Characterising Amygdala Activation During Emotion Processing in a Sub-Clinical Anxiety Cohort

by

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Abstract

Emotions play a pivotal role in guiding our behaviour within society and our environment. In particular, emotions enable interpersonal social interactions through non-verbal communication that may be below conscious awareness. However, when there is some disruption to normal emotional processing, such as in anxiety disorders, quality of life of the individual can be severely disrupted. Anxiety disorders account for nearly a quarter of all mental health diagnoses, however the aetiology and underpinning neural correlates of anxiety are still not fully understood. This thesis sought to investigate the neurobiological mechanisms of emotion processing, specifically in the amygdala, in a healthy sub-clinical cohort. Six different studies are presented using quantitative methodology to explore amygdala activation and connectivity during emotion processing, and structural differences, as modulated by gender and sub-clinical anxiety. Overall results reveal a modulating effect of sub-clinical anxiety on amygdala habituation, fronto-amygdala connectivity (at rest and during emotion processing) and neural structure. In addition, results presented in this thesis suggest that there may be an attentional component to characteristic hyper-responsivity of the amygdala during emotion processing seen in clinical anxiety patients that should be incorporated into future models of maladaptive emotion. Furthermore, various different chapters in this thesis present evidence that the left amygdala appears to be more specialised for responses to more socially salient stimuli and the right amygdala appears to be more responsive to threat related stimuli indicating that key theoretical models of emotion (the dual processing model, and the salience detection model) should be integrated into one cohesive model of emotion processing. In addition to these theoretical implications, results demonstrating the modulating effect of anxiety and gender presented in this thesis suggest that research on emotion should account for individual differences as a matter of standard practice. This thesis also supports the use of resting state -functional magnetic resonance imaging (fMRI) as a low cost, valid alternative, to task based fMRI within the study of anxiety. Finally, results suggest that investigation of structural differences in sub-clinical populations, and the use of analytical methods such machine learning classification techniques, could aid the development of diagnostic tools that can track disease progression and identify individuals at risk of developing anxiety disorders. The possibility of identifying such neural biomarkers will allow research to look for therapeutic treatments and interventions, which could prevent individuals from transitioning from sub-clinical anxiety to chronic anxiety disorders.
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Declaration of originality

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service TurnitinUK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.

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Table of Contents

Chapter 1: Literature Review ............................................................................................................ 1
  1. Overview .................................................................................................................................... 1
    1.1 Introduction: Why is emotion research important? ................................................................. 3
      1.1.1 What are emotions? ........................................................................................................... 4
    1.2 Physiological and psychological measurement of emotion .................................................. 9
      1.2.1 Generating an emotion: stimuli ......................................................................................... 10
      1.2.2 Measuring the emotion: Response ................................................................................... 14
      1.2.3 Summary ......................................................................................................................... 16
    1.3 Measurement of emotion in the brain .................................................................................. 17
      1.3.1 Animal models ................................................................................................................ 17
      1.3.2 Research in human participants ....................................................................................... 19
    1.4 The biology of emotion ....................................................................................................... 25
      1.4.1 Neuropsychological models of emotion ........................................................................ 25
      1.4.2 Biological Circuitry of Emotion ..................................................................................... 28
    1.5 The Amygdala ...................................................................................................................... 39
      1.5.1 Models of direct amygdala activation ............................................................................. 41
      1.5.2 Dual Route of Fear .......................................................................................................... 42
      1.5.3 What are the key characteristics of the amygdala? ......................................................... 45
      1.5.4 Resting state analyses of amygdala connectivity ............................................................. 54
    1.6 Anxiety, abnormal anxiety and emotional disorders ................................................................. 54
      1.6.1 Behavioural Inhibition System (BIS)/ Behavioural Approach System (BAS) models of
            anxiety disorders .............................................................................................................. 56
      1.6.2 Putative Neurocognitive Correlates of BIS/BAS ............................................................ 56
      1.6.3 Neural Basis for Anxiety Disorders .................................................................................. 59
    1.7 Chapter Summary ................................................................................................................ 60

Chapter 2: Research Aims .............................................................................................................. 62
  2.1 Aims of thesis .......................................................................................................................... 62
  2.2 Plan of thesis ........................................................................................................................... 62

Chapter 3: Method .............................................................................................................................. 65
  3.1 Overview ................................................................................................................................. 65
    3.1.1 Recruitment Overview ...................................................................................................... 65
    3.1.2 Study Design Overview .................................................................................................... 65
    3.1.3 Behavioural Materials and measures ................................................................................ 66
    3.3.4 The Mood Online Experience Survey: MOOX .............................................................. 73
10.2 Summary of results by study ................................................................. 254
10.3 Implications of results ........................................................................ 257
  10.3.1 Theoretical Implications ............................................................... 257
  10.3.2 Methodological Implications ......................................................... 261
  10.3.3 Practical Implications ................................................................ 262
10.4 Methodological limitations ................................................................. 265
10.5 Conclusion ....................................................................................... 267
References .............................................................................................. 269
Appendices ............................................................................................. 305
  Appendix A .......................................................................................... 305
List of Tables

Chapter 4 Mood Online Experience Survey (MOOX)

Table 4.1 Percentage breakdown of demographic information across all participants, and within each demographic (by anxiety group or gender). 95
Table 4.2 Cross tabulation results (count) for overall group results 96
Table 4.3 Percentage of participants who would not participate in each study by anxiety group. ‘N.S.’ denotes non-significant (p>0.05) relationships between high and low anxiety participants 98
Table 4.4 Percentage of participants who would not participate in each study by gender group. ‘N.S.’ denotes non-significant (p>0.05) relationships between male and female participants 99
Table 4.5 Inferential results of the three-way cross tabulations of gender/age/English as a first language by anxiety group by different study design types. 102

Chapter 5: General Linear Model

Table 5.1 Table showing different Contrasts that were considered in the second level regression 116
Table 5.2 Overview of descriptive results for the male and female participants and the age/ hospital anxiety and depression ranges, mean scores and standard deviations 117

Chapter 6: PsychoPhysiological Interaction

Table 6.1 Main effect and interaction results of the two-way ANOVA looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral amygdala as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala). 151
Table 6.2 Main effect and interaction results of the mixed ANOVA with gender as a between groups variable looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral amygdala as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala). 153
Table 6.3 Main effect and interaction results of the mixed ANOVA with anxiety as a between groups variable looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral amygdala as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala). 155
Table 6.4 Main effect and interaction results of the two- ANOVAs looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral fusiform gyrus as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala). 156
Table 6.5 Interaction results of the mixed ANOVA with gender as a between groups factor looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral fusiform gyrus as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala) and gender.

Table 6.6 Interaction results of the mixed ANOVA with anxiety as a between groups factor looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral fusiform gyrus as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala) and gender.

Chapter 7: Categorisation Analysis

Table 7.1 Classification accuracy for data from GLM analysis and PPI analysis

Chapter 8: Cortical Thickness Analysis

Table 8.1 Showing scores for the 90th percentile state and trait anxiety scores from normative data in the Spielberger STAI manual
Table 8.2 ANCOVA results showing group differences for every ROI, when using HADS_A
Table 8.3 Overview of the current study in relation to four previous studies looking at anxiety and neural structure in sub-clinical population

Chapter 9: Resting state and Parcellation of the Amygdala

Table 9.1 List of brain regions showing significant positive relationship with the left and right amygdala
Table 9. 2 List of brain regions whose connectivity with the left or right amygdala is significantly (positively or negatively) correlated with HADS_A
Table 9.3 List of brain regions whose resting functional connectivity with the amygdala (left/right) is significantly different between high and low anxiety groups
Table 9.4 List of brain regions whose resting functional connectivity with the amygdala (left/right) is significantly different between male and female groups.
Table 9.5 List of brain regions showing a significant positive or negative relationship with the right and left laterobasal (LB) sub-region of the amygdala.
Table 9.6 List of brain regions showing a significant positive or negative relationship with the right and left laterobasal (LB) sub-region of the amygdala compared to the centromedial (CM) and superficial (SF) sub-regions.
Table 9.7 List of brain regions showing a significant positive or negative relationship with the right and left centromedial (CM) sub-region of the amygdala
Table 9.8 List of brain regions showing a significant positive or negative relationship with the right and left centromedial (CM) sub-region of the amygdala compared to the laterobasal (LB) and superficial (SF) sub-regions.

Table 9.9 List of brain regions showing a significant positive or negative relationship with the right and left superficial (SF) sub-region of the amygdala.

Table 9.10 List of brain regions showing a significant positive or negative relationship with the right and left superficial (SF) sub-region of the amygdala compared to the laterobasal (LB) and centromedial (CM) sub-regions.

Table 9.11 List of brain regions showing significant positive or negative convergences across all three amygdala sub-divisions.
List of Figures

Chapter 1 Literature Review

Figure 1.1 Schematic providing an overview of structure of the literature review detailed in this thesis. 2
Figure 1.2 Image of the bidirectional continuum of emotion 4
Figure 1.3 Seyle’s Generalise Adaptation Syndrome (Gas) model of stress 6
Figure 1.4 Chronology of theoretical models of emotion 9
Figure 1.5 Schematic of the emotion experience as informed by appraisal models of emotion 10
Figure 1.6 Examples of Friedman and Ekman (1975) emotional face stimuli 13
Figure 1.7 Default mode network as identified by Shulman et al. (1997) 23
Figure 1.8 Schematic of Papez’s original circuit of emotion. 26
Figure 1.9 Image showing where the amygdala is in the brain 40
Figure 1.10 Image showing the two main efferent pathways from the amygdala 41
Figure 1.11 Schematic of the dual route of fear 44
Figure 1.12 Image highlighting four key interacting factors suggested to modulate amygdala activation during emotion processing 45
Figure 1.13 Figure showing the changes in brain activation (including amygdala) during an emotion recognition task during the different phases of the menstrual cycle. 52

Chapter 3: Methods

Figure 3.1 Diagram providing an overview of data collection in this thesis 66
Figure 3.2 Schematic showing procedural overview of data collection 67
Figure 3.3 Schematic showing the breakdown of participants in the mood experience survey (MOOX) 74
Figure 3.4 Schematic showing the MOOX procedure 76
Figure 3.5 Schematic showing the experimental phase of data collection 77
Figure 3.6 Schematic indicating distribution of participants in the experimental phase of data collection 79
Figure 3.7 Schematic showing distribution of participants in each experimental chapter 80
Figure 3.8 Schematic of exemplar MRI machine with viewing mirror. 81
Figure 3.9 Examples of the NimStim emotional face stimuli used within this thesis 82
Figure 3.10 Example of the backward masking stimuli and timings using in the experimental paradigm 83
Figure 3.11 Sequence of stimuli presentation and timings during the backwards masked emotion paradigm 84
Chapter 4 Mood Online Experience Survey (MOOX)

Figure 4.1 Graph comparing willingness to take part in a research design where performance is measured by willingness to take part in a study where performance is not measured 97

Figure 4.2 Graph comparing willingness to take part in a research design where brain based measures (neuroimaging techniques) are used, compared to willingness to participate in studies not using brain based measures 97

Figure 4.3 Graph to show the interaction between gender and anxiety in participants willingness to participate in a brain based study where performance is measured 100

Figure 4.4 Graph to show the interaction between English as a first language and anxiety in participants’ willingness to participate in a brain based study where performance is measured 101

Chapter 5: General Linear Model

Figure 5.1 Whole brain activation for main effect of faces across all participants, activation of fear against baseline, activation of happy against baseline and activation of neutral against baseline 118

Figure 5.2 Graphs showing group level results for the interaction of habituation, valence and lateralisation on amygdala activation during emotion processing 119

Figure 5.3 Graph showing the interaction of gender by valence in amygdala activation 120

Figure 5.4 Graphs representing amygdala activation change over time showing habituation patterns in the left amygdala and right amygdala in difference anxiety groups 121

Figure 5.5 Graph showing mean fusiform activation over runs showing habituation in the left and right hemisphere across all participants 123

Figure 5.6 Graph representing mean fusiform activation over time in male and female participants 124

Chapter 6: PsychoPhysiological Interaction

Figure 6.1 Results of whole brain connectivity with the left and right amygdala during fear conditions 146

Figure 6.2 Results of whole brain connectivity with the left and right amygdala during happy conditions 147

Figure 6.3 Results of whole brain connectivity with the left and right amygdala during neutral conditions 148

Figure 6.4 Results of whole brain connectivity with the left and right fusiform gyrus during happy and fear conditions 149

Figure 6.5 Results of whole brain connectivity with the left and right fusiform gyrus (FFG) during neutral condition 150

Figure 6.6 Graphs showing mean coupling between (a) left amygdala and dmPFC, and (b) right amygdala and dmPFC in difference valence conditions (fear, happy and neutral) 152

Figure 6.7 Graph showing the gender by valence interaction in amygdala-dmPFC coupling 154
Figure 6.8 Graphs showing the hemisphere by gender interaction between the amygdala and dmPFC, vmPFC and Precuneus

Figure 6.9 Graph showing the significant interaction of seed ROI hemisphere (left and right fusiform gyrus; FFG) and connectivity between three secondary regions of interest: the anterior cingulate cortex (ACC), the vmPFC (ventromedial prefrontal cortex) and the Precuneus.

Figure 6.10 Graphs showing the valence by hemisphere interaction between the left and right fusiform gyrus and Precuneus in difference emotion conditions (fear, happy and neutral).

Figure 6.11 Graph showing the main effect of gender on Fusiform gyrus – Precuneus connectivity

Figure 6.12 Graph to show numerical differences between the high (orange) and low (green) anxiety groups in fusiform gyrus – Anterior cingulate cortex connectivity

Figure 6.13 A visual representation of the results presented in section 6.4.2

Figure 6.14 A visual representation of the results presented in section 6.4.3

Figure 6.15 Schematic showing connections observed in the PPI and ROI analysis grouping activation patterns into two parallel roles – the left amygdala is suggested to be involved in salience detection as a result of apparent responsivity during fear

Figure 6.16 Image showing amygdala-dorsomedial prefrontal cortex coupling described in section 6.4.3.1. Here it the coupling patterns observed is considered in light of results when accounting for gender (see section 6.4.3.2).

Chapter 7: Categorisation Analysis

Figure 7.1 Most discriminant (top 10%) voxels for distinguishing between fear and happy conditions, using the PPI results from the left amygdala

Chapter 8: Cortical Thickness Analysis

Figure 8.1 Volume differences between high and low anxiety groups categorised using the HADS_A.

Figure 8.2 Partial correlation scatterplot showing negative correlation controlling for effect of age between HADS_A scores and left amygdala left vmPFC right vmPFC and right dmPFC and between STAI scores and right FFG

Chapter 9: Resting state and Parcellation of the Amygdala

Figure 9.1 Whole amygdala functional connectivity across participants (N=57).

Figure 9.2 Areas where resting functional connectivity correlates with anxiety. Patterns of significantly positive (red) and negative (blue) correlations for the left and right amygdala
Figure 9.3 Differences in resting functional connectivity of the amygdalae between high (N=18) and low (N=39) anxiety participants

Figure 9.4 Differences in resting functional connectivity of the amygdalae between male (N=25) and female (N=32) participants

Figure 9.5 Functional connectivity of amygdala regions of interest at rest. Patterns of significantly positive (red) and negative (blue) relationships for the laterobasal (LB), centromedial (CM) and superficial (SF) sub-regions of the amygdala

Figure 9.6 Direct comparisons of the functional connectivity of each sub-division of the amygdala in comparison to the other two sub-divisions.
Chapter 1: Literature Review

1. Overview
The purpose of this chapter is to present the current literature, historical and theoretical perspectives that contribute to the field of affective neuroscience, with particular focus on the role and characteristics of the amygdala in emotion processing. It will start by clarifying the impact and importance of such research. Next the wider picture will be considered, presenting the historical perspectives that have led to current theoretical considerations in both psychology and affective neuroscience. Then emerging methods and techniques which aid study in the field will be discussed. The outline of the literature review is presented schematically in Figure 1.1.
Figure 1.1. Schematic providing an overview of structure of the literature review detailed in this thesis. Section 1 addresses why studying emotion is important, section 2 and 3 introduce methods of studying emotion using behavioural, physiological and brain based techniques, section 4 and 5 present literature on the underlying neurocircuity involved in emotion processing, with particular focus on the amygdala, finally section 6 introduces literature on abnormal emotion processing, in particular anxiety disorders.
1.1 Introduction: Why is emotion research important?

Emotions are a universal phenomenon, shared not only across cultures but also across species. They can be relayed as the primary form of non-verbal communication and thus serve a vital role in terms of quality of life and in extreme cases survival of an organism in a shared environment. The integral importance of emotions becomes particularly apparent when we observe atypical or maladaptive emotions. Results from a National Survey Comorbidity Replication in America stated that mood disorders were ‘highly prevalent, highly persistent, and highly impairing’ (Kessler, Merikangas, & Wang, 2007, pp150). In addition, the most recent World Mental Health survey (2009) of community epidemiological studies stated that anxiety disorders are ‘the most prevalent class of mental disorders in the general population’ (Kessler et al., 2009, pp 4). Anxiety disorders account for an annual estimated 16% global prevalence of all mood disorders (Kessler et al., 2009) with estimates of the economic burden of anxiety disorders in the U.S. alone over a decade from 1990 to be in excess of $42 billion (Greenberg et al., 1999). Despite the clear impact of mood disorders on the global population, in particular that of anxiety, we still do not fully understand the underlying neuro-behavioural emotion mechanisms that are involved (Dunsmoor, Åhs, & Labar, 2011; Sladky et al., 2013). To start to unravel such mechanisms we must first take a step back and try to fully understand what is involved in typical emotion processing and anxiety in non-clinical populations. The amygdala has been shown to play a pivotal role in emotion processing (for a review see Phelps & LeDoux, 2005). As a result, the primary focus of this thesis is to characterise amygdala activation involved in emotion processing, in particular focussing on mechanisms involved in fear processing and anxiety with the aim of contributing to our understanding of underlying mechanisms.

Cognitive neuroscience typically investigates attention, memory, perception and language, avoiding the key consideration as to how these aspects of cognition interact with something as fundamental as emotions. In the last twenty years interest has increased exponentially into the cognitive neuroscience of emotion, in an attempt to investigate these difficult questions. Indeed, in noting that emotion clearly interacts with cognitive theories of memory, Phelps wrote ‘it has become increasingly clear that we can no longer neglect the exploration of emotion, as it is rarely absent from our daily functions’ (Phelps, 2004, pp201). The reluctance to study emotion in the past has largely come from the difficulty in defining clear concepts of what emotions are, or even how many emotions there are, and creating rigorous
1.1.1 What are emotions?

It is understood that emotions arise from a combination of cognitive, motor and visceral sensory elements through autonomic and endocrine responses yet there are no concrete scientific definition of emotions as a whole resulting in ambiguity in how to define emotions. However, there is agreement over the existence of some more common emotions, which appear to be universal both cross-culturally and across species. There are two key approaches to categorising such emotions, some researchers measure them on a bidirectional continuum of affect (Figure 1.2). ‘Affect’ is considered emotion as it is represented in language, though even in this definition there is still some debate (for further details see Barrett and Russel, 1999). Within the bidirectional model emotions are considered in terms of their valence (i.e. pleasantness) and arousal levels (for review see Mauss & Robinson, 2009) resulting in either positive or negative affect.

