachycardia syndrome, in which the overlap with hypermobility is high. One study has examined twelve patients with postural tachycardia syndrome and twelve matched controls (Umeda et al., 2009). Half the postural tachycardia syndrome patients had joint hypermobility. Using functional imaging they examined the processing of emotional and neutral pictures. Physiologically, postural tachycardia syndrome patients show exaggerated orientating responses compared to controls. Controls increase their heart rate when processing the visual stimuli by about 1.5 beats per minute peaking around 2-3 seconds after presentation. Postural tachycardia syndrome patients had a higher resting heart rate (76 compared to 65 beats per minute), yet showed an increase in heart rate to the pictures of 4 to 5 beats per minute, peaking around 4-5 seconds after stimulus onset. The orientating response in postural tachycardia syndrome patients also did not show the same degree of
sensitivity to the emotional content of the pictures observed in controls. This group difference was also reflected in differences in regional brain responses to the pictures. The deactivation of ventromedial prefrontal cortex, typically reflecting engagement during processing of external stimuli (and also implicated in 'antisympathetic' autonomic control) was accentuated in postural tachycardia syndrome patients irrespective of which emotion or neutral stimulus was presented. Also across both groups a region of the basal ganglia (globus pallidus) predicted state anxiety scores. In these postural tachycardia syndrome patients, connectivity between basal ganglia, orbital and dorsolateral prefrontal cortices reflected the interaction of anxiety state and physiological responsivity.

These findings endorse the proposal that postural tachycardia syndrome patients are constitutionally vulnerable to anxiety symptoms through abnormalities in central mechanisms controlling autonomic reactions. Postural tachycardia syndrome patients showed generalized stimulus-evoked cardiovascular responses and did not show the distinct differences across emotion categories that was observed in controls (cardiac acceleration to anger and blunted cardiac response to disgust stimuli) and reported in previous studies (Critchley, 2005, Ekman et al., 1983). Thus the exaggerated cardiovascular response observed in the postural tachycardia syndrome patients abolishes the emotion-specificity of the psycho-physiological reactions. Overall, these findings suggest that hyper-reactive bodily states can underlie the disruption of emotional state through attenuation of activity within ventromedial pre-frontal cortex. This study is small and replication in a larger group would be of great import to the field.

1.2.12 Further research
Although it seems likely from both the phenomenology of anxiety and the above data, that abnormalities of the autonomic nervous system mediate the relationship between joint hypermobility and anxiety disorder, the precise neurobiological mechanisms underpinning this vulnerability remain unclear.
For example it is uncertain whether the differences in amygdala structure represent pathogenesis or resilience.

Functional neuroimaging, particularly of patient groups, coupled with detailed autonomic monitoring may elucidate these neurovisceral processes and vulnerabilities further. I hypothesise that traits of autonomic reactivity may affect the expression of psychiatric symptoms and vulnerability may be linked to inefficient coordination of efferent autonomic drive with sensitive interoceptive afferent representations autonomic prediction error in hypermobile individuals. I propose a model in Figure 1.8.
Figure 1.8: Proposed model of the neurobiological mechanisms underpinning association between joint hypermobility and anxiety.
If rates of hypermobility are as high as discussed in the literature above, I would argue that there is considerable value in screening anxiety disorder patients for both joint hypermobility and the attendant hypermobility syndrome, considering the impact of its extra-articular manifestations which could lead not only to increased recognition and understanding of this important condition within the psychiatric field but also to individualized treatment strategies, including medication and cognitive therapy.

**1.2.13 Conclusions**

Influential theories argue that bodily states of arousal are a key component to emotions, and are the basis to emotional feeling states. Emotional processes are intrinsically coupled to autonomic bodily responses through shared neural substrates. Exaggerated patterns of autonomic responsivity can enhance the expression of panic or anxiety symptoms. Some of the vulnerability to psychological symptoms, particularly anxiety, originates in constitutional differences in the control of bodily states of arousal such as joint hypermobility, postural tachycardia Syndrome. The mechanisms underlying these brain-body interactions can be defined by combining brain imaging with detailed physiological monitoring of psychiatric and neurological patients and healthy controls. Ultimately, these interactions are relevant to the recognition, understanding and treatment of individuals with anxiety, and also for stress-sensitive medical disorders. These data argue for the appreciation of variants in physiological state that may underlie psychological susceptibility to anxiety symptoms and that reflect the interaction and emergence of emotional feelings with representations and control of bodily arousal. These data implicate dysautonomia and a discrete set of brain regions that include the amygdala, cingulate and insula cortex along with specific levels of the brainstem, basal ganglia and ventromedial prefrontal cortex. Further work is needed to extend what is already known regarding the contribution of these regions to subjective feelings of anxiety, perception and misperception of bodily arousal, and generation of stereotyped patterns of affective reactions. These data reveal how brain-body mechanisms underlie individual differences in psychophysiological reactivity that can be
important for predicting, stratifying and treating individuals with anxiety disorders and related conditions.

The association between joint hypermobility and psychiatric disorders other than anxiety relatively under investigated. This PhD hopes to characterize the relationship between hypermobility, autonomic dysfunction and psychiatric symptoms, filling a significant gap in the literature. Firstly, I aim to investigate whether rates of joint hypermobility and autonomic symptoms are overrepresented in psychiatric populations. If this proves to be correct it will highlight the need for screening patients with psychiatric disorder for joint hypermobility and autonomic dysfunction. Secondly, I aim to investigate whether constitutional variants in autonomic reactivity predispose to the expression of particular psychiatric symptoms and whether signs and symptoms of autonomic hyperactivity mediate the relationship between joint hypermobility and anxiety. If this proves to be correct it could help guide future medical treatments, for example the use of adrenoceptor blockers in the treatment of anxiety in hypermobile individuals. Thirdly, I aim to investigate whether a neural basis to these associations lies in inefficient coordination of efferent autonomic drive with sensitive interoceptive afferent representations and whether the amygdala and insula are likely neural substrates. If this proves to be correct, it has implications for possible future cognitive treatments.
1.3 Aims of the project

1.3.1 Hypotheses to test

1. Rates of joint hypermobility and autonomic symptoms are overrepresented in psychiatric populations (Chapter 3 and 4).

2. Constitutional variants in autonomic reactivity predispose to the expression of particular psychiatric symptoms. Symptoms and signs of autonomic hyperactivity mediate the relationship between joint hypermobility and anxiety (Chapter 4 and Chapter 5).

3. A neural basis to these associations lies in inefficient coordination of efferent autonomic drive (i.e. autonomic prediction error) with sensitive interoceptive afferent representations. The amygdala and insula are likely neural substrates (Chapter 6 and 7).

1.3.2 Key Objectives

In this programme of doctorate work, I aimed to extend my previous work (structural imaging study of joint hypermobility) into clinical psychiatric populations and integrate structural and functional neuroimaging with psychophysiological monitoring:

1. To characterize the real-world relevance of joint hypermobility and autonomic symptoms to clinical psychopathology, particularly anxiety, through conducting a clinical survey of 400 patients accessing local mental health services.

2. To delineate physiological and neural mechanisms through which traits of autonomic reactivity affect the expression of psychiatric symptoms. These studies attempt to test empirically sophisticated theoretical models (notably an interoceptive predictive coding model (Seth et al., 2011)) that extends psychophysiological models of anxiety (Paulus and Stein, 2006, Gray et al., 2007) and symptom expression (Edwards et al., 2012)).

3. To use findings of above to characterize patterns of vulnerability and resilience to psychiatric symptoms and differential responses to medication to inform better treatments and selection of medication.
Chapter 2: Materials and Methods
2.1 Background

In order to test the hypotheses presented above (in Chapter 1), I undertook three experimental studies.

The first (Study 1) was a cross sectional epidemiological study of 400 patients attending local mental health services exploring prevalence of joint hypermobility and symptoms of autonomic dysfunction. This sample size was determined as follows. With a sample size of 400 individuals I have 90% power to detect a difference of 10% in the prevalence of hypermobility (between general psychiatry population and general population) at the 5% level of significance. This large sample size accounts for the use of non-parametric tests, which are often mandated as the distribution of hypermobility scores is skewed.

The second (Study 2) was a behavioural study designed to probe the relationship between signs of autonomic dysfunction and vulnerability to anxiety associated with hypermobility. 60 individuals (four experimental groups (2 x 2 factor design: presence/absence of hypermobility; presence/absence of anxiety)) were recruited and underwent anxiety and hypermobility screening and specific tests of autonomic dysfunction. For convenience the same sample served as the basis for recruitment to Study 3 (below).

The third study (Study 3) was a functional MRI and behavioural study designed to probe emotional processing and vulnerability to anxiety associated with hypermobility and relationship to autonomic dysfunction and interoception. Sample size was determined using existing sample sizes (n=12) in the literature that related to healthy individuals performing the same tasks (Gray et al., 2007, Umeda et al., 2009). This size was increased to 15, consistent with more recent clinical studies, then multiplied by 4 to account for the four experimental groups (2 x 2 factor design: presence/absence of hypermobility; presence/absence of anxiety). An additional 10 participants were recruited to minimise effects of drop out, structural, artefact or movement abnormalities, leading to a sample size of 70.
All of these studies were ethically approved by the Brighton and Hove NRES committee (ref 12/LO/1942) and were sponsored by Sussex Partnership NHS Foundation Trust.

2.2 Participants

2.2.1 Study 1

Study 1 forms the basis of experimental chapters ‘Is hypermobility more common in the general psychiatric population?’ and ‘Is hypermobility associated with symptoms of autonomic dysfunction in the psychiatric population?’

All patients attending Assessment and Treatment Services and Neurobehavioral Service in Sussex Partnership Trust NHS Foundation Trust (Brighton and Hove) were consecutively invited by an ethically approved letter (please see appendices) to take part in the questionnaire survey between February 2013 and March 2014 (n=1856). In addition to the letter a copy of the patient information leaflet was enclosed (see appendices). As a result of this letter some potential participants approached the research team directly and asked to take part. In addition to this a member of the research team sat in waiting room and approached patients who had already been sent a letter to invite them to take part. In parallel adverts giving information about the study were displayed in clinical sites (see appendices).

If the potential participant decided to take part the patient was taken to a private clinic room and the study was further explained to them. If agreeable, participants gave written informed consent to take part in the study and for the research team to have access to their patient notes to confirm their diagnosis (see appendices for consent form). As such the researcher was blind to the diagnosis at the time of the study.

2.2.1.1 Inclusion and exclusion criteria

Patients aged 16 – 65 years old, with an ICD-10 diagnosis of mental disorder were suitable for this study. There were no exclusion criteria other than age.
### 2.2.2 Study 2 and 3

Study 2 forms the basis of experimental chapters ‘What is the relationship between hypermobility, anxiety and autonomic dysfunction?’ Study 3 forms the basis of experimental chapters ‘What are the affective neural correlates of the association between joint hypermobility and anxiety: Neuroimaging of emotional faces?’ and ‘What is the effect of interoceptive influence on affective processing in joint hypermobility and anxiety: Neuroimaging of false physiological feedback?’

These studies was designed to be analysed in a factorial model, as described above and shown in Table 2.1 below. 70 participants aged 18-65 were recruited to this study, half of whom suffered from generalized anxiety disorder (factor 1 – anxiety status). The remainder were free from psychiatric illness and served as controls. Additionally half of each group scored 4 or more on the Beighton Scale and as such met criteria for generalised joint laxity (factor 2 – hypermobility status). Controls were age and sex matched to patient participants.
<table>
<thead>
<tr>
<th>FACTOR 1</th>
<th>FACTOR 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety (-)</td>
<td>Hypermobility (-)</td>
<td>No anxiety</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non hypermobile</td>
</tr>
<tr>
<td>Anxiety (+)</td>
<td>Hypermobility (+)</td>
<td>No anxiety</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anxiety</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non hypermobile</td>
</tr>
</tbody>
</table>

Table 2.1: Table showing factorial design of participants for study 2 and study 3.
2.2.2.1 Inclusion and exclusion criteria

Inclusion criteria for patients included ICD-10 diagnosis of generalized anxiety disorder. Controls needed to be free from psychiatric disorder. General exclusion criteria included MRI incompatibility, presence of neurological illness, and presence of psychiatric illness other than anxiety or co-morbid depression in patients, presence of any psychiatric illness for controls. Patients were recruited either from Sussex Partnership NHS Trust after inclusion in Study 1 or via bulletin boards at University of Sussex and University of Brighton. Controls were recruited via bulletin boards at University of Sussex and University of Brighton.

2.3 General Methods

2.3.1 Study 1

2.3.1.1 Diagnostic criteria

All patients required an ICD-10 diagnosis of mental disorder. This was established from clinical notes.

2.3.1.2 Questionnaire methods

All patients completed the Autonomic Symptoms and Quality of Life Scale (ASQoLS), designed by the Autonomic Medicine Unit at Imperial College (Iodice et al., in preparation). This incorporates assessment of two features, firstly self-report of symptoms suggestive of autonomic dysfunction and secondly clinical assessment (by clinician) of the Beighton scale (Beighton et al., 1973) for assessment of hypermobility. Symptoms suggestive of autonomic dysfunction incorporate orthostatic, gastrointestinal, bladder, secretomotor, sudomotor and sleep domains and combines presence of symptoms with both frequency and impact on life. A copy of the questionnaire is in the appendix.

The Beighton scale explores the joint mobility range of 5 body areas: wrists/thumb, knees, spine, paired elbows and fifth meta-carpo-phalangeals. The highest score is nine and an accepted cut-off point is 4, e.g. (Clinch et al., 2011). The Beighton assessment was conducted by the research
clinician after formal training in the assessment of hypermobility by myself. Inter-rater reliability (Kappa value) for this study was determined to 0.78.

The Beighton score is one tool, amongst several, for rating hypermobility. It was chosen for this study for several reasons. Firstly, it is the method by which Clinch and colleagues report hypermobility in the largest clinician assessed prevalence study of hypermobility to date, which serves as the reference population in chapter 3 (Clinch et al., 2011). Secondly, Bulbena and colleagues found that correlation was good between the Beighton scale and other, less widely used, methods of assessing hypermobility such as Carter Wilkinson, Rotes-Querol and Hospital del Mar methods (Bulbena et al., 1992). Thirdly it has been found that the inter-rater reliability of the Beighton scale is generally good with a kappa value of 0.74 (Juul-Kristensen et al., 2007).

The ASQoLS is in the process of validation (Iodice et al., in preparation) and has been designed by one of leading international clinical centres of assessment of autonomic dysfunction.

\[2.3.2\] \textit{Study 2 and Study 3}

\subsection*{2.3.2.1 Diagnostic criteria}

Generalized Anxiety disorder was established or refuted using the MINI International Neuropsychiatric Interview (Sheehan et al., 1998). The presence or absence of generalised joint laxity was established using Beighton Scale (Beighton et al., 1973), where a cut off of 4 out of 9 was used in line with the literature e.g. (Clinch et al., 2011). Presence of hypermobility syndrome was confirmed or not using Brighton Criteria (Grahame et al., 2000)

\subsection*{2.3.2.2 Questionnaire measures}

Beck Anxiety Inventory was used to assess severity of anxiety symptoms (Beck et al., 1988). ASQoLS was used to assess symptoms suggestive of autonomic dysfunction, Porges Body Questionnaire (Porges, 1993) was used to measure subjective report of autonomic and related internal feelings. This
assesses the concept of interoceptive sensibility (Garfinkel et al., 2015) (see below).

2.3.2.3 Assessment of autonomic function

The integrity and function of the autonomic nervous system can be tested by a variety of laboratory investigations. Typically these involve measurement of cardiovascular changes to certain procedures including orthostasis (either standing or head up tilt) and changes in breathing (Mathias et al., 2013, Wieling and Karemaker, 2013).

2.3.2.3.1 Orthostasis (active standing from supine)

Changing posture from supine to standing leads to a rapid pooling of 300 to 800 ml of blood in the lower extremities and to the pelvic region causing thoracic hypovolemia due to an abrupt drop in venous return to the heart hence decreasing the ventricular preload. This fall in preload leads to a decrease in cardiac output and to less distention of the aortic arch and carotid sinus baroreceptors and subsequently reduced afferent baroreflex traffic to the brainstem. The unloading of the baroreceptors triggers reciprocal changes in autonomic activity with parasympathetic inhibition and sympathetic activation resulting in an increase in both heart rate and total peripheral resistance in order to minimize orthostatic reduction in blood pressure. Normally, orthostatic stress evokes compensatory vasoconstriction across multiple vascular beds including the skeletal muscle, which can be recorded as muscle sympathetic nerve activity (MSNA) in humans (Lambert and Lambert, 2014). As such cardiovascular responses to standing, are frequently used assess autonomic function (Plash et al., 2013, Low et al., 2014, Romero-Ortuno et al., 2011) and is commonly called the active stand. Initial peak in heart rate occurring within 15 seconds of standing is thought to be vagally mediated (i.e parasympathetic withdrawal), after this initial peak, rises in heart rate are thought to be due to increased sympathetic outflow to sinus node. The heart rate increase at one minute of standing indicates a strong adrenergic drive to the sinus node (Wieling and Karemaker, 2013). In a study combining baroreflex sensitivity with MSNA analysis Schwartz and colleagues have shown that sympathetic baroreflex is augmented by standing (Schwartz and Stewart, 2012).
2.3.2.3.2 Deep Breathing

The measurement of heart rate variation during forced breathing at a frequency of 6 cycles/min is a well-known clinical test of parasympathetic function. It is also known that normal values of heart rate variation to this test are strongly dependent on age (Diehl et al., 1997, Mathias et al., 2013, Wieling and Karemaker, 2013). Analysis of over 21,000 individuals also demonstrates that deep breathing at such a rate can also induce significant changes in systolic and diastolic blood pressure from baseline (Mori et al., 2005) an effect attributed to relative increase of vagal (parasympathetic) activity, and decreased sympathetic activity (Mori et al., 2005, Bernardi et al., 2002).

Vagus nerve-mediated autonomic control of the heart, can also be measured by Root Mean Square of the Successive Differences (RMSSD), a measure of heart rate variability (Stein et al., 1994).

2.3.2.3.3 Paradigm for assessment

In order to assess autonomic nervous system dysfunction cardiovascular responses were monitored during the two above tests (which were picked for their brevity and simplicity) and conducted as per the method commonly described by Mathias et al (Mathias et al., 2013). The participant was connected to a Finometer (Finapres Medical Systems, Amsterdam, The Netherlands), which is simple non-invasive method of monitoring beat-to-beat heart rate and blood pressure and recommended for detailed non-invasive autonomic monitoring, the Finapres Technique (Mathias et al., 2013, Wieling and Karemaker, 2013), as conventional syphygmomanometry cannot inform the investigator about beat-to-beat fluctuations in arterial pressure, and as such cannot measure transient changes in circulation. The Finometer measures changes in heart rate and arterial pressure through a cuff applied to the finger and is connected to a height correction unit and a visual display unit which shows heart rate and blood pressure in real time. Recordings can be exported for analysis using Beat Scope Easy Software (Finapres Medical Systems, Amsterdam, The Netherlands).
After the Finometer was connected, the machine was allowed to calibrate and patient lay supine on a couch. Once calibrated, the participant continued to lay supine in a quiet room for two minutes without talking while baseline heart rate and blood pressure was determined. After two minutes of continuous baseline measurement the patient was instructed to engage in deep breathing at rate of 6 cycles a minute for 30 seconds (as per method of Mori et al (Mori et al., 2005)). Patients were instructed for each 10 s cycle as follows ‘Deep breath in, 1, 2, 3, 4; deep breath out, 1,2,3,4) After the assessment of the effect of deep breathing the participant was instructed to remain supine and silent for a further two minutes. After two minutes the patient was instructed to stand and to remain standing for one minute. Heart rate and blood pressure were recorded throughout. See Figure below:
Figure 2.1: Paradigm for assessment of autonomic function, including assessment of cardiovascular responses to a deep breathing challenge and a standing challenge. Participant is connected to Finometer supine and after calibration remains supine for two minutes at which point they are instructed to engage in deep breathing for 30 seconds at a rate of 6 cycles/min. They then remain supine for a further two minutes at which point they are instructed to stand for 60 seconds.
2.3.2.4 Measures of interoception

There is a theoretical prediction that individual differences in emotional reactivity may in part be explained by differences in individual sensitivity to states of internal bodily arousal (interoception i.e. signals from the body). Methods for assessing this have centred, largely for practical reasons, on assessments of whether people can count or judge the timings of their individual heart beats at rest. Interoceptive accuracy (Garfinkel et al., 2015) was assessed using the mental tracking task. In the heartbeat tracking task, participants were connected to a pulse oximeter (NONIN, Nonin Medical, Minnesota, USA) and given the following instructions: ‘Without manually checking, can you silently count each heartbeat you feel in your body from the time you hear “start” to when you hear “stop”’. This task was repeated six times to form six trials, using time-windows of 25, 30, 35, 40, 45 and 50 s, presented in randomized order.
Figure 2.2: Graphic showing method of mental tracking task.
2.3.2.5 Brain imaging tasks
The tasks (designed to probe emotional processing) were presented using Cogent (http://www.vislab.ucl.ac.uk/) running in Matlab R2013a (Mathworks). Participants were placed in a supine position, connected to a pulse oximeter (NONIN, Nonin Medical, Minnesota, USA). Visual stimuli were projected on a screen behind the scanner, which the participant could view through a mirror mounted in the head coil. Auditory stimuli were played through in-ear head-phones. Full detail of each task is presented in the appropriate experimental chapter.