Figure 1.2: Image showing the bidirectional continuum of emotions. Emotions relating to states of pleasure (e.g. happy) and displeasure (e.g. sadness) are places along the valence dimension. The position along the arousal dimension relates to the level of energy related to the emotion (e.g. a low arousal emotion could be considered calm, compared to a high arousal state –surprised).

Alternatively, researchers have used more discrete forms of categorisation. This started with Darwin’s investigation of the evolution of human emotions from animal emotions, concluding that humans have a finite discrete set of biologically salient emotions (1872 cited in Hess & Thibault, 2009). How to categorise and divide emotions has been heavily debated, and as a result there have been a variety of suggestions as to how many emotions exist (for
discussion see Scherer, 2005). However, observation of shared facial expressions (Ekman & Friesen, 1971) led to a general consensus that there are six basic emotions. These are considered to be happiness, sadness, surprise, fear, disgust and anger (Ekman, 1992). Evidence of shared emotions with the animal kingdom can provide vital insight into possible underlying physiological mechanisms for negatively valenced emotions (often linked to threat responses and survival mechanisms), and to a lesser extent positively valenced emotions (related to social well-being rather than survival). However, conscious awareness transforms these shared basic emotions into the experience of ‘feelings’ which are thought to be a more uniquely human attribute. Affective neuroscience must therefore not only use translational animal models to guide research, but also look beyond these to human models to understand the complexities of more subtle emotions and feelings (for more details see section 1.3.2).

1.1.2 Psychophysiological Models of Emotion

1.1.2.1 Historical Models

Despite ambiguity over how to define emotions and how many emotions there really are, many theories and models have been put forward over time that have tried to encapsulate the concept of emotions. Early theories such as that by James (1884), and at a similar time, Lange (1885; cited in Dalgleish, 2004), suggested emotions are the result of an individual experiencing a bodily sensation and then the brain detecting and perceiving these physiological changes and assigning an appropriate emotion (for full review see Dalgleish, 2004). However, these theories could not account for how an individual can experience the same physiological responses from very different emotions. Bard (1928), and later his colleague Cannon (1931), proposed an alternative theory of emotion where cognitive experiences of emotions and physiological experiences occur in parallel but are independent of each other. This notion is almost reminiscent of the dual theory of body and mind (Descartes, 1984), where the mind and body are two separate entities that can exist without the other. The concept of cognitive and physiological parallel processing was largely accepted, and provided research into emotion with a new framework from which further models could develop.

Alternative theories to Cannon and Bard’s parallel processing models of emotion arose in a post-world war II climate from stress and coping research (see Lazarus, 1993 for full review).
A key focus of psychology at that time was understanding the effects of psychological strain on the returning military, and increasing numbers of physiological and mental dysfunctions, such as ‘shell shock’. In particular investigating why some individuals exhibited these dysfunctions, yet others who experienced similar situations did not, eventually resulted in the development of the General Adaptation Syndrome (GAS) model of stress (Selye, 1973, see Figure 1.3). This model moved away from simple stimulus – response mechanisms towards a model that allowed for individual differences (stimulus – organism – response), a notion largely ignored up until that point. The GAS model of stress is a three stage model of how different stressors could impact an organism’s internal environment and result in a largely standardised physiological stress response (see Figure 1.3).

![Selye's General Adaptation Syndrome](image)

Figure 1.3 Selye’s GAS model starts with the alarm stage, whereby a stimulus is recognised as posing a threat to the organism's homeostatic balance and triggers an alarm mechanism. This stage is associated with the initiation of autonomic or endocrine responses, such as the release of stress hormones. During the second "resistance" stage, a behavioural response will have been elicited to reduce or remove the stressors. As the stressor is attenuated, the defence ("resistance") mechanisms also reduce. Stress hormone production lessens, and focus turns to repairing any damage the body has endured. The body will still be on alert in the resistance stage, ready for any return of the stressor. If the stressor persists, it enters the final "exhaustion" stage. In this stage, the body’s ability to remain on alert reduces, as the long-term build-up of stress hormones can cause damage to the body.

Seyle’s GAS model of stress provides a clear framework for physiological aspects of the stress response, and in particular has moved forward understanding of stress hormones and possible negative impact the stress response can have. For example, cortisol is a key stress hormone which acts to mobilise energy reserves and suppress the immune system. This enables the body to spend all necessary energy on dealing with the stressor in the short term, but can have negative repercussions in the long-term (the effects of cortisol will be discussed further in section 1.6). As noted by Lazarus (2009), traditionally stress and emotion research are often treated as two interdependent fields; when there is stress there is concomitant
emotional responses, and often the reverse is true, but not in all cases. However, despite allowing for the organism and individual differences, the GAS model is lacking in detail on cognitive and psychological aspects of the stress response which would be associated with emotional responses, and is too rigid in its expected inputs and outcomes.

1.1.2.2 Appraisal theories and Modal Models
To counter such strict models, modern emotion theories take a more cognitive mediational approach, with the concept of cognitive appraisals at the fore. This arose from work by Schachter & Singer (1962) who suggested that the experience of an emotion requires not only the physiological experience itself (as in Selye's GAS model), but also an interpretation of these physiological responses (reminiscent of Cannon and Bard’s models). This interpretation, or appraisal, depends on the current external context or situation, and thus cognitive factors become important in the emotion experience. Individual differences and behavioural outcomes when faced with the same stressor, as introduced in the GAS model, are included in this model under the concept of ‘psychological coping’. Early cognitive mediational approaches to stress suggested an interplay between appraisals and coping mechanisms during a stress response. Thus, cognitive interpretation of physiological responses varies between individuals, and this appraisal mediates the individual reaction to stress (see Lazarus, 1993 for more information). Through this type of model, coping mechanisms were largely viewed as adaptive, whereas the defence mechanisms within the GAS model were considered maladaptive (Parker and Endler, 1996).

Gross & Thompson (2007) built on the earlier appraisal theories and proposed a ‘modal model’ of emotion. In this model, as observed in appraisal theory, there is a context-dependent interaction between the organism and the environment. The interaction is therefore flexible, orienting attention to objects high in individual salience and generating a number of appropriate responses dependent on the changing context over time. Despite similarities to previous models, Gross and Thompson (ibid.) identified three key ways in which accounts of emotion regulation by the modal model differs from earlier theories of the stress response. Firstly, the model looks at emotions in general, and not just stress responses. There are no a priori assumptions of whether an emotion is good or bad, as seen in the appraisal/ coping dichotomy (Thompson & Calkins, 1996). Secondly, the model proposes that individuals can down- or up-regulate their emotional experience (positive or negative), often as a result of
social context (e.g. see Goldin, McRae, Ramel, & Gross, 2008 for recent neuroimaging evidence). Thirdly, the modal model proposes that there is automatic, non-conscious emotional regulation in addition to conscious regulation. This proposal is supported by a growing body of research (e.g. Williams, Bargh, Nocera, & Gray, 2009; Hopp, Troy, & Mauss, 2011).

In summary, modern appraisal theories maintain that emotions are adaptive responses to environmental cues relating to the well-being of the organism (Moors, Ellsworth, Scherer, & Frijda, 2013). These cues can be external, or associated with the internal state of the organism. Thus, a potentially threatening stimulus will be perceived by the organism, whereby the nature of the threat will be assessed and an appropriate reaction chosen (fight or flight response). In such scenarios we may only see a transient alarm reaction which dissipates if the appraisal is that there is no immediate danger to the organisms’ well-being (e.g. LeDoux, 1998; Somerville et al., 2012). Rachman (1980) defined this system of appraisal as ‘emotion processing’ where an organism experiences an emotional disturbance (be it positive or negative) and this emotional disturbance is processed and eventually assimilated such that the organism can carry on behaving as normal. Appraisal theory therefore has the potential to explain the complexities of emotions, as well as the individual differences observed in response to the same stimulus. It may also explain how prior experiences can greatly influence, and reshape, emotional processing and responses, and how these can change in the same individual over time. Therefore, the working definition of emotion used in this thesis is largely grounded in the appraisal theories of emotion, which continue to provide a framework for emotion research today (see Figure 1.4).
Figure 1.4 Timeline showing the development of the main theories of emotion over time. From left to right; James (1884) and Lange (1885) whereby emotion was said to result from perception of internal physiological states; Cannon (1928) and Bard (1931) where cognitive experiences of emotions and physiological experiences occur in parallel but are independent; Seyles (1973) GAS model based on research into the stress response which is a three stage model of stress and emotion; Schachter and Singer (1962) developed appraisal theories at the same time as Seyle’s GAS model suggesting that emotion requires both physiological experience and an interpretation of these physiological responses and Gross and Thompson (2007) modal model of emotion which is built on appraisal models allowing for modulation individual differences in the stimulus-response process. The final box indicates that the framework used in this thesis arises from modern appraisal theories such as that of Gross and Thompson.

1.2 Physiological and psychological measurement of emotion

According to appraisal theory models, the emotional response generated after an appraisal is considered to result from an interaction between experiential, behavioural and neurobiological response systems. These models expand the stimulus – organism- response model, resulting in a more dynamic model that accommodates the different elements of an emotional experience. They usually contain a stimulus or trigger, an internal assessment of the stimulus and the response, with continuous updating throughout the process (Figure 1.5). There are two steps involved in studying physiological and psychological aspects of emotions; researchers need to utilise methods that enable manipulation of these stimuli to generate an emotion experience and consequently they must observe and measure the resulting behavioural responses. These two phases involved in the study of emotions will be considered in turn, as each has different considerations.
Figure 1.5. Schematic of experiences of emotions based on appraisal theories of emotion. Here it is suggested that the experience of emotion contains a stimulus or trigger followed by an internal assessment of the stimulus and then response. In addition there is continuous updating throughout the process in order for an appropriate reaction (i.e. if a stimulus is incorrectly perceived (a stick is seen to be a snake) the fear response may be triggered but with updating (the stimulus is identified as a stick, not a snake) the reaction can be moderated accordingly (no longer reacting with a fear response)).

1.2.1 Generating an emotion: stimuli

Early work into emotion relied on using animals and used predominantly fear or anxiolytic stimuli (e.g. sudden loud noises, sounds and/or smells of predators and electric shocks) to generate what was believed to be a reliable fear response. However, novel, more sensitive, techniques and more complex paradigms have been established with which to study emotions in human participants. The primary aim of these paradigms is to be able to create an emotional event that the participant actually experiences, rather than presenting a stimulus which they can perceive to have emotional content but do not personally engage with (does not result in a physiological emotion experience). There are two key groups of methods detailed below by which researchers try to create an environment which captures a ‘real’ emotional experience; these are learning paradigms and induction paradigms.

1.2.1.1 Learning paradigms

In learning paradigms, reward and punishment are used to experimentally manipulate (both activating and inhibiting) participant’s emotions. Such paradigms can use motivation for reward/punishment to study emotional reactions (for example much research looking at decision making and the relationship between emotion and rationality uses monetary reward/loss, for review see Pham, 2007). However, the most well-known example of learning
The paradigm used in emotion research is conditioning paradigms, especially those looking at fear conditioning which have been used in animal studies in rats and other mammals (e.g. see Delgado, Olsson, & Phelps, 2006; LeDoux, 2000; LeDoux, 1996). The use of fear conditioning paradigms in emotion research are most commonly associated with the work by Joseph LeDoux who revived interest in understanding the brain basis of emotion and in particular the amygdala’s role in the fear response (Ledoux, 1995; Joseph LeDoux, 1998b; R. G. Phillips & LeDoux, 1992). Fear conditioning uses Pavlovian concepts of conditioning; a mundane stimulus (e.g. turning on a light) is paired with an unpleasant fear-inducing stimulus (e.g. a small electric shock). The pairing is repeated until eventually the mundane stimulus alone generates a fear response akin to that of the noxious stimulus. For example in the work by Phillips and LeDoux (1992) the potential differences between cued (a tone was paired with foot shocks) and contextual (presence in the rodent conditioning chamber where tone-foot shocks were administered) fear conditioning in rats was investigated. Time spent freezing (absence of all movement except for respiratory related movement) in each condition was used as an index of fear conditioning. Three groups of rats were included in the study, these were un-operated rats (controls), those with lesions to the amygdala and those with lesions to the hippocampus. In comparison to the controls, successful fear conditioning in both the cue and context condition were impaired in those with amygdala lesions, however in rats with hippocampal lesions only, context fear conditioning was impaired. This work highlighted the primary role of the amygdala in fear conditioning in both simple (cue) and more complex (context based) situations and added momentum to the use of fear conditioning paradigms in emotion research. More recent studies in humans and fear conditioning have supported and extended much of the evidence from animal studies (e.g. Adolphs et al., 2005; Morris et al., 1998; Phelps & LeDoux, 2005; Phelps et al., 2000). Fear conditioning paradigms have provided the bulk of evidence to show the pivotal role of the amygdala in the fear response, as well as the amygdala’s association with other brain structures involved in emotional learning and memory (for details see Rodrigues, Schafe, & LeDoux, 2004). Within emotion research, such methods are useful to understand where learning and habituation occurs in the organism and in what context(s), and have been vital in the progression of emotion research and affective neuroscience. They also provide insight into preventing emotions disorders and possible treatments. However, they do not tap into the participant’s innate emotional experience in a naturalistic setting.
1.2.1.2 Induction Paradigms

An alternative method to learning and motivational paradigms used to stimulate an emotion experience is mood induction, which can be split into direct and indirect mood induction paradigms. Direct mood induction involves researchers directly asking participants to ‘feel’ a specific emotion. A common type of direct mood induction was developed by Velten (1968) which is based on participants reading positive or negative statements, such as ‘I feel cheerful and lively,’ reflecting on these statements, and then trying to feel the mood described in the statement. However, a meta-analysis assessing the effectiveness of mood induction paradigms concluded that the effects of demand characteristics cannot be ruled out when using direct mood induction paradigms such as the Velten paradigm (Westermann, Spies, Stahl, & Hesse, 1996). The lack of experimental control and reliance on participant compliance and honesty involved in this type of mood induction paradigm has led to the use of this type of tool in such studies falling out of favour in recent emotion research.

Indirect mood induction uses emotionally evocative stimuli, with participants asked to report what they feel. Occasionally, participants are explicitly instructed to feel what is portrayed by the stimulus. Indirect mood induction paradigms currently account for much research in the field (Tottenham et al., 2009; Dyck, Loughead, Gur, Schneider, & Mathiak, 2014). The type of medium used to evoke mood varies from music mood induction (where participants listen to mood-suggestive music and are instructed to feel the mood portrayed or report what the music makes them feel) to film and story mood induction paradigms (e.g. Gross & Levenson, 1995, for a comprehensive review of various different mood induction paradigms see Westermann et al., 1996). One of the most widely used methods for emotion induction uses emotional pictures (Uhrig et al., 2016). Studies often favour the use of emotional face stimuli in particular, such as those by Ekman & Friesen, 1975 (Figure 1.6), and more recently a set developed by Professor Nim Tottenham in association with the MacArthur Foundation Research Network on Early Experience and Brain Development (the NimStim Set of Facial Expressions — available to the scientific community at http://www.macbrain.org/resources.htm).
Figure 1.6 Example of the Friedman and Ekman, 1975 emotion face stimuli. From left to right; fear, happy and neutral.

Such stimuli are commonly used in laboratory studies of emotion and have been shown to be both reliable and valid (Tottenham et al., 2009), and almost as effective at mood induction as paradigms that use story-based induction (Westermann, Stahl, & Hesse, 1996). A recent study of 144 participants investigated the effectiveness of films clips and pictures in inducing emotion states (Uhrig et al., 2016). The participants rated emotion and arousal states following stimulus presentation of either positive or negative film clips. The results revealed that though films and pictures both induced the corresponding mood being investigated on absolute scales; only negative stimuli were seen to significantly modulate emotion state compared to baseline. In addition, it was found that pictures were more effective in evoking negative emotion states than short film clips.

Film and pictures have been shown to be particularly useful tools to induce emotion states in participants and are still widely used today in emotion research. A key critique of such studies is the risk of inflated findings resulting from demand effects whereby the participants form an interpretation of the experimental purpose and perhaps subconsciously, or deliberately, alter their behaviour to demonstrate what they believe the researcher is looking for. In particular, in order to determine the effectiveness of such induction paradigms participants are often asked to complete self-report measures of emotion state. The use of such measures is discussed further in section 1.2.2, however when investigating emotion which is such an internal, and individual, experience, the risk of demand effects is a key concern. However, one way in which such bias could be avoided would be through the use of deception, so that participants do not know the true aim of the research. Emotional stimuli can also be easily manipulated, for example using backward masking which prevents conscious perception of the stimulus, to combat any demand effects. This allows the researcher to directly tap into the emotional response of the participants, without other cognitive manipulation which could confound the study. Many studies investigating emotion have used backwards masking methods to reduce the possibility of such biases (e.g.
Dannlowski et al., 2007; Morris, Ohman, & Dolan, 1998; Rauch et al., 2000; Whalen et al., 1998; Winkielman, Berridge, & Wilbarger, 2005). Such studies have further built on knowledge gained from fear conditioning paradigms, revealing underlying mechanisms in emotion processing and in particular in conjunction with neuroimaging techniques (discussed in section 1.2.2) has allowed the study of automatic emotion responses in the amygdala (e.g. Rauch et al., 2000).

The research presented in this thesis uses backwards masking of the NimStim face stimuli to induce emotion in the participants. Both learning and induction paradigms are currently used in emotion research and both have strengths and limitations as discussed in more detail above. In particular learning paradigms can be used to directly manipulate a participant’s emotional response to a particular stimulus. This is beneficial in terms of introducing a level of control in that researchers can be reasonably sure they are studying the emotion of interest and associated processes involved in learning emotion. However, this is low in ecological validity and can be time consuming to build emotional associations. There are also ethical considerations in terms of building negative associations with a specific stimulus. In the present thesis indirect mood induction was selected as it allows a level of control over the stimulus and the emotional experience generated can be maintained with as little manipulation as possible. This allows researchers to tap into the innate experience of emotion whilst preserving a level of mundane realism which is perhaps the closest we can get to understanding emotional experiences in the individual.

1.2.2 Measuring the emotion: Response

After successful induction of emotion in participants, the outcome must be captured and measured in a meaningful way. As discussed in the previous section, a critical issue in the study of emotion is the confidence we can have that participants truly are experiencing the emotions we are trying to capture. This issue is particularly problematic when we consider that if the stimulus presented or the emotions themselves can be unconsciously processed, then even the participants may be unable to reliably confirm that they experienced the emotion. This will be discussed further in the following sections. Emotion responses can be measured using both direct and indirect methods.
1.2.2.1 Self-reports
The most straight-forward method is to use self-report from participants which is considered to directly measure emotional response. This provides researchers with clear responses, and also a rich insight into the qualitative experience of the participant. It also directly confronts the issue of the level of confidence we can have that the intended emotion has been generated. However, self-report is not an absolutely direct measurement of emotion and has the potential for bias and erroneous subjective interpretation. Demand effects were discussed in section 1.2.1.2 where participants do not directly feel an experience, but report that they have under the assumption that is what the researcher is looking for. In reality they may have just perceived, and correctly identified, the emotional content of a stimulus without direct physiological arousal. Direct measurement only taps into conscious experience of emotions, but as mentioned previously emotions can be experienced both consciously and unconsciously (Gross & Thompson, 2007). Another potential bias is that participants may hide or downplay how a particular image or scenario has made them feel if they believe it is socially unacceptable. Such is the nature of emotion that these risks are unavoidable however, some solutions to overcome the risk of bias in using self-report measures in emotion research were discussed in section 1.2.1.2. For example, not revealing the true intention of the study to the participant, or even using masked stimuli in order to prevent conscious manipulation of the stimuli and responses by participants are methods to reduce bias in emotion research. Despite short-comings, self-report is used widely in research as it remains the clearest direct indicator of whether we are studying the emotion of interest.

1.2.2.2 Physiological and behavioural measures
The clear weaknesses of direct measures warranted the development of alternative, indirect measures. There are a variety of indirect measures researchers can employ, such as the choices participants make in a reward and punishment task. These choices indicate the values that the participant has assigned to a particular selection, and how strongly they feel about obtaining the reward/avoiding the punishment. Alternatively, researchers can obtain a measure of the alteration in reaction time induced by an emotional stimulus. The most well-known of these tasks is the modified emotional stroop task (based on the original Stroop Task; Stroop, 1935). In this task, participants are required to identify the ink colour of a printed word. Some of these words are emotionally loaded (e.g. ‘violence’, ‘torture’, ‘scream’ etc.), whilst others are neutral (e.g. ‘cloud’, ‘tree’, ‘Table’). There is evidence to show that
response times vary depending on the emotional content of the words, as well as individual differences in the degree to which people attend their own emotional state and the values they assign to them (e.g. Coffey, Berenbaum, & Kerns, 2003).

Another way of indirectly measuring emotional response is by using psychophysiological measures of the autonomic nervous system (ANS), such as heart rate increases, pupil dilation, increased sweating and endocrine responses (e.g. cortisol release). For example, researchers use measures of galvanic skin response (GSR) in lab settings to measure arousal and emotional reaction. Animal studies have also measured ANS response elicited from foot shock (e.g. Davis, 2001) as an indication of fear potentiated startle. These psychophysiological measures are commonly used in emotional conditioning experiments, even in humans (e.g. Grillon & Davis, 1997). Collectively, they give insight into the underlying physiological responses to emotions, and therefore increase our understanding of emotional experience and subsequent response.

Finally, while psychophysiological measures offer a measure by which emotion responses in the body can be observed, in order to glean a picture of emotion in the brain researchers must turn to alternative methods. These methods are also an indirect measure of emotional response, and are considered in detail in section 1.3. This section considers what comes between the emotional stimulus and the response - emotion processing or appraisal. In brief, these methods involve novel neuroscience and neuroimaging techniques which allow insight into how the areas of the brain act in isolation and in concert to process an emotional stimulus. The magnitude of the neural response allows some indication of the magnitude of the emotional affect.

Indirect emotion measurements not only offer the opportunity to overcome potential biases in participants’ responses, but also allow researchers access to unconscious emotional processes that are unavailable to direct measurement techniques. As such, the more intricate aspects of emotional experience must be researched using human participants.

1.2.3 Summary

Direct and indirect measures clearly complement each other and perhaps it is best to use them in combination as this may give a more holistic picture of the emotional experience. For these reasons, the data presented in this thesis employs a combination of indirect (self-report measures of anxiety) and direct measures (using neuroimaging techniques discussed in
1.3 Measurement of emotion in the brain

It is clear that in the field of cognitive psychology researchers have established a variety of rigorous and reliable methods by which we can both stimulate and measure behavioural outcomes of emotion. As these measures have become more established, it has enabled research into what happens in the middle of an emotional experience – the emotion processing. We know how stimuli are attended to and detected, and to an extent we know what physiological mechanisms are involved in behavioural responses, but what actually facilitates those responses and how they are produced within the brain are less clear. Affective neuroscience uses a variety of methods and techniques to investigate neural substrates of emotion including behavioural, psychophysiological and cognitive experiments, pharmacological studies, lesion studies (temporary and real), behavioural genetics, electrophysiological recordings and functional neuroimaging (Dalgleish, 2004). Initial research predominately used animal models or patients with brain damage. However, neuroimaging techniques offered a means to extend this investigation of the motor, sensory and visceral elements of emotion processing non-invasively in the human brain. There has therefore been an exponential growth in research utilising neuroimaging techniques for the study of emotion over the last twenty years.

This section briefly considers animal models and current neuroimaging in turn discussing the advantages and limitations of the different neuroimaging techniques, and their utility over alternative methods in neuroscience. The validity of using fMRI as a measure of emotion processing is demonstrated. Details on the various analysis methods of the fMRI data used in this thesis will be given in the methods chapter (Chapter 3).

1.3.1 Animal models

Animal models have been of particular importance in emotion research. In the past they have provided insight into behavioural changes associated with emotions, causes and manifestations of emotion disorders in the brain, and indicated possible pharmacological treatments for specific brain based emotion disorders (Campos, Fogaça, Aguiar, &
Guimarães, 2013). In particular, a key benefit of using animals in emotion research is that researchers are able to investigate emotion processes using targeted lesion techniques, which ethically is not possible in human studies. Though animal research provides a relatively low cost, quick method to study dimensions of emotion, there is the consideration of the ecological validity of directly applying this research to the understanding of human emotions. There are some key brain homologies between animal models and lesion studies or neuroimaging in human participants with regards to emotion processing (see section 1.4 for review of key emotion brain areas). This is especially the case in primate species; however, there remains considerable uncertainty about how accurate we can be mapping areas from primates to humans. Researchers applying such translational knowledge should therefore be careful in drawing definitive conclusions without testing (Sereno & Tootell, 2005).

A key focus of this thesis is the biological basis of anxiety in humans. Steimer (2011) identified that the majority of animal models of anxiety are based on evidence from rats and mice, making the problem of translation of knowledge more acute. Despite this, there are similarities between rodents and humans in emotion processing. Mice in particular share neuroanatomical, neurochemical and behavioural characteristics with humans, and there are only a small number of cases where no equivalent mouse gene can be found for a specific human gene (Carver & Stubbs, 1997). There are also phylogenetic overlaps between mouse and human brains. Both structural divisions (e.g. cerebellum, midbrain etc.) and subcortical structures identified in human studies that are said to be involved in anxiety processing (e.g. hippocampus and amygdala), appear to be represented in the mouse brain (though clearly at a smaller scale; Sartori, Landgraf, & Singewald, 2011). Additionally, Sartori and colleagues (2011) emphasise that the endocrine systems involved in anxiety processing in mice also appear to be functionally synonymous with those in humans.

Consequently, animal models have been useful in laying down foundations for emotion processing and anxiety research in terms of brain structures, endocrine function and basic behaviours. They can be used to look into more reflexive emotion responses, as well as conditioning (section 1.2.1.1), and can to some extent capture the survival mechanisms involved in the emotion response. However, there are huge cognitive differences between animal models and human emotions and emotion disorders. Animal models cannot capture the complexities involved in the aetiology, development and maintenance of dysfunctional emotion responses (Campos et al., 2013). Therefore, in considering higher level psychophysiological implications there is a need to expand and transfer the knowledge of
these previous studies and apply it to the investigation of emotion processing in human participants.

1.3.2 Research in human participants

1.3.2.1 Brain damage/ Virtual lesion studies

The first research carried out on human participants’ involved behavioural and cognitive investigation of patients with brain damage and lesions. These studies allowed researchers to bridge the gap between animal models and understanding of emotions and emotion dysfunctions in early affective neuroscience research. The example of Roger, a brain damaged patient presented in section 1.4.2, demonstrates how informative case studies of such patients can be, and case studies still provide insight into understanding underlying mechanisms. However, the rarity of case studies with specific damage to an area of interest and no comorbid damage or symptomatology limits the use of such data. For obvious ethical reasons it is not possible to use the invasive techniques seen in animal models to gather samples (e.g. brain tissue or blood) and investigate functional connectivity directly (Sartori, Landgraf, & Singewald, 2011b).

However, it is possible to use transcranial magnetic stimulation (TMS) to stimulate or inhibit activation in an area, effectively creating temporary hyperactivity or a temporary lesion. This technique has gone some way to enhancing our understanding of behavioural correlates of brain regions or connected regions. For example, Balconi, Canavesio, & Finocchiaro (2014) applied repeated TMS to induce activity in the left premotor region of high anxiety participants and low anxiety (control) participants. Stimulation occurred whilst they made a two-alternative forced choice response relating to whether they had seen an emotion or not. As a result of this frontal motor area potentiation, decreased reaction times to happiness across all participants were observed, inferring greater recognition. Furthermore, in highly anxious participants this improvement in performance in the happiness condition with stimulation was greater than controls. In contrast, without stimulation the highly anxious participants exhibited a typical negative-valence bias, with faster reaction times to negative emotions in comparison to positive and neutral conditions. This finding is supported by previous research which suggests that anxiety is associated with hypervigilance and increased or more sensitive threat detection (e.g. see Etkin & Wager, 2007; Holzschneider & Mulert, 2011).
The use of TMS in studies of emotion has clear value in the field but the application of such a technique is limited in isolation. Some of the key regions implicated in emotion processing are deep brain structures, yet the fields induced by TMS decay as they go deeper meaning TMS can only be reliably used to target cortical structures (Daskalakis, Christensen, Fitzgerald, & Chen, 2002; Zangen, Roth, Voller, & Hallett, 2005). It may be possible to stimulate some subcortical structures with TMS, but targeting is not refined and it is impossible to do so without also effecting cortical structures (Bolognini & Ro, 2010). In addition, it is impossible to specify which neurons in the target region have been effected, and Ziemann (2010) showed that TMS may have a combination of excitatory, inhibitory and state-dependent effects on targeted neurons. As a result, the use of TMS in isolation is not common in affective neuroscience.

1.3.2.2 Neuroimaging

Research investigating integrated networks and neural circuitry in the brain involved in emotion processing rather than specific regions associated with emotion (as in TMS/brain damage studies) use in vivo neuroimaging techniques such as positron emission tomography (PET) and fMRI.

PET uses radioactively labelled ligands to measure the movement and alteration of various molecules within the brain, which can also be used as an indication of neural activity in the brain. For example, Rauch, Savage, Alpert, Fischman, & Jenike (1997) used PET to measure regional cerebral blood flow in patients suffering from three different anxiety disorders (obsessive-compulsive disorder, simple phobia and posttraumatic disorder) during symptom provocation tasks in order to determine what common brain systems are involved in such disorders. Researchers were able to identify a set of structures that appeared to be common across the different disorders including the paralimbic belt; right inferior frontal cortex and bilateral brain stem (see Rauch et al., 1997 for more information). Despite PET’s high sensitivity in terms of measuring concentrations of neurochemical compounds it is less widely used in current emotion research. The use of radioactivity and the relatively limited spatial and temporal resolution limit the use of PET. Non-radioactive, non-invasive methods have gained favour more recently, with magnetic resonance spectroscopy (MRS) used as an alternative method of measuring neurochemistry (Stern & Silbersweig, 2001), and functional magnetic resonance imaging is a more common measure of neural activity.
Another advantage of fMRI over PET is that it is relatively rapid, and has very good spatial resolution with which to study functional neuroanatomy. In order to determine whether fMRI is as effective as PET at imaging midbrain and brainstem structures during emotion processing, Wager, Barrett, et al., (2008) performed a meta-analysis comparing density maps acquired using both techniques. In comparison to PET, fMRI is more prone to distortions and artefacts as a result of local field inhomogeneity’s, especially in areas closer to air sinus spaces at the base of the brain. This variability could account for relatively low reporting of brain stem regions in emotion neuroimaging research compared to animal studies. However, the authors determine that there are no significant differences between PET and fMRI for imaging deeper structures. It is suggested that this may be due to the better spatial resolution offered by fMRI offsetting the greater distortions and artefacts. Therefore, fMRI appears to be a useful, and appropriate, tool when it comes to investigating emotions in the brain in real time. Furthermore, it is currently the most appropriate method for identifying brain networks and interactions during emotion processing.

It must be noted that there are other tools which have greater temporal resolution that PET and fMRI, providing information on timing and neuronal firing in the order of milliseconds. Electroencephalography (EEG) measures electrical potentials at the surface of the brain, and many studies investigate differences in the evoked potential (EP) seen milliseconds after a stimulus. This technique has been used in affective neuroscience studies to investigate brain dynamics with regards to immediate responses to emotion stimuli (e.g. for review see Kim, Kim, Oh and Kim, 2013; Yuvaraj et al., 2014). Such EEG studies have reported correlates of emotion based on two dimensions – valence and arousal. In particular, P1, N1 short latency components, and N2 and P2 in middle latency have been shown to correlate with valence, however P3 (long latency) has been shown to correlate with arousal (e.g. Bernat, Bunce, & Shevrin, 2001; Hu et al., 2011; Kim, Kim, Oh, & Kim, 2013; Olofsson, Nordin, Sequeira, & Polich, 2008; Olofsson & Polich, 2007). However, despite EEG allowing the study of emotions in real time, there are a number of criticisms that can be levelled against EEG and it is not commonly used in isolation in emotion research. For example, the EEG signal comes predominately from the surface of the brain, with deeper sources more difficult to study without complicated analysis or the combination of other techniques limiting the scope of its application in emotion research. In addition, the spatial resolution of EEG is poor, with anatomical location problematic at times. It is therefore becoming common to use complementary imaging techniques such as fMRI to identify neural substrates of emotion a
priori (Stern and Silbersweig, 2001, see section 1.4 for details on biological circuitry of emotion).

This thesis is concerned with understanding underlying mechanisms involved in emotion processing with particular focus on the role and characteristics of the amygdala. The amygdala is a small, deep brain structure (as discussed in section 1.5). It is clear from the discussion above that methods such as TMS and EEG would not be suitable tools to measure such a brain structure; fMRI allows imaging for deeper structures and greater spatial resolution compared to these other techniques. In addition to conventional fMRI, the newer technique of resting state fMRI (Rs-fMRI) will also be used in this thesis to look at neural connectivity at rest. The assumptions and utility of this technique will be introduced in the next section, discussion of resting state research and connectivity during emotion processing using Rs-fMRI is discussed in section 1.5.4.

1.3.2.3 Resting state fMRI
Resting state functional magnetic resonance imaging (Rs-fMRI) is a relatively new technique within neuroscience. Standard Rs-fMRI procedure involves individuals laying in an fMRI scanner and either fixating their gaze on a cross-hair or closing their eyes. The individual is instructed to not do, or think of, anything in particular. That is to say, they are asked to not actively focus on the outside world but remain in a passive state during the scan. As such, Rs-fMRI measures levels of spontaneous brain activity at rest (i.e. no active task is carried out) to allow observation of functional brain networks independent of task-induced correlations and a priori predictions. This is assumed to represent baseline activation in the brain, or underlying brain activation unique to the individual at rest. When Rs-fMRI is utilised in affective neuroscience, it is usually put at the start of a typical task-related fMRI study in order to enhance and supplement the traditional findings.

The technique itself is inherently linked to the study of low frequency resting state networks (RSNs) in the brain. These RSNs are suggested to reflect ‘the intrinsic energy demands of neuron populations that, via firing together with a common functional purpose, have subsequently wired together through synaptic plasticity’ (Cole, Smith, & Beckmann, 2010, pp.1). Thus the resting state is said to be where salient information from experiences (emotional, cognitive etc.) are processed, understood and stored in a system of adaptive learning (Albert, Robertson, & Miall, 2009; Lewis, Baldassarre, Committeri, Romani, &
Corbetta, 2009). The potential of studying such systems of adaptive learning means that resting state research has the potential to offer an insight into emotional processing, as well as individual differences in this processing dependent on previous experience and learning (e.g. in anxiety disorders).

The original RSN was discovered as a result of a meta-analysis in which it was observed that specific regions of the brain consistently deactivated during goal-directed task imaging (Shulman et al., 1997; see Figure 1.7). Areas showing consistent decreases during active tasks (meaning any study whereby some kind of stimulus is being presented) included posterior cingulate/precuneus, bilateral inferior parietal cortex, left dorsolateral frontal cortex, left lateral inferior frontal cortex, left inferior temporal gyrus, a strip of medial frontal regions running along a dorsal-ventral axis, and the right amygdala (ibid). Collectively these areas have become known as the Default Mode Network (DMN; Raichle et al., 2001). Raichle and colleagues advocated that the DMN is an organised baseline mode of brain function that deactivates during specific goal-directed behaviours (ibid).

Since the introduction of the DMN in neuroscience a number of other specialised low-frequency RSNs have been observed including networks associated with vision, motor learning and also auditory processes (Li et al., 2011). Research has indicated that RSNs can be reliably identified using Rs-fMRI (for example see Damoiseaux et al., 2006; Lowe, Dzemidzic, Lurito, Mathews, & Phillips, 2000). It is believed that the identification of such
networks without the need to actively manipulate variables (such as using an experimental paradigm) allows researchers to access the brain’s ‘natural state’ activation and connectivity. Tapping into potentially subconscious, innate processes offers valuable insight into the underpinning neural mechanisms at play in vivo in the brain. In addition, in the case of emotion research, such techniques overcome some of the limitations in measuring an emotional response such as potential demand effects in self-report measures (see section 1.2.2.1 for further discussion). There are no experimental stimuli, and no behavioural responses; only neurophysiological activity is measured. Just as connectivity across individuals at rest can enlighten us as to the baseline connectivity in the brain, individual differences in this resting connectivity can indicate functional alterations related to demographic and clinical factors. Furthermore, all of this can be achieved without the confounds of whether an emotion was truly experienced (section 1.2.1) or whether a response is truly indicative of internal state (section 1.2.2).

Rs-fMRI places minimal cognitive burden on the individual, and the lack of a task means that a full dataset can be collected in as little time as five minutes. This makes it an ideal tool to investigate emotion processing, especially within groups of participants with mood disorders, in particular those with anxiety disorders. As the scanner environment has been shown to be stressful to participants (e.g. McIsaac, Thordarson, Shafran, Rachman, & Poole, 1998; Rosen & Gur, 2002), such groups pose greater ethical considerations. Furthermore, this population tends to be less willing to participate in imaging studies (e.g. see Stein, Simmons, Feinstein and Paulus, 2007). A short session fMRI (e.g. 5 minutes), with no cognitive task would limit the stress posed to such participants as much as possible. By recording demographics representing mood or anxiety levels, one could then look at connectivity differences related to these conditions.

As a result of the possibility of collecting full data sets in very short periods of time, with potentially problematic cohorts (e.g. children, individuals with movement disorders involving spasms or muscle twitches, individuals who suffer from claustrophobia), the application of Rs-fMRI has increased exponentially in the last decade. However, despite evidence indicating that our emotional response evolves through adaptive learning and the applicability of Rs-fMRI to participants with mood disorders, very little research has used this technique to study emotional processing.
1.4 The biology of emotion

As previously discussed, the focus of affective neuroscience has shifted from measures of the psycho-physiological responses (section 1.2.2.2) towards investigating the neural networks associated with different emotion states. This latter strand of research has been enabled by the advanced neuroimaging techniques discussed in the previous section. In this section, models of emotion presented in section 1.1.2.1 are briefly considered from a more neuropsychological perspective. Following on from this, a brief review of the biological circuitry of emotion will be presented (for a detailed review of the history of neuroanatomical theories see Dalgleish, 2004). As the characteristics and connectivity of amygdala is the main focus of this thesis, this brain area will be considered in more detail in section 1.5.