2.4 Implementation

2.4.1 Study 2

2.4.1.1 Autonomic (cardiovascular) recording
Heart rate and blood pressure were monitored on a beat-to-beat basis using a Finometer PRO (Finapres medical systems, Amsterdam, The Netherlands). Data was exported into BeatScope Easy software and R-R intervals were calculated.

As described above for the deep breathing test patients lay supine for two minutes prior to an instruction to take deep breaths in and out. Maximum and minimum heart rate during the deep breathing epoch was calculated (Mathias et al., 2013). Systolic and diastolic blood pressures were recorded during this period and the average of the last four recordings prior to instruction represented baseline blood pressure. After 30 seconds, the last four recordings of systolic and diastolic blood pressure were averaged to constitute a post-baseline reading.

For the active stand test baseline heart rate was calculated by averaging the four R-R intervals prior to instruction to stand, the peak heart rate was determined as smallest R-R interval once standing and the four R-R intervals were averaged at one minute of standing to determine heart rate at one minute of active stand.

An index of heart rate variability Root Mean Square of the Successive Differences (RMSSD) was calculated during the 30 seconds of deep

2.4.2 Study 3

2.4.2.1 Brain activity: BOLD contrast

The MR (magnetic resonance) imaging method most often used to produce information relating to brain function is called BOLD (blood oxygenation level dependent) contrast imaging. This method is based on MR images made sensitive to changes in the state of oxygenation of the haemoglobin (Ogawa et al., 1990). This molecule has different magnetic properties depending on the concentration of Oxygen: when it is fully saturated with oxygen (oxyhaemoglobin) it behaves as a diamagnetic substance, while when some oxygen atoms have been removed (deoxyhaemoglobin) it becomes paramagnetic. Within any particular imaging voxel (representing a small part of the brain) the proportion of deoxyhaemoglobin relative to oxyhaemoglobin dictates how the MR signal will behave in a BOLD image: areas with high concentration of oxyhaemoglobin give a higher signal (brighter image) than areas with low concentration. The increase in blood flow related to neuronal function is also accompanied by a relative increase in oxyhaemoglobin and hence BOLD is used as a proxy of brain activity in functional neuroimaging (Amaro and Barker, 2006).

One of the main external factors known to co-vary with BOLD measurement are physiological parameters (e.g. perspiration, respiration, heart rate and blood pressure) reflecting activity in the autonomic nervous system (Iacovella and Hasson, 2011). For example, Fan and colleagues (Fan et al., 2012) find that spontaneous fluctuations of BOLD signals in key nodes of resting state networks are associated with changes in nonspecific skin conductance response, a sensitive psychophysiological index of autonomic arousal. Some lines of research have treated autonomic nervous system activity as noise, and have devised methods to reduce this ‘physiological noise’ from analyses, however alternative research approaches have demonstrated the interaction between the autonomic nervous system and cortical and sub cortical systems involved in the
regulation, monitoring and/or generation of autonomic nervous system activity such as those involved in decision making, conflict resolution and the experience of emotion, e.g. (Harrison et al., 2010, Critchley, 2005, Critchley, 2009, Critchley et al., 2013, Critchley and Harrison, 2013).

2.4.3 Data analysis and statistics

2.4.3.1 Study 1

2.4.3.1.1 Questionnaire data
All data was analysed in SPSS (IBM, New York, USA). The database contains both scale and category data. For categorical analysis (i.e. prevalence of joint hypermobility) contingency tables were constructed using cross tabs and reported using Chi squared when the assumptions of the test were met, otherwise Fishers Exact Test was used. To compare means of normally distributed data, such as autonomic dysfunction scores, independent sample t tests were performed. To correlate parametric scale data, Pearson correlation co-efficient was used; to correlate non parametric or ordinal data, Spearman Rho co-efficient was used. Univariate analysis in the general linear model was used to explore interactions (specifically of gender), and residuals generated using linear regression were used to correct the effects of age on scale data in the model for parametrically distributed variables. To correct for the effects of age on non-parametrically distributed variables, partial correlation was used. Unless specified otherwise two-tailed tests of significance were used and a p value of p<0.05 was used a significance threshold. Mediation analysis was performed using the method of Baron and Kenny (Baron and Kenny, 1986).

2.4.3.2 Study 2 and 3

2.4.3.2.1 Autonomic and questionnaire data
As in study 1, all questionnaire and behavioural data was analysed in SPSS. The database contained both scale and category data. For categorical analysis (i.e. prevalence of joint hypermobility) contingency tables were constructed using cross tabs and reported using Chi squared when the assumptions of the test were met, otherwise Fishers Exact Test was used.
To compare means of normally distributed data, such as autonomic dysfunction scores, independent sample t tests were performed. To correlate parametric scale data, Pearson correlation co-efficient was used; to correlate non parametric or ordinal data, Spearman Rho co-efficient was used. Univariate analysis in the general linear model was used to explore interactions (specifically of gender), and residuals generated using linear regression were used to correct the effects of age on scale data in the model for parametrically distributed variables. To correct for the effects of age on non-parametrically distributed variables, partial correlation was used. Unless specified otherwise two-tailed tests of significance were used and a p value of p<0.05 was used a significance threshold. Mediation analysis was performed using the method of Baron and Kenny (Baron and Kenny, 1986).

To determine autonomic function in relation to active standing the following measures were used: initial heart rate, peak heart rate and heart rate at one minute of standing. In addition changes (peak and at one minute of standing) were expressed as both absolute changes and as proportion change from baseline. To determine autonomic function in relation to deep breathing the following was measured baseline diastolic and systolic blood pressure, maximum and minimum heart rate during deep breathing epoch and post-epoch measure of diastolic and systolic blood pressure. Variations in heart rate (representing respiratory sinus arrhythmia) were expressed as the minimum/maximum ratio. A correction for resting heart rate is not required (Wieling and Karemaker, 2013) for this test. Differences in blood pressure were expressed as both absolute changes and as a proportion change from baseline. RMSSD was calculated during the 30 seconds of deep breathing as described above.

Autonomic prediction error (i.e the mismatch between signs and symptoms of orthostatic intolerance) was calculated by z- transforming the mean proportional rise in heart rate on active stand (orthostasis) and subtracting the z transformed orthostatic sub-scale of the ASQoLS for each participant.
2.4.3.2.2 Interoception data

Interoceptive sensitivity scores were calculated across the mental tracking trials using the following equation:

\[ 1 - \frac{|n\text{beats}_{\text{real}} - n\text{beats}_{\text{reported}}|}{(n\text{beats}_{\text{real}} + n\text{beats}_{\text{reported}})/2} \]

(Hart et al., 2013, Garfinkel et al., 2015). For the heartbeat detection trials a score was calculated as follows (no of correct trials/number of total trials). Interoceptive sensibility was assessed using Porges Body Perception Questionnaire. To determine difference between accuracy and sensibility the two scores were z transformed (x – mean/standard deviation) and one subtracted from the other this represents Interoceptive trait prediction error (i.e. the mismatch between objective interoceptive accuracy and subjective interoceptive sensibility) (Garfinkel et al., 2015).

2.4.3.3 Study 3

2.4.3.3.1 Brain data

2.4.3.3.1.1 Pre-processing

FMRI data were analysed using Statistical Parameteric Mapping (SPM8) software (www.fil.ion.ucl.ac.uk/spm) and Matlab R2012a (Mathworks). Pre-processing was performed so data would at least approximate the following assumptions – all voxels in any given image of the series of images taken over time were acquired at the same time; each data point in the time series from a given voxel was collected from that voxel only; residual variance will have a Gaussian distribution; when carrying out analyses across different subjects any given voxel will correspond to the same brain structure in all the subjects in the study (Hernandez, 2002).

2.4.3.3.1.2 General Linear Model

Statistical analyses were performed on the basis of the general linear model framework and details of which are provided in the experimental chapters.

2.4.3.3.1.3 Covariates

Age and gender were entered as co-variates of no interest in all brain imaging analyses. Beighton score or Anxiety score was also entered as an explanatory variable of interest for certain specific analyses. Weighting the
second level design to this regressor then highlights any activation that
varies proportionally with the regressor in the direction specified, i.e. as a
positive or negative relationship. This enables the identification of areas of
activation that are significantly predicted by Beighton Score or Anxiety score.
Chapter 3: Is hypermobility more common in the general psychiatric population?
3.1 Introduction

A number of investigations observe an over representation of hypermobility in anxiety populations, e.g. (Martin-Santos et al., 1998, Bulbena et al., 1993, Bulbena et al., 2004b, Bulbena et al., 2011) and in depression, e.g. (Smith et al., 2014). However, the relevance of joint hypermobility to the general psychiatric population and to many other specific psychiatric disorders is poorly appreciated: for example, very little if any data is available characterising the relationship between neurodevelopmental conditions (such as ADHD or ASD), bipolar affective disorder, personality disorder or eating disorder and joint hypermobility. For example, the only studies of neurodevelopmental conditions such as ADHD are in children, and the only evidence linking ASD to joint hypermobility comes from case studies (Koldas Dogan et al., 2011, Tantam et al., 1990). To my knowledge no studies have investigated the relationship with bipolar disorder.

3.2 Aims and hypothesis

The aim of this chapter is firstly to determine the prevalence of joint hypermobility in a general psychiatric population. Secondly, I aim to determine the prevalence of joint hypermobility in a subset of other psychiatric disorders.

I hypothesise that joint hypermobility will be significantly more prevalent in the general psychiatric population compared to the general population, e.g. (Clinch et al., 2011). In addition, I hypothesise that joint hypermobility will be significantly more prevalent than the general population in specific disorders, such as neurodevelopmental conditions where affective symptomatology is grounded on trait like features that plausibly may have a systemic / constitutional basis and previous studies, in children, have suggested a link (Koldas Dogan et al., 2011, Tantam et al., 1990).

3.3 Patient demographics

Questionnaires were collected from 416 patients attending adult psychiatric clinics in Brighton and Hove since February 2013. Thirty-nine were excluded due to missing or incomplete data. 181 (48%) of respondents were male,
196 (52%) were female. Ages ranged from 18 – 65 years old, mean age was 38.9 (±SEM 0.61) years old. There was no significant difference in ages between the two sexes. In addition, data was compared to two reference populations. Firstly, the ALSPAC population cohort data (Clinch et al., 2011) and secondly the healthy controls used to validate the ASQoLS questionnaire (Iodice et al., in preparation).

3.3.1 Patient diagnoses
All patients had at least one psychiatric diagnosis: 99 (26.7%) had two diagnoses and 14 had three (3.7%). Depression was the most common diagnosis, followed by anxiety disorder, bipolar affective disorder, attention deficit hyperactivity disorder, schizophrenia, personality disorder, autism spectrum disorder, obsessive compulsive disorder, and eating disorder. Table 3.1 illustrates frequency of diagnoses.
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>129 (34.2%)</td>
</tr>
<tr>
<td>Anxiety Disorder</td>
<td>77 (20.4%)</td>
</tr>
<tr>
<td>Bipolar Affective Disorder</td>
<td>66 (17.5%)</td>
</tr>
<tr>
<td>Attention Deficit Hyperactivity Disorder</td>
<td>60 (14.4%)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>38 (10.1%)</td>
</tr>
<tr>
<td>Personality Disorder</td>
<td>45 (11.9%)</td>
</tr>
<tr>
<td>Autism Spectrum Disorder</td>
<td>21 (5.6%)</td>
</tr>
<tr>
<td>Obsessive Compulsive Disorder</td>
<td>15 (4%)</td>
</tr>
<tr>
<td>Eating Disorder</td>
<td>7 (1.9%)</td>
</tr>
</tbody>
</table>

*Table 3.1: Frequency of diagnoses in the general psychiatric population.*
3.3.2 Gender
The effect of gender on diagnosis was explored. Contingency tables were constructed for each diagnosis by gender and there were significant effects of gender on depression, anxiety disorders, ADHD, schizophrenia and personality disorder. Full results are described in Table 3.2.
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N (male)</th>
<th>% male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>52</td>
<td>40.3%</td>
</tr>
<tr>
<td>Attention Deficit Hyperactivity Disorder</td>
<td>38</td>
<td>63%</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>31</td>
<td>81.6%</td>
</tr>
<tr>
<td>Bipolar Affective Disorder</td>
<td>27</td>
<td>40.9%</td>
</tr>
<tr>
<td>Anxiety Disorder</td>
<td>27</td>
<td>35.1%</td>
</tr>
<tr>
<td>Autism Spectrum Disorder</td>
<td>15</td>
<td>71.4%</td>
</tr>
<tr>
<td>Personality Disorder</td>
<td>11</td>
<td>23.9%</td>
</tr>
<tr>
<td>Obsessive Compulsive Disorder</td>
<td>5</td>
<td>33.3%</td>
</tr>
<tr>
<td>Eating Disorder</td>
<td>1</td>
<td>14.2%</td>
</tr>
</tbody>
</table>

Table 3.2: Gender differences in psychiatric disorder. Significant gender differences are highlighted in bold.
3.4 Results

3.4.1 Distribution and prevalence of joint hypermobility in the psychiatric population

The 9 point Beighton scale was used to assess joint hypermobility. The fifth metacarpophalangeal joint was scored as hypermobile if it could be extended >90°, the thumb was scored as hypermobile if it could be opposed to the wrist, the elbows and knees were scored as hypermobile if they could be extended >10°, and the trunk was scored as hypermobile if both palms could be placed flat on the floor with the knees straight. Scores were recorded for the individual joints, and a total score (of a maximum of 9) was ascertained. See Figure 1.6 in Chapter 1. Two hundred and fifty three (67.1%) patients scored 1 or more on the scale. A cut-off of ≥4 hypermobile joints was used to define generalized joint laxity, based on the cut-off most commonly cited in the literature and the method used to assess the two reference populations (see Chapter 1). The distribution of scores amongst the whole psychiatric population studied is shown in Figure 3.1A.

One hundred and forty two patients (37.7%)% scored 4 or more and as such were classed as hypermobile (Figure 3.1B). The effects of gender and age (previously documented in general population) on hypermobility were explored in this cohort and as expected, an effect of both was found. Hypermobile subjects were significantly more likely to be female (male hypermobile 24.9%, female hypermobile 49.5%; $\chi^2$ (1, N=377)=24.309) and younger: ((mean age(years), ±SEM)) hypermobile (35.01,0.92), non-hypermobile ((41.23,0.80, t(375)=5.08)). The distribution of Beighton scores by gender is illustrated in Figure 3.1C and mean Beighton score was significantly higher in women (3.53, 0.20) than men ((1.85,0.20), t(375)= 6.42). A significant correlation was found between Beighton score and age ($r$(375)=0.26). This remained after correction for gender.
Figure 3.1: Hypermobility in the patient group. A: Distribution of hypermobility score in the general psychiatric population. B: Distribution of hypermobility by gender. C: Significant differences in mean Beighton score in men and women.
3.4.2 Prevalence of joint hypermobility compared to reference populations

Prevalence estimates of joint hypermobility vary widely, including by ethnicity, gender and age and assessment criteria (see Chapter 1). By far and away the largest epidemiological study of clinician-assessed joint hypermobility to date comes from the UK and studied 6,022 adolescents as part of the ALSPAC birth cohort, finding 10.6% of males with a Beighton score of 4 or more and 27.6% of females with a Beighton score of 4 or more (in total 19.19%). Although the populations are not directly comparable due to differences in age, I believe the sample size and the method of assessment makes this the most suitable comparator of available data sets. This rate is equivalent to that found in a large self-report questionnaire survey (Mulvey et al., 2013) which surveyed 12,853 adult participants (median age 55, range 25-107) and found 18.3% to be hypermobile.

Twenty nine control participants, who were recruited as part of a separate study (to validate the ASQoLS) using the same tools (Iodice et al., in preparation), served as an additional reference population. There were no differences in sex or age compared to the psychiatric sample in this population. Three of these 29 control participants (10.3%) were hypermobile. There was no significant difference in rates of hypermobility in the ASQoLS control group compared to the general ALSPAC population.

Contingency tables were used to explore whether rates of hypermobility differed between the psychiatric population and the reference populations. There was a significant difference found between the reference populations and the general psychiatric population, with higher rates of joint hypermobility in the psychiatric population compared to general population (ALSPAC cohort) (χ²(1, N=6339)=74.85) and controls (ASQoLS validation study) (χ²(1, N=406)=8.76) (Figure 3.2). This difference remains statistically significant when taking into account the effects of gender (see below).
Figure 3.2: Rates of hypermobility in controls, the general population and the general psychiatric population. This demonstrates statistically significantly higher rates of hypermobility in the psychiatric population compared to both controls (Iodice et al., in preparation) and the general population (Clinch et al., 2011)
3.4.3 Differences in rates of joint hypermobility by diagnosis

As discussed in the literature review above, hypermobility has mainly been studied in anxiety disorders. Little is known about rates of hypermobility in other psychiatric disorders. Rates of hypermobility by diagnosis were compared to the ALSPAC cohort. Significant differences were found in whole group ($\chi^2(1,N=6399)=74.85$), depression ($\chi^2(1,N=6151)=66.27$), anxiety disorder ($\chi^2(1,N=6099)=60.19$), bipolar disorder ($\chi^2(1,N=6088)=12.31$), ADHD ($\chi^2(1,N=6082)=28.60$), ASD ($\chi^2(1,N=6043)=14.79$) and eating disorder ($\chi^2(1,N=6029)=12.27$). Schizophrenia was negatively associated with hypermobility ($\chi^2(1,N=6060)=4.74$). However the sample sizes for ASD and eating disorder were significantly underpowered to explore this effect. Rates of hypermobility in obsessive compulsive disorder and personality disorder were no different to the (ALSPAC) reference population. Full results are reported in Table 3.3 and Figure 3.3.
<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Hypermobile</th>
<th>Non-hypermobile</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALSPAC</td>
<td>6022</td>
<td>1156 (19.19%)</td>
<td>4866 (80.81%)</td>
<td></td>
</tr>
<tr>
<td>Psychiatric population</td>
<td>377</td>
<td>142 (37.7%)</td>
<td>235 (62.3%)</td>
<td>2.38(1.95-2.90)</td>
</tr>
<tr>
<td>Depression</td>
<td>129</td>
<td>62 (48.1%)</td>
<td>67 (51.9%)</td>
<td>3.75(2.67-5.26)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>77</td>
<td>42 (54.5%)</td>
<td>35 (45.5%)</td>
<td>4.90(3.15-7.65)</td>
</tr>
<tr>
<td>Bipolar</td>
<td>66</td>
<td>24 (36.4%)</td>
<td>42 (63.6%)</td>
<td>2.38(1.45-3.91)</td>
</tr>
<tr>
<td>ADHD</td>
<td>60</td>
<td>28 (46.7%)</td>
<td>32 (53.3%)</td>
<td>3.62(2.19-5.99)</td>
</tr>
<tr>
<td>Personality disorder</td>
<td>46</td>
<td>13 (28.3%)</td>
<td>33(71.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>38</td>
<td>2 (5.3%)</td>
<td>36 (94.7%)</td>
<td>0.24(0.06-0.098)</td>
</tr>
<tr>
<td>OCD</td>
<td>15</td>
<td>4 (26.7%)</td>
<td>11(73.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>ASD</td>
<td>21</td>
<td>11(52.4%)</td>
<td>10 (47.6%)</td>
<td>4.60(1.96-10.80)</td>
</tr>
<tr>
<td>Eating Disorder</td>
<td>7</td>
<td>5 (71.4%)</td>
<td>2 (28.6%)</td>
<td>10.48(2.04-53.96)</td>
</tr>
</tbody>
</table>

Table 3.3: Rates of hypermobility in the psychiatric population, with significant differences from the reference population (Clinch et al., 2011) in bold.
3.4.4 Effect of gender

As gender was associated both with hypermobility and diagnosis, separate analyses were performed by gender, using only the same gender as a reference population (see Figure 3.3B).