1.4.1 Neuropsychological models of emotion

The first real steps towards explaining the brain mechanisms involved in emotion were taken by Cannon and Bard (Bard, 1928; Cannon, 1931) in their lesion work in cats. As previously mentioned (section 1.1.2.1) they theorised that there was mutually exclusive, parallel processing of the cognitive and physiological experiences of emotion. Furthermore, they determined that the hypothalamus was the region primarily involved in emotion responses by observing the occurrence of sham rage after systematic removal of portions of cortex (Bard, 1928; Cannon, 1931). An alternative theory of the underlying neural mechanisms of emotion was proposed by James Papez soon after Cannon and Bard’s theories were published (Papez, 1937). This theory proposed that a network of brain structures were involved in emotions, rather than just one specific region. These areas included the hypothalamus, but also other areas such as the anterior thalamus, cingulate gyrus, and hippocampus. It soon became referred to as the ‘Papez-Circuit’ (Papez 1937, see Figure 1.8).
Other structures were also implicated as being involved in the emotion neuro-circuitry. For example, bilateral removal of the temporal lobes led to a reduction in emotional reactivity in monkeys (Kluver & Bucy, 1939). Maclean’s 1949 review of the literature on emotion circuits suggested the addition of the amygdala, orbitofrontal cortex and parts of the basal ganglia to Papez’s Circuit (MacLean, 1949). The legacy of MacLean’s newly represented emotional brain network, ‘the limbic system’, is still used as a rough framework in affective neuroscience today, and is almost synonymous with lay definitions of the emotional brain.

1.4.1.1 Locationist and Psychological Constructionist models

MacLean’s concept of the limbic system provided a framework for subsequent research, which in turn stimulated the development of the currently used models of emotions (MacLean, 1949). These can broadly be split into locationist and psychological constructionist models.

Locationist models have been the most dominant paradigm for the neural basis of emotions since the re-emergence of this topic in affective neuroscience. Within this category are both the ‘modal model’ mentioned in section 1.1.2.2 (Gross and Thompson, 2007; also see Barrett & Wager, 2006; Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012) and the ‘natural kind model’ (Barrett, 2006b; Lindquist et al., 2012). Fundamentally, locationist models come from a ‘natural-kind’ approach to emotion (Barrett, 2006a), which presumes each emotion category has a characteristic cluster of properties and distinct categorical boundaries. There may be some shared properties of different emotions (e.g. increased heart rate in happiness...
and fear), however the overall pattern of characteristics can clearly be categorised as different, unique emotions. Furthermore, locationist models assume that these distinct emotion types are associated with discrete causal mechanisms within neural areas or networks. The notion that there are basic, distinct categories of emotions with associated underlying brain mechanisms makes them inherently easier to investigate. If it is assumed that observable physiological outputs have a shared emotional cause, researchers are able to measure one response output as an indicator that a certain emotion had occurred. Without such an approach, the study of emotion may have been overlooked as far too complex to examine in a scientifically rigorous fashion. As a result, most of the research into emotion has arisen from locationist ideals, and this model therefore accounts for a vast proportion of our current understanding about emotional processing within the animal and human brain. However, it has become apparent with further research that emotion is a far more complex and multifaceted process, spanning contextual, behavioural, experiential and physiological response systems. The locationist model may be too restrictive an approach when trying to incorporate these elements, as well as individual differences in emotional processing and response.

In a series of papers, Barrett (Barrett & Wager, 2006; Barrett, 2006a, 2006b) proposed an alternative framework for emotion ontology. The psychological constructionist model was developed from Barrett’s Conceptual Act Model, with empirical evidence for the model provided by a meta-analysis by Lindquist and colleagues (Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012). The model by Lindquist et al. (2012) proposes that emotions are simply psychological events that arise from non-specific psychological operations. These psychological events are not specific to any discrete category of emotion, as is assumed in the locationist model. Furthermore, as noted by Lindquist and colleagues, they are not even specific ‘to the category of emotion itself’ (Lindquist et al., 2012, pp. 124). At a neural level, it is suggested that there is no one specific emotion region, or network, in the brain. In brief, the model (Lindquist et al., 2012) proposes that emotional experiences are generated from the interplay between ‘core affect’ (the mental representation of sensory input from the body), ‘conceptualisation’ (the contextual understanding of these sensations arising from prior experience and knowledge), ‘executive attention’ (the modulation of attention given to some representations over others based on all incoming information from internal and external sources) and ‘emotion words’ (or ‘essence placeholders’, words which we use to verbally anchor the experience and share with others). Therefore, the exact networks and regions
involved in generating the emotional response are highly context-dependent. Despite the complexity of the model, it cannot account for all current research findings. For example, it neglects evidence for the lateralisation of emotion domains in the brain (Sackeim et al., 1982). However, the model is in its infancy and draws its empirical evidence from studies based on testing the locationist model (as these studies make up the majority of the research field). Lindquist and colleagues (Lindquist et al., 2012) state that further research that allows more direct testing of the psychological constructionist model is needed, and that this research would enable further refinement of the model.

There is evidence to support both the locationist and psychological constructionist models of emotion, and neither offers a complete explanation of emotional processing in the brain. However, they provide a framework for future research, and both agree that this research should be looking for a range of underlying neural systems depending on the different emotion experienced rather than one unanimous circuit as MacLean suggested in the mid-1950s. For the purpose of this thesis a more locationist stance is adopted since it has been established in the field. However if and where appropriate a psychological constructionist perspective will be considered.

1.4.2 Biological Circuitry of Emotion
The large scale neural distribution of emotional processing described in the locationist and psychological constructivist approaches is aptly demonstrated by the case of ‘Roger’ as described by Feinstein (2013). Roger suffered from extensive brain damage following illness, affecting areas of bilateral anterior cingulate cortex, medial prefrontal cortex, amygdala, hippocampus and 90% of his insula cortex (see Feinstein, 2013 for further details). Despite this damage, he exhibited intact emotional functionality, claiming to understand and experience emotions (Feinstein, Adolphs, Damasio, & Tranel, 2011). The amount of damage observed, in combination with the report of intact emotion processing, suggests a dynamic network of brain structures similar to those regions identified in MacLean’s limbic system which are distributed such that behaviour can appear to be normal despite damage to component parts.

Wager et al., (2008) identified the neural areas most consistently observed in emotion research, providing a succinct and concise snapshot of areas currently understood to be involved in emotional experiences (for more details see Wager, Barrett, et al., 2008;
Lindquist et al., 2012). They termed these areas their ‘observed neural reference space’. This section will discuss the current understanding of the role of a number of these component areas, finishing with consideration of a key area involved in emotion processing and response generation; the medial prefrontal cortex. The focus of this thesis is the key characteristics of the amygdala, but this area does not work in isolation, rather as part of a network. In order to investigate the role of the amygdala in emotion processing, there must be an awareness of all the biological mechanisms involved in this network. As stated previously, the amygdala will be considered in detail in a separate section (section 1.5).

1.4.2.1 Diencephalon
The diencephalon contains two key regions involved in emotion: the hypothalamus and thalamus. As mentioned in section 1.1, there is a visceral sensory component to emotions, produced by autonomic and endocrine responses. These visceral sensory processes are largely generated from the hypothalamus, which regulates endocrine responses through pituitary function and is heavily connected to the brainstem. The effect of hormone levels on emotion has been researched extensively (for a detailed review, see Wirth, Gaffey, & Work, 2013). A full discussion of the area is beyond the scope of this thesis, but section 1.5.3.4 will consider hormonal interactions in the context of maladaptive emotion processing of fear and anxiety.

The thalamus is predominantly implicated in the role of sensory processing. As discussed later in section 1.5.1, it plays a key role in projecting sensory information for further processing in associated emotion regions in the cortex and amygdala.

1.4.2.2 Subcortical Telencephalon
1.4.2.2.1 Hippocampus
The hippocampus has long been associated with the neuro-circuitry of emotion. Ferrier (1886; cited in Papez, 1937) experimentally destroyed the hippocampal regions in monkey brains and suggested the resulting depressive mood was related to the damage to this region. Papez (1937) concluded the hippocampus plays an important role in emotion processes based on Ferrier's work, as well as observations of exaggerated fear and aggression in cats and dogs affected by rabies (an infection known to predominantly manifest in hippocampal neurones of these animals; e.g. Stein, Rech, Harrison, & Brown, 2010). The role of the hippocampus in
emotion is likely to be related to memory. Research has evidenced multiple memory systems, each modulated by specific neural substrates (e.g. see Squire, Stark, & Clark, 2004), and the hippocampus has been shown to play a role in the formation and consolidation of long term declarative memory. However, the hippocampus is not necessary for certain types of emotion-related memory formation. A clear double dissociation between the hippocampus and the amygdala has been identified by looking at focal lesions, where memory formation is still possible after damage to either area. This indicates that there are two independent memory systems involving these brain regions, which are moderated by emotion (Bechara et al., 1995). The amygdala’s role in emotion will be discussed in section (section 1.5), and the roles of both the amygdala and hippocampus in maladaptive anxiety responses will be considered in section 1.6. However, in terms of emotion and memory, the amygdala is involved in the acquisition of fear and fear conditioning (Philips and LeDoux, 1992). It is suggested that the hippocampus codes for emotional relevance and interpretations of specific events (Bechara et al., 1995) through its involvement in acquisition and formation of declarative memory. There is some evidence that these two independent memory systems (amygdala and hippocampus) only interact and influence each other in cases where an emotional situation was involved (Phelps, 2004). However, Wager and colleagues (2008) note that though the hippocampus appears to be reliably activated in humans when performing emotion-related tasks, this appears to reflect perception of the emotional stimulus rather than the emotional experience itself. Clearly, further research needs to be conducted into the involvement of the hippocampus in emotion-related memory as well as its interaction with other neural areas during this process.

1.4.2.2.2 Basal ganglia
The basal ganglia are a set of subcortical structures including the striatum (caudate nucleus and the putamen), the globus pallidus and the subthalamic nucleus. They appear to be highly connected to the cerebral cortex, and have historically been associated with motor systems in the brain. However, recent anatomical studies have revealed discrete circuits, or ‘loops,’ with connectivity to a variety of areas suggesting that the basal ganglia may be involved in a number of processes beyond motor responses. For example, basal ganglia loops with connections to the prefrontal cortex play a role in cognitive functioning (Middleton & Strick, 2000). An indication of the role of the basal ganglia in emotion is given by a study of Parkinson’s patients (an illness with a motoric deficit, thought to be due to basal ganglia
dysfunction) which found that patients had decreased ability to detect differences in emotional speech tone compared to controls (Pell & Leonard, 2003). The basal ganglia has also been implicated in emotion processing through its involvement in the dopamine signalling network. Wager et al, (2008) suggested through this network it may be involved in planning and initiating motivationally relevant behaviours, though further research would be needed to corroborate these suggestions.

1.4.2.3 Paralimbic Cortex

The paralimbic cortex is a region of the brain closely associated with the structures of the limbic system, with extensive connectivity between these areas meaning it is sometimes difficult to disentangle the two. In particular, the boundaries between these two areas are hard to distinguish when structures have many bidirectional connections, such as the amygdala (Mesulam, 2000). The key areas in the paralimbic system primarily associated with emotion are the orbital frontal cortex, the anterior insula and rostral anterior cingulate. The paralimbic system is involved in a diverse array of emotion processes, reflective of the level of connectivity it has with a variety of cortical and subcortical structures. The paralimbic cortex (sometimes referred to as the mesocortex) is the layer between the allocortex (limbic cortex structures) and neocortex, and has been shown to play a role in direct emotion responses (such as the role of the dorsal anterior cingulate cortex) as well as more top-down executive functions in emotion regulation (i.e. orbitofrontal cortex contributions). As such, the paralimbic system is a good example of why emotion research must consider the neural underpinnings of emotion at a network level, and not just as areas in isolation.

1.4.2.3.1 Orbitofrontal Cortex

Evidence for the role of the orbitofrontal cortex (OFC, also see section 1.4.2.4.3) in emotion has come from animal models, lesion studies and neuroimaging. A role in behavioural modulation in response to threat (fear response) was evidenced by a lesion study in monkeys (Fox et al., 2010). In this study the monkeys’ OFC appeared to modulate the bed nucleus if the stria terminalis (BNST) activity which has been associated with behavioural inhibition (N. A. Fox, Henderson, Marshall, Nichols, & Ghera, 2005). Disruption to the modulation of the fear response could highlight a potential connection between the OFC and the
development of anxiety and affective disorders which are associated with dysfunctional emotion responses (discussed in section 1.6).

Observations of behaviour changes following damage or lesions to areas of the paralimbic cortex tentatively suggest that dysfunction in the paralimbic cortex as a whole is associated with psychopathy (Kiehl, 2006). In particular, damage to the OFC has been associated with increased anger and hostility (Mattson & Levin, 1990), intermittent explosive disorder (Best, Williams, & Coccaro, 2002), impulsivity, reduction in empathy, guilt, a general reduction in emotional responses, as well as uninhibited social behaviour (e.g. Elliott, 1978; Hornak et al., 2003a; Rolls, 2004).

The OFC also appears to be involved in sensory integration, with animal studies revealing that it receives inputs from different sensory modalities including olfactory, gustatory and auditory areas (e.g. Hackett, Stepniewska, & Kaas, 1999; Price, 2006; Reep, Corwin, & King, 1996). In addition to sensory areas, the OFC has connectivity with various cortical regions, including other areas of the prefrontal cortex (PFC), temporal and parietal cortices and subcortical structures in the striatum and midbrain (Kahnt, Chang, Park, Heinzle, & Haynes, 2012).

Evidence for orbitofrontal involvement in emotion response in humans has primarily come from neuroimaging studies. For example, Blair, Morris, Frith, Perrett, & Dolan (1999) conducted a study in which PET scans were obtained from thirteen volunteers whilst they viewed emotionally valenced images depicting angry faces of increasing intensity. Participants were asked to categorise the stimulus based on gender, but were not asked to judge or recognise the emotional content of the images so that this information was perceived passively. The imaging results showed that OFC activation increased with greater intensity of anger depicted in the face. Based on their findings, and evidence from the animal model and lesion studies presented previously, the authors suggest that the OFC’s role is more of a social emotion regulator, involved in behavioural extinction. Specifically, they suggest that when viewing angry faces, the OFC acts to suppress behaviour either through inhibition (behavioural extinction) or by activating alternative behavioural response mechanisms (role reversal learning). This is based on the premise that an angry reaction or facial expression is displayed in social situations in order to curb inappropriate or socially unacceptable behaviours in others, where these behaviours are considered to break social norms based on some kind of ‘value judgement’ (Averill, 1983; Keltner & Haidt, 1999). The premise that
OFC is involved in behavioural inhibition is corroborated by a plethora of evidence, summarised in a review of OFC research conducted by Rolls (2004). Having assessed the literature, Rolls proposed that the OFC plays a primary role in stimulus-reinforcement. Damage to this area therefore results in impaired learning, and reversal (extinction), of such associations which could result in psychopathological symptoms observed in psychopathy (Best et al., 2002; Elliott, 1978; Hornak et al., 2003b; Mattson & Levin, 1990; Rolls, 2004).

1.4.2.3.2 Anterior Insula
The anterior insula (AI) cortex has been shown to be one of the most consistently activated regions in neuroimaging studies of emotion (Kober et al., 2008). The insula cortex as a whole is implicated in interoceptive (internal) awareness, and (Craig, 2002, 2009) put forward a framework for understanding insula activation where it is involved in integrating representations of interoceptive signals (e.g. muscular and visceral sensations, heart rate and arousal etc.) from the posterior and anterior insula and making them consciously accessible.

Like the OFC, the anterior insula in particular has also been implicated in a more social emotional role. Activation is commonly associated with vicarious emotions such as empathy, as well as more basic emotions such as anger or sadness. A review by Lamm & Singer (2010) suggested that the AI plays an important role in predicting emotion states which arise from interpersonal interactions and social context, thus representing ‘social emotions’, as well as its established role in interoceptive awareness. Evidence for AI involvement in social emotions first came from the work of Singer and colleagues (Singer et al., 2004; later solidified in a model by Singer, Critchley, & Preuschoff, 2009), where it was demonstrated that AI was engaged in both the processing of pain experienced by the self and empathetically for others thus demonstrating AI involvement in social emotion processing as well as interoceptive processes.

1.4.2.3.3 Anterior Cingulate
The anterior cingulate (ACC) has subdivisions which have different patterns of connectivity, which are reflected in their distinct functional roles. In particular, the rostral (rACC) portions are more highly connected with core regions in the limbic cortex, and as a result have been implicated in regulating emotion responses. The dorsal (dACC) portions have greater connectivity with frontal areas of the brain, and are therefore suggested to be involved in top-
down emotion processing (e.g. Mansouri, Tanaka, & Buckley, 2009; Polli et al., 2005). In keeping with this notion Etkin, Egner, & Kalisch (2011) suggested a framework specifically focusing on negative emotion processing in which the dACC is more involved in appraisal and expression of negative emotion, and the rACC is involved in generating emotion responses through the limbic system. Etkin and colleagues (2006) conducted an fMRI study in which participants performed an emotional conflict resolution task based on the emotional stroop task. They found that resolution of conflict was associated with activation in the rACC. Furthermore, this rACC activation was dependent on prior conflict levels and the associated amygdala deactivation. This provides evidence for the suggestion the rACC is involved in emotion regulation, in the form of rACC regulated inhibition of the amygdala in emotional conflicts.

1.4.2.4 Prefrontal Cortex

The prefrontal cortex (PFC) is implicated in playing a higher level role in cognitive processing, executive control and decision making (e.g. Euston, Gruber, & McNaughton, 2012; Ongür & Price, 2000; Tanji & Hoshi, 2008). Early association of the PFC with memory systems was largely influenced by work of Baddeley and his working memory hypothesis in the early nineties (Baddeley, 1992). Though this function for the PFC is particularly enduring, there has been a diversification of the PFC’s role over time, with evidence for its involvement in a myriad of cognitive processes. In more recent years evidence for the role of the PFC in cognitive regulation of emotion has come from patient, PET and fMRI research, and as such the PFC is now considered a key proponent in emotion research alongside the amygdala. It is perhaps no surprise that the PFC plays a role in emotion processing when looking at the type and number of connections it has within the brain. In particular, is it highly connected with inputs from various different structures found in the diencephalon, mesencephalon and limbic system, all of which, as discussed in this section, are implicated in emotion processing. The prefrontal cortex can be subdivided into three different sub-regions; the dorsal (dPFC), medial (mPFC) and orbitofrontal (OFC) regions. A further division can be made bilaterally (Left/Right; Ongür and Price, 2000), but hemispheric specialisation in PFC receives little focus in the emotion research literature. However, the three sub-regions are of particular importance with regards to involvement in emotion processing, so this section will concentrate on these divisions in turn.
1.4.2.4.1 The Orbitofrontal Subdivision

The orbitofrontal subdivision has been discussed earlier in the paralimbic cortex section (for more detail section 1.4.2.3.1). Though the OFC has long been established as playing a role in terms of reward-guided learning and decision-making (Rushworth, Noonan, Boorman, Walton, & Behrens, 2011) more pertinent to this thesis, the literature currently suggests that the role of the OFC in emotion processing is that of an emotion regulator. It is involved in inhibiting certain emotions depending on social context in order to preserve greater long term benefits (see Rolls, 2004 for detailed review). A recent review of the brain basis of emotion (Lindquist et al., 2012) supports this perspective of the OFC’s role in emotions, and refines it further. In their meta-analysis, Lindquist and colleagues concluded that current evidence suggests that the OFC plays a major role in emotional regulation by guiding behaviour through the integration of exteroceptive (external) and interoceptive (internal, visceral) sensory information. This emotion regulation role therefore combines both the sensory integration and decision-making functions of the OFC.

1.4.2.4.2 The Medial Prefrontal Cortex subdivision

The medial prefrontal cortex (mPFC) is also implicated in interoceptive or visceromotor processing of emotion. The ventral mPFC in particular shows high levels of connectivity with emotional and autonomic systems in the brain (Ongur and Price, 2000). Notably, it has bidirectional connectivity with the amygdala, and is also connected to dorso-and ventromedial striatum, anterior insula, periaqueductal gray (PAG) and hypothalamus as well as further connections within neuromodulatory systems (e.g. Roy et al., 2009, for details see Euston, Gruber, McNaughton, 2012). It is as a result of this connectivity that the ventral mPFC is considered to be a key output area of the ‘visceromotor’ system (Ongur and Price, 2000). Lindquist and colleagues (2012) provide support for this functional specialisation of the mPFC when discussing the ‘conceptualisation network’ (Buckner, Andrews-Hanna, & Schacter, 2008), which also includes dorsal mPFC, the medial temporal lobe and the retrosplenial cortex. They suggest that this conceptualisation network is involved in the experience and perception of different emotions, and enables their translation into a meaningful personal experience. Taken together, this research reinforces the notion that the
mPFC contributes in visceromotor processing of emotion, and higher-level interpretation of these interoceptive stimuli as emotions is achieved in concert with a larger network.

1.4.2.4.3 The mPFC and OFC
An alternative proposal for the role of the mPFC is that it is involved in the orchestration of appropriate adaptive, and particularly emotional, responses based on learned associations between context (be it situation, or emotion, based), locations and events (Euston, Gruber and McNaughton, 2012). This higher-level function may be possible in concert with the OFC, as these two areas process emotion-related sensory (OFC) and visceromotor (mPFC) inputs. If this is the case it could be considered vital in guiding emotional behaviour in the manner suggested by Euston and colleagues (2012). Some support for the involvement of these regions in such a function comes from research looking at changes in social status in animals after frontal lobe lesions (Nauta, 1971). Lesioned animals were seen to lose their hierarchical status; Nauta argued that the interruption in social status was due to an inability of these animals to monitor internal states and react appropriately in the least risky or most desirable social contexts. As such, it seems from this research that these regions of the PFC are vital in monitoring internal states and continuously updating these states based on bodily reactions and bodily feedback in a system of reward and feedback. Early studies involving human patients also provided support for notions of internal monitoring and reward learning within OFC and mPFC. For instance, Bechara, Damasio, Damasio, and Anderson (1994) observed a lack of appropriate visceral response to negative or excitatory images in a patient (E.V.R.) with specific orbital and medial PFC damage. This suggests that these areas are required for internal monitoring of visceral response. In addition, E.V.R and other such patients typically present with an inability to perform well in reward based learning (Bechara et al., 1994).

1.4.2.4.4 Dorsolateral Prefrontal Cortex subdivision
Unlike the mPFC and OFC, the dorsolateral prefrontal cortex (dlPFC) is not directly considered to be involved in emotion processing, and is instead implicated in top-down processes and goal-directed control of attention (Miller, 2000). It shares many of the same connections as the ventral mPFC, but exhibits comparatively weaker connectivity with emotional and autonomic systems and greater connectivity with motor related systems (Schultz, Tremblay, & Hollerman, 1998). Fuster (1997) suggested that there is a motor-
sensory transfer system in place in which sensory information converges on the dIPFC, where it is translated into behavioural responses and outputs. Lindquist et al. 2012 meta-analysis presented evidence of concurrent dIPFC activation in emotion processing, in particular dIPFC activity increased during perception of anger over and above any other emotion. As such, although dIPFC may not be directly involved in emotion processing, it may be involved in formulating the response subsequent to processing.

1.4.2.5 Prefrontal connectivity with the Amygdala

As mentioned previously, there has been a shift in research focus from individual sites in the brain being responsible for discrete emotions to multiple regions working together in a network, with patterns of activity across regions relating to subjective emotion. This concept has led to an expansion of research into the role of the PFC and its subdivisions in emotion processing. Of particular interest to this thesis is the evidence that the PFC plays a key role in terms of its modulatory effect on the amygdala through the neural connections previously discussed. Our understanding of the exact nature of this modulatory effect has advanced via research into mood disorder and anxiety disorders (Etkin & Wager, 2007), where disrupted fronto-amygdala connectivity has been observed. Investigation into fronto-amygdalar connectivity and its disruption in mood disorders, has been crucial to recent advances in the field of emotion research.

Quirk, Likhtik, Pelletier, & Paré (2003) investigated suggestions that mPFC reduced, or even inhibited, the effects of fear conditioning through its connectivity to the amygdala (M. A. Morgan & LeDoux, 1995; Royer & Pare, 2002). Quirk and colleagues investigated the effect of mPFC pre-stimulation on the responsiveness of the central nucleus of the amygdala to synaptic input, using extracellular recordings from both rat and cat amygdala. They determined that mPFC stimulation did indeed decrease responsiveness of the amygdala, providing evidence for an inhibitory role of the mPFC on amygdala function which has since become established in the field (e.g. Akirav & Maroun, 2007; Hare et al., 2008; Hackjin Kim, Somerville, Johnstone, Alexander, & Whalen, 2003; Milad, Vidal-Gonzalez, & Quirk, 2004; Phelps, Delgado, Nearing, & Ledoux, 2004).

More recently, development in neuroimaging techniques have allowed researchers to look into this functional connectivity in human participants at rest (see section 1.5.4). As previously mentioned, the use of resting state fMRI has allowed observations of the brain’s
‘natural state’ connectivity free of a priori predictions. A resting state study of 29 healthy participants investigated the connectivity between the amygdala and the mPFC, using anxiety scores as a predictor of resting-state connectivity (M. J. Kim, Gee, Loucks, Davis, & Whalen, 2011a). The authors found that functional connectivity between the amygdala and dorsal and ventral mPFC altered as a function of anxiety levels. Based on previous literature (Roy et al., 2009), the expectation would be that there is positive coupling of the amygdala and vmPFC and negative coupling of the dmPFC at rest. However, Kim and colleagues (Kim et al., 2011) found that the expected positive vmPFC and negative dmPFC connectivity was only observed in low anxiety individuals, with high anxiety participants showing negative connectivity for vmPFC and no effect for dmPFC. This suggests that although mPFC does modulate amygdala activity, the nature of its effect may be altered by state (long-term) anxiety.

The modulatory role of the mPFC is also indicated by a study which investigated neural activity in four patients with bilateral vmPFC lesions whilst they performed a task rating aversive and neutral pictures for negativity. In addition to amygdala functional connectivity during the task, a resting state fMRI acquired prior to the task was also collected (Motzkin, Philippi, Wolf, Baskaya, & Koenigs, 2014). Patients had significantly elevated right amygdala reactivity to aversive stimuli compared to controls, despite there being no difference in ratings of the images between the groups. Furthermore, there was greater amygdala functional connectivity during rest in patients, particularly between the right amygdala and anterior temporal cortex. The results support a modulatory, inhibitory role for vmPFC as without patients with vmPFC lesions have greater amygdala activity during an emotion task, as well as greater connectivity at rest compared to controls. However, patients do not report any difference in perception of the aversive stimuli, and do not exhibit typical anxious traits. This is somewhat contradictory to the findings of Kim and colleagues (Kim et al., 2011) that the inverse coupling between vmPFC and the amygdala is disrupted in mood and anxiety disorders. However, previous research indicates that damage to the vmPFC may actually insulate against mood disorders, resulting in personality changes more akin to psychopathy (e.g. low emotional expressivity) with notably reduced physiological reactions to aversive stimuli (e.g. Barrash, Tranel, & Anderson, 2000; Koenigs, Huey, Calamia, Raymont, & Grafman, 2009). It is apparent from these various findings that the vmPFC may not simply inhibit the amygdala to modulate negative emotion; its role may be more intricate than that and depend on the individual characteristics and current circumstances. The field of emotion research is still evolving as new techniques are developed, and it seems more likely
from these studies that emotion processing occurs as a cascade of processes involving multiple areas in the brain working in parallel. Evidently, the PFC and amygdala play a major role in emotion processing. However, the intricacies of each area’s involvement, and indeed the synergistic relationship between the two regions, remains relatively unclear in the current literature.

1.5 The Amygdala
In this section the connectivity and role of the amygdala will be discussed in detail, with consideration of the amygdala pathways and current understanding of key characteristics in terms of its involvement in emotion processing and the fear response. Finally, a brief overview of the utility of resting neural networks in researching amygdala connectivity will be examined.

The focus on the role of the amygdala (or ‘amygdaloid complex’) in the brain has predominantly emerged from extensive research into the emotional fear response (for review see Phelps & LeDoux, 2005). Contemporary models are based on the notion that the amygdala plays a pivotal role in emotion processing in terms of its involvement in signal detection, consolidation of emotional memories and learning, emotion regulation, and mediating the emotional response (e.g. Adolphs & Spezio, 2006; Amunts et al., 2005; Büchel & Dolan, 2000; LeDoux, 2000).

The amygdala is a deep brain structure; it consists of two almond-shaped masses of neurones situated on either side of the thalamus, beyond the hippocampus (see Figure. 1.9). Within non-human organisms, these structures consist of a complex of multiple separate distinct nuclei. Each of which divide further into sub-nuclei with distinct functions, structures, chemical signature and histological appearance (e.g. LeDoux, 2000; Freese & Amaral, 2009; Joseph LeDoux, 2007; Styliadis, Ioannides, Bamidis, & Papadelis, 2014). These nuclei interact with each other and various different cortical and subcortical structures in the brain to varying degrees. Much of what we understand about the divisions of the amygdala, and how they interact, comes from animal research and as a result of different organisms being tested the exact number of nuclei and sub-nuclei varies (for a full review see LeDoux, 2000; Freese and Amaral, 2009). The continuing debate relating to the subdivisions of the amygdala leads to regular overhaul of the names, divisions, and terms, used to classify separate nuclei. However, in humans there is somewhat more clarity in defining these areas, this lucidity
comes from the limitations of the technology we currently have available to image the human amygdala in vivo. For example, the low spatial resolution of fMRI limits the depth and detail in which divisions can be observed compared to more invasive techniques available in animal research resulting in fewer observable divisions. As a result, three key subdivisions are identified in human amygdala based on cytoarchitectonic maps: the laterobasal, centromedial and superficial subdivisions (Amunts et al, 2005). Despite these three key areas being identified, much research in the field continues to report findings from the amygdala as one structure (or two bilaterally). However, this allows for more standardised comparison between studies and techniques. Roy and colleagues (2009) noted this aggregation can severely limit the scope of our understanding of the intricacies of interactions within the amygdala. Roy and colleagues used the anatomical subdivisions specified by Amunt and colleagues (2005) to investigate amygdala connectivity at rest. This was the first study to attempt to apply such techniques to the amygdala at rest, and was successful in tentatively identifying distinctly different patterns of connectivity from the subdivisions. However, despite the success of this study, it remains typical to treat the amygdala as one entity in human neuroimaging studies. Nevertheless, awareness of these emerging post-hoc parcellation techniques does allow future studies to advance our understanding of amygdala function and connectivity. In keeping with typical affective neuroimaging studies, most of the data presented in this thesis treats the amygdala as a whole, however Roy et al’s (2009) parcellation technique has been applied and explored in Chapter 9.

Figure 1.9 Images showing the position of the amygdala in the brain. Images generated by Life Science Databases (LSDB). [CC-BY-SA-2.1-jp (www.creativecommons.org/licenses/by-sa/2.1/jp/deed.en)]
1.5.1 Models of direct amygdala activation

Neuro-anatomically, the amygdala has sensory inputs to detect incoming emotional stimuli, and outputs to other areas in the brain which cause emotion specific changes in the individual. The central role of the amygdala in emotion processing and regulation is reflected in its level of connectivity. It is situated in the limbic cortex (or ‘allocortex’), receiving inputs from all senses (olfaction via the olfactory bulb and auditory/visual information from the temporal and anterior cingulate cortices), as well as visceral input pathways (via the hypothalamus, septal area, orbital cortex and parabrachial nucleus). Virtually all input pathways are bidirectional and thus also represent output pathways. There are three major efferent pathways from the amygdala as well as a number of direct connections from the amygdala. Direct connections are found between the amygdala and the hippocampus, entorhinal cortex, dorsomedial thalamus and brainstem (Price & Amaral, 1981; Rajmohan & Mohandas, 2007). The three major efferent pathways are the ventral amygdalofugal pathway, the stria terminalis and the anterior commissure. The latter is believed to represent a route via which information passes contralaterally between the two amygdalae.

![Image showing two of the main efferent pathways from the amygdala. As indicated the green line shows the (ventral amygdalofugal pathway) VAP, the red line shows the stria terminalis. Image from page 456 Siegel & Sapru (2006).](image)

1.5.1.1 Ventral Amygdalofugal Pathway (VAP)

The VAP represents connectivity primarily between the central and basolateral nuclei of the amygdala and cortical structures (Figure 1.10). This pathway is predominantly involved in emotion learning, with responses generated as a result of internal motivations or drives generated within the limbic system.
1.5.1.2 Stria Terminalis
The bed nucleus of the stria terminalis (BNST) has been implicated as a key region involved in fear and anxiety processes (Crestani et al., 2013; Y. Lee & Davis, 1997) and is associated with autonomic and neuroendocrine systems (Ulrich-Lai & Herman, 2009). The stria terminalis predominately exhibits connectivity with subcortical structures and the centromedial nuclei of the amygdala. There is some overlap between the VAP and the stria terminalis, with both possessing connections between the hypothalamus and septal nucleus. However, the major connections between these two areas and the amygdala are through the stria terminalis.

Recently, Somerville, Whalen, & Kelley (2011) conducted a neuroimaging study of healthy participants with varying levels of trait anxiety. A group of the participants took part in an environmental threat-monitoring task whilst in the fMRI scanner. Results indicated that BNST activation is correlated with levels of threat monitoring and hypervigilance, which is a key symptom of anxiety disorders (Etkin & Wager, 2007; Holzschneider & Mulert, 2011; Ventura-Silva et al., 2012). As presented later (section 1.6), in line with this observation, there is a large quantity of literature showing that the BNST plays a key role in the regulation of hypothalamic-pituitary-adrenal stress responses (HPA stress response, see Crestani et al., 2013 for review). The BNST is primarily involved in the inhibitory modulation of HPA axis output neurones (Cullinan, Herman, & Watson, 1993; see section 1.6 for HPA axis and anxiety). However, it must be noted that the most recent review of the literature on the HPA stress response by Crestani and colleagues (ibid) states that research into the neurochemical mechanisms underlying the stress response has yet to provide conclusive results and the mechanisms are not yet fully understood.

1.5.2 Dual Route of Fear
Focusing specifically on the incoming signal to the amygdala, LeDoux (Ledoux, 1995; LeDoux, 1996; Morgan & LeDoux, 1995; Romanski & LeDoux, 1992) proposed a dual pathway model of fear stimuli as a result of his work looking into fear conditioning (section 1.2.1.1). There is a growing body of evidence for this dual route model (e.g. Armony, Servan-Schreiber, Cohen, & LeDoux, 1995; Garrido, Barnes, Sahani, & Dolan, 2012, see Figure 1.11). According to this model information is first processed by the thalamus in the brain and
then branches off down two pathways in order to process an incoming fear stimulus. These pathways have gone by various names but for clarity are here referred to as ‘reflexive’/‘reflective’ pathways intoning the functionality and purpose of the two routes. The reflexive pathway is a direct pathway whereby information reaches the basolateral nucleus of the amygdala directly from the thalamus. This pathway is involved in rapid detection of basic, simple, information from the stimulus, and the more instinctual, cognition-free, response to any danger cues picked up from the basic signal that conveys threat to the organism. As such, this is a very fast route resulting in a reflexive response. Conversely, the reflective pathway travels indirectly to the amygdala via the sensory cortex. This reflective pathway is slower than the reflexive route and involves cognitive assessment, allowing for a more complex examination of the incoming sensory elements. Where the reflexive pathway responds to basic stimulus features, the reflective pathway can process perceptually complex stimuli. Evidence from animal lesion and patient studies indicate that these two routes work in parallel in response to a stimulus (e.g. Adolphs, Tranel, Damasio, & Damasio, 1994; Feinstein et al., 2011; Garrido et al., 2012). The reflexive pathway orients to the potential danger and may elicit an autonomic nervous system "fight or flight" response, whereas the reflective pathway will process the stimulus further and determine whether a true threat is detected or whether to quell the alarm response. LeDoux’s early work largely focuses on the fear response, which can be misleading suggesting that the amygdala is specialised for the fear response however, research has started to re-evaluate this role with evidence coming to light that the amygdala may activate in response other emotions (e.g. see Cunningham & Kirkland, 2014; Iidaka et al., 2002; Yang et al., 2002). This is discussed further in Chapter 4 however, the idea of a dual route of emotion processing, particularly in the fear response, endures. Thus in studying the amygdala’s role when looking at fear processing, it is important to observe the chronometry of the neural response from initial stimulus detection through to a return to baseline in activity in order to capture the activity related to both these pathways.
Figure 1.11. Schematic of dual route of fear. The reflexive pathway is a direct pathway between the basolateral nucleus of the amygdala and the thalamus said to be involved in rapid detection of simple stimulus information conveying threat to the organism. As such, this is a very fast route resulting in a reflexive response. Conversely, the reflective pathway travels indirectly to the amygdala via the sensory cortex which is thought to be slower than the reflexive route allowing for cognitive assessment of incoming sensory elements.

There is some evidence of lateralised specialisation with the right amygdala being implicated in the fast reflex route, and the left amygdala being involved more in the slow reflective route (see also section 1.5.3.2). For example Morris, Öhman, & Dolan (1999) investigated the effect of masked, or ‘unseen’, emotion stimuli on amygdala connectivity. They collected PET scan data from ten healthy participants who viewed masked fear conditioned faces and compared the amygdala connectivity when viewing seen and ‘unseen’ fear stimuli. The left amygdala activation and connectivity did not discriminate between masked and unmasked target images. However, the right amygdala exhibited differential activation and connectivity for the seen and unseen faces, supporting the notion that the right amygdala is involved in the reflex pathway and may be involved in more unconscious processing of emotion (also see Morris et al., 1999; Whalen et al., 1998). Further evidence for this dual route comes from a case study of a cortically blind patient who had damage to both his left and right visual cortices following two strokes. A series of behavioural tests showed he could correctly identify different emotional faces despite a lack of conscious visual experience, but could not differentiate other visual stimuli (either emotional stimuli or non-emotional; Pegna, Khatheb, Lazeyras and Seghier, 2005). Functional neuroimaging data showed activation solely in the right amygdala, with strongest effect to fear faces. The results also tentatively indicated that the dual route into the amygdala is not restricted to just fear processing but that it has a role in processing the overall emotional relevance of face images (Pegna, Khatheb, Lazeyras, & Seghier, 2005).
1.5.3 What are the key characteristics of the amygdala?

Despite vast quantities of research being conducted, and an ever expanding knowledge base about the structure and functionality of the amygdala there is still controversy surrounding specific factors and their impact on the magnitude of amygdala activation during emotion processing (for a full reviews see Chochol, & Armony, 2008; Wager, Phan, Liberzon, & Taylor, 2003; Zald, 2003). In brief, there are four key factors at the centre of the debate: valence specialisation, lateralisation of functionality, habituation rates and the modulating effects of gender (see Figure 1.12).