Results for women are shown in Table 3.4. Significant differences in rates of hypermobility were found in the psychiatric population ($\chi^2(1, N=3257)=43.38$), depression ($\chi^2(1, N=3136)=38.10$), anxiety ($\chi^2(1, N=3111)=36.06$) and ADHD ($\chi^2(1, N=3083)=22.24$).
<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Hypermobile</th>
<th>Non hypermobile</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALSPAC</td>
<td>3061</td>
<td>842 (27.5%)</td>
<td>2219 (72.5%)</td>
<td></td>
</tr>
<tr>
<td>Psychiatric population</td>
<td>196</td>
<td>97 (49.5%)</td>
<td>99 (50.5%)</td>
<td>2.42 (1.85-3.17)</td>
</tr>
<tr>
<td>Depression</td>
<td>75</td>
<td>45 (60%)</td>
<td>30 (40%)</td>
<td>3.80 (2.41-5.60)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>50</td>
<td>33 (66%)</td>
<td>17 (34%)</td>
<td>4.96 (2.78-8.86)</td>
</tr>
<tr>
<td>Bipolar</td>
<td>39</td>
<td>16 (41%)</td>
<td>23 (59%)</td>
<td>NS</td>
</tr>
<tr>
<td>Personality disorder</td>
<td>35</td>
<td>11 (31.4%)</td>
<td>24 (68.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>ADHD</td>
<td>22</td>
<td>16 (72.7%)</td>
<td>6 (27.3%)</td>
<td>6.91 (2.72-17.61)</td>
</tr>
<tr>
<td>OCD</td>
<td>10</td>
<td>3 (30%)</td>
<td>7 (70%)</td>
<td>NS</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>7</td>
<td>1 (14.3%)</td>
<td>6 (85.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Eating Disorder</td>
<td>6</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>ASD</td>
<td>6</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 3.4: Rates of hypermobility in the female psychiatric population, with significant differences from the female ALSPAC cohort (Clinch et al., 2011) in bold.
When analysing women separately, the significant difference between rates in eating disorder and autism spectrum disorder and the general population is lost, this is likely because the sample size is too small.

Significantly higher rates of hypermobility in men (Table 3.5) were established in the psychiatric population as a whole ($\chi^2(1,N=3142)=34.26$), depression ($\chi^2(1,N=3013)=21.30$), ADHD ($\chi^2(1,N=2999)=17.04$), bipolar disorder ($\chi^2(1,N=2988)=10.07$), anxiety ($\chi^2(1,N=2986)=7.82$), and ASD ($\chi^2(1,N=2976)=20.17$).
<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Hypermobile</th>
<th>Non-hypermobile</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALSPAC</td>
<td>2961</td>
<td>314 (10.6%)</td>
<td>2647 (89.4%)</td>
<td></td>
</tr>
<tr>
<td>Psychiatric population</td>
<td>181</td>
<td>45 (24.9%)</td>
<td>136 (75.1%)</td>
<td>2.57 (1.87-3.53)</td>
</tr>
<tr>
<td>Depression</td>
<td>52</td>
<td>16 (30.8%)</td>
<td>36 (69.2%)</td>
<td>3.61 (2.03-6.44)</td>
</tr>
<tr>
<td>ADHD</td>
<td>38</td>
<td>12 (31.6%)</td>
<td>26 (68.4%)</td>
<td>3.78 (1.93-7.43)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>31</td>
<td>1 (3.2%)</td>
<td>30 (96.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Bipolar</td>
<td>27</td>
<td>8 (29.6%)</td>
<td>19 (70.4%)</td>
<td>3.49 (1.54-7.90)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>25</td>
<td>7 (33.3%)</td>
<td>18 (66.7%)</td>
<td>3.22 (1.36-7.67)</td>
</tr>
<tr>
<td>Personality disorder</td>
<td>11</td>
<td>2 (18.2%)</td>
<td>9 (81.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>ASD</td>
<td>15</td>
<td>4 (46.7%)</td>
<td>8 (53.3%)</td>
<td>7.24 (2.64-19.82)</td>
</tr>
<tr>
<td>OCD</td>
<td>5</td>
<td>1 (20%)</td>
<td>4 (80%)</td>
<td>NS</td>
</tr>
<tr>
<td>Eating Disorder</td>
<td>2</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 3.5: Rates of hypermobility in the male psychiatric population, with significant differences from the male ALSPAC cohort (Clinch et al., 2011) in bold.
In general, the same diagnoses have significantly higher rates of hypermobility as men in the general population, but the proportion of hypermobile individuals is different between sexes. This is significantly true for anxiety, depression, and ADHD.
Figure 3.3: Rates of hypermobility in the ALSPAC cohort (general population) and psychiatric patients. 3A demonstrates the different rates of hypermobility in psychiatric population and 3B by gender.
3.4.5 Effect of adult neurodevelopmental conditions

Patients were sampled from adult psychiatric clinics. A secondary hypothesis was to explore the anecdotal link e.g (Kirby and Davies, 2007, Koldas Dogan et al., 2011, Tantam et al., 1990) between adult neurodevelopmental conditions (ADHD and ASD) and hypermobility. As such patients attending the specialist neurodevelopmental service were also invited to take part. I have observed significantly high rates of hypermobility in ADHD and ASD. In order to explore whether the purposeful sampling of patients from this clinic were driving the high rates of hypermobility found in the general psychiatric population, I first tested to see if the remaining psychiatric population (i.e. excluding all those with a diagnosis of a neurodevelopmental condition) exhibited higher rates of hypermobility than the ALPSAC cohort, and indeed they did: across the whole group (35.2%); males (21.5%) and females respectively (45.8%) ($\chi^2(1,N=6318)=45.73$, $\chi^2(1,N=3089)=13.19$, $\chi^2(1,N=3229)=17.19$). Secondly, I looked to see if there was a significant difference in the rates of hypermobility between the two patient populations, and at whole group level there was none. However, there were significantly higher rates of hypermobility in the female neurodevelopmental population than in the general psychiatric population (70.3% and 46.2% respectively).
3.5 Discussion

It has been known for some time that hypermobility is more overrepresented in certain psychiatric conditions, e.g. anxiety and depression (Sanches et al., 2012, Smith et al., 2014). However, for the first time I have demonstrated that hypermobility is significantly more prevalent in the psychiatric population attending secondary mental health services, than in the general population with an OR of 2.38 (1.95-2.90) for risk of hypermobility in the psychiatric population. Moreover, there are significantly higher rates with particular diagnoses, e.g. anxiety disorder and ADHD. Although some of this consistent with previous studies e.g. (Martin-Santos et al., 1998, Koldas Dogan et al., 2011), much of my data is novel, for example the association between ADHD and hypermobility in adults. A complicating factor is the presence of multiple psychiatric diagnoses in the same person, for example symptoms of depression and anxiety are commonly found together and anxiety symptoms are common across almost all psychiatric diagnoses.

I also show there to be significant effects of gender, for example 72.7% of women with ADHD are hypermobile compared to 31.6% of men, which is perhaps consistent with the gender effects found in the large survey (2,600 participants) of Brazilian students conducted by Sanches et al. This demonstrated for the first time gender specific effects of hypermobility on anxiety: finding that hypermobile women score significantly higher on anxiety scales than hypermobile men (Sanches et al., 2014).

Although the numbers are small, I have explored the relationship between joint hypermobility and conditions such as bipolar disorder, autism spectrum disorder which have not previously been studied systematically. I replicate for the first time existing work that demonstrates high rates of hypermobility in eating disorder patients (Goh et al., 2013). Replication of these findings in larger studies would be of particular importance to the field.
Given the high rates of hypermobility in this population, in the next chapter, I go on to explore whether, as hypothesised in Chapter 1, this is associated with autonomic dysfunction, and, whether there are, again, specific effects of gender.
Chapter 4: Is hypermobility associated with symptoms suggestive of autonomic dysfunction in the psychiatric population?
4.1 Introduction and aims

Emotion is dynamically coupled to the autonomic nervous system (Critchley et al., 2013), yet surprisingly there is little systematic evaluation of dysautonomia in psychiatric disorder. This chapter seeks to address this. Additionally, it is known that hypermobility is associated with autonomic dysfunction (Gazit et al., 2003, Hakim and Grahame, 2004), typically postural tachycardia syndrome (Mathias et al., 2012), in which there is a phenomenological overlap with anxiety disorder. These investigations typically focus on orthostatic intolerance, whereas this chapter takes a multi-system approach.

4.2 Hypotheses

1. Symptoms suggestive of autonomic dysfunction will be higher in the psychiatric population compared to general population controls.

2. Symptoms suggestive of autonomic dysfunction will be greater in hypermobile psychiatric patients compared to non-hypermobile psychiatric patients.

3. Symptoms suggestive of autonomic dysfunction will positively correlate with degree of hypermobility as determined by the Beighton score.

4. Symptoms suggestive of orthostatic intolerance will mediate the relationship between anxiety and hypermobility.

5. Patients taking psychotropic medication will have higher autonomic dysfunction.

6. Hypermobile participants will demonstrate higher symptoms suggestive of autonomic dysfunction even if medicated and participants with hypermobility will show a differential effect of medication on their symptoms.
4.3 Patient demographics

Questionnaires were collected from 416 patients attending adult psychiatric clinics in Brighton and Hove since February 2013. Forty-one were excluded due to missing or incomplete data. Forty-eight percent of respondents were male, 52% were female. Ages ranged from 18 – 65 years old, (mean age (years), ±SEM) (38.9, 0.61). There was no significant difference in ages between the two sexes. In addition, data was compared to reference data collected from 29 healthy controls in the validation process of the ASQoLS (Iodice et al., in preparation); there were no significant differences in gender (15, 51.7% male) and age (range 19-68 (37.9, 3.32)) between patients and controls.

4.4 Results

4.4.1 Prevalence of core symptoms suggestive of autonomic dysfunction and effect of gender

Patients were also asked about symptoms suggestive of autonomic dysfunction, including dizziness, fainting and symptoms of pre-syncope (Table 4.1). Dizziness was reported more frequently in women than men ($\chi^2(1,N=375)=7.90$).
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Total yes</th>
<th>Male yes</th>
<th>Female yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you feel dizzy or lightheaded?</td>
<td>287 (76.5%)</td>
<td>127 (70.2%)</td>
<td>160 (81.9%)</td>
</tr>
<tr>
<td>Have you ever passed out/lost consciousness?</td>
<td>145 (38.7%)</td>
<td>60 (33.1%)</td>
<td>85 (43.8%)</td>
</tr>
<tr>
<td>Have you ever nearly fainted/swooned (not unconscious but blacked out or fell down from dizziness)?</td>
<td>210 (56%)</td>
<td>94 (51.9%)</td>
<td>116 (59.8%)</td>
</tr>
</tbody>
</table>

Table 4.1: Percentage of patients reporting common symptoms of autonomic dysfunction with significant gender differences in bold.
Rates of symptoms suggestive of autonomic dysfunction appear high in this population, with 38.7% of patients having fainting and 56% experiencing presyncope. Females experienced dizziness more frequently than men. For example, Serletis and colleagues estimate that fainting has a prevalence of 32% in the general population (Serletis et al., 2006). Our rates of fainting are significantly higher than this population. Additionally, the expression of syncopal symptoms may be complicated by use of psychotropic and sedative medication. Very few patients in our sample were medication free.

There was no statistically significant effect of age on autonomic symptoms.

4.4.2 Effect of hypermobility on common symptoms suggestive of autonomic dysfunction

Across the whole group, hypermobility had no association with common symptoms suggestive of autonomic dysfunction, however when considering individual diagnoses there were effects regardless of gender, with frequent dizziness and hypermobility in depression ($\chi^2(1,N=129)=3.90$). In patients with diagnosis of a neurodevelopmental condition, there were higher rates of fainting in the hypermobility group ($\chi^2(1,N=79)=6.71$).

4.4.3 Symptoms suggestive of autonomic dysfunction – type and frequency

Symptoms suggestive of autonomic dysfunction were assessed by the Autonomic Symptoms and Quality of Life Score (ASQoLS), (Iodice et al., in preparation). The questionnaire incorporates orthostatic, gastrointestinal, bladder, secretomotor, sudomotor and sleep domains and combines presence of symptoms with both frequency and impact on life. For the purposes of this analysis only, frequency (rather than quality of life) components were analysed and the musculoskeletal measure was removed, to produce a single autonomic measure (the musculoskeletal domain includes a Beighton Scale assessment, which would confound correlations with hypermobility score due to their direct association).
4.4.4 Prevalence of symptoms suggestive of autonomic dysfunction in psychiatric population

Patients reported significantly higher symptoms suggestive of total autonomic dysfunction ((mean, ±SEM: 59.0, 1.83) compared to controls, ((8.59, 1.62), t(402)=5.04)) (Figure 4.1A). This was true for all domains of the ASQoLS (orthostatic, t(402)=7.25; gastrointestinal, t(402)=5.78; bladder, t(402)=3.83; secretomotor, t(402)=5.60; sudomotor, t(402)=5.17; sleep, t=(402)4.69).

In the patients, total autonomic symptom score was not significantly affected by gender or by age.

4.4.5 Association between symptoms suggestive of autonomic dysfunction and psychiatric diagnosis

A sub analysis of the larger patient groups was undertaken.

4.4.5.1 Anxiety

Patients with anxiety reported significantly higher total symptoms suggestive of autonomic dysfunction (i.e. without musculoskeletal component) (mean, ±SEM; 69.50, 3.80) compared to those patients without anxiety ((56.14, 2.06), t(373)=5.04)). This was true for the following domains of the ASQoLS: orthostatic, (t(373)=4.28) (see Figure 4.1B), gastrointestinal (t(373)=2.35) and secretomotor (t(373)=2.13). Although there was no significant difference in mean autonomic symptom score between men and women, women reported more symptoms suggestive of orthostatic intolerance (40.80, 2.74) than men ((28.60, 2.40), t(75)=2.30)), illustrated in Figure 4.1B.

4.4.5.2 Depression

Although there was no significant difference in mean autonomic symptom score between patients with depression and those without, depressed patients reported more symptoms suggestive of orthostatic intolerance (31.67,1.72) (Figure 4.1C) than those without ((27.19, 1.15), t(373)=2.23)). Again, although there was no significant difference in mean autonomic symptom score between depressed men and women, women reported more symptoms suggestive of orthostatic intolerance (35.50, 2.37) than men ((26.15, 2.27), t(127)= 2.74)).
4.4.5.3 Adult patients with a diagnosis of a neurodevelopmental condition

As a group, adult patients with neurodevelopmental condition do not have greater symptoms suggestive of autonomic dysfunction than other patients, however there are interesting sex differences. Women with neurodevelopmental diagnoses report greater total symptoms suggestive of autonomic dysfunction and orthostatic intolerance than men ($t(75)=2.16$, $t(75)=1.60$).
Figure 4.1: Symptoms suggestive of autonomic dysfunction in the psychiatric population. A. Significant differences in autonomic symptoms in patients compared to controls. B. Significant differences in orthostatic intolerance scores in patients with anxiety compared to those without and differences in gender. C. Significant differences in
orthostatic intolerance scores in patients with depression compared to those without and differences in gender.
4.4.6 Association between symptoms suggestive of autonomic dysfunction and hypermobility

There was trend towards higher total symptoms suggestive of autonomic dysfunction in hypermobile patients compared to non-hypermobile patients (p=0.06) (Figure 4.2A). However, across all diagnoses, hypermobile compared to non-hypermobile patients reported significantly higher scores for symptoms suggestive of orthostatic intolerance, and gastrointestinal disturbance, (t(373)=2.64, t(373)=1.89). This is illustrated in Figure 4.2B. Although age is not significantly associated with symptoms suggestive of autonomic dysfunction, hypermobility is. These relationships remain after correction for age.

A significant effect of gender was seen (Figure 4.2B) (F(2,373)=23.87), with hypermobile males not having any statistically significantly higher rates of symptoms suggestive of autonomic dysfunction in any domain. Hypermobile women on the other hand reported significantly higher symptoms of orthostatic intolerance than non-hypermobile women ((30.86, 1.90), t(192)=2.01)) and higher symptoms suggestive of gastrointestinal disturbance (12.90, 1.13) compared to non-hypermobile women ((10.51, 0.67), t(192)=1.81)) again these relationships remain after correction for age.

Across all patients, symptoms suggestive of autonomic dysfunction increase with degree of hypermobility as assessed by Beighton score, with significant associations with total symptoms suggestive of autonomic dysfunction score, orthostatic intolerance and gastrointestinal disturbance, (r(373)=0.13, r(373)=0.18, r(373)=0.17). These survive corrections for age. Again a significant interaction of gender, was seen (F(2, 373)=16.10).
Figure 4.2: Relationship between hypermobility status and scores of symptoms suggestive of autonomic dysfunction. A: Higher total symptoms suggestive of autonomic dysfunction in hypermobile patients compared to non-hypermobile patients. B: Significantly higher symptoms suggestive of orthostatic intolerance in hypermobile patients compared to non-hypermobile patients with an effect of gender.
4.4.7 Association between symptoms suggestive of autonomic dysfunction and hypermobility in particular diagnoses

4.4.7.1 Anxiety
Across the whole group of anxiety patients there was no significant difference in total symptoms suggestive of autonomic dysfunction. However, there was a significantly greater difference in symptoms suggestive of orthostatic intolerance (Figure 4.3A): in hypermobile (41.21, 18.99) compared to non-hypermobile patients with anxiety ((30.89, 15.60), t(75)=2.58)) This survives correction for age and is associated with an interaction of gender (F(2,75)=5.25).

In men with anxiety, symptoms suggestive of autonomic dysfunction were not significantly higher in the hypermobile group, however women with hypermobility reported significantly higher symptoms suggestive of gastrointestinal disturbance (13.51, 1.51) than those without hypermobility ((9.88, 1.26), t(25)=1.76)).

Across the Anxiety group, Beighton Score was positively correlated with orthostatic intolerance symptom score, (r(75)=0.29) (Figure 4.3B) . There was a significant interaction between gender and Beighton score on total symptoms suggestive of autonomic dysfunction (F(2,75)=3.09). Although the correlation remains after correction for age, the interaction does not.

4.4.7.2 Depression
There were no significant differences in symptoms suggestive of autonomic dysfunction between the hypermobile group compared to the non-hypermobile group. When correcting for age, symptoms suggestive of gastrointestinal disturbance are significantly higher in the hypermobile group, (t(127)=1.70). When assessing the relationship between Beighton score and symptoms suggestive of autonomic dysfunction, significant effects were found in females only, in both total autonomic symptoms score, and orthostatic intolerance (r(73)=0.241, r(73)=0.287). Moreover, a significant
interaction with gender was observed (F(2,127)= 0.26) (Figure 4.3). These associations remain after correction for age.

4.4.7.3 Adult patients with a diagnosis of a neurodevelopmental condition

No significant differences in total symptoms suggestive of autonomic dysfunction between non-hypermobile and hypermobile were found in this group, however gastrointestinal disturbance was associated with Beighton score (r(75)=0.25). This effect was rendered non-significant when looking at males and females separately. However, when looking only at those who are hypermobile significant correlations were found with Beighton Score. Across this group there were correlations with total symptoms of autonomic dysfunction score, and symptoms of gastrointestinal disturbance (r(34)=0.38, r(34)=0.35). This survives correction for age.
Figure 4.3: Relationship between symptoms of orthostatic intolerance and hypermobility status in particular diagnoses. A: Significantly higher rates of symptoms of orthostatic intolerance in patients with anxiety who are hypermobile compared to patients with anxiety who are not hypermobile. B: In patients with anxiety, degree of hypermobility is positively correlated with symptoms of orthostatic intolerance. C: In patients with depression, degree of hypermobility is positively correlated with symptoms of orthostatic intolerance with a significant effect of gender.
4.4.8 Mediation between hypermobility, symptoms suggestive of autonomic dysfunction and anxiety.

It has been well established by several separate lines of research that joint hypermobility is related to anxiety (Smith et al., 2014). It has also been established joint hypermobility is related to signs and symptoms of autonomic dysfunction, typically orthostatic intolerance (De Wandele et al., 2013, Gazit et al., 2003, Mathias et al., 2012). Linking these themes it seems that perhaps autonomic dysfunction might underpin the relationship between joint hypermobility and anxiety and as such I wished to explore the whether the established relationship between joint hypermobility and anxiety is in fact mediated by the relationship between joint hypermobility and symptoms of autonomic dysfunction. Using my dataset I used mediation analysis (method of (Baron and Kenny, 1986) to test this hypothesis.
Figure 4.4: Proposed mediation model to test whether orthostatic intolerance mediates the relationship between joint hypermobility and anxiety
Mediation analysis was used to explore pathways linking hypermobility as predictor (X) to symptoms of orthostatic intolerance as mediator (M) and Anxiety diagnosis as dependent variable (Y). Mediation first requires that the predictor is significantly and independently related to all mediators and to the dependent variable. To establish mediation, the direct relationship between predictor and outcome (hypermobility and Anxiety) must lose significance when a mediator is entered. All associations were corrected for age, given the relationship between hypermobility and age.

### 4.4.8.1 Mediation across the whole psychiatric population between hypermobility status, symptoms suggestive of orthostatic intolerance and anxiety.

#### 4.4.8.1.1 Main effect
In this model hypermobility status (our predictor) was related to anxiety (our dependent variable) ($r(372)=0.19$). It was also significantly related to mediator (symptoms of orthostatic intolerance) ($r(372)=0.13$). Symptoms of orthostatic intolerance were also significantly related to anxiety, ($r(372)=0.22$). Hypermobility status was then entered in a multiple regression model along with symptoms of orthostatic intolerance to explore their relationships to anxiety when assessed together. To establish mediation, the direct relationship between predictor and outcome (hypermobility status and anxiety) must lose significance when a mediator is entered. In this model, hypermobility status effects on anxiety were rendered less significant ($r(371)=0.17$). Thus, symptoms of orthostatic intolerance met criteria for partial mediation of the relationship between hypermobility status and anxiety (Figure 4.5 A).

#### 4.4.8.1.2 Effect of gender
Mediation analysis was not possible in males as all the assumptions could not be met. However in females, in the mediation model, hypermobility status effects on anxiety were rendered less significant by symptoms of orthostatic intolerance ($r(190)=0.18$). Thus symptoms of orthostatic
intolerance met criteria for partial mediation of the relationship between hypermobility status and anxiety in females. (Figure 4.5B)

**4.4.8.2 Mediation across the whole psychiatric population between degree of hypermobility, symptoms of orthostatic intolerance and anxiety**

**4.4.8.2.1 Main effect**
In this model, hypermobility score (our predictor) was related to Anxiety (our dependent variable) \( r(372)=0.27 \). It was also significantly related to a mediator, symptoms of orthostatic intolerance \( r(372)=0.18 \). In this model, hypermobility status effects on anxiety were rendered less significant \( r(371)=0.24 \). Thus symptoms of orthostatic intolerance met criteria for partial mediation of the relationship between hypermobility status and anxiety. (Figure 4.5C)

**4.4.8.2.2 Effect of gender**
Mediation analysis was not possible in males as all the assumptions could not be met. However in females, in this mediation model, hypermobility status effects on anxiety \( r(191)=0.31 \) were rendered less significant by symptoms of orthostatic intolerance \( r(190)=0.29 \), thus symptoms of orthostatic intolerance met criteria for partial mediation of the relationship between hypermobility status and anxiety in females (Figure 4.5D).
Figure 4.5: Mediation analyses demonstrating the effect of symptoms of orthostatic intolerance on the relationship between hypermobility and anxiety, corrected for age. A: Partial mediation by symptoms of orthostatic intolerance on relationship between hypermobility status and anxiety in the whole psychiatric group. B: Partial mediation by symptoms of orthostatic intolerance on relationship between hypermobility status and anxiety in females only. C: Partial mediation by symptoms of orthostatic intolerance on relationship between hypermobility score and anxiety in the whole psychiatric group. D: Partial mediation by symptoms of orthostatic intolerance on relationship between hypermobility score and anxiety in females only.
4.4.9 Association between medication and symptoms suggestive of autonomic dysfunction

The expression of autonomic symptoms may be complicated by use of psychotropic and sedative medication. Complete medication data was available for 348 participants, of whom 266 (76.4%) were taking medication. The mean number of classes of medication was 1.8 and the distribution is shown in Figure 4.6A. The frequency of classes of medication is shown in Table 4.2 below:
<table>
<thead>
<tr>
<th>Class</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical antipsychotic</td>
<td>138</td>
<td>35.5</td>
</tr>
<tr>
<td>SSRI</td>
<td>108</td>
<td>27.8</td>
</tr>
<tr>
<td>Benzodiazapine</td>
<td>76</td>
<td>19.5</td>
</tr>
<tr>
<td>Mood stabilizer</td>
<td>52</td>
<td>13.4</td>
</tr>
<tr>
<td>SNRI</td>
<td>46</td>
<td>11.8</td>
</tr>
<tr>
<td>NASSA</td>
<td>41</td>
<td>10.5</td>
</tr>
<tr>
<td>Opiate</td>
<td>40</td>
<td>10.3</td>
</tr>
<tr>
<td>Anxiolytic</td>
<td>38</td>
<td>9.8</td>
</tr>
<tr>
<td>Stimulant</td>
<td>30</td>
<td>7.7</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>22</td>
<td>5.7</td>
</tr>
<tr>
<td>Anti-histamine</td>
<td>21</td>
<td>5.4</td>
</tr>
<tr>
<td>Typical antipsychotic</td>
<td>8</td>
<td>2.1</td>
</tr>
<tr>
<td>Tricyclic antidepressant</td>
<td>7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table 4.2: Frequency of prescription of different classes of psychotropic medication in sample.
The use of medication was associated with both common symptoms suggestive of autonomic dysfunction such as experiencing dizziness daily, and greater autonomic dysfunction symptom score ($\chi^2(1,N=348)=3.83$), ($t(346)=5.42$) (Figure 4.6B). The presence of SSRIs, benzodiazepines, opiates, anti-histamines and anxiolytics only was associated with greater total autonomic symptom score ($t(346)=3.93$, $t(346)=2.67$, $t(346)=5.53$, $t(346)=3.27$, $t(346)=3.13$), particularly orthostatic intolerance.

4.4.9.1 Associations with hypermobility

There was no association between medication status (medication or not) and hypermobility. There was no difference in number of classes of medications taken by those with hypermobility compared to those without. However, participants with hypermobility were significantly more likely to be taking SSRIs, compared to those without, ($\chi^2(1,N=348)=5.69$)

4.4.9.2 Interaction with hypermobility on symptoms suggestive of autonomic dysfunction

In terms of experiencing dizziness daily, hypermobility had a significant interaction with medication status ($F(2, 346)=4.67$) (Figure 4.7C). For both medicated and un-medicated individuals greater total symptoms suggestive of autonomic dysfunction and symptoms suggestive of orthostatic intolerance were associated with hypermobility status (Figure 4.6D). Additionally, there was positive correlation with total symptoms of autonomic dysfunction, orthostatic intolerance and hypermobility score.
Figure 4.6: Medication use in the psychiatric population. A: Frequency plot of number of classes of medication. B: Plot showing significantly higher symptoms suggestive of autonomic dysfunction in those taking medication. C: Plot showing effect of medication on experience of feeling dizzy daily, with significant interaction of hypermobility status. D: Plot showing significantly higher symptoms suggestive of autonomic dysfunction in hypermobile group regardless of medication use.
Interestingly the presence or absence of a particular class of medication interacted with hypermobility on the total degree of symptoms suggestive of autonomic dysfunction for some classes of medications, such that the relationship between symptoms suggestive of autonomic dysfunction and hypermobility was significantly different when taking the medication compared to not taking the medication.

For example, of patients not taking an SSRI, hypermobile patients demonstrated significantly higher total symptoms suggestive of autonomic dysfunction and symptoms suggestive of orthostatic intolerance than non-hypomobile, (t(240)=3.22, t(240)=3.02). However, there was no association between symptoms suggestive of autonomic dysfunction and hypermobility if taking an SSRI. The difference of these is significant (F(2,346)=8.31) (Figure 4.7A). This divergent relationship is significant in the same direction for opiates, antihistamines and anxiolytics (F(2,346)=27.18, F(2,346)=8.83, F(2,346)=7.64). Conversely, the relationship between symptoms suggestive of autonomic dysfunction and hypermobility is greater in those taking atypical antipsychotics and beta-blockers than those not (F(2,346)=4.53, F(2,346)=10.41) (Figure 4.7B; C)
Figure 4.7: The effect of medication on the relationship between hypermobility and symptoms suggestive of autonomic dysfunction. A: Plot showing effect of SSRI medication with significant interaction of medication. B: Plot showing effect of atypical antipsychotic medication with significant interaction of medication. C: Plot showing effect of beta-blocker medication with significant interaction of medication.
4.5 Discussion

Emotion is dynamically coupled to the autonomic nervous system (Critchley et al., 2013), and we know that the experience of worry (perseverative cognition), typical of anxiety disorder, is itself associated with abnormal autonomic profiles (Brosschot et al., 2006, Ottaviani et al., 2015). Yet surprisingly, there is little systematic evaluation of dysautonomia in psychiatric disorder. It is known that hypermobility is associated with symptoms of autonomic dysfunction (Hakim and Grahame, 2004) and that hypermobility is associated with certain psychiatric disorders, but the direct relationship between joint hypermobility, autonomic dysfunction and psychiatric symptoms is underappreciated.

I demonstrate that symptoms suggestive of autonomic dysfunction are higher in psychiatric patients compared to controls. I find for the first time that symptoms suggestive of autonomic dysfunction are greater in general psychiatric patients with hypermobility compared to those without. I show that symptoms of autonomic dysfunction correlate with degree of hypermobility, even in patients who are not classified as hypermobile. I discover that symptoms suggestive of orthostatic intolerance partially mediate the relationship between anxiety and hypermobility, both status and score. These last three findings show interesting gender differences. However, this is in keeping with recent work in a non-clinical sample in which Sanches and colleagues show significantly higher symptoms of autonomic dysfunction (as demonstrated on BAI autonomic subscale) in women with hypermobility, compared to men with hypermobility (Sanches et al., 2014).

I demonstrate that medicated patients have higher total symptoms suggestive of autonomic dysfunction, and this varies by class of medication. However, from cross-sectional data, and without illness severity measures, it is impossible to know whether this is cause or effect, but is consistent with the side-effect profile of many psychotropic medications. The two most commonly prescribed psychotropic medications in my sample were atypical antipsychotics and SSRIs. Many commonly prescribed antipsychotic drugs
interact with numerous receptors both centrally and peripherally. These include dopaminergic, serotonergic, histaminergic, a-adrenergic and muscarinic receptors. The non-specific nature of their pharmacological action may result in adverse cardio-vascular side effects such as orthostatic hypotension and syncope (Mackin, 2008). It is thought that up to 75% of patients receiving anti-psychotic medication may experience hypotension (Stanniland and Taylor, 2000) and Mackin reports that the incidence of syncope varies from around 0.2% in olanzapine and risperidone-treated patients, 1% in patients treated with quetiapine to 6% following exposure to clozapine (Mackin, 2008). Frequent side effects of anti-depressants (e.g SSRI) include dry mouth, sweating, dizziness and gastrointestinal disturbance (Ferguson, 2001). It is also known that different classes of antidepressants have differential effects on sympathetic control, e.g. patients on SSRIs show increased sympathetic activity compared to those on SNRIs (Licht et al., 2012). As such data presented on symptoms suggestive of autonomic dysfunction may be confounded by medication use as the common side-effects are often the very same symptoms suggestive of autonomic dysfunction measured in this chapter. However it is important to note that there were no general differences in the medication status of hypermobile participants compared to non-hypermobile participants, i.e there was no association between medication status (medication or not) and hypermobility. There was no difference in number of classes of medications taken by those with hypermobility compared to those without. And as such although medication status may confound the expression of symptoms suggestive of autonomic dysfunction this would apply to both hypermobile and non-hypermobile and yet I still demonstrate a difference in symptoms suggestive of autonomic dysfunction between hypermobile and non-hypermobile patients and, crucially a differential of medication of symptoms suggestive of autonomic dysfunction.

I also demonstrate that my main finding of symptoms suggestive of higher autonomic dysfunction in hypermobility is not confounded by medication use. Interestingly, however I show that hypermobility interacts with specific medication on symptoms of autonomic dysfunction in different ways with
different classes of medication. For example if taking atypical antipsychotics (the most frequently prescribed medication in this sample (commonly included drugs such as quetiapine and risperidone)) or beta-blockers (typically propranolol in this sample) the difference in symptoms suggestive autonomic dysfunction between hypermobile individuals and non-hypermobile individuals becomes significantly greater. Whereas in individuals medicated with SSRIs, who display higher symptoms suggestive of autonomic dysfunction that those not taking SSRIs, there is no difference between hypermobile and non-hypermobile in terms of symptoms of autonomic dysfunction. Unfortunately my data is not organised to explore the effects of individual medications rather than classes of medication, but these findings endorse the need for further work that will ultimately lead to personalised pharmacological medicine.

It must however be borne in mind that the scale for assessing symptoms suggestive of autonomic dysfunction, the ASQoLS, is likely non-specific as many symptoms are common and frequent to many psychiatric disorder and the side-effects of psychiatric medication and this is a potential limitation. However despite this, I have shown clear differences in symptoms suggestive of autonomic dysfunction between patients with hypermobility and those without.

This novel set of data has several implications. Firstly, it will be important to screen psychiatric patients for possible symptoms of autonomic dysfunction, particularly orthostatic intolerance and gastro-intestinal disturbance. Secondly, this may have direct benefit in terms of stratifying individuals for particular treatment strategies, both pharmacological and psychotherapeutic. It also gives direct insight into (autonomic) factors potentially mediating the relationship between joint hypermobility and anxiety. Having determined the relationship between symptoms of autonomic dysfunction, psychiatric symptoms and joint hypermobility, in the next chapter, I will go on to explore the relationship between signs of autonomic dysfunction, joint hypermobility and anxiety.
Chapter 5: What is the relationship between hypermobility, anxiety and autonomic dysfunction?
5.1 Introduction and aims

Previous studies suggest that hypermobility is associated with both signs and symptoms of autonomic dysfunction (De Wandele et al., 2013, Gazit et al., 2003, Mathias et al., 2012). However, the relationship between this and anxiety remains unexplored, particularly in relationship to parasympathetic function. Existing research focuses only on hypermobility diagnoses rather than taking into account the relationship with potential psychiatric co-morbidities, such as anxiety. The aim of this chapter is to address this gap in the literature.

5.2 Hypotheses

1. Anxious and hypermobile participants will show greater sustained heart rate on standing suggestive of sympathetic activation and reduced heart rate and blood pressure changes during deep breathing suggesting parasympathetic dysfunction.

2. This pattern of dysfunction will be preferentially expressed in hypermobility, such that if not hypermobile there will be no difference in function between anxious and non-anxious participants.

3. Signs of autonomic dysfunction will correlate with degree of hypermobility.

5.3 Sample characteristics and patient demographics

Sixty-six participants were studied: of whom 29 (43.9%) were classified as anxious using DSM – IV criteria for Generalised anxiety disorder (GAD). 37 (56.1%) did not meet criteria for GAD: and thus served as controls. Of those who met criteria for GAD, 16 (55.2%) were classified as hypermobile (Beighton score ≥ 4) and 16 (55.2%) met Brighton criteria for Hypermobility syndrome. Of the controls, 18 (48.6%) were classified as hypermobile and 13 (44.8%) met criteria for Hypermobility syndrome. There was no significant difference in age or gender between the two groups. In terms of anxiety symptoms there was a significant difference in score (mean, ±SEM) on the Beck Anxiety Inventory between GAD patients and controls ((22.2,
2.07) (6.03, 0.86), t(64)=7.83), illustrated in Figure 5.1. If anxious there was no significant difference in anxiety scores between hypermobile participants and non-hypermobile participants or hypermobility syndrome participants and non-hypermobility syndrome participants.
Figure 5.1: Anxiety characterisation. This figure demonstrates significant difference in mean anxiety score between anxiety patients and controls regardless of hypermobility status. Error bars represent one standard error of the mean.
5.4 Results

In terms of symptoms of autonomic dysfunction ASQoL data was available for 59 participants. In terms of signs of autonomic dysfunction heart rate and blood pressure data was available for 60 participants during autonomic function challenge.

5.4.1 Symptoms suggestive of autonomic dysfunction

5.4.1.1 Symptoms suggestive of autonomic dysfunction: main effect of anxiety

There were significant differences in total symptoms suggestive of autonomic dysfunction as characterised by the ASQoL (mean, ±SEM) between patients and controls ((58.78, 6.49) (21.25, 2.92), t(57)=5.56)), illustrated in Figure 5.2A. Patients reported significantly higher symptoms of orthostatic intolerance, and gastrointestinal disturbance than controls ((31.22, 3.60) (10.62, 1.61), (8.63, 1.04) (2.9, 0.57), t(57)=5.52, t(57)=5.03)).

5.4.1.2 Symptoms suggestive of autonomic dysfunction: main effect of hypermobility

Regardless of anxiety status, there were significant differences in autonomic symptoms between those participants with hypermobility compared to those without ((46.1, 6.11) (29.93, 5.16), t(57)=2.01)), illustrated in Figure 5.2B. Again all hypermobile participants, regardless of anxiety status, reported significantly higher symptoms of orthostatic intolerance and gastrointestinal disturbance compared to those without hypermobility ((25.1, 3.36) (14.46, 2.74), (6.77, 1.10) (4.15, 0.67), t(57)=2.42, t(57)=2.00)). Across the whole group degree of hypermobility as assessed by the Beighton scale was positively correlated with autonomic dysfunction symptoms, and orthostatic intolerance (r(57)=0.33, r(57)=0.45), see Figure 5.2C.

If hypermobile, anxious patients demonstrated significantly higher autonomic dysfunction orthostatic intolerance and gastrointestinal disturbance compared to non-anxious controls (t(29)=3.60, t(29)=3.67, t(29)=3.29). There was an interaction between hypermobility and anxiety on orthostatic intolerance score (F(2,57)=5.08), illustrated in Figure 5.2D.
Figure 5.2: Symptoms suggestive of autonomic dysfunction. A. Significant differences in total symptoms of autonomic dysfunction score between generalised anxiety disorder patients and controls. B. Significant differences in total symptoms of autonomic dysfunction...
score between hypermobile participants and non-hypermobile participants. C. Significant positive correlation between symptoms of orthostatic intolerance and Beighton score. D. Significant interaction of hypermobility and anxiety on orthostatic intolerance score.
5.4.1.3 Correlations with symptoms suggestive of autonomic dysfunction

Across the group, reported symptoms suggestive of autonomic dysfunction (particularly orthostatic intolerance, see Figure 5.3A) were positively correlated with an index suggestive of sympathetic hyperactivity: the one minute active stand (lying to standing heart rate measurement, see below), \( r(54)=0.432 \), \( r(54)=0.452 \). Again symptoms of orthostatic intolerance were positively correlated with degree of anxiety, \( r(54)=0.749 \) (Figure 5.3B), and a significant interaction with hypermobility was found \( F(2,54)=5.31 \) (Figure 5.3C). They also correlated with a measure of interoceptive sensibility (Porges Body Perception Questionnaire), and correlated with interoceptive trait prediction error - the mismatch between interoceptive accuracy and sensibility \( r(53)=0.53 \), \( r(37)=0.36 \), see Figure 5.3D.
Figure 5.3: Correlations with symptoms suggestive of autonomic dysfunction.  
A: Demonstration of the relationship between signs and symptoms of autonomic dysfunction. B: Significant correlation between orthostatic intolerance score and anxiety score. C: The interaction in this correlation by hypermobility status. D: Significant
correlation between orthostatic intolerance score and interoceptive trait prediction error.
5.4.1.4 Effect of gender

Across the whole group women reported significantly greater total symptoms suggestive of autonomic dysfunction, orthostatic intolerance, and gastrointestinal disturbance. In looking at the relationship between anxiety and total symptoms of autonomic dysfunction, a significant effect of gender was found, (F(2,57)=5.29), but not in the domains of orthostatic intolerance or gastrointestinal disturbance. When looking at the relationship between hypermobility and symptoms of autonomic dysfunction, a significant interaction of gender was found for total autonomic dysfunction, gastrointestinal disturbance, (F(2,57)=3.27, F(2,57)=4.77), but not for orthostatic intolerance. This is illustrated in Figure 5.4.
Figure 5.4: Significant differences in symptoms suggestive of autonomic dysfunction between males and females. A: Higher total symptoms suggestive of autonomic dysfunction in females B: Greater orthostatic intolerance in females C: Greater gastrointestinal disturbance in females. Significant interaction of gender when exploring D: the relationship between symptoms suggestive of
autonomic dysfunction and anxiety and E: the relationship between symptoms suggestive of autonomic dysfunction and hypermobility.
5.4.2 Signs of autonomic dysfunction

5.4.2.1 Effect of age on autonomic parameters and associated corrections
Age not associated with baseline heart rate, but was associated with higher baseline systolic and diastolic blood pressure (r(58)=0.34, r(58)=0.29). Age, however was associated with initial peak in heart rate (r(58)=-0.54) but not heart rate at one minute on standing. Age was not associated with relative change in blood pressure during deep breathing, but was associated with change in heart rate during deep breathing (r(58)=-0.44). As such analyses where there is an effect of age are corrected for age.