![Figure 1.12 Image highlighting four key interacting factors that literature has suggested moderate amygdala connectivity during emotion processing.](image)

1.5.3.1 Valence Specialisation

As mentioned previously (section 1.3.1) much of the research into amygdala functionality in emotion processing comes from animal research, predominantly using fear conditioning. As a result of this work, it was initially concluded that the amygdala was specialised for processing the emotion fear (e.g. Davis, 1992). Though this notion continues to receive backing with continued observations of rapid automatic amygdala activation in response to a general ‘threatening’ or dangerous stimulus (e.g. Adolphs, 1999; Bishop, 2008; Ohman, 2005), key supporters have had to re-evaluate the specificity of the amygdala in terms of fear detection alone. Researchers have started to suggest that the amygdala plays a role in processing signals of distress, and even in processing signals which potentially purvey threat...
but remain ambiguous without further information (e.g. signals of surprise or anger from gaze not specifically directed at the organism; Adams, Gordon, Baird, Ambady, & Kleck, 2003; Wager et al., 2003; Whalen et al., 2001; Zald, 2003). Even the limited view of the amygdala solely being involved in threat detection has been undermined. Lesion studies in patients with bilateral amygdala damage (e.g. patients E.P. and G.T. (Hamann et al., 1996); patient S.M (Adolphs, Tranel, Damasio, & Damasio, 1995); patient G.P. (Schmolck & Squire, 2001)) report that such individuals exhibit impairments in emotion detection and recognition extending beyond just threat related stimuli to other negative emotions such as sadness.

An emerging perspective is that the amygdala plays more of a role as a ‘relevance detector’ based on salience irrespective of the valence of a stimulus (Michael Davis & Whalen, 2001; Sander, Grafman, & Zalla, 2003). Work such as that by Santos, Mier, Kirsch, and Meyer-Lindenberg (2011) add weight to this argument. Santos and colleagues contrasted amygdala reactivity to matched emotional stimuli and non-emotional stimuli of equal salience in a visual search task finding amygdala activation correlated with salience, but not the emotional content per se, of the stimulus. They concluded that their study provided clear evidence in favour of the amygdala as a salience detector. This notion has gained favour with many other researchers in the field who have found the amygdala activates more to salient features than valence specifically - e.g. elevated amygdala activation has been seen in relation to eye gaze (Adams et al., 2012), facial attractiveness (Winston, O’Doherty, Kilner, Perrett, & Dolan, 2007), positively valenced emotions such as happiness (Cunningham & Kirkland, 2014; Iidaka et al., 2002), general emotional faces (Yang et al., 2002) and social emotional stimuli regardless of sensory modality (Scharpf, Wendt, Lotze, & Hamm, 2010). This growing body of evidence bolsters the claim that the amygdala is specialised for emotional faces, specifically responding to the most biologically and socially salient information (for further details see Adolphs, 2008; Fitzgerald, Angstadt, Jelsone, Nathan, & Phan, 2006; Pegna et al., 2005). The most recent meta-analysis in the field also supports this idea (Sergerie et al, 2008), however there remains contention in the literature which largely comes from discrepancies in the methods and techniques used. Low methodological homogeneity means comparisons are very difficult to make and can lead to erroneous interpretation of findings. Continuing work needs to be conducted with more systematic examination of the amygdala’s role in emotion processing to allow for succinct comparisons to be made.
1.5.3.2 Lateralisation of functionality

Several different accounts have been given relating to possible hemispheric differences or specialisations within the amygdala. These date back to early proposals based on basic understanding of general brain lateralisation which would suggest each hemisphere lends itself to a different function. For example, early accounts of the brain suggested that the right side of the brain was the primary seat of emotion, regardless of valence (e.g. Schwartz, Davidson, & Maer, 1975). Conversely, other accounts based lateralisation of function on our understanding of language stating that the left side of the brain was more associated with linguistic elements of emotion processing. For example, Erhan, Borod, Tenke, & Bruder (1998) investigated lateralised emotion using reaction times and ERP measurements in participants in an auditory target detection task. They found that emotional intonation (variations in pitch in the voice) was more accurately recognised when presented to the left ear thus supporting the notion of right hemisphere dominance. Further evidence supporting the right hemisphere hypothesis comes from evidence showing that right hemisphere damage is linked to reduced accuracy in emotional face recognition (Mandal, Mohanty, Pandey, & Mohanty, 1996; Weddell, 1994). An alternative argument to the right hemisphere hypothesis is the valence hypothesis. This suggests that there is valence specific lateralisation – the right amygdala is specialised for negative emotions; the left is for positive. This argument was extrapolated from the work by Sackheim and colleagues (1982) who conducted three retrospective studies looking into brain damage data and mood impact, which evidenced clear lateralization and valence associations (Sackheim et al., 1982). This lateralisation of amygdala function argument has received backing from other patient, and also imaging studies, however support has been varied. A meta-analysis by Wager, Phan, Liberzon and Taylor (2003) looking at 65 PET and MRI studies showed a confused picture with some studies clearly supporting the argument and others undermining it.

1.5.3.3 Interaction between lateralisation of function and habituation

Recent studies show a more intricate story relating to differing temporal dynamics for the left and right amygdala in terms of lateralisation and habituation in relation to the dual processing model of emotion (Section 1.5.2). Breiter and colleagues (Breiter, Rauch, Kwong, 1996) measured amygdala activation in response to rapidly presented fearful, happy and neutral visual stimuli from which they concluded that the amygdala favourably responds to emotional faces, and habituates quickly to repeated exposure. This idea of amygdala
habituation has received much support (e.g. Fischer, Furmark, Wik, & Fredrikson, 2000; Wright et al., 2001) and continues to develop in the literature. In the current prevailing theory, the suggestion is that neither amygdala is dominant in emotion processing. Rather they work in parallel in a synergistic relationship which is modulated by the perceived amount of direct threat to the organisms’ well-being. The right amygdala is involved in rapid stimulus detection of crude stimuli, as such it habituates faster to stimuli. On the other hand, the left amygdala is said to represent a more sustained response involving more detailed stimulus evaluation (e.g. Gläscher & Adolphs, 2003; Wright et al., 2003). In a meta-analysis of the literature, Baas et al. 2004 suggested that there was little evidence for this temporal lateralisation of function based on a review of 54 PET and MRI studies. However, in a more recent review of the literature by Sergerie and colleagues (2008) it was suggested that this may be due to the ramifications of using different study designs. Similar to the theories based on valence specialisation, a great deal of the disagreement between studies comes from the variety of measures and techniques used, as well as the exact method of classification of amygdala regions. In addition, variability in outcome may come from trying to observe the characteristics of the amygdala in isolation rather than in parallel processing systems.

1.5.3.4 Modulating effects of gender
Sergerie, Chochol, & Armony (2008) touch upon sex differences in relation to amygdala responsivity in their review of the literature. Despite conducting a comprehensive meta-analysis of the research they were unable to draw concrete conclusions, encapsulating the prior state of the literature as yielding ‘contradictory results’ (Pg.812). In particular, the modulating effects of gender on amygdala activation is a difficult characteristic to tease out from the literature. Similar to the research on valence, lateralisation and habituation, this difficulty arises from the various different techniques, paradigms, and stimuli that have been used. Historically, the lay notion that women are more emotional than men has driven forward research looking into sex differences (e.g. Birnbaum & Croll, 1984; Kring & Gordon, 1998; Schwartz, Brown, & Ahern, 1980; Shields, 1991; Stevens & Hamann, 2012). The current perspective is that women tend to show stronger, more bilateral amygdala activity in comparison to men. Indeed, this view is endorsed by a relatively large body of evidence (e.g. Domes et al., 2010; Hall & Matsumoto, 2004; Hofer et al., 2006; Kring & Gordon, 1998). In research specifically looking at gender differences in emotion, and amygdala reactivity, findings putatively suggest a female bias towards overt emotional
reactivity. Yet when key amygdala characteristics, and their interactional relationships, are considered holistically these clear cut findings become lost, suggesting there is a far more complex picture to be considered. Wager, Phan, Liberzon, and Taylor (2003) conducted a meta-analysis of the field and found no key gender differences in the literature they compared. This conclusion was affirmed by Sergerie and colleague’s (2008) more recent assessment of the literature, in which the authors found that despite current perspectives in the field, there was little evidence for the suggested female bias.

As yet there is only a very limited body of work that has attempted to tease apart all the key characteristics of amygdala reactivity (habituation, lateralisation, valence and gender) and examine their interactions within one succinct dataset. Andreano, Dickerson, and Barrett (2013) conducted a study in which they observe these different factors interacting. However, their focus was on the differences between familiar and novel stimuli presentation rather than determining the interactions of these key factors. Increasing research in this area seems the logical step towards pulling together the strands of understanding from meta-analyses and independent studies looking into amygdala reactivity and individual interacting characteristics.

Furthermore, in considering the modulating effect of gender on amygdala activation researchers should not assume that there will be clear cut differences. The idea that woman show more bilateral amygdala activation compared to men is a sweeping generalisation and needs to be considered in more detail. Sergerie and colleagues (2008) not only found no support for a female bias in emotional amygdala activation, they actually observed significantly greater mean amygdala effect sizes in studies involving male participants over those involving female. Andreano, Dickerson, and Barrett’s, (2013) research supported sex differences in emotional brain response. However, this was a specific difference relating to the interaction between valence and gender, and the valence-gender specificity also interacted with amygdala habituation and lateralisation. Specifically, they found that women (gender) showed sustained activation (reduced habituation) in bilateral amygdala compared to men for negative stimuli (valence). Furthermore, the sustained activation was greater in the left over the right amygdala (lateralisation). Reduced habituation for negative face stimuli in female participants has also been shown in different age groups in other studies (e.g. Thomas et al., 2001; Williams et al., 2005). The lateralisation of sustained amygdala activation is in accordance with a meta-analysis looking into sex differences and emotional stimuli conducted by Stevens & Hamann (2012). Stevens and Hamann’s work extracted and
compared the findings specifically from neuroimaging studies of brain activation and emotions which reported data from female only and/ or male only populations (totalling 44 studies each). The authors observed that across these studies women showed greater left amygdala activation to negative stimuli, whereas men exhibited greater left amygdala activation for positive stimuli compared to women. Andreano and colleagues (2013) state that they support this bias of male left amygdala activation to positive stimuli based on the observation of a non-significant trend towards significance in men relative to women ($t(43)=-1.449, p=.146$).

1.5.3.4.1 Negative Bias

For clarity it must be noted that there is a greater body of work focusing on sex differences and negative stimuli compared to research focusing on positive stimuli. This largely stems from clinical evidence for a greater prevalence of depression and anxiety disorders in women. For example women are more likely than men to experience generalized anxiety disorder in their lifetime (Solomon & Herman, 2009). Research has even shown that when exposed to the same type of traumatic event women are more likely to develop posttraumatic stress disorder compared to men (e.g. Hourani, Williams, Bray, & Kandel, 2015; Luxton, Skopp, & Maguen, 2010). It has been suggested that the potentially exaggerated response to negative stimuli and stressors observed in the female left amygdala is indicative of underlying mechanisms contributing to these emotion disorders (Leach, Christensen, Mackinnon, Windsor, & Butterworth, 2008; Thomsen, Mehlisen, Viidik, Sommerlund, & Zachariae, 2005). Due to the clear health implications of these emotion disorders, research into negative stimuli has been prioritised while the possible disparity between the sexes and positive stimuli has largely been neglected. Furthermore, Stevens and Hamann (2009) note that the results indicating a gender difference in positive stimulus processing are predominately drawn from research demonstrating that male participants show greater emotional arousal to visual erotica compared to women (e.g. Bradley, Codispoti, Sabatinelli, & Lang, 2001). As the limited amount of research in this area focuses on gender differences in sexual arousal to visual stimuli, perhaps these results should not be considered indicative of a bias towards the experience of all positive emotions. Indeed, Bradley and colleagues (ibid) only observed sex differences in response to visual erotica, but not with regards to other positively valenced stimuli that they presented to their participants. Though Stevens and Hamann’s 2012 meta-analysis suggests there is this interaction between male emotional arousal and positive
stimuli, the non-significant results evidenced by Bradley and colleagues (2001) suggests that perhaps such conclusions should be restricted until further independent research looking at the interaction of gender with both positive and negative valence has been conducted.

1.5.3.4.2 Beyond Imaging
Finally, functional MRI is only one way to investigate gender differences in emotion processing. Research in the field can benefit from the acquisition and interpretation of hormone and volumetric data. These have been largely neglected in affective neuroscience research, as they are only considered of importance in biological and physiological research domains. Sergerie and colleagues (2008) observed that most neuroimaging studies do not fully account for the influence of hormones. In particular, they often overlook the phase of the female participants’ menstrual cycle at the time of testing. If mentioned at all, the lack of control of menstrual cycle is simply given as a limitation of the study (as in the case of Andreano et al., 2013). There is evidence that the levels of fear and anxiety-like behaviour exhibited in female rats is dependent on the phase of their cycle (Frye, Petralia, & Rhodes, 2000; Toufexis, Myers, & Davis, 2006). This is backed up by human studies where it has been shown that amygdala responsivity changes over the female menstrual cycle (e.g. Andreano & Cahill, 2010; Derntl et al., 2008; Goldstein et al., 2005; Ossewaarde et al., 2010; see Figure 1.13 for hormone fluctuations over the cycle and amygdala reactivity during phase).
In a review of the literature, Van Wingen, Ossewaarde, Bäckström, Hermans, and Fernández (2011) assess the few neuroimaging studies that have focussed on the modulating effects that hormone changes across the cycle have on the underpinning emotion neuro-circuitry. They found that there is a clear interaction between gonadal hormone concentrations and emotion circuitry (specifically coupling between the amygdala and medial prefrontal cortex is enhanced by progesterone). Evidently, this alteration in top down inhibition of the amygdala could contribute to the sex differences in amygdala reactivity we see in the literature. Van Wingen et al., (2008) found that during the luteal phase, increased progesterone levels in women resulted in escalation of amygdala reactivity to emotional stimuli. In addition, observations on the modulating effect of testosterone levels associated with increased amygdala reactivity to threatening stimuli (e.g. (Bos, van Honk, Ramsey, Stein, & Hermans, 2013; Hermans, Ramsey, & van Honk, 2008; Van Wingen et al., 2008) led Van Wingen and colleagues to postulate that testosterone decreases coupling between the amygdala and OFC (Van Wingen, Ossewaarde, Backstrom, Hermans and Fernandez, 2011), and that this decoupling could contribute to increased impulsivity and violence typically found in males over females (Antonucci et al., 2006; Coccaro, McCloskey, Fitzgerald, & Phan, 2007).
Gender differences have also been observed in the volumetric and fine-scale structure of the brain. Relatively larger volumes of grey matter have been observed in the amygdala of men compared to women, even when controlling for overall brain size (Goldstein et al., 2001; see Brierly, Shaw and David, 2002 for review). These basic volumetric differences could in part explain sex differences seen in amygdala activation levels, and therefore warrants not only further investigation but acknowledgement when interpreting such data. For instance, Sergerie and colleagues (2008) suggest that greater activation in male amygdala to emotional stimuli could be explained by findings from animal studies showing greater dendritic density (indicating increased excitatory synapses) in male animals (for review see Cooke & Woolley, 2005). An interaction between hormones and structure is suggested by studies showing that the gonadal hormones can influence structural plasticity and volume changes in regions of the brain (e.g. hippocampus (Protopopescu et al., 2008); and parahippocampus (Pletzer et al., 2010). This is especially relevant to the female menstrual cycle, as putative plasticity changes over the cycle could explain the varied findings in the literature, and highlights the importance of controlling for these factors in research and showing awareness when drawing inferences from the data.

### 1.5.3.5 Summary of amygdala characteristics

This section has attempted to present current understanding, and more importantly current conflict, in the role and characteristics of the amygdala in emotion processing. It is clear there is large disagreement though tentatively this conflict is being resolved with the aid of technological advances in imaging tools as discussed in section 1.3.2. These advances go beyond physiological investigations measuring emotion behaviour outputs, towards observation of emotion processing as it is occurring. As stated previously, there is only a very limited body of work that has attempted to tease apart the key characteristics of amygdala reactivity namely habituation, lateralisation, valence (both positive and negative) and gender, and examine their interactions within one succinct dataset. This thesis attempts to fill the gap in the research using the technological advances detailed above in functional neuroimaging during emotion processing and at rest. Furthermore, it will investigate how these mechanisms are altered in those with high and low anxiety, as an insight into the abnormal processing underlying anxiety disorders.
1.5.4 Resting state analyses of amygdala connectivity

The technique of resting state fMRI (Rs-fMRI), and its use in investigation of emotion processing has been discussed in detail in section 1.3.2.3. Despite its potential utility, there are only a small number of studies that use Rs-fMRI to investigate emotional processing and the functional connectivity of the amygdala at rest (e.g. Baur, Hänggi, Langer, & Jäncke, 2012; Kim, Gee, Loucks, Davis, & Whalen, 2011; Rabinak et al., 2011; Roy et al., 2009). Two of these studies were examined in the section on the prefrontal cortex (section 1.4). In brief, the first study demonstrated that connectivity between the amygdala and the mPFC altered as a function of anxiety levels, with low anxiety individuals showing positive coupling of ventral mPFC and negative coupling of dorsal mPFC (Kim et al., 2011). High anxiety individuals exhibited the opposite coupling for vmPFC, and no coupling for dmPFC. The second study reported increased amygdala functional connectivity during rest in patients with vmPFC lesions, particularly between the right amygdala and anterior temporal cortex (Motzkin et al., 2014). However, patients did not exhibit any typical anxious traits. These are potentially contradictory findings about the role of the vmPFC, but demonstrate the utility of the Rs-fMRI technique in emotion research. This is particularly important as it has been made clear in this chapter that emotion processing, particularly that of fear, does not solely rely on the amygdala, though it does clearly play a pivotal role. Rather it has been demonstrated it works in parallel with a network of areas in the brain which modulate each other.

It should be clear that in order to unravel the intricacies of individual differences in emotion processing future research must pay due attention to the networks in the brain as well as attempting to explore the functionality of the amygdala itself. In order to address this gap in the field this thesis is applying traditional fMRI techniques alongside Rs-fMRI to the investigation of emotional processing looking specifically at the amygdala and individual differences.

1.6 Anxiety, abnormal anxiety and emotional disorders

After reviewing the regions of the brain implicated in typical emotion processing in section 1.4 and focused on the amygdala in section 1.5 which is identified as a key neuroanatomical area involved in maladaptive emotion processing, this section focuses on the current understanding of what happens in the brain in atypical or maladaptive emotion processing. Firstly, the theoretical underpinnings of such concepts are considered. This is then followed
by a discussion of the current understanding of the neural basis of abnormal anxiety and fear responses.

Emotions often serve a purpose, allowing the individual to function within a social context and to adequately respond to the environment in order to survive and prosper. Therefore, the experience of fear or anxiety should not solely have negative connotations, but rather it contributes to successful adaptation and survival (Thompson & Calkins, 1996). As considered in section 1.1.2, a stress response to a threatening stimulus allows the organism to ready itself to react to the situation, for instance a primed autonomic nervous system may enable an athlete to run faster and win a race. Another example is that although a child crying out may be viewed as maladaptive, it gains the child attention that was the goal of the behaviour (Gross and Thompson, 2006). Evidently the experience of fear or anxiety in itself, in certain situations, can be beneficial to the organism.