5.4.2.2 Signs of baseline autonomic dysfunction
There were no significant differences between groups in baseline heart rate. However anxious participants had significantly higher (mean, ±SEM mmHg) baseline systolic blood pressure compared to non-anxious participants ((114.21, 4.79) (100.97, 3.16), t(58)=2.35)). They also had significantly elevated baseline diastolic blood pressure compared to non-anxious participants ((71.75, 3.56) (62.97, 1.54), t(58)=2.35)). These effects remained significant after correction for age. Across the group, there was no main effect of hypermobility or hypermobility syndrome status on baseline blood pressure.

5.4.2.3 Signs of autonomic dysfunction after autonomic challenge

5.4.2.3.1 Autonomic function: Orthostasis

5.4.2.3.1.1 Orthostasis: effect of anxiety
Regardless of correction for age, there was no difference in initial peak heart rate, either absolute or relative change from baseline, between anxious and non-anxious participants, and degree of initial heart rate rise does not correlate with anxiety score. Anxious patients demonstrate a greater absolute (mean, ±SEM beats per minute) change in heart rate at one minute compared to non-anxious participants ((16, 1.86) (9.34, 1.37), t(58)=2.94)). They demonstrated significantly higher proportional change (%) from
baseline at one minute, \( t(58)=2.90 \), see Figure 5.5A. The degree of heart rate rise correlates with the degree of anxiety \( r(58)=0.38 \).

**5.4.2.3.1.2 Orthostasis: effect of hypermobility**

After correction for age, hypermobile participants do not show any differences in initial peak heart rate, either absolute or relative from baseline. Proportion of initial change however does correlate with Beighton score, even after correction for age \( r(58)=0.35 \).

Hypermobile participants showed a greater (mean, \( \pm \)SEM beats per minute) absolute (bpm) and proportional sustained rise in heart rate (%) at one minute compared to non-hypermobile participants ((14.87, 1.77) (9.64, 1.46), \( t(58)=2.23 \), (22.4, 15.44) (14.21, 11.12), \( t(58)=2.322 \)), illustrated in Figure 5.5B. This change in heart rate correlated with degree of hypermobility as assessed by Beighton scale, \( r(58)=0.34 \), illustrated in Figure 5.5D. Additionally there was a significant interaction with anxiety, \( F(2,58)=4.63 \). If hypermobile, anxious participants showed a greater proportional rise than non-anxious participants, \( t(30)=0.19 \). If not hypermobile there was no difference between anxious and non-anxious participants. See Figure 5.5C for illustration. All these findings were also significant when looking at the difference between participants who had hypermobility syndrome and those without.
Figure 5.5: Signs of autonomic dysfunction as illustrated by proportional change in heart rate on active stand. A: Significantly greater proportional change in heart rate at one minute in anxious versus non-anxious participants and B: Significantly greater proportional change in heart rate at one minute in hypermobile versus
non hypermobile participants, C: Interaction between hypermobility and anxiety, D: Degree of hypermobility is correlated with degree of rise.
5.4.2.3.2 Autonomic function: Deep Breathing challenge

5.4.2.3.2.1 Autonomic function: Deep Breathing challenge: effect of anxiety

Respiratory Sinus Arrhythmia in Deep Breathing

After correction for age there was no difference in change in heart rate during deep breathing in anxious compared to non-anxious participants. There was no correlation between change in heart rate and anxiety scores.

Heart rate variability in Deep Breathing - Root Mean Square of the Successive Differences (RMSSD)

There were no differences in RMSSD between anxious and non-anxious participants during deep breathing.

Change in blood pressure in Deep Breathing

There were no significant differences in blood pressure change, either absolute or proportional between anxious and non-anxious participants. There was no correlation with anxiety score across the group.

5.4.2.3.2.2 Autonomic function: Deep Breathing challenge: effect of hypermobility

Respiratory Sinus Arrhythmia in Deep Breathing

After correction for age, there was no difference in change in heart rate during deep breathing in hypermobile compared to non-hypermobile participants

Heart Rate Variability in Deep Breathing - Root Mean Square of the Successive Differences (RMSSD)

There were no differences in RMSSD between hypermobile and non-hypermobile participants

Change in blood pressure in Deep Breathing

Participants with hypermobility syndrome (rather than hypermobility), compared to those without, showed a reduced proportional change (%) in
diastolic blood pressure following deep breathing ((0.35, 2.5) (8.34, 2.8), (t(58)=2.0), see Figure 5.6. Beighton score did not correlate with any measures of blood pressure change.

Figure 5.6: Signs of autonomic dysfunction as illustrated by deep breathing challenge. A: significant differences in baseline systolic blood pressure between anxious and non-anxious participants, B: Blunted change in diastolic blood pressure during deep breathing in
hypermobility syndrome participants compared to non-hypermobility syndrome participants as evidenced by a reduced change from baseline (1.00) compared to non-hypermobility syndrome participants.
5.4.2.3.3 Effect of gender

Females showed a greater sustained proportional change in heart rate on active stand compared to males ($t(58)=5.95$). There was a significant interaction of gender on the relationship between sustained proportional change in heart rate and anxiety ($F(2,58)=4.19$) and a significant effect of gender on the relationship between sustained proportional change in heart rate and hypermobility ($F(2,58)=4.92$). See Figure 5.7 for illustration.
Figure 5.7: The effect of gender on active stand. A: Significantly higher mean proportional heart rate rise in women compared to men. B:
Interaction of gender and anxiety on active stand. C: Interaction of gender and hypermobility on active stand.
5.5 Discussion

Previous studies suggest that hypermobility is associated with both signs and symptoms of autonomic dysfunction (De Wandele et al., 2013, Gazit et al., 2003, Mathias et al., 2012). However, the relationship between this and anxiety remains previously unexplored.

I demonstrate the effect of hypermobility on both symptoms suggestive of autonomic dysfunction and signs of autonomic dysfunction. For example I show a significant interaction of hypermobility on the relationship between symptoms of orthostatic intolerance and anxiety and symptoms suggestive of autonomic dysfunction correlate with Beighton score.

I demonstrate that both hypermobile and anxious participants show heightened height rate responses to sustained standing and that degree of response correlates both with Beighton scale and anxiety scores on BAI. This is suggestive of sympathetic overdrive, because whilst initial peaks in heart rate are thought to be vagally-mediated, a sustained rise suggests increased sympathetic outflow to sinus node (Wieling and Karemaker, 2013) and an overall shift to sympathetic baroreflex activity (Schwartz and Stewart, 2012).

There are interesting effects of gender which require further explanation but are in keeping with work by Sanches and colleagues that finds differential expression of autonomic symptoms between hypermobile men and women (Sanches et al., 2014).

I show that there are interactions between anxiety and hypermobility suggestive of sympathetic over-activity. Firstly, hypermobile anxious participants show significantly heightened sustained heart rate rise at one minute) compared to non-hypermobile and there is a significant interaction with hypermobility, which suggests sympathetic overdrive as described above. Interestingly, if participants are non-hypermobile, there is no difference in this activity between anxious and non-anxious participants. Secondly, I demonstrate that the degree of this sympathetic activity
corresponds with Beighton scale, again with a significant interaction of anxiety.

In general there seems to be only minimal evidence for parasympathetic differences in this sample. After appropriate correction for age no differences in peak heart rate on standing were observed and no changes in heart rate or heart rate variability on deep breathing. However, of note, patients with hypermobility syndrome show a blunted blood pressure response following a deep breathing challenge, normally a reduction is observed which normally suggests heightened parasympathetic and reduced sympathetic activity (Mori et al., 2005) – this appears to be diminished in the hypermobility syndrome participants, suggesting diminished parasympathetic activity and or heightened parasympathetic activity.

These findings build on the results of the previous chapter where I demonstrate the relationship between symptoms suggestive of autonomic dysfunction and hypermobility. This chapter provides the first objective evidence of the link between signs of autonomic dysfunction and joint hypermobility in anxiety, namely increased sympathetic activity and possibly decreased parasympathetic activity, building on and adding to previous work that shows dysautonomia in hypermobility generally. Again this helps with potentially identifying novel treatment targets, e.g. specific medications, in the treatment of anxiety in hypermobile individuals and cognitive behavioural approaches centred on physiological interpretation. Further work is required to explore the effect of hypermobility and anxiety on other parameters of autonomic dysfunction such as pressor tests, hyperventilation, Valsalva manoeuvre and head up tilt. Additionally given the findings in the previous chapters of the interaction of hypermobility on medication and symptoms of autonomic dysfunction, an analysis is required taking into account the effects of medication on this sample.

In the next chapters I will explore whether either signs or symptoms of autonomic dysfunction correlate with brain activity in affective processing or mediate the relationship between brain activity and anxiety.
Chapter 6: What are the affective neural correlates of the association between joint hypermobility and anxiety: Neuroimaging of emotional faces?
6.1 Introduction and Aims

No functional neuroimaging exists exploring the relationship between clinical anxiety and joint hypermobility. This chapter seeks to address this, building on earlier work that implicates the amygdala and insula (Eccles et al., 2012, Mallorqui-Bague et al., 2014) as likely neural substrates mediating the relationship between joint hypermobility and anxiety.

6.2 Hypotheses

1. As per previous studies of anxiety, anxious participants will show differences in patterns of reactivity in areas of brain including insula and related brain areas (Paulus and Stein, 2006, Stein et al., 2007, Klumpp et al., 2013, Shah et al., 2009).

2. Participants will show differences in brain reactivity according to anxiety group and hypermobility status. There is no specific previous functional imaging work specifically addressing the link between hypermobility and clinical anxiety to lead to a specific hypothesis, yet nevertheless I predict from earlier structural imaging of healthy hypermobile participants (Eccles et al., 2012) and from functional imaging of healthy hypermobile participants (Mallorqui-Bague et al., 2014) that this would be involve the amygdala and insula.

3. Previous work in postural tachycardia syndrome (in which hypermobility is overrepresented) (Umeda et al., 2009) suggests that hypermobile participants will show exaggerated reactivity to even neutral faces with deactivation of ventromedial prefrontal cortex to neutral images.
6.3 Specific methods

6.3.1 Imaging data acquisition
Whole brain fMRI data was acquired on a 1.5 T Siemens Avanto scanner. To minimise signal artefacts originating from the sinuses, axial slices were tilted 30° from the intercommissural plane. Thirty-four slices (3mm thick, 0.6 mm interslice gap) were acquired with an in plane resolution of 3 x 3 mm (repetition time =2.52s per volume, echo time = 43ms).

6.3.2 Experimental task
In this event-related task, an emotional faces task modified from Umeda and colleagues (Umeda et al., 2009), 5 classes of images of emotional faces from the the Karolinska Directed Emotional Faces set (KDEF) (classes: angry, afraid, disgusted, neutral and happy) (Goeleven et al., 2008), for examples see figure below, were presented in a randomised order. Null events were presented as fixation cross. These were also included to facilitate the identification of haemodynamic responses to stochastically ordered stimuli. There were 15 trials of each emotion category and each of the 96 trials (of which 21 (21.9%) were null events) lasted 4 seconds each. During each presentation participants were asked to make an incidental judgement of whether they could see teeth or not.
Figure 6.1: Graphic showing stimuli used in functional neuroimaging task. A: Example faces used in the emotional faces task, selected from the Karolinska Directed Emotional Faces set (Goeleven et al., 2008).
6.3.3 Pre-processing

Standard spatial pre-processing [realignment of all EPI images using a six parameter rigid body transformation to a mean EPI image, segmentation, normalisation to Montreal Neurological Institute (MNI) space, and smoothing with an 8-mm FWHM Gaussian Kernel] was performed. Voxel size was interpolated during pre-processing to isotropic 3 x 3 x 3 mm.

6.3.4 General Linear Model

Statistical analyses were performed on the basis of the general linear model framework within SPM 8. Models were estimated at the first level with the restricted maximum likelihood approach to provide parameter estimates for each condition and enable generation of relevant contrast images. The image realignment parameters were included as regressors of no interest in each first level model to account for variance associated with participant motions. These statistical parametric maps of contrast estimates of experimental effects from individual participant analyses were entered into second-level group analyses. A full factorial model was used to analyse the results. A factorial model is an alternative to the previously common subtraction approach and this allows for analysis of interaction between each component (Friston et al., 1996).

At the second level three factorial models were used. In all age and gender were added as co-variates. The first model (2x2x6) allowed the study of main effects and interactions of hypermobility status and anxiety status (1st factor hypermobility status (two levels); 2nd factor anxiety status (two levels) 3rd factor stimulus type (six levels)). The 2nd model (2x6) (1st factor anxiety status (2 levels); 2nd factor stimulus type (six levels)) included Beighton score as an additional co-variate, so that the interaction of Beighton score on anxiety status could be explicitly modelled as could the main effect of Beighton score. The 3rd model (2x6) (1st factor hypermobility syndrome status (two levels); 2nd factor stimulus type (six levels)) was designed to model the interaction of anxiety level on hypermobility syndrome status and as such included BAI score as an additional co-variate. All co-variates were mean centered around zero.
6.3.5. Contrast design
Main effects were calculated using the subtraction method e.g. activations in anxious>non anxious. Interactions between groups were calculated as the difference of differences. Interactions of co-variate on group were modelled into the design matrix as appropriate.

6.3.6 Statistical threshold
All neuroimaging results are reported at the cluster level with correction for multiple comparisons (FWE p<0.05) unless otherwise stated.

6.4 Sample characteristics
Seventy participants underwent functional neuroimaging as described in chapter 2. Of those, 10 participants were excluded due to the presence of notable structural abnormalities or artefact (n=4), excessive movement (n=1) or missing data (n=5). Of the 60 remaining, 26 (43.3%) participants met criteria for generalized anxiety disorder and 34 acted as controls (56.7%). Across the whole group, 32 (53.3%) were classified as hypermobile and 22 (36.7%) additionally met criteria for hypermobility syndrome. Of those with generalized anxiety disorder, 15 (57.7%) were hypermobile, of those without generalized anxiety disorder 17 (50%) were classified as hypermobile. See table below. There was no significant difference in gender between the groups. In terms of age, the hypermobile group was significantly younger (mean, ±SEM: years) than non-hypermobile ((35.53, 2.09) (45.29, 2.57), t(60)=2.975). There was no difference in age between the generalized anxiety disorder group and the controls. However, age and gender were entered as co-variates of no interest in the model.
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<td>FACTOR 1</td>
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<tr>
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</tr>
<tr>
<td>Anxiety (+)</td>
<td>11</td>
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</tbody>
</table>

Table 6.1: Factorial design of the sample.
6.5 Results

6.5.1 Behavioural Data
Anxiety and Beighton scores were available for all participants. Full autonomic data was available for 54 participants, as was interoceptive sensibility data. Interoceptive accuracy data was available for 42.

6.5.1.1 Interoception
There were no differences in interoceptive accuracy between the groups. However, the main effect of anxiety was greater (mean, ±SEM) interoceptive sensibility scores ((112.36, 7.29) (87.34, 6.44), t(52)=2.54). Interoceptive sensibility positively correlated with anxiety score (r(52)=0.48). Anxious participants showed greater interoceptive trait prediction error (the mismatch between interoceptive accuracy and sensibility) than non-anxious ((-0.45, 0.31) (0.39, 0.28), t(36)=2.02)). There was no interaction of hypermobility on these associations.

The main effect of hypermobility was greater interoceptive sensibility ((107.23, 6.46) (84.44, 7.47), t(52)=2.31), and this correlated with Beighton score (r(52)=0.287). There was no interaction of anxiety on these associations.

6.5.1.2 Autonomic prediction error
There was no difference in autonomic prediction error (mismatch between signs and symptoms of orthostatic intolerance) between the groups. Across the whole group anxiety score positively correlated with autonomic prediction error (r(48)=0.40), when looking at hypermobility status, this effect was only significant in the hypermobile group (r(24)=0.44), however there was no formal interaction of hypermobility on this relationship.

6.5.2 Neuroimaging data
A discrete set of regions were activated during the task and are reported in full below (Table 6.2).
6.5.2.1 Main Effects

6.5.2.2 Main effect of anxiety
Across all face conditions the main effect of anxiety was to activate insula cortex (Figure 6.2A,B), inferior parietal lobule, frontal lobe and anterior cingulate (Figure 6.2D). Activation in anterior insula correlated with autonomic prediction error, \( r(46)=0.35 \), illustrated in Figure 6.2C. Activation in anterior cingulate negatively correlated with interoceptive accuracy, \( r(40)=0.43 \) (Figure 6.2E)
Figure 6.2: T-Contrast estimates showing main effect of anxiety on presentation of all stimuli (p<0.05 FWE corrected). A: Brain activation greater in anxious patients than non-anxious patients demonstrating mean activity in insula cluster. B: Correlation of mean activity in insula cluster with autonomic prediction error. C: Plot (error bars represent 1 standard error of mean) demonstrating differential response of brain activity centred at insula cluster. D: Brain activation greater in anxious patients than non-anxious patients demonstrating mean activity in anterior cingulate cluster. E: Plot showing negative correlation between interoceptive accuracy and activity in anterior cingulate cluster.
6.5.2.3 Main Effect of hypermobility

Main effect of hypermobility across all face conditions was to activate parahippocampus, hippocampus, caudate, precuneus and posterior insula. The main effect of hypermobility score, i.e. across the task the degree to which brain activation correlated with degree of hypermobility, was to activate anterior insula (Figure 6.3A), primary motor cortex, supplementary motor area, fusiform, parietal cortex and hippocampus.
Figure 6.3: T Contrast estimates showing main effect of hypermobility on presentation of all stimuli (p<0.05 FWE corrected). A: Brain activation greater in hypermobile patients than non-hypermobile patients demonstrating mean activity in insula cluster B: Plot positive correlation between activity in insula cluster and Beighton score.
The main effect of hypermobility syndrome was to activate insular (Figure 6.4A), temporal and frontal cortices. This activation in insula also correlated with the degree of sustained heart rate rise on active stand (sympathetic activation), \( r(52)=0.29 \). On mediation analysis, sympathetic activation partially mediates the relationship between hypermobility syndrome and insula activation (Figure 6.4B), reducing the correlation between the two from \( r(60)=0.46 \) to \( r(51)=0.43 \).
Figure 6.4: T-Contrast estimates showing main effect of hypermobility syndrome on presentation of all stimuli (p<0.05 FWE corrected). A: Brain activation greater in hypermobility syndrome patients than non-hypemobility syndrome patients demonstrating mean activity in insula cluster. B: Graphic showing partial mediation of the
relationship between hypermobility syndrome and insula activation by sympathetic reactivity (one minute of active stand).
6.5.2.4 Main effect of emotion
The effect of viewing emotional faces, compared to fixation cross, activated large areas of the occipital lobe (Figure 6.5 A), supplementary motor cortex (Figure 6.5A: and anterior insula (Figure 6.5B).
Figure 6.5: T-Contrast estimates showing main effect of viewing emotional faces rather than fixation cross (p<0.05 FWE corrected). A: Brain activation greater in emotional faces rather than fixation cross demonstrating mean activity in occipital lobe and supplementary motor area cluster and B: Insula cluster. C: Brain activation in lingual lobe cluster showing interaction between emotion and hypermobility (p<0.001 uncorrected).
When looking at the difference between the specific classes of emotional faces and neutral faces no significant activations were found.

6.5.2.2 Interactions

6.5.2.2.1 Interaction hypermobility and anxiety
A set of interactions were explored to examine the relationship between hypermobility and anxiety. I.e. I was interested to see which areas were activated firstly in anxious hypermobile participants compared to anxious non-hypermobile participants (i.e. interaction of hypermobility on anxiety) and secondly in anxious hypermobile participants compared to non-anxious hypermobile participant (i.e. interaction of anxiety on hypermobility).

In the interaction of hypermobility on anxiety anxious hypermobile participants compared to anxious non-hypermobile participants activated parahippocampus, caudate, mid-cingulum and posterior insula. In the interaction of anxiety on hypermobility, hypermobile anxious participants, compared to hypermobile non-anxious participants, activated posterior insula (Figure 6.6A) and activation in this area of insula correlated significantly with sympathetic activation on active stand ($r(52)=0.377$) and orthostatic intolerance score, ($r(52)=0.419$) (Figure 6.6B). They also activated lingual gyrus, superior temporal lobe, orbitofrontal cortex and posterior cingulate gyrus.
Figure 6.6: T-Contrast estimates showing main effect of interaction between hypermobility status and anxiety status across all conditions (p<0.05 FWE corrected). A: Brain activation greater in hypermobile anxious participants compared to hypermobile non-anxious participants in insula cluster. B: Plot showing positive correlation between activation in insula cluster and symptoms of orthostatic intolerance.
6.5.2.2.2 Interaction anxiety status and hypermobility score

I wished to explore the positive interaction between anxiety status and hypermobility score, i.e. the interaction testing activation in which areas are more positively correlated with Beighton score in those who are anxious compared to those who are not anxious, (Figure 6.7B). The effect of this was to activate amygdala and parahippocampus (Figure 6.7A), inferior temporal lobe, anterior cingulate and anterior insula.
Figure 6.7: T-Contrast estimates showing main effect of interaction between anxiety status and hypermobility score across all conditions (p<0.05 FWE corrected). A: Brain activation in amygdala cluster positively correlating with Beighton score in anxious participants, but not in non-anxious participants. B: Plot showing interaction between Beighton score and anxiety status in the amygdala cluster.
6.5.2.2.3 Interaction hypermobility syndrome and anxiety score

The positive interaction (i.e. the interaction testing which areas are more positively correlated with anxiety score in those with hypermobility syndrome than those without (Figure 6.8 B): activated posterior insula, superior temporal gyrus, caudate and parahippocampus including amygdala (Figure 6.8 A). In hypermobility syndrome, this activation correlated with sympathetic activation on active stand and interoceptive sensibility (r(52)=0.36, r(52)=0.39). A graphic of this interaction at the level of the amygdala is shown below:
Figure 6.8: T-Contrast estimates showing main effect of interaction between hypermobility syndrome status and anxiety score across all conditions (p<0.05 FWE corrected). A: Brain activation in amygdala cluster positively correlating with anxiety score in hypermobility syndrome participants, but not in non-hypermobility syndrome participants. B: Plot showing interaction between anxiety score and hypermobility syndrome status in the amygdala at the cluster level.
6.5.2.2.4 Interaction between emotion and hypermobility status

Hypermobile participants, compared to non-hypermobile participants, activated lingual gyrus (Brodmann area 18) (Figure 6.5C) when viewing emotional faces compared to fixation cross.
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**Hypermobility syndrome>no hypermobility syndrome**

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**FACES>FIXATION**

**Main effect faces>fixation**

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**INTERACTION**

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Table 6.2: Table showing significant activations with location, cluster size, co-ordinates and z values. 334 degrees of freedom from 360 images.
6.6 Discussion

There have been no previous neuroimaging studies exploring the relationship between clinical anxiety and hypermobility, previous studies have focused on healthy volunteers (Eccles et al., 2012, Mallorqui-Bague et al., 2014). I have shown in this chapter that this task successfully activates areas such as anterior insula and anterior cingulate in anxiety, which could be expected from previous work (Paulus and Stein, 2006, Stein et al., 2007, Klumpp et al., 2013, Shah et al., 2009) and is consistent with the work that suggests anterior insula is associated with subjective feeling states, see (Craig, 2015). I show that such activations are directly linked to autonomic and interoceptive processes, for example activity in anterior insula, in this contrast, correlates with mismatch between signs (i.e. that which is observed) and symptoms (i.e. that which is expected) of orthostatic intolerance (autonomic prediction error) and activity in anterior cingulate correlates with interoceptive accuracy. The former finding fits with predictive coding models e.g. (Edwards et al., 2012, Seth et al., 2011) that are based on the assumption that unexpected information (i.e. the difference between observed and expected) requires processing and signals deviation from the anticipated (i.e. expected) state. This then may be signalled as a symptom, namely anxiety.

Unfortunately, I fail to replicate the work of Umeda et al (Umeda et al., 2009) and do not demonstrate exaggerated reactivity to neutral faces in hypermobility with associated deactivation of ventromedial prefrontal cortex as I had previously hypothesised. This may be that although many hypermobile participants demonstrate orthostatic tachycardia it did not meet threshold for PoTS. Additionally I have not investigated the relationship between heart rate reactivity and emotional processing.

I do however, show for the first time in a clinical group that there are specific areas activated in hypermobility in an emotional processing task including posterior insula, and that activation in anterior insula correlates with Beighton score. Activation within both posterior insula, in people with hypermobility
syndrome, was partially mediated by sympathetic hyperactivity. I demonstrate specific interactions between hypermobility and anxiety, including the observation that hypermobile anxious participants, compared to non-anxious hypermobile participants, activated posterior insula and this activation correlated with sympathetic activity, as demonstrated by sustained change in heart rate on active stand. This posterior activation perhaps relates to greater interoceptive sensitivity in hypermobile individuals.

The interaction between anxiety status and hypermobility score activated amygdala, such that amygdala activation showed no correlation with hypermobility score in non-anxious participants, but was strongly associated with hypermobility score in anxious participants. I showed a similar pattern of activation in posterior insula in hypermobility syndrome, whereby activation in insula showed significant correlation with anxiety score in hypermobility syndrome participants alone.

The insula and amygdala are not only implicated not only in anxiety, but also in social emotional processing (Lamm and Singer, 2010, Boddaert et al., 2004, Bickart et al., 2011). Crucially the insula plays a fundamental role in homeostasis and autonomic control through mapping and regulation of sympathetic and parasympathetic activity (Critchley and Harrison, 2013, Gianaros et al., 2012, Critchley, 2005). The amygdala also implicated in autonomic control: The amygdala is one region involved in translating psychological stress into bodily arousal (Gianaros et al., 2008). This relationship is bidirectional (Garfinkel et al., 2014). Afferent signals of physiological arousal are represented within the amygdala and integrated with the processing of threat stimuli.

These findings suggest that there is a particular pattern of affective reactivity in anxiety patients who have hypermobility and may provide insight into cognitive treatment targets. The next chapter will explore how this affective reactivity is altered by false physiological feedback which is particularly important given the abnormalities in sympathetic function noted in chapter 5 and the associations with autonomic function and brain activity in this chapter.
Chapter 7: What is the effect of interoceptive influence on affective processing in joint hypermobility and anxiety: Neuroimaging of false physiological feedback?
7.1 Introduction and Aims

As mentioned in the previous chapter no functional neuroimaging studies exist exploring the relationship between joint hypermobility and clinical anxiety. To test the concept that this relationship maybe mediated by autonomic hyperactivity and interoceptive/autonomic prediction error this task incorporated false physiological feedback (Gray et al., 2007). This task was motivated by the idea that anxious people with hypermobility will show altered autonomic dysfunction and thus their affective processing may be altered by perceived physiology and/or interoceptive processes.

7.2 Hypotheses

1. Participants will rate the images with different intensity ratings during the two feedback conditions (Gray et al., 2007).

2. As hypothesised in the previous chapter anxious participants will show differences in patterns of reactivity in areas of brain including insula and related brain areas (Paulus and Stein, 2006, Stein et al., 2007, Klumpp et al., 2013, Shah et al., 2009) and participants will show differences in brain reactivity according to hypermobility status (Eccles et al., 2012, Mallorqui-Bague et al., 2014).

3. The main effect of false physiological feedback will be to activate insula (Gray et al., 2007). There is no previous work to support a particular hypothesis as to how this will be affected by hypermobility but it is anticipated that insula will be implicated, due to both its representation of bodily physiology and its activation in previous functional imaging of emotional processing in hypermobility (Mallorqui-Bague et al., 2014).

7.3 Specific Methods

7.3.1 Imaging data acquisition

Whole brain fMRI data was acquired on a 1.5 T Siemens Avanto scanner. To minimise signal artefacts originating from the sinuses, axial slices were tilted 30º from the intercommissural plane. Thirty-four slices (3mm thick, 0.6 mm
interslice gap) were acquired with an in plane resolution of 3 x 3 mm (repetition time =2.52s per volume, echo time = 43ms).

7.3.2 Experimental task

In this task, the physiological feedback task, modified from Gray and colleagues (Gray et al., 2007), participants were shown three classes of images (positive, neutral and negative) from the International Affective Picture System library (Lang et al., 2008). This task embodied both event and block related design: images were shown in an event related design, in a randomised order in blocks of 6 under two randomised heart rate conditions. In the first heart rate condition ‘true feedback’, the participant viewed the images whilst was played the sound of their heartbeat in real time at its natural rate as determined by a pulse oximeter (NONIN, Nonin Medical, Minnesota, USA) connected to the patient in the MRI scanner. In the second condition, ‘false feedback’, whilst the participant viewed the same images the participant’s heart rate was experimentally increased by 20%. Participants were not made aware of this manipulation. After each task they were asked to rate how intense they found each image from zero to extreme on a visual analogue scale. Each class of image was presented 12 times under the two conditions constituting 72 trials in total. Each image was presented for 2 seconds. See Figure 7.1 for illustration of the experimental paradigm.
Figure 7.1: Graphic showing stimuli used in functional neuroimaging task. A: Visual stimuli: example affective images used in the false physiological feedback task, selected from the international affective pictures library (Lang et al., 2008). B: Auditory stimuli: Affective images were displayed whilst auditory feedback of heart rate (tones) was presented in a block design, either true (actual heart rate) or false (accelerated heart rate).
7.3.3 General Linear Model
Statistical analyses were performed on the basis of the general linear model framework within SPM 8. Models were estimated at the first level with the restricted maximum likelihood approach to provide parameter estimates for each condition and enable generation of relevant contrast images. The image realignment parameters were included as regressors of no interest in each first level model to account for variance associated with participant motions. These statistical parametric maps of contrast estimates of experimental effects from individual participant analyses were entered into second-level group analyses. A full factorial model was used to analyse the results. At the second level three factorial models were used. In all age and gender were added as co-variates. The first model (2x2x2x3) allowed the study of main effects and interactions of hypermobility status and anxiety status (1\textsuperscript{st} factor hypermobility status (two levels); 2\textsuperscript{nd} factor anxiety status (two levels) 3\textsuperscript{rd} factor feedback type (six levels), 4\textsuperscript{th} factor emotional category (three levels). The 2\textsuperscript{nd} model (2x3x2) (1\textsuperscript{st} factor hypermobility syndrome status (two levels); 2\textsuperscript{nd} factor feedback type (two levels); 3\textsuperscript{rd} factor emotional category (three levels)) included Beighton score as an additional co-variate, so that the interaction of Beighton score on anxiety status could be explicitly modelled as could the main effect of Beighton score. The 3\textsuperscript{rd} model (2x2x3) (1\textsuperscript{st} factor hypermobility syndrome status (two levels); 2\textsuperscript{nd} factor feedback type (two levels); 3\textsuperscript{rd} factor emotional category (three levels)) was designed to model the interaction of anxiety level on hypermobility syndrome status and as such included BAI score as an additional co-variate. All co-variates were mean centered around zero.

7.3.4 Contrast design
Main effects were calculated using the subtraction method e.g. activations in anxious>non anxious. Interactions between groups were calculated as the difference of differences. Interactions of co-variate on group were modelled into the design matrix as appropriate.

7.3.5 Statistical threshold
All neuroimaging results are reported at the cluster level with correction for multiple comparisons (FWE p<0.05) unless otherwise stated.
7.4 Sample characteristics

70 participants underwent functional neuroimaging as described in chapter 2. Of those, 13 participants were excluded due to structural abnormalities or artefact (n=4), excessive movement (n=1) or incomplete timing data set (n=8). Of those 57 remaining 26 (45.6%) met criteria for generalized anxiety disorder and 31 acted as controls. Across the whole group 30 (52.6%) were classified as hypermobile and 21 (36.8%) met criteria for hypermobility syndrome. Of those with generalized anxiety disorder, 15 (57.7%) were hypermobile, of those without generalized anxiety disorder 15 (48.4%) were classified as hypermobile. See table below. There was no significant difference in gender between the anxious and non-anxious groups or the hypermobile and non-hypermobile groups. In terms of age there was no significant difference in age between anxious and non-anxious groups. However, the hypermobile group was significantly (mean, ±SEM) younger (35.53, 2.04) than non-hypermobile ((45.74, 2.62), t(55)=3.1).

<table>
<thead>
<tr>
<th>FACTOR 1</th>
<th>FACTOR 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypermobility (-)</td>
</tr>
<tr>
<td>Anxiety (-)</td>
<td>16</td>
</tr>
<tr>
<td>Anxiety (+)</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 7.1: Factorial design of the sample.
7.5 Results

Anxiety and Beighton scores were available for all participants. Complete autonomic data was available for 51 participants, as was interoceptive sensibility data. Interoceptive accuracy data was available for 47 participants.

7.5.1 Behavioural Data

7.5.1.1 Interoception data

Across the whole group anxious participants demonstrated greater (mean, ±SEM) interoceptive sensibility compared to non-anxious ((112.67, 6.46) (81.03, 4.64), t(49)=4.07). Interoceptive sensibility positively correlated with anxiety score (r(49)=0.569) Additionally, hypermobile participants demonstrated greater interoceptive sensibility compared to non-hypermobile ((103.17, 5.17) (85.92, 7.13) t(49)=2.00. A significant interaction with anxiety was found (F(2,49)=13.60). Beighton score correlated with interoceptive sensibility and interoceptive accuracy (r(49)=0.28, r(45)=0.30). There was a significant interaction of anxiety on the relationship between interoceptive sensibility and Beighton score (F(2,49)=6.50)

7.5.1.2 Autonomic prediction error

There were no group differences in autonomic prediction error, however autonomic prediction error positively correlated with anxiety score (r(43)=0.40). However this effect is only significant for hypermobile individuals (r(22)=0.44), although there is no significant interaction of hypermobility on this association.

7.5.1.3 Task behavioural data

No differences were found in valence ratings of images under the different feedback conditions. No differences were found in between groups (anxious, hypermobile) in ratings of emotional pictures under the true and false physiological feedback conditions.
7.5.2 Neuroimaging Data
A discrete set of regions were activated during the task and are reported in full below (Table 7.2).

7.5.2.1 Main Effects

7.5.2.1.1 Main effect of anxiety
In this task across all feedback conditions the main effect of anxiety (i.e. areas activated in anxious participants rather than non-anxious participants, regardless of hypermobility status) was to activate bilateral anterior insula (Figure 7.2A), parietal lobe, frontal lobe, lingual gyrus and parahippocampus (Figure 7.2C,D), including amygdala. The activation in left anterior insula correlated with interoceptive sensibility (Porges Body Perception Questionnaire score), and with anxiety score ($r(49) = 0.24$, $r(55) = 0.38$). Anxiety score was also correlated with interoceptive sensibility ($r(49) = 0.61$). The correlation between this insula activation and anxiety score was found to be fully mediated by interoceptive sensibility (Figure 7.2B), reducing the correlation between insula and anxiety score to a non-significant $r(48) = 0.19$. 


Figure 7.2: T Contrast estimates showing main effect of anxiety on presentation of all stimuli (p<0.05 FWE corrected). A: Brain activation greater in anxious patients than non-anxious patients demonstrating mean activity in insula cluster. B: Graphic showing mediation of relationship between anxiety score (Beck Anxiety Inventory, BAI) and insula activation by interoceptive sensibility. C: Brain activation greater in anxious patients than non-anxious patients demonstrating activity in parahippocampal/amygdala cluster. D: Plot (bars represent one standard error of the mean) showing differences in activity in parahippocampal/amygdala cluster in anxious patients compared to controls.
7.5.2.1.2 Main effect of hypermobility
Hypermobile compared to non-hypermobile participants, regardless of anxiety status, across all feedback conditions, activated mid insula (Figure 7.3A), occipital lobe, cuneus, lingual gyrus and parahippocampus including amygdala.

7.5.2.1.2.1 Effect of Beighton score
Areas of activation that correlated with Beighton score included mid insula (Figure 7.3B), middle occipital gyrus and cuneus.
Figure 7.3: T Contrast estimates showing effect of hypermobility on presentation of all stimuli (p<0.05 FWE corrected). A: Brain activation greater in hypermobile patients than non hypermobile patients demonstrating mean activity in insula cluster. B: Brain activation correlating with Beighton score across participants and conditions demonstrating mean activity in insula cluster.
7.5.2.1.2.2 Effect of hypermobility syndrome

Hypermobility syndrome participants, compared to non-hypermobility syndrome participants, activated lingual (Figure 7.4A) and middle occipital gyri. Activity in lingual gyrus correlated with anxiety score, r(55)=0.234 and sustained heart rate rise on standing (sympathetic activation), r(49)=0.247. Mediation analysis (Figure 7.4B) shows that the relationship between anxiety and activation in lingual lobe is full mediated by sympathetic activation, rendering the correlation between anxiety score and lingual activation non-significant.
Figure 7.4: T Contrast estimates showing main effect of hypermobility syndrome on presentation of all stimuli (p<0.05 FWE corrected). A: Brain activation greater in hypermobility syndrome participants than non-hypermobility syndrome participants demonstrating mean activity in lingual lobe cluster B: Graphic showing full mediation of relationship between anxiety score and lingual activation by sympathetic activation (proportional change in heart rate after one minute).
7.5.2.1.3 Main effect of feedback

The main effect of viewing images under false physiological feedback rather than true physiological feedback was to activate posterior and mid insula (Figure 7.5A), this activation with posterior insula correlated positively with autonomic prediction error (r(43)=0.27) (Figure 7.5B).
Figure 7.5: T Contrast estimates showing main effect of false physiological feedback versus true physiological feedback (p<0.001 uncorrected). A: Brain activation greater in false feedback in insula cluster B: Plot showing correlation between insula activity and mismatch between signs and symptoms of orthostatic intolerance (autonomic prediction error).
7.5.2.2 Interactions

7.5.2.2.1 Interaction hypermobility and anxiety
A set of interactions were explored to examine the relationship between hypermobility and anxiety. I.e. I was interested to see which areas were activated firstly in anxious hypermobile participants compared to anxious non-hypemobile participants (i.e. interaction of hypermobility on anxiety) and in anxious hypermobile participants compared to non-anxious hypermobile participant (i.e. interaction of anxiety on hypermobility)

In the interaction of hypermobility on anxiety, anxious hypermobile participants activated fusiform and mid occipital love compared to anxious non hypermobile participants.

In the interaction of anxiety on hypermobility anxious hypermobile participants compared to hypermobile non-anxious participants activated lingual lobe and anterior insula.

7.5.2.2.2 Interaction anxiety status and Beighton score
I wished to explore the positive interaction between anxiety status and Beighton score, i.e. the interaction testing activation in which areas are more positively correlated with Beighton score in those who are anxious compared to those who are not. There were no significant activations for this contrast.

7.5.2.2.3 Interaction hypermobility status and anxiety score
I wished to explore the positive interaction between hypermobility status and anxiety score, i.e. the interaction testing activation in which areas are more positively correlated with anxiety score in those who have hypermobility syndrome compared to those who do not. This interaction, (Figure 7.6B, D) activated parahippocampus including amygdala (Figure 7.6A); lingual lobe, mid-cingulum and mid insula (Figure 7.6C). Activity in mid insula negatively correlates with blood pressure changes to deep breathing, r(49)=-.33.
Figure 7.6: T Contrast estimates showing interaction of hypermobility syndrome on the relationship between brain activation and anxiety (p<0.001 uncorrected). A: interaction between hypermobility syndrome and anxiety score demonstrating activity in amygdala cluster B: Plot showing interaction C: Interaction between hypermobility syndrome and anxiety score demonstrating activity in insula cluster D: Plot showing interaction.
7.5.2.2.4 Interaction between hypermobility status and feedback

Based on the hypothesis that hypermobile individuals will differentially respond to false physiological feedback, I tested whether hypermobile individuals responded differently to false feedback. There were no significant results for this contrast.