The ability of some people to ‘bounce back’ from adverse emotional experiences is sometimes called emotional resilience and is defined as ‘the maintenance of positive adaptation by individuals despite experiences of significant adversity’ (Luthar, Cicchetti, & Becker, 2000, pp 1). Research into emotional resilience, and in particular the fear response, has largely been in the field of social psychology and has focused on the impact of positive peer and family relationships. However, as highlighted by appraisal theories and the modal models of emotion, positive family and peer relationships are only two of the three contributing factors to emotional resilience, the third factor being individual differences (Werner, 1995). The lack of investigation into individual differences is somewhat striking, especially as abnormal variations in the emotional processing and subsequent response to fear can lead to pathological anxiety disorders. These disorders are likely to occur in 28% of the population over a lifetime, and are thought to be the most common psychiatric disorder (Kessler et al., 2005). Research into individual differences in emotion processing, and the interaction between basic emotions and affective traits (such as anxiety), will further understanding of emotional resilience and could be extremely valuable in the development of preventative mechanisms or treatment interventions for anxiety disorders.

An emotional trait is an emotion or set of emotions which frequently reappear during a period, or across the lifespan of an individual (Ekman, 1984). Traits are key components of an individual’s unique personality and can be adaptive in some circumstances. For example, having an anxious personality may help an airline pilot remain hypervigilant, maintain their
focus and regulate their responses when flying in order to do their job efficiently and safely. However, when certain emotions are no longer transient, and start to occur outside the contexts in which we would expect them, they may start to be considered maladaptive. In the specific case of anxiety, persistent experiences of anxiety and fear response can start to be detrimental to the organisms’ well-being. When a participant does not exhibit emotional resilience to fear individual differences emerge. These differences are of particular interest to researchers in the context of understanding emotional resilience and investigating possible mechanisms by which affective disorders can be identified and treated. They also highlight the importance of integrating cognitive psychological research with neuro-scientific methods as used within this thesis.

1.6.1 Behavioural Inhibition System (BIS)/ Behavioural Approach System (BAS) ¹
models of anxiety disorders
One of the dominant theories of personality and psychological dysfunctions is the BIS/BAS model first proposed by Gray in 1975 (cited in Amodio, Master, Yee, and Taylor, 2008). Gray’s theory is grounded in the idea that there are two internal systems that exist in a state of dynamic equilibrium. The BIS system serves to disengage behaviour and promote the monitoring of incoming information to detect threat cues and aversive stimuli. The second system, the BAS, works in opposition to the BIS and is largely involved in motivation, reward, behavioural engagement and directing behaviour towards positive outcomes. These two systems loosely relate to approach and avoid mechanisms. Though there appear to be parallels, these systems do not correspond to the reflexive fight/flight mechanisms related to threat detection, but represent psychological internal systems (McNaughton & Gray, 2000).

1.6.2 Putative Neurocognitive Correlates of BIS/BAS
Gray’s BIS/ BAS model provides a framework for anxiety models, with high BIS characterised by hypervigilant behaviour and increased arousal. Very strong BIS is associated with anxiety-related disorders (e.g. Amodio, Master, Yee and Taylor, 2008, Morgan et al., 2009). On the other hand, the BAS is not directly related to anxiety disorders, and so is not interesting in the context of this thesis. A growing body of research has established that the

¹ On occasion this system is referred to as the Behavioural Activation System (Fowles, 1980)
neurocognitive correlate of the BAS is primarily driven by the dopaminergic neurotransmitter system associated with prefrontal cortex (e.g. McNaughton & Gray, 2000; Moghaddam, 2002; Takahata & Moghaddam, 1998) and implicated in the reward network. Asymmetry in the PFC has been implicated in the BAS, with greater activation in the left hemisphere playing an important role (e.g. Amodio, Master, Yee, & Taylor, 2008; M Balconi & Cobelli, 2015; Harmon-Jones, 2003).

In contrast to the BAS, research into the underlying neurocognitive correlates of the BIS remain relatively sparse. Gray based the BIS on observations that there is a group of pharmacological drugs which appear to reduce anxiety via inhibiting behaviour in response to punishment/ noxious stimuli (e.g. barbiturates, alcohol; Fowles, 1980; Gray, 1990)). Gray (1982, cited in Gray and McNaughton, 2000) associated the BIS with the hippocampus which was supported by findings that anxiolytic drugs do act on the hippocampus (e.g. Buzsáki, 2002; McNaughton & Gray, 2000). Levita and colleagues (Levita et al., 2014) also provide evidence in support of hippocampal involvement in the BIS in a neuroimaging study of thirty participants. Participants completed measures associated with BIS (e.g. sensitivity to punishment scale), and volumetric data for the hippocampus and amygdala were collected using T1-weighted structural functional magnetic resonance imaging. The authors found an association between greater hippocampal volume and greater associated indicators of BIS scores. They note that similar findings have been reported in two other studies to date (Barrós-Loscertales et al., 2006; Cherbuin et al., 2008), providing more concrete evidence for hippocampal involvement than Gray's initial observations.

1.6.2.1 The HPA, Hippocampus and Amygdala

Further evidence for neurocognitive correlates of BIS comes from the stress response literature discussed in section 1.1.2. A number of different brain regions have been implicated in a general response to acute stressors, regardless of the type of stressor. These regions include the hippocampus, amygdaloid nuclei and several brain stem nuclei (Campeau, Akil, & Watson, 1997; Cullinan et al., 1993; for further details and an exhaustive list see López, Akil, & Watson, 1999). López and colleagues (ibid) note that the holistic stress response does not simply rely on these brain regions, but arises from their integration with other peripheral systems. It is the interaction between brain circuits and these peripheral body systems that can often result in maladaptive responses. In particular, there is one primary neuroendocrine
circuit which has been associated with the stress response, the hypothalamic-pituitary-adrenal (HPA) axis or the limbic- hypothalamic-pituitary-adrenal (LHPA) axis (Keller-Wood & Dallman, 1984; Keller-Wood, Shinsako, & Dallman, 1984; Vázquez, 1998). As a result of threat detection, the stress response results in secretion of the stress hormone cortisol from the adrenal cortex. As mentioned earlier (section 1.1.2), circulating cortisol acts throughout the body to prepare the body for action as part of the autonomic nervous system response. This autonomic nervous system response, and its regulation, is critical for an organism’s ability to adapt and survive in stressful and threatening situations. The exact mechanism by which the neuronal stress response is integrated with these peripheral endocrine components is not fully understood (López et al., 1999). Nonetheless, there is an indication that cortisol release into the blood stream results from elevated concentrations of adrenocorticotropic hormone (ACTH), which is in turn released from the anterior pituitary gland in response to corticotropin-releasing hormone (CRH) from the parvocellular neurons of the paraventricular nucleus in the hypothalamus. In essence this system represents the HPA axis.

Critically, HPA axis activity has been shown to be modulated by the amygdala and hippocampus (Bratt et al., 2001; Laryea, Arnett, & Muglia, 2012; Yoshida, Takayanagi, & Onaka, 2014). In particular, the hippocampus has been implicated in the suppression, or inhibition, of cortisol secretion. This is achieved through the binding of cortisol to receptors in the hypothalamus, which then acts to inhibit CRH and thus ACTH release in feedback mechanisms (e.g. Jacobson & Sapolsky, 1991). Therefore the influence of steroidal cortisol hormones on HPA activity is primarily generated by mineralocorticoid receptors located in the hippocampus (Gesing et al., 2001). This is in line with Gray's suggestion of hippocampal involvement in the BIS.

In contrast to the inhibitory effects of the hippocampal CRH system, the amygdala CRH system has been shown to have an excitatory effect on the stress response. Reduced corticosterone and ACTH secretion has been shown in rats with bilateral lesions to the medial amygdala compared to controls (Gray et al., 1993; Masini et al., 2009).

The amygdala has also been implicated in the stress response more directly, rather than as a mediator of the HPA axis. There is evidence that the central nucleus of the amygdala (CEA) plays a specific role in more fear-related stress responses. Makino and colleagues (Makino et al., 1999) looked into the impact of psychological stressors on the hypothalamic and amygdala CRH systems in rats. Their evidence suggested that the amygdala CRH system is
more sensitive to psychological stressors than the CRH system associated with the hypothalamus. The authors note that previous research has also found evidence that the amygdala CRH system can be activated by psychological stressors, even in absence of the HPA (Britton, Varela, Garcia and Rosenthal, 1986, cited in Makino et al., 1999). This indicates a possible route through which the amygdala can be a threat detector and mediate a stress response through the CRH system.

1.6.2.2 Insula and ACC

There are two more structures that are putative neural correlates of the BIS. The insula and anterior cingulate cortex (ACC) have also been shown to be involved in emotion processing (see section 1.4.2.3), and in particular these have been associated with maintenance of anxiety disorders (Holzschneider and Muler, 2011). Both areas seem to be consistently co-activated during symptom provocation in studies of clinical anxiety (Etkin and Wager, 2007), and both appear to be functionally connected to the amygdala. In general, the insula has been widely implicated in interoceptive awareness, and the ACC which has been shown to play a key role in approach/ avoid behaviours and conflict resolution. A recent study using multimodal magnetic resonance imaging approach (resting state MRI and diffusion tensor imaging (DTI)) has shown that the anterior insula and basolateral amygdala are functionally and structurally connected, and that this connectivity was related to state (functional) and trait (structural) anxiety (Baur, Hänggi, Langer, et al., 2012). This finding is in line with animal studies (e.g see Stein et al., 2007) but is the first to explicitly look into this connectivity in humans. In addition research by Etkin and colleagues (Etkin, Egner, Peraza, Kandel, & Hirsch, 2006) suggests that part of the ACC acts as a top down regulator of amygdala inhibition. Etkin and Wager (2007) later identified that in mood disorders this fronto-amygdala connectivity is disrupted suggesting this relationship is key to understanding dysfunctional emotion responses in individuals with clinical mood disorders.

1.6.3 Neural Basis for Anxiety Disorders

So far this section has discussed the relationship between putative neural mechanisms of the BIS and their relationship with anxiety. As mentioned earlier activation of the stress response to acute stimuli can be beneficial to an organism in the short term, and has an adaptive function. However, a key characteristic of anxiety disorders is an inappropriate stress
response (Campos et al., 2013; Charmandari, Tsigos, & Chrousos, 2004). This inappropriate response is likely to be caused by alterations in the underlying neural mechanisms of the stress response. In particular, Selyes GAS model of stress (section 1.1.2.1) suggests that the inhibitory role of the hippocampal CRH system is particularly important to counteract inflated levels of circulating corticosteroids following continued or sustained stress responses. Persistently high levels of circulating corticosteroids can have harmful effects on the organism (López et al., 1999). In particular, research shows that such chronic stress responses and increased cortisol can cause hippocampal cells to atrophy (Lee, Jarome, Li, Kim, & Helmstetter, 2009; Magariños, McEwen, Flügge, & Fuchs, 1996). Reduced hippocampal volume has been shown in neuroimaging studies of patients with post-traumatic stress disorder (PTSD) compared to controls (e.g. Bremner et al., 1995; Gurvits et al., 1996), and also in relation to higher anxiety in healthy participants (e.g. Levita et al., 2014). As the hippocampal cells degenerate, a vicious cycle can ensue as there are fewer receptors to bind to the cortisol and less inhibition of the HPA axis, causing the system to become flooded with cortisol (e.g. Lee et al., 2009; Magariños et al., 1996). In addition to structural changes, there is evidence linking anxiety disorders to both hyperactivity of the amygdala and/ or diminished hippocampal activity (e.g see Etkin and Wager, 2007; Holzschneider and Mulert, 2011). This should come as no surprise as the HPA axis, the primary stress response system, is clearly modulated by the dynamic equilibrium between amygdala and hippocampal activity.

1.7 Chapter Summary

After reviewing the literature this thesis sought to investigate the neurobiological mechanisms in healthy populations involved in emotion processing specifically in the amygdala using the exemplar emotion fear. Evidence has emerged in affective neuroscience literature relating to a number of key interacting factors which modulate amygdala activation during emotion processing, these are discussed in section 1.5. However historically cognitive research into emotion has shown a reluctance to study multiple interacting factors, and in particular individual differences are often bypassed for more demonstrable factors in the literature. Here the present a body of work attempts to assess these key interacting factors in combination in a sub-clinical population in order to attempt to tease out the interplay between them. This is done using stimuli such as those discussed in section 1.2.1, and both direct and indirect measurements in conjunction as discussed in section 1.2.2. Furthermore, Rs-fMRI
was acquired, as discussed in section 1.3.2.3, in order to investigate the spontaneous connectivity patterns with areas involved in emotion processing such as those discussed in section 1.4 across gender and sub-clinical anxiety groups. Parcellation techniques as discussed in section 1.5 are applied to the resting state data post-hoc in order to investigate the worth of studying whole amygdala connectivity with other brain regions or looking at such connectivity using individual sub-nuclei as investigated by Roy et al. (2009). It is evident from the literature that the underlying neurobiological factors involved in maladaptive anxiety are not fully understood, and it is hoped that findings here can shed light on these mechanisms and help build towards not only a better understanding of such mood disorders, but also contribute to treatments and potential interventions in the future. The structure of the thesis will be defined in the following chapter, with precise research aims informed by the theoretical outlooks presented in this literature review.
Chapter 2: Research Aims

2.1 Aims of thesis

Based on the research discussed in the literature review, there are still open questions with regards to the nature of amygdala activation in isolation and within a network in the brain in response to emotional stimuli and the modulating influence of individual differences such as anxiety and gender. This thesis focuses on characterising amygdala activation during emotion processing in a healthy population of participants. In particular, the potentially modulating influence on amygdala activation, connectivity and structure at rest, and during task, of individual differences such as sub-clinical anxiety and gender will be explored. In this way it will contribute to growing understanding and enable further research within the field. In particular, the data presented within this thesis comes from one comprehensive dataset (n=50+ ²). This approach increases the chance of observing subtle differences and interactions due to the sample size, as well as allowing direct comparison of different analyses from the same dataset enabling observation of a holistic picture of amygdala activation during emotion processing.

2.2 Plan of thesis

This thesis consists of 6 empirical studies using quantitative methodologies.

Study 1: Mood online experience survey (MOOX)

Aims:

- To look at whether sub-clinical anxiety or gender modulates willingness to volunteer in studies which could be considered 'high stress'; those involving performance measures, as well as those using neuroimaging.

Study 2: General Linear Model

² Specific sample sizes are detailed within the Methods Chapter 3 and also within each study chapter. The sample size varies slightly depending on the type of analysis being conducted and specific exclusion criteria
Aims:

- To investigate the individual impact of discrete factors involved in amygdala reactivity during emotion processing (valence, habituation, lateralisation of amygdala, gender and anxiety).

Specific study questions:

- Does the amygdala activate only for fearful faces or for happy and neutral face stimuli as well?
- Is amygdala activation lateralised?
- Does amygdala activation change over time?
- Is amygdala activation lateralisation modulated by time?
- Is amygdala activation modulated by gender?
- Is amygdala activation modulated by state anxiety?

Study 3: Psychophysiological Interaction

Aims:

- To assess the modulating impact of gender and sub-clinical anxiety on fronto-amygdala connectivity.

Study 4: Categorisation Analysis

Aims:

- To investigate whether emotion condition can be predicted by brain activation maps using SVM and MDLA classification methods.

Study 5: Cortical Thickness Analysis

Aims:

- To assess amygdala, prefrontal and hippocampal structural alteration in a subclinical population.
Study 6: Resting state connectivity and Parcellation of the amygdala

Aims:

• Study A: Whole amygdala resting state
  o To determine the resting state connectivity pattern of the amygdala across participants
  o To determine whether this connectivity pattern is modulated by anxiety
  o To determine whether there are group differences in fronto-amygdala connectivity between anxiety and gender groups

• Study B: Parcellated amygdala resting state.
  o To replicate Roy et al. (2009) parcellation methods and determine resting state connectivity of the amygdala subdivisions.
Chapter 3: Method

3.1 Overview
This chapter presents the methods and analysis used in each of the studies conducted for this thesis. The participants, design, materials and procedure will be discussed here. For conciseness, analysis techniques relating to individual studies will be discussed in detail in the relevant chapters.

The methods described here were used to conduct the following studies:

- Chapter 4: Mood Online Experience Survey (MOOX)
- Chapter 5: General Linear Model
- Chapter 6: PsychoPhysiological Interaction
- Chapter 7: Categorisation Analysis
- Chapter 8: Cortical Thickness Analysis
- Chapter 9: Resting State Connectivity and Parcellation of the Amygdala

3.1.1 Recruitment Overview
Data described here was collected from opportunity sampling. Participants were recruited through University e-mails or fliers based on University of Surrey or Royal Holloway University campuses. In addition, the online survey which was distributed via University of Surrey e-mail allowed for additional recruitment based on participants’ responses.

3.1.2 Study Design Overview
The overall design of this study was implemented as such that various different analyses could be run on one set of data to answer multiple questions. Figure 3.1 shows how data were collected in order to maximise on ways in which the data could be analysed. The online survey was collected independently of other recruitment methods and has a dual purpose in order to answer questions relating to non-participation in fMRI studies as well as to provide a potential source of informed and willing participants.
Figure 3.1 Diagram providing an overview of data collection in this thesis. The Mood Online Experience Survey (MOOX) represents a stage of data collection which was also used as an avenue for participant recruitment to feed into the experimental phase. The experimental phase comprises two different scans, a six minute resting fMRI scan which is used in studies characterising amygdala activation at rest, and a 50 minute backwards masking scan used to look at amygdala activation during emotion processing. In addition behavioural measures were collected before and after scanning as well as during the MOOX survey.

3.1.3 Behavioural Materials and measures
An overview of the order in which participants completed behavioural measures is shown in Figure 3.2. Basic demographic information was collected (which is anonymously coded for all analysis) in the online survey and in the participant information questionnaire (PIQ) in the experimental phase. In addition, a number of behavioural measures have been used in the studies presented in this thesis. Information on each measure is detailed below.
3.3.3.1 Handedness

The Edinburgh inventory of handedness (Oldfield, 1971) is a self-report ten item measure used to gauge hand dominance in participants. Participants simply indicate preference for hand use in relation to different tasks (e.g. writing, using a toothbrush etc.). Participants have to indicate strength of preference and can indicate equal preference for both hands using this measure. Strength of handedness is calculated by the researcher through calculation of difference in preference for hand, divided by the cumulative total and multiplied by 100. Scores > 40 indicate right handedness, < -40 indicate left hand preference and between these values are suggested to be interpreted as ambidexterity. Handedness was measured as a control variable in later analysis, not as exclusion criteria.
3.3.3.2 Hospital Anxiety and Depression Scale (HADS)

The Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983; Zigmond, 2013) is a self-report measure that was developed in order to measure general levels of anxiety and propensity to depression symptoms. The measure is commonly used in order to screen for individuals who are experiencing/ or are at risk of developing clinically significant symptomology and takes no more than five minutes for participants to complete. This is a fourteen item scale comprising of an anxiety sub-scale (seven questions; HADS_A) and a depression sub-scale (7 questions; HADS_D). In response to each question participants are asked to identify on a four-point Likert scale, how they are currently feeling. The responses are coded from 0-3, the coding is dependent on the direction of the response, e.g. ‘I get sudden feelings of panic’ scores 0 for ‘Not at all’ and 3 for ‘very often indeed’, whereas ‘I can sit at ease and feel relaxed’ scores 0 for ‘Definitely’, and 3 for ‘Not at all’. In order to calculate final scores, the researcher simply tallies up the overall scores from the HADS for each sub-scale; a minimum of zero is possible, with a total score of 21 for each. There are guidelines provided which indicate a score of 0-7 would be considered normal to no anxiety/depression, 8-10 is borderline or mild anxiety/depression and ≥11 is indicative of severe levels of anxiety/depression (note that Zigmond & Snaith, (1983) identify 8+ as ‘possible cases’ of clinical anxiety/depression).