7.5.2.3 Conjunctions

I wished to explore which areas of the brain activity that correlated with hypermobility were also activated in the false feedback compared to true feedback condition. The area of the brain correlated with Beighton score that also was activated in true versus false feedback was insula (Figure 7.7A). Activity in this area correlated positively with sympathetic activation on active stand (Figure 7.7B) and negatively with difference in rating of positive images in the two feedback conditions, i.e. the tendency to rate positive images less positively under false feedback \((r(55)=0.23, r(55)=-.42))\).
Figure 7.7: T Contrast estimates showing conjunction of true versus false feedback and Beighton score (p<0.001 uncorrected). A: Brain activation (insula cluster) in conjunction of true versus false feedback and Beighton score B: Plot showing correlation between insula activity and sympathetic activation
<table>
<thead>
<tr>
<th>Location</th>
<th>K</th>
<th>Co-ordinates (x,y,z)</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EFFECT OF ANXIETY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect anxiety&gt;non anxiety</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>-36 16 13</td>
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<td>7.33</td>
</tr>
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<td></td>
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<tr>
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<td>Middle occipital gyrus</td>
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<td>-33 -87 30</td>
<td>Inf</td>
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<tr>
<td>Hypermobile anxious&gt;anxious non-</td>
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### Table 7.2: Table showing significant activations with cluster size, co-ordinates and z values. 316 degrees of freedom from 342 images.

<table>
<thead>
<tr>
<th>Region</th>
<th>Cluster Size</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Z Value</th>
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<td><strong>Interaction between hypermobility syndrome and anxiety score</strong> (p&lt;0.001 uncorrected)</td>
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</table>
7.6 Discussion

There have been no previous neuroimaging studies exploring the relationship between clinical anxiety and hypermobility, previous studies have focused on healthy volunteers (Eccles et al., 2012, Mallorqui-Bague et al., 2014). This task incorporates false physiological feedback in an emotion processing task. In line with previous work I have shown that this emotional task as whole reliably activates areas of brain involving affective processing in anxiety including anterior insula (Paulus and Stein, 2006) and amygdala (Fox et al., 2015). I have shown that the relationship between anxiety and activation in the anterior insula is mediated by interoceptive sensibility.

I have shown for in a clinical group of anxiety patients that the main effect of hypermobility was to activate mid insula lobe and that these activations correlated with hypermobility score. The interaction between hypermobility syndrome and anxiety score correlated with activations in amygdala and mid insula, and correlated with failure to enact a parasympathetic response.

I fail to demonstrate altered behavioural valence of emotional pictures during false physiological feedback as hypothesised. However, I demonstrate that in the brain the main effect of feedback was to activate posterior insula; this correlated with interoceptive mismatch which is perhaps unsurprising given the role of posterior insula in interoceptive processes (Seth et al., 2011, Critchley, 2004, Paulus and Stein, 2006). Although I do not show any specific differences in the false-feedback task between individuals with hypermobility and those without, activation in this area also correlated with hypermobility score and correlated with sympathetic activation and autonomic prediction error.

The results of this task demonstrate for the first time the effect of false physiological feedback on the relationship between anxiety and hypermobility and its relationship with interoceptive and autonomic processes. For example activation in posterior and mid insula in the false feedback condition is predicted by degree of autonomic prediction error, in keeping with the role of posterior insula as interoceptive cortex (Craig, 2015). The activation in the
area of insula that is conjointly activated by hypermobility and false feedback correlates with sympathetic autonomic dysfunction.

This data implicates altered interoception and autonomic prediction error in the relationship between joint hypermobility and anxiety, building on the work of previous chapters that shows that signs and symptoms of autonomic dysfunction are higher in hypermobility and that symptoms of autonomic dysfunction partially mediate the relationship between joint hypermobility and anxiety. It also builds on the earlier imaging chapter showing altered affective processing in key emotional brain regions demonstrating amygdala and insula as the likely regions underpinning the association between joint hypermobility and anxiety.
Chapter 8: **Summary and conclusions**
8.1 Overview

The importance of extra-articular manifestations, including dysautonomia, of joint hypermobility is increasingly recognised. The link with anxiety, the most common psychiatric symptom and disorder, has been repeatedly highlighted and is further demonstrated by this body of work, however little is known of the underlying mechanisms and also the relevance of joint hypermobility to other psychiatric disorders. This set of novel experiments sought to address this substantial gap in the literature and has additionally demonstrated the importance of joint hypermobility to disorders such as ADHD, bipolar and eating disorders. I have demonstrated for the first time that autonomic dysfunction, particularly sympathetic activation, partially mediates the relationship between anxiety and joint hypermobility. I believe that the biological mechanism underpinning the relationship between hypermobility, autonomic dysfunction and the expression of anxiety is a consequence of abnormal peripheral vasoconstriction, consequent upon variant connective tissue, e.g. collagen within the vasculature. It is likely that reduced venous return during standing due to venous pooling may be responsible for an increased sympathetic state – as the body attempts to compensate for these abnormalities - resulting in orthostatic intolerance and associated symptoms (Bohora, 2010, Benarroch, 2012, Mathias et al., 2012). I also show an interaction of hypermobility on the relationship between common classes of medication and autonomic dysfunction, which is particularly important when considering potential treatment targets.

I also delineate the likely neural substrates underpinning the association between joint hypermobility and anxiety, e.g. amygdala and insula, and additionally their correlations in activation with interoceptive indices and dysautonomia including autonomic prediction error. I believe this is crucial for understanding possible psychosomatic mechanisms for this association as these findings fit with existing predictive coding models e.g. (Edwards et al., 2012, Seth et al., 2011, Paulus and Stein, 2006) that are based on the assumption that unexpected information (i.e. the difference between observed and expected) requires processing by the brain and signals
deviation from the anticipated (i.e. expected) state. I believe in this group of patients this then may be signalled as a symptom, namely anxiety.
Figure 8.1: Proposed model of the neurobiological mechanisms underpinning association between joint hypermobility and anxiety.
The work contained within this thesis has great potential impact, by enhancing the wider recognition of this common but underdiagnosed condition, and facilitating paths toward personalised medical treatments. This could be both pharmacological (for example, medications that lessen symptoms and signs of orthostatic intolerance) and psychotherapeutic (for example, therapies that act to reduce autonomic or interoceptive prediction error by reframing or refocusing the salience of physiological arousal and processes). Further work needs to be done to systematically test these approaches.

8.2 Is joint hypermobility over-represented in psychiatric populations?

In chapter 3 I demonstrated, in a large patient survey, that joint hypermobility is over-represented in the general psychiatric population regardless of diagnosis, with an OR of 2.38 (1.95-2.90). I also demonstrate for the first time in adults very high rates of hypermobility in patients with neurodevelopmental disorders. 73% of women with ADHD are classified as hypermobile and 67% of women with ASD. Although lower, significantly high rates are found in men also.

I suggest that the association between hypermobility and neurodevelopmental conditions may contribute to the generation and maintenance of psychological and behavioural symptoms of neurodevelopmental disorders in adulthood. Putatively, the psychological vulnerabilities commonly arising in people with this constitutional variation in connective tissue may represent a lifelong contextual influence on the expression of core and comorbid symptoms of neurodevelopmental disorders.

The mechanisms underlying the association between joint hypermobility and neurodevelopmental conditions may be the same as those accounting for enhanced vulnerability to anxiety disorders among the general population. Differences in the structural integrity of brain regions supporting emotional arousal, mood regulation, fear learning and social processing are observed in individuals with hypermobility and in people with autism (Boddaert et al., 2004, Eccles et al., 2012). Abnormal regulation of emotion-related
physiological arousal may represent one particular mediator: Joint hypermobility syndrome is linked to postural tachycardia syndrome, an autonomic disorder (Mathias et al., 2012). Interestingly, patients with postural tachycardia syndrome score significantly higher than controls for symptoms of ADHD (Raj, 2006), an effect that has been ascribed to abnormalities in the norepinephrine transporter molecule (Faraone and Mick, 2010, Raj, 2006). Moreover, abnormal peripheral vasoconstriction, consequent upon variant connective tissue, e.g. collagen within the vasculature, may underlie in episodic tachycardia and related autonomic symptoms associated with postural tachycardia, and shared by anxiety disorders through interaction with central interoceptive, cognitive and emotional processes. This physiological mechanism might also explain increased vulnerability to psychosomatic stress-sensitive somatic disorders in both hypermobile patients and people with ADHD or ASD (Hodgkins et al., 2011), for example, as discussed earlier, through imprecise autonomic or interoceptive prediction error.

My observation of an association between joint hypermobility and neurodevelopmental conditions such as ADHD and ASD was particularly strong for females. Putatively, females with hypermobility may represent a sub-phenotype within neurodevelopmental disorders, an effect that may contribute to gender differences in prevalence rates and symptom expression in both ADHD and ASD. More generally, our findings suggest potential value in screening individuals presenting with neurodevelopmental disorders for hypermobility (perhaps also dysautonomia) to anticipate psychological vulnerabilities and mitigate physical health problems. Novel therapeutic approaches may emerge with increasing mechanistic knowledge about the association between psychological symptoms and constitutional physical traits, with the potential to optimise and personalise symptom management.
8.3 Is joint hypermobility related to symptoms suggestive of autonomic dysfunction in the psychiatric population?

In chapter 4 I demonstrate that symptoms suggestive of autonomic dysfunction, particularly orthostatic intolerance and gastro-intestinal disturbance are particularly high in the general psychiatric population. I discover that not only are symptoms suggestive of autonomic dysfunction substantially higher in those who are classified as hypermobile, but also degree of symptoms suggestive of autonomic dysfunction correlates with Beighton score, even if patients are not classified as hypermobile. This finding is important in further understanding the neurobiological mechanisms underpinning the association between joint hypermobility and anxiety. I demonstrate that symptoms suggestive of orthostatic intolerance not only partially mediate the relationship between both joint hypermobility and anxiety but also the relationship between degree of hypermobility and anxiety. Interestingly although this finding held for the whole group, it was driven by an effect in females only, replicating an earlier finding of gender differences by Sanches et al (Sanches et al., 2014). A previous body of work demonstrates abnormal autonomic function in hypermobility (Gazit et al., 2003, Mathias et al., 2012), typically postural tachycardia syndrome; however this is the first experimental work to directly explore the link between symptoms suggestive of autonomic dysfunction and anxiety. Additionally my work suggests that hypermobility has differential interactions on the relationship between medication and symptoms suggestive of autonomic dysfunction, highlighting the need for further research in this area to elucidate potential treatment targets.

8.4 Do constitutional variants in autonomic reactivity predispose to the expression of anxiety?

In chapter 5 I have demonstrated that both hypermobile and anxious participants show heightened sympathetic activation. I have shown that there are interactions between anxiety and hypermobility in terms of sympathetic-over activity. Firstly, hypermobile anxious participants show significantly heightened sympathetic over-activity compared to non-
hypermobile anxious participants and there is a significant interaction with hypermobility on the relationship between anxiety and sympathetic over-activity. Interestingly if participants are non-hypermobile there is no difference in sympathetic activity between anxious and non-anxious participants. I demonstrate that degree of heart-rate rise is directly linked to both anxiety and Beighton score.

I have shown that anxious participants have a higher baseline blood pressure and that if hypermobile there is a significant difference in baseline blood pressure between anxious and non-anxious participants – if not hypermobile there is no significant difference. Patients with hypermobility syndrome show a blunted blood pressure response following deep breathing, suggesting blunted parasympathetic activation.

These findings provide the first direct evidence for the association between joint hypermobility and anxiety and show the importance of the interaction between emotion and the autonomic nervous system. It is likely that the underlying mechanism for this relationship between autonomic dysfunction and anxiety lies in the connective tissue abnormalities underpinning joint hypermobility. For example collagen is also present in blood vessels and it is likely that reduced venous return during standing due to venous pooling or denervation causing low plasma volume may be responsible for an increased sympathetic state – as the body attempts to compensate for these abnormalities - resulting in orthostatic intolerance and associated symptoms (Bohora, 2010, Benarroch, 2012, Mathias et al., 2012).

8.5 What are the neural bases to the association between joint hypermobility and anxiety? Is this association driven by inefficient coordination of efferent autonomic drive (i.e. autonomic predication error) with sensitive interoceptive afferent representation?

Building on previous work in healthy volunteers with hypermobility (Eccles et al., 2012, Mallorqui-Bague et al., 2014), in chapter 6 and 7 I demonstrate, in a clinical population, the likely neural substrates underpinning the association between joint hypermobility and anxiety, e.g. amygdala and insula. I demonstrate that my emotional processing tasks activated areas typically
associated with anxiety such as insula and anterior cingulate and amygdala which would be expected from previous work e.g. (Paulus and Stein, 2006, Fox et al., 2015). I have shown that such activations are linked to interoceptive and autonomic processes, for example activation in insula in anxiety in the emotional faces task correlated with autonomic prediction error. In the false feedback task the main effect of anxiety was to activate insula and the correlation between insula activation and anxiety score was found to be fully mediated by interoceptive sensibility.

I discover that there are specific areas activated in hypermobility in an emotional processing task including insula, and that activation in this area is correlated with Beighton score. Activation in insula in hypermobility syndrome was partially mediated by sympathetic over-activity. Additionally, I also identify interactions between hypermobility and anxiety, for example hypermobile anxious participants compared to non anxious hypermobile participants activated insula and this activation correlated with sympathetic activity.

I find an interaction between anxiety status and Beighton score such that amygdala activation showed no correlation with hypermobility score in non-anxious participants but was strongly activated with hypermobility score in anxious patients. I showed similar interaction of hypermobility status on anxiety score in insula and amygdala, whereby activation in insula showed significant correlation with anxiety score in hypermobility syndrome participants alone.

The main effect of false physiological feedback was to activate insula which is line with previous studies (Gray et al., 2007), this correlated with autonomic mismatch which unsurprising given the role of insula in interoceptive processes (Seth et al., 2011, Critchley, 2004). Activation in this area also correlated with hypermobility score and correlated with sympathetic activation.

My data implicate the amygdala, insula and anterior cingulate cortex as a likely neural substrate mediating previously reported clinical associations between hypermobility, anxiety and psychosomatic conditions. Speculatively,
potential mechanisms include heightened susceptibility of individuals with hypermobility to (threat of) pain and/or a perturbation of autonomic control, and I have demonstrated that activity in insula in hypermobility syndrome was partially mediated by sympathetic over-activity in the false feedback task and in the emotional processing task hypermobile anxious participants compared to non-anxious hypermobile participants activated insula and this activation correlated with sympathetic activity. I have also demonstrated differences in interoceptive sensibility in hypermobility, with heightened awareness of internal bodily sensations. The central interaction of processes supporting the generation and the representation of autonomically-mediated changes in visceral state may be the critical mediator of autonomic influences on cognition and emotion. Central viscerosensory and visceromotor representations are exchanged as afference and efference copies to allow error signalling. Where there is mismatch between intended and actual autonomic state, corrective efferent reactions are accompanied by interpretative processes. The unconscious operation of the autonomic nervous system can be interrupted by deviations from expected state, i.e. we become aware of our autonomic bodily state when we experience changes in internal state that are ‘unpredicted’ by control centres (Critchley et al., 2013, Seth et al., 2011). I believe in this population such prediction error signals a symptom (as in Bayesian models of hysteria (Edwards et al., 2012)) and I have shown that autonomic mismatch (between signs and symptoms of orthostatic intolerance) predicts activity in insula during false feedback and in the main effect of anxiety in insula.

Differences in amygdala activity occur in pain disorders (chronic widespread pain is a feature of hypermobility) including fibromyalgia, irritable bowel syndrome and chronic regional pain syndrome (Tracey and Bushnell, 2009). Anxiety itself is also linked theoretically to the abnormal generation and mapping of bodily arousal through the engagement of amygdala and insula. Crucially the insula plays a fundamental role in homeostasis and autonomic control through mapping and regulation of sympathetic and parasympathetic activity (Critchley and Harrison, 2013, Gianaros et al., 2012, Critchley, 2005). The amygdala also implicated in autonomic control: The amygdala is one
region involved in translating psychological stress into bodily arousal (Gianaros et al., 2008). This relationship is bidirectional (Garfinkel et al., 2014). Afferent signals of physiological arousal are represented within the amygdala and integrated with the processing of threat stimuli.

It is also noteworthy that functional differences within anterior cingulate cortex correlated with hypermobility, a central driver of autonomic arousal and a region implicated in the cognitive control of pain and negative emotions (Critchley, 2009, Tracey and Bushnell, 2009). Enhanced interoceptive sensitivity also points to a more finely tuned sensory representation of internal bodily signals within the hypermobile group, who show increased interoceptive sensibility. Heightened interoceptive awareness is coupled to exaggerated cardiovascular arousal responses (Herbert et al., 2010). Moreover, in postural tachycardia syndrome, which commonly occurs with hypermobility (and may have a common basis in connective tissue variants), heart rate acceleration compensates for dysfunctional vasoconstriction giving rise to physiological symptoms (e.g. palpitations and light-headedness) that are shared with panic and anxiety states (Mathias et al., 2012). Such deregulated responses are likely to affect neural processes supporting emotional feelings, and we have shown not only heightened sympathetic activity and blunted parasympathetic activity in hypermobility, but also that this correlates with activity in key emotion processing areas.

This functional neuroimaging of patient groups, coupled with detailed autonomic monitoring has elucidated the neurovisceral processes underlying vulnerabilities to psychological distress in joint hypermobility further. Traits of autonomic reactivity and interoceptive awareness affect the expression of anxiety and vulnerability may be linked to inefficient coordination of efferent autonomic drive with sensitive interoceptive afferent representations and autonomic prediction error in hypermobile individuals.
If rates of hypermobility are as high as discussed in these experiments above, I would argue that there is considerable value in screening anxiety disorder patients for both joint hypermobility and the attendant hypermobility syndrome, considering the impact of its extra-articular manifestations which could lead not only to increased recognition and understanding of this important condition within the psychiatric field but also to individualised treatment strategies, including medication and cognitive therapy. Given the interactions of hypermobility found on the association between medication and autonomic dysfunction, further work is required to determine exactly which medications may be beneficial to people with hypermobility.

8.6 Limitations and further work

There are several limitations to this work. In chapter 3 and 4, I do not have full information about Brighton Criteria and as such cannot make any inferences about the prevalence of hypermobility syndrome. Similarly in chapter 3 and 4 I only have categorical, rather than scale measures of anxiety, so are unable to fully explore the relationship between hypermobility and psychiatric diagnosis in terms of potential anxiety co-morbidity and also the relationship between degree of anxiety and degree of symptoms of suggestive of autonomic dysfunction. This is addressed in a smaller sample in chapter 5, however I did not undertake exhaustive tests of autonomic dysfunction, e.g Valsalva manoeuver, pressor tests, head up tilt. In chapters 5, 6 and 7 I also focused only on anxiety and it is clear that autonomic dysfunction and hypermobility play a role in other psychiatric disorders, which is certainly worth further exploration. I fail to behaviourally show a difference in ratings of images under false physiological feedback, however I do show activations in insula. The neuroimaging group of anxiety without hypermobility was smaller than the others, perhaps representing the difficulty in finding anxious participants who are not hypermobile. It is apparent from chapters 3, 4 and 5 that there are significant effects of gender both on the expression of hypermobility and its associated impact on autonomic dysfunction. Although gender was included as a co-variate in the brain
imaging analyses in chapters 6 and 7, effect of gender and interactions with
gender on hypermobility and anxiety would be very useful.

It is hoped that future work would address these limitations and go on to pin-
point precise treatment targets for the future.
Bibliography


Appendices

Want to take part in research investigating the relationship between mental health symptoms and the body?

We are looking for people who attend mental health clinics in Brighton and Hove to complete a short questionnaire to explore the relationship between mental health and the body. This project is a collaboration between the Sussex Partnership NHS Trust & Brighton and Sussex Medical School (BSMS).

We are seeking volunteers aged 18 to 65 to participate by completing a short questionnaire.

If you are interested please contact Dr Jessica Eccles or pick up a leaflet at reception and ask if a member of the research team is available.

jeccles@bsms.ac.uk  01273 873818

Version 1 25/10/2012
Want to take part in research investigating the relationship between anxiety and the body?

We are looking for patients who suffer from anxiety to undertake research exploring the relationship between the body and brain. This involves coming for a brain scan and completing tasks on the computer and/or blood pressure and heart rate measurements. This project is a collaboration between the Sussex Partnership NHS Trust & Brighton and Sussex Medical School (BSMS).

We are seeking volunteers aged 18 to 65. You will be reimbursed for your time.

If you are interested please contact: Dr Jessica Eccles

j.eccles@bsms.ac.uk  01273 873833

Version 1 14/10/2014
Want to take part in a brain imaging research investigating the relationship between mental health symptoms and the body?

We are looking for healthy volunteers to participate in a research project which is exploring the relationship between mental health symptoms and the body. The study will happen at the University of Sussex and will last approx. 2 hours and will involve having a brain scan and completing some questionnaires. You will be reimbursed for your time and receive a copy of your brain scan. This project is a collaboration between the Sussex Partnership NHS Trust & Brighton and Sussex Medical School (BSMS).

We are seeking volunteers aged 18 to 65.

If you are interested please contact: Dr Jessica Eccles

j.eccles@bsms.ac.uk 01273 873818

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Version 1 26/10/2012
Dear __________

I understand that you are due to attend the Hove Polyclinic for an appointment. We would like to invite you to participate in a research study. We are interested in how the body and the brain interact and are studying the relationship between mental health symptoms and the body. If you are interested we would like you to complete a questionnaire which includes questions about fainting and related symptoms and also questions about your joints.

Participation in the study is entirely voluntary and a decision to take part will not affect your clinical care in any way. I enclose a copy of the participant information leaflet which gives more information about the study.

If you are interested please contact below or speak to reception on the day of your appointment.

Thank you very much for your time.

Dr Jessica Eccles  lecless@bms.ac.uk or 01273 873818
Hypermobility and autonomic hyperactivity: Relevance for the expression of psychiatric symptoms

PARTICIPANT INFORMATION SHEET – Questionnaire Survey
Version 3 · 28/01/2013

We would like to invite you to take part in a research study investigating the relationship between mental health symptoms and the body. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Part 1 explains the purpose of the study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. This project is a collaboration between Sussex Partnership NHS Trust and Brighton and Sussex Medical School. This research is being undertaken as part of an educational qualification (PhD).

Take time to decide whether or not you wish to take part and please feel free to discuss your participation with friends and family. Please remember your decision whether to take part or not will not affect your care in any way.

PART 1

What is the purpose of the study?

The way in which people differ physically can affect how much their body reacts and this can influence how likely they are to experience mental health symptoms such as anxiety. Some of these differences run in families, such as having flexible 'hypermobile' joints (double-jointed) or a tendency to faint at the sight of blood. More people who are double-jointed or who faint experience problems with anxiety or panic than you would expect by chance. Knowing how and why this is the case is important as it may improve ways of treating anxiety and other symptoms, and help doctors pick the right medicines to avoid side effects.

We are interested in this relationship between certain physical symptoms (unusual joint flexibility, fainting and migraines) and mental health symptoms. Most people know if they have fainted or have had a migraine in the past, but most are unaware of their own joint flexibility. We want to invite all people using assessment and treatment mental health services in Brighton and Hove to answer a questionnaire to help us explore this relationship further.

Why have I been invited to take part?

You have been invited to take part as you use mental health services in Brighton and Hove. We would like to invite all people experiencing mental health problems and using Assessment and Treatment Services to answer a short questionnaire to explore the relationship between symptoms in the mind and symptoms in the body.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form to keep. If you decide to take part you are still free to withdraw at any time, without needing to give a reason.
What will happen to me if I take part?
If you decide to take part, you will be asked to complete a short questionnaire. This should take about 15 minutes. All data will be anonymised. These questionnaires do not diagnose a physical problem, but if you are concerned about joint/fainting or migraine symptoms please contact the research doctor or ask your CP for further advice. At the end of the study you will be debriefed - you will be asked about your experience and have the opportunity to ask any questions.

As part of this study you may be asked if we can contact you to see if you wish to participate in further research studies. In one study we would ask you to perform some tasks on the computer that involve looking at emotive pictures or listening to emotive sounds. Whilst you are performing these tasks we will monitor some responses in your body: heart rate, blood pressure and sweating in your fingers. We will also see how your heart rate and blood pressure responds to lying and standing. We will ask you to also complete some short questionnaires. Another study involves brain scanning. This is safe and non-invasive. You would lie in the scanner (you lie on a soft bed and your head is in a small tunnel) which allows us to measure brain activity. You will be able to contact the researchers at any time if you feel uncomfortable. Whilst in the scanner you will be looking at pictures and words on a screen and making judgements. You will also listen to the sound of your own heartbeat and other sounds. It is absolutely up to you whether you agree to be contacted about participating in further research and your decision will not affect your clinical care in any way. You may also change your mind at any point.

What are the possible risks in taking part?
There are no risks to completing the questionnaire. It should only take a few minutes to complete. Information from the study will be protected and anonymous, so that people will not have access to the information about who took part or find out results.

What are the possible benefits of taking part?
There are no immediate benefits to you of taking part in this study. Although this research may not directly benefit you, it could result in a new ways of treating anxiety and other mental health symptoms in the future.

Will my taking part in this study be kept confidential?
Yes. We want to emphasise that all results obtained will be strictly confidential and will only be used for research purposes. Further details of how this information will be stored are detailed in part 2 below.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

PART 2

What will happen if I don't want to carry on with the study?
You may withdraw at any point during the study. If you withdraw from the study we will destroy all data and results associated with your participation. This will not affect your future care in any way.
What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers (Dr Jessica Eccles and Prof Hugo Critchley) in the first instance, who will do their best to answer your questions (01273 873818). If you remain unhappy and wish to complain formally, you can do this either by contacting the Research and Development department at Sussex Partnership NHS Trust (01273 265896) or the Service Experience Team - also known as PALS (01903 843028).

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If taking part in this research project harms you, there are no special compensation arrangements, but if you are harmed by someone else's negligence, then you may have grounds for legal action and you are free to complain.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be secured against any unauthorised access. Although the overall results will be published in medical journals, no individual subjects will be identifiable from this. Confidential information regarding identity of participants will be kept secure for 10 years, and you may be contacted for follow-up studies related to this project in the future.

Involvement of the General Practitioner (GP)
Your GP will not routinely be notified that you have taken part in this study.

What will happen to the results of the research study?
The questionnaires will be anonymised (removed of identifying information) and kept in a locked office at Brighton and Sussex Medical School.

The results of the questionnaire, along with all other information collected from you in the course of this research will be kept strictly confidential. The results will be statistically analysed and findings subsequently published in peer reviewed journals. You will not be identified in any publication. You are more than welcome to a copy of any publication resulting from this work which can be obtained by giving us your email address.

Who has funded this study?
This study is funded by a grant from the Medical Research Council (MRC).

Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests.

Contact for further Information
Many thanks for reading this. We hope you feel able to take part in our study. If you have any questions, please contact the following people:

Dr Jessica Eccles J.Eccles@bsms.ac.uk 01273 873818
Prof Hugo Critchley H.Critchley@bsms.ac.uk 01273 873818
Hypermobility and autonomic hyperactivity: Relevance for the expression of psychiatric symptoms

PARTICIPANT INFORMATION SHEET – MRI study
Version 2 - 7/01/2013

We would like to invite you to take part in a research study investigating the relationship between the mind and the body. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Part 1 explains the purpose of the study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. This project is a collaboration between Sussex Partnership NHS Trust and Brighton and Sussex Medical School. This research is being undertaken as part of an educational qualification (PhD).

Take time to decide whether or not you wish to take part and please feel free to discuss your participation with friends and family. Please remember your decision whether to take part or not will not affect your care in any way.

PART 1

What is the purpose of the study?
We hope to explore the relationship between anxiety, responses in the body (e.g. heart rate and joint hypermobility). Previous studies have shown a link between anxiety and joint hypermobility and we hope to explore this relationship further, particularly in relationship to brain activity (using MRI – magnetic resonance imaging – brain scans). This will hopefully help to better understand the relationship between the mind and the body and may help determine which medications are more suitable for certain groups of people.

Why have I been invited to take part?
You have been invited to take part for one of three reasons. You may be experiencing anxiety symptoms, you may also have unusually flexible joints. We are also asking people who do not suffer from anxiety or have unusually flexible joints to take part for comparison purposes.

Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form to keep. If you decide to take part you are still free to withdraw at any time, without needing to give a reason.

What will happen to me if I take part?
If you decide to take part, you will be asked to come to the University of Sussex, where you will be met by a researcher. We will ask you a few basic health questions, explain everything again and give you the opportunity to ask any further questions. We will ask you to undertake some light exercise to raise your heart rate, whilst we make a recording of your heart rate. We will take you through to the MRI scanner.
The MRI examination is performed in a special room that houses the MR system or “scanner”. The scanner consists of a circular magnetic tunnel which contains the radio coils. During your scan you will lie on a padded bed, which will move slowly into the scanner. Only your head will be in the scanner. Here is a picture of the scanner:

Before going into the scanner we will ask you to remove any metal items (e.g., watch, earring, necklace). If you wear glasses we will ask you to take them off and we will give you a pair of MRI glasses of a similar strength to wear in the scanner. We will then ask you to lie down on the scanner bed. As the scanner is very noisy when it is running we will also give you ear plugs and headphones to wear. We will ask you to lie still. Whilst you are in the scanner we will put a sensor on your finger to monitor your heart rate. We will also show you words and pictures in the scanner and play some sounds and ask you to make some responses by pressing buttons. We will also play you sounds of your own heartbeat. Whilst you are doing this we will measure brain activity using the scanner. The scanning session will last 1 hour. After a short break we will ask you to complete some questionnaires. At the end of the study you will be debriefed - you will be asked about your experience and have the opportunity to ask any questions.

Expenses and payments
You will be reimbursed £25 for your participation.

What are the possible risks in taking part?
There are no risks to taking part. All procedures are safe and non-invasive. MRI scanning has been used extensively in medicine and research for over 20 years and no side-effects have been reported. However, MRI scanning does involve lying in a high strength magnetic field. If you have a cardiac pacemaker, inner ear/cochlear implants, or if you may have metal fragments in your eye (for example from welding) you should not undergo MRI. This could be dangerous. Also, any metal objects such as pens, coins or keys should not be taken into an MRI room.

If you experience any discomfort or distress, you are free to stop at any point. If you experience any adverse effects from the study, clinical doctors will be available on site for you to talk to.

Information from the study will be protected and anonymous, so that people will not have access to the information about who took part or find out results.

What are the possible benefits of taking part?
There are no immediate benefits to you of taking part in this study. Although this research may not directly benefit you, it could result in better understanding of the relationship between the mind and the body and new ways of treating anxiety and other mental health symptoms.
What mechanisms are in place if my answers to questionnaires suggest my current mental health is of particular concern?

It is possible that your answers to some of the questionnaires may indicate that your symptoms are worse than usual. The researcher is a trained psychiatrist and if this is the case we will contact your mental health team to inform them of this.

Will my taking part in this study be kept confidential?

Yes. With the exception of the above scenario we want to emphasise that all results obtained from the study will be strictly confidential and will only be used for research purposes. Further details of how this information will be stored are detailed in part 2 below.

What will happen if an abnormality is found on my brain scan?

Very rarely an incidental abnormality is found during brain scanning. Prior to enrolment in the study we will therefore request your permission to contact your GP should any abnormality be found on your brain scan. In the unlikely event that an abnormality is found, we will explain the finding to you and with your permission will refer you on to your GP.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

PART 2

What will happen if I don’t want to carry on with the study?

You may withdraw at any point during the study. If you withdraw from the study we will destroy all data and results associated with your participation. This will not affect your future care in any way.

What if there is a problem?

If you have a concern about any aspect of this study, you should talk to the researchers (Dr Jessica Eccles and Prof Hugo Critchley) in the first instance, who will do their best to answer your questions (01273 873818). If you remain unhappy and wish to complain formally, you can do this either by contacting the Research and Development department at Sussex Partnership NHS Trust (01273 265696) or the Service Experience Team - also known as PALS (01903 843026).

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be secured against any unauthorised access. Although the overall results will be published in medical journals, no individual subjects will be identifiable from this. Confidential information regarding identity of participants will be kept secure for 10 years, and you may be contacted for follow-up studies related to this project in the future.

Involvement of the General Practitioner (GP)

Your GP will not routinely be notified that you have taken part in this study.

What will happen to the results of the research study?
The recordings of bodily responses, brain activity during tasks and questionnaires will be anonymised (removed of identifying information) and kept in a locked office at Brighton and Sussex Medical School.

The results of the questionnaires, along with all other information collected from you in the course of this research will be kept strictly confidential. The results will be statistically analysed and findings subsequently published in peer reviewed journals. You will not be identified in any publication. You are more than welcome to a copy of any publication resulting from this work which can be obtained by giving us your email address.

Who has funded this study?
This study is funded by a grant from the Medical Research Council (MRC).

Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests.

Contact for further information
Many thanks for reading this. We hope you feel able to take part in our study. If you have any questions, please contact the following people:

Dr Jessica Eccles J.Eccles@bsms.ac.uk 01273 873818
Prof Hugo Critchley H.Critchley@bsms.ac.uk 01273 873818
CONSENT FORM - Confidential

Title of project: Hypermobility and autonomic hyperactivity: Relevance for the expression of psychiatric symptoms (Questionnaire Survey)

Project ID: 

Form: Version 2. 07/01/2013

Name of chief investigator: Dr Jessica Eccles

Named researchers: Dr Neil Harrison, Prof Hugo Critchley

1 I confirm that I have read and understood the information sheet ‘Hypermobility and autonomic hyperactivity: Relevance for the expression of psychiatric symptoms (Questionnaire Survey)’ and have had the opportunity to ask questions.

2 I confirm that I have had sufficient time to consider whether or not I want to be included in the study.

3 I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.

4 I understand that any information I give is completely confidential and will not be stored in such a way that it can be traced back to me. I agree that the data that I provide will be anonymised and stored for further analysis.

5 I understand that research data collected during the study may be looked at by individuals from the sponsor organisation, from regulatory authorities, and from the NHS Trust where it is relevant to my taking part in the study. I give permission for these individuals to have access to my records.

6 I do/do not (please delete as appropriate) agree to be contacted in the future to consider participating in further related research if I am suitable for such studies. I understand that I am under no obligation to be contacted or participate in further research.

7 I do/do not (please delete as appropriate) wish to receive a copy of the final results of the study. If so, I would like to receive this by post/email (please delete as appropriate) and I agree to provide my contact details. These will be kept separately from the research data.

8 I agree to take part in the above study

Name of participant ____________________________ Date ____________ Signature ____________________________

Name of person taking consent ____________________________ Date ____________ Signature ____________________________

1 Form for participant, 1 Form for study documentation

Version 2 07/01/2013
CONSENT FORM - Confidential

Title of project: Hypermobility and autonomic hyperactivity: Relevance for the expression of psychiatric symptoms (MRI study)

Project ID: Version 2, 07/01/2013

Name of chief investigator: Dr. Jessica Eccles
Named researchers: Dr Neil Harrison, Prof Hugo Critchley

1. I confirm that I have read and understood the information sheet ‘Hypermobility and autonomic hyperactivity: Relevance for the expression of psychiatric symptoms (Brain imaging study)’ and have had the opportunity to ask questions.

2. I confirm that I have had sufficient time to consider whether or not I want to be included in the study.

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.

4. I understand that any information I give is completely confidential and will not be stored in such a way that it can be traced back to me. I agree that the data that I provide will be anonymised and stored for further analysis.

5. I understand that research data collected during the study may be looked at by individuals from the sponsor organisation, from regulatory authorities, and from the NHS Trust where it is relevant to my taking part in the study. I give permission for these individuals to have access to my records.

6. I agree to allow you to contact my GP should any abnormality be found on my brain Scan (for MRI studies only).

7. I do/do not (please delete as appropriate) wish to receive a copy of the final results of the study. If so, I would like to receive this by post/email (please delete as appropriate) and I agree to provide my contact details. These will be kept separately from the research data.

8. I agree to take part in the above study.

Name of participant __________________________ Date ___________ Signature ___________

Name of person taking consent __________________________ Date ___________ Signature ___________

1 Form for participant, 1 Form for study documentation

Version 2 07/01/2013
Hypermobility and autonomic hyperactivity: Relevance for the expression of psychiatric symptoms (Questionnaire Survey)

Instructions to research team on use of the Questionnaire (Study 1 - ASQoL5)

- Explain to the participant that the questionnaire is about common symptoms such as feeling faint. It also includes physical examinations of the joints.
- Explain that on the left hand side they must indicate whether they have the symptom and how frequently they have the symptom. Options for frequency include:
  - Less than once a once a month
  - 1-4 times a month (i.e. more than monthly, but less than weekly)
  - More than once a week
  - Daily
- On the right hand side they must indicate the impact the symptom has on their life using the different boxes
- For question 22 – 26 please perform the Brighton Assessment as indicated in training

For internal use only
### Autonomic Symptoms and Quality of Life Score

#### Table 1:
<table>
<thead>
<tr>
<th>No.</th>
<th>Autonomic Symptom</th>
<th>Severity</th>
<th>Impact on Daily Life</th>
<th>Emotional Impact</th>
<th>Action Taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Heart rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>Respiratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>Urinary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>Sexual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Table 2:
<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment Plan</th>
<th>Date of Start</th>
<th>Date of End</th>
<th>Outcome</th>
<th>Action Taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>Lifestyle Changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>Therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Graph 1:
- Autonomic symptoms are represented using smiley faces indicating severity.
- High severity symptoms are marked with a red smiley face.
- Low severity symptoms are marked with a green smiley face.
- Moderate severity symptoms are marked with a yellow smiley face.

#### Graph 2:
- The graph shows the progression of symptoms over time.
- Changes in symptom severity are indicated by changes in the color of the smiley face.
- The timeline indicates the duration of treatment and associated outcomes.
THANK YOU
BODY PERCEPTION QUESTIONNAIRE

Read the instructions for each sub-test and answer (a - e) next to each item

I: AWARENESS (Image how aware you are of your body processes)

Select the answer that most accurately describes you. Rate your awareness on each of the characteristics described below using the following 5-point scale:

a) never  b) occasionally  c) sometimes  d) usually  e) always

During most situations I am aware of:

1. swallowing frequently
2. a ringing in my ears
3. an urge to cough to clear my throat
4. my body swaying when I am standing
5. my mouth being dry
6. how fast I am breathing
7. watering or tearing of my eyes
8. my skin itching
9. noises associated with my digestion
10. eye fatigue or pain
11. muscle tension in my back and neck
12. a swelling of my body or parts of my body
13. an urge to urinate
14. tremor in my hands
15. an urge to defecate
16. muscle tension in my arms and legs
17. a bloated feeling because of water retention
18. muscle tension in my face
19. goose bumps
20. facial twitches
21. being exhausted
22. stomach and gut pains
23. rolling or fluttering my eyes
24. stomach distension or bloatedness
25. palms sweating
26. sweat on my forehead
27. clumsiness or bumping into people
28. tremor in my lips
29. sweat in my armpits
30. sensations of prickling, tingling, or numbness in my body
31. the temperature of my face (especially my ears)
32. grinding my teeth
33. general jitteriness
34. muscle pain
35. joint pain
36. fullness of my bladder
37. my eye movements
38. back pain
39. my nose itching
40. the hair on the back of my neck "standing up"
41. needing to rest
42. difficulty in focusing
43. an urge to swallow
44. how hard my heart is beating
45. feeling constipated
Below is a list of common symptoms of anxiety. Please carefully read each item in the list. Indicate how much you have been bothered by that symptom during the past month, including today, by entering the number in the corresponding space in the column next to each symptom.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Not At All</th>
<th>Mildly but it didn't bother me much</th>
<th>Moderately - it wasn't pleasant at times</th>
<th>Severely - it bothered me a lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbness or tingling</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Feeling hot</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Wobbliness in legs</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Unable to relax</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Fear of worst happening</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Dizzy or lightheaded</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Heart pounding/racing</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Unsteady</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Terrified or afraid</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Nervous</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Feeling of choking</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hands trembling</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Shaky / unsteady</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Fear of losing control</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Difficulty in breathing</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Fear of dying</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Scared</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Indigestion</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Faint / lightheaded</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Face flushed</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hot/cold sweats</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

**Column Sum**