Julian (2011) used literature in the field to conclude that the HADS_A is a suitable stand-alone subscale of the HADS. This is backed up by evidence from a study (Bjelland, Dahl, Tangen, and Neckelmann 2002) which assessed the validity of the HADS by reviewing 747 papers, and determined that the HADS_A subscale had high internal validity with Cronbach’s alpha coefficient ranging from .68-.93 (average .83). Further Bjelland and colleagues found concurrent validity with other measures of state anxiety (for example correlations between HADS_A and Spielberger’s State-Trait Anxiety Inventory (STAI) ranging from .64-.81, and .69-.75 for the Clinical Anxiety Scale (CAS); see Bjelland et al., 2002 for more information). Although the anxiety sub-scale is of particular interest in this research, observations have been made from the HADS_D and also of the total HADS score (HADS_T; aggregate scores of the two sub-scales). Both the depression subscale and the overall HADS scores have also been shown to be reliable and valid measures (Bjelland et al., 2002; Michopoulos et al., 2008).

It is of note that a more recent interrogation of the structure of the HADS has somewhat called the specificity of the measure into question (Cosco, Doyle, Ward, & McGee, 2012;
Norton, Cosco, Doyle, Done, & Sacker, 2013). Using confirmatory meta-analyses and a systematic review, the authors revealed that potentially the HADS is not as sensitive as originally thought in terms of dissociating the anxiety and depression constructs. This finding is conceivably not surprising in that literature has shown that anxiety and depression are strongly comorbid, e.g. Hirschfeld, 2001). As a result of their studies Cosco and colleagues (2012, 2013) suggested that perhaps the HADS is better suited as an overall measure of general emotional distress. However, in both studies they did highlight that the results in previous literature from which their assessments were drawn, as well as their own findings, appeared to be sensitive to the type of statistics used. In light of the fact that the structure of the HADS is potentially sensitive to the type of statistics used, interpretation of the subscales within this thesis will be considered with due caution. Despite identifying possible cross correlation between the two dimensions of the HADS (anxiety and depression sub-scales), Cosco and colleagues (2012, 2013) reiterate that the HADS continues to be particularly useful as a classification and case-finding tool and is suitable with the remit of research presented here.

3.3.3.3 Six-item Short-form of the Spielberger State-Trait Anxiety Inventory (STAI-6)

The short form of the STAI was developed in 1992 (Marteau & Bekker, 1992). This measure is adapted from the full forty item Spielberger STAI (Spielberger, 1983, Spielberger, 2010). The key purpose of the STAI and STAI-6 is to identify via self-report the presence and severity of current anxiety symptomology and propensity towards anxiety. The STAI-6 comprises anxiety-present and anxiety-absent measures which identified as being particularly sensitive to low and high stressors (Spielberger, 1983; Marteau and Bekker, 1992). When completing the STAI-6, participants respond to six descriptive statements (e.g. ‘ I feel calm’) and are asked to assess the intensity of their current feelings ‘right now, at this moment’. Responses are given on a four-point Likert type scale ranging from ‘Not at all’ to ‘very much’. The total STAI-6 score can then be calculated by summing all values, multiplying by twenty and diving by six (note the anxiety-absent items; calm, relaxed and content, are reverse scored prior to summing). The range of scores is from 20-80, with a ‘normal’ score suggested to be approximately 34-36 (Spielberger, 1983; Spielberger, 2010). Two STAI-6 measures were taken during the experimental phase and also during the online survey.
The STAI-6 has demonstrated good internal consistency across both clinical and non-clinical populations yielding a Cronbach’s alpha coefficient of .82 for the six items in Marteu and Bekker’s validation study (1992). The STAI-6 has been shown to yield similar scores to the original measure whilst also providing a platform by which researchers can assess state and trait anxiety within a short time frame reducing non-completion due to time constraints and lends itself to studies whereby participants must complete a battery of tests (Marteau and Bekker, 1992).

Note the STAI-6 was not consistently measured during data collection – a number of male participants did not complete the measure pre-and post-scan (only 8 males completed both STAI-6 measures; all female participants completed both). For those who did complete the STAI-6, the scores were compared to results for the HADS_A measure, which all participants did complete. These two measures were found to be highly consistent ($r_s = .72$, $p<0.01$). Consequently, results reported in this thesis predominantly focus on scores from the HADS. This is with the exception of chapter 8 which specifically looks at both measures.

3.3.3.4 Profile of Mood States (POMS)

The POMS (first developed by McNair, Lorr, & Droppleman, 1971) is a self-report questionnaire which yields a six-factor measure of mood disturbance (anger, confusion, depression, fatigue, tension and vigour) and can also be used to calculate a total mood disturbance (TMD) score. Participants respond to a series of 65 adjectives rating how they have been feeling during the week before and on the day they completed the inventory which takes no more than ten minutes to complete. Each adjective has a five-point scale which ranges from ‘not at all’ to ‘extremely’. Responses are coded by the researcher from 0-4 (typically 0 = ‘not at all’, 4 = ‘extremely’) however some items are reverse coded, for example ‘Panicky’ uses typical scoring however participants’ response to the adjective ‘Relaxed’ results in a score of 4 for ‘not at all’ and 0 for ‘extremely’. Once scores for each mood dimension have been calculated a cut-off point of 1-1.5 standard deviations above the mean is suggested to be indicatory of non-clinical and clinical scores (in line with other psychological measure guidelines, Nyenhuis, Yamamoto, Luchetta, Terrien, & Parmentier, 1999).

This thesis only addresses the tension/ anxiety subscale of the POMS consisting of nine items in total with a total score range of 0-36 and uses the instructions ‘right now’ rather than
‘during the last week’/ ‘today’. Though the standard instructions for the test are considered to be a week/ the actual test day, shorter time frames have been used when investigating fluctuations in mood in relation to situation or experimental manipulation. In a comparative review of state anxiety measures Rossi and Pourtois (2011) reported that specifically the use of the ‘right now’ instructions led to higher sensitivity particularly on the tension-anxiety subscale.

The internal consistency for this subscale is relatively high (Cronbach’s alpha coefficients of .90 -.92; McNair & St Heuchert, 2005). Nyenhuis and colleagues (1999) endorse the validity of the POMS and in particular the tension-anxiety subscale having found high correlations of the POMS and its subscales with other measures aimed at capturing state anxiety fluctuations (such as the state trait anxiety inventory). In Rossi and Pourtois’ 2005 review, the authors found similar evidence for external validity of this subscale through high correlations with other state anxiety measures. Overall evidence in the literature shows that the POMS, and the tension-anxiety sub-scale, a reliable and valid method by which to measure state dependent fluctuations in mood.

*Note the POMS tension-anxiety scores before scanning were compared to the HADS_A measure and found to be highly correlated (r_r= .76, p<0.01). However, the POMS scores were more variable and since there is a directly defined cut-off for abnormal/ normal anxiety in the HADS_A measure, only the HADS_A measure is reported within this thesis.*

### 3.3.3.5 Karolinska Sleepiness Scale (KSS)

The Karolinska Sleepiness Scale (Åkerstedt & Gillberg, 1990, reprinted 2009) is a brief measure of the subjective level of state sleepiness participants are experiencing at a specific time point. It consists of a nine-point Likert type scale in which participants indicate how sleepy they currently feel from ‘very alert’ to ‘very sleepy, great effort to keep awake’. The KSS has been shown to be sensitive to fluctuations in day-time sleepiness (Shahid, Shen, & Shapiro, 2010). Numerous validation studies have been conducted looking into the sensitivity of the KSS, it has been shown to have high levels of external validity with significant relations with other measures of state sleepiness (with correlation coefficients ranging from .65 -.81 between the KSS and the Accumulated Time with Sleepiness Scale and a Visual Analogue Scale, Gillberg, Kecklund, & Akerstedt, 1994). The KSS has also been found to be highly correlated with electroencephalography as well as behavioural measures of sleepiness.
in more recent studies (e.g. see Kaida et al., 2006; Putilov & Donskaya, 2013). In the experimental phase of the study the KSS was administered pre-, and post-scan to all participants. Overall the KSS is a reliable, objective and user-friendly measure of state sleepiness and suitable for use in the research presented in this thesis.

3.3.3.6 Post-Scan Interview

In order to gain a more holistic insight into the experience of the participants whilst in the scanner a brief structured post-scan interview was conducted that was designed specifically for this research (appendix A). This was a brief interview conducted with participants’ permission at the end of data collection prior to debriefing whereby participants were given the opportunity to share their experiences of the study. Furthermore, this interview was deemed a vital part of ensuring that the nature of the experimental paradigm was not compromised. The experimental phase of the research presented in this thesis relies on processing of emotion to be ‘unseen’ or subconscious in the backwards masking paradigm. Participants were questioned initially about what different aspects of the faces they saw and whether anything stood out in particular. They were asked if any features stood out and whether there were any emotional aspects of the faces they would like to comment on (this is similar to methods described in Whalen et al., 1998). In addition, participants were asked for comments on their overall experience and whether they were able to rest and ‘think of nothing in particular’ in rest phases as they had been instructed to do so (this was deemed important for the resting aspects of data collection to ensure genuine resting state had been observed). Any participants who were able to identify that they had seen happy and/or fearful faces and not just observed neutral faces would have been excluded from further analysis. Though a minority participants mentioned that they felt the images were moving (an effect of the change between two images presented, the ‘flicker effect’ discussed in section 3.3.5.4.2), none gave responses that alerted the researcher to the possibility that they had correctly identified emotional aspects of the masked faces presented and thus all were put through for further analysis.
3.3.4 The Mood Online Experience Survey: MOOX

3.3.4.1 Design and Recruitment

A cross-sectional survey using internal e-mail at the University of Surrey, word of mouth and the social media platform Facebook to recruit participants was conducted. The purpose of the survey was two-fold, to collect information on the impact of individual differences and how they modulate willingness to participate in research and to potentially recruit anxious participants for an fMRI study. Participant recruitment e-mails were distributed to University of Surrey staff and student e-mail lists and information was placed on Facebook on 11th April 2014 and was available for completion until 12th May 2014. The online survey was programmed using Qualtrics Research Suite (Qualtrics software, Version 04-05-2014 of Qualtrics. Copyright © 2014). This is an online survey tool that allows researchers to build, distribute and perform basic analysis on surveys (see Appendix B for the full survey). The survey contained items on participant demographics, anxiety measures (Hospital Anxiety and Depression Scale and the short version of the State Trait Anxiety Inventory) and questions on willingness to participate. For those participants who showed interest in participating in neuroscience research they were shown an additional set of slides with information on an fMRI research study and gave them the opportunity to provide their e-mail address to be contacted further should they wish to find out more information.

Participants received no financial or other incentives to participate, were assured of anonymity (except in those cases where they wanted to be contacted further) and were informed they could withdraw at any time. Before filling in the questionnaire participants were also provided with a brief summary of the study aims and how long the questionnaire should take to complete. Their informed consent was obtained by ticking a box if they agreed to continue and participate in the study. The study received favourable ethical opinion from the University of Surrey ethics committee in April 2014. An overview of participants is given in Figure 3.3.
Three hundred and ninety-five participants started the online survey, of those 348 participants voluntarily completed the online survey (aged 20-45; $\bar{x}=29.56\pm6.42$; 135 male, 213 female). Participants completed the survey in response to an e-mail inviting them to take part online and by word of mouth thereafter, the survey was active for one month between April and May 2014. Using the HADS_A scores participants were categorised into the high (a score of 11+) or low anxiety (0-10) group. With 87 participants in the high anxiety group (29 male; aged 21-45, $\bar{x}=29.13\pm6.14$) and 261 participants in the low anxiety group (106 male; aged 20 to 45, $\bar{x}=29.71\pm6.51$).

Three hundred and thirty-three participants who completed the survey were offered the chance to participate in a follow up fMRI study as a result of them indicating they would be willing to take part in neuroimaging based studies. One hundred and thirty-one consented to being contacted further about participation in this study (57 males; aged 21 to 43, $\bar{x}=28.80\pm5.78$). Of these, twenty-nine were highly anxious (9 males; aged 21 to 42, $\bar{x}=26.90\pm5.30$). In follow up communication with the highly anxious participants, 18 did not respond, of the remaining 11 no one met scanning criteria/ were available during the study duration so these participants were not put into the experimental phase.

Figure 3.3. Schematic showing the breakdown of participants in the online survey. *Although the low anxiety individuals were not recruited for this study, they consented to their contact details being stored for future fMRI studies.
3.3.4.3 Measures and Materials

Online demographic information was collected from participants in the MOOX as well as the self-report STAI-6 and HADS measures as described above. Since the study aim was to investigate willingness to participate in different types of research, a set of five polar yes/no questions:

1. A study where you have to complete a task where your performance is being assessed?
2. A study where your brain function is being measured whilst your performance on a task is assessed?
3. A study where your brain function is being measured whilst you are not performing an assessed task (at rest)?
4. A study where your behaviour is observed but your performance is not being measured?
5. An interview study?

These study designs were reviewed in an initial pilot study to ensure suitability of the wording used. The first two questions relate to task-active scenarios with performance measures, the second two questions should be relative controls offering task-negative scenarios with no measure of performance, the final design offers a control design within the context of the other design types.

3.3.4.4 Procedure

A schematic of the procedure is given below (Figure 3.4). Participants initially saw an information screen where they were briefed on what the survey would entail. Once they had consented to take part they were firstly asked about demographic information, this was followed by an online version of the Hospital Anxiety and Depression questionnaire (HADS), then the short version of the State Trait Anxiety Inventory. Participants were then asked about willingness to participate in various different research designs (detailed above, section 3.3.4.3) to which they responded ‘yes’ or ‘no’. Finally, any participants who responded positively to questions relating to studies involving brain imaging techniques were given on-screen information on the experimental MRI studies detailed in this thesis (and detailed in
section 3.3.5) and asked to provide their e-mail addresses if they would like further information about participating. All participants were thanked for completing the survey at the end. Please see appendix B for a print out of the online survey.

Figure 3.4 Schematic showing overview of the procedure in the Mood online experience survey. As indicated any participants who were highly anxious and that were willing to be contacted about participation in neuroimaging studies were later contacted after the MOOX survey had closed. Twenty-nine participants were contacted however none were put through to the experimental phase due to non-response/ not meeting scanning criteria or availability issues.
3.3.5 Experimental Phase

3.3.5.1 Overview

Chronologically, participants first had structural scans taken whilst in the scanner for normalisation processes as well as use in chapter 8. They then underwent a six minute resting scan (see section 1.3.2.3). When they were ready to continue this was followed by the 50 minute backwards masking paradigm (see section 1.2.1). After exiting the scanner participants completed post scan paperwork which was followed by a brief post-scan interview regarding their experiences whilst in the machine (see section 3.3.3.6; see Figure 3.5 for overview). Participants were then debriefed and the study procedure was complete.

Figure 3.5 Schematic showing the experimental phase of data collection. The experimental phase comprises two different scans, the six minute resting fMRI scan which is used in studies characterising amygdala activation at rest, and the 50 minute backwards masking scan used to look at amygdala activation during emotion processing.

3.3.5.2 Design and Recruitment

Participants were recruited in response to either flyers distributed at the Freshers Fayre, e-mails sent out to students and staff within the Psychology Department at the University of Surrey, an advertisement on the Royal Holloway University intranet or through a university based recruitment website (SONA systems; http://surrey-uk.sona-systems.com/). As discussed earlier, the MOOX was also used as a potential recruitment strategy, however no suitable/viable respondents were recruited through this avenue for the experimental phase. To limit attrition and familiarise participants with the scanning environment prior to data collection participants were given the opportunity to attend a replica scanner based at the University of Surrey campus. All suitable participants met strict screening criteria including reporting no history of claustrophobia or neurological disorders and all had normal or
corrected-to-normal vision. Use of the contraceptive pill was not used as exclusion criteria however, further inclusion criteria for all female participants had to be met whereby they were only scanned during the first fourteen days of their menstrual cycle (whether on the pill or not) to account for possible hormonal variability compromising male and female comparisons (see Section 1.5.3.4). Participants who passed screening criteria were scheduled in to complete pre-scan paperwork and final screening checks prior to scanning at the CUBIC MRI unit (http://www.pc.rhul.ac.uk/sites/CUBIC/).

Participants provided informed written consent to take part and all were fully debriefed after participation. It was made clear that they could withdraw at any time without any repercussions and all data collected was coded so that it is anonymous and confidential. Volunteers were not reimbursed for participation so as a token of thanks, participants were given a printout image of their brain to keep. The study received favourable ethical approval from the University of Surrey Ethics Committee.

On follow up interview one participant revealed use of illicit substances which had not been revealed earlier and was removed from all data analysis (see Figure 3.6). Another two participants were removed from all data analysis due to incomplete paperwork meaning participants could not be categorised as high or low anxiety for analysis. A further seven participants were excluded from the backward masking analysis (see section 3.3.5.3) due to movement artefacts across the fifty minute scan and prior knowledge of the paradigm. However, data for these participants was not compromised for the resting scan stage and were included in these analyses.
3.3.5.3 Participants

Sixty participants volunteered to take part in the experimental phase of the study (aged 19-45 years, $\bar{x} = 24.72 \pm 5.44$; 28 male, 32 female; excluding those who volunteered through the MOOX who are described previously). See Figure 3.6 for a breakdown of participants who took part in the experimental phase.

![Image of a flowchart showing the distribution of participants in the experimental phase of data collection. Grey boxes indicate stages at which participants were lost/removed from further analysis.]
3.3.5.3.1 Resting State Participants
Fifty-seven participants were suitable for analysis from the resting scan (aged 19-45 years, $\bar{x} = 24.72, \pm 5.54$; 25 male, 32 female). When categorised using the HADS scores (as described in section 3.3.3.2) 18 participants were considered high anxiety (aged 19-30, $\bar{x} = 24.78 \pm 3.78$; 6 male, 12 female) and 39 were categorised as the low anxiety group (aged 19-45, $\bar{x} = 24.79 \pm 6.23$; 19 male, 20 female).

3.3.5.3.2 Backwards Masking Participants
Fifty datasets from participants who completed this stage of the experimental phase were suitable for further analysis (aged 19-45 years, $\bar{x} = 24.66 \pm 5.38$; 21 male, 29 female). Using the HADS_A scores 16 participants were in the high anxious group (five male, eleven female; mean age 24.63 ±3.98) and thirty-four participants in the low anxious group (sixteen male, eighteen female; mean age 24.68 ±5.98).

Further breakdown of numbers of participants is shown in Figure 3.7 and then discussed in detail in the relevant chapters.

![Figure 3.7 Schematic showing the breakdown of participants from experimental data collection. The schematic indicates which chapters within this thesis are associated with various sub-groups of participants.](image)

3.3.5.4 Task Display and procedure overview
The experimental phase was designed using Presentation 15.0 software (available for download from [http://www.neurobs.com/index_html](http://www.neurobs.com/index_html)). High resolution colour images were projected onto an adjustable mirror attached to the head coil so that participants could see the
experiment during scanning (see Figure 3.8). All stimuli were centred in the participant’s field of view in order to give participants something to focus on and to minimise the possibility of motion artefacts in both stages of data collection. Prior to entering the scanner room participants completed a final safety checklist to ensure there were no contraindications to their being suitable to scan, they also completed pre-scan paperwork (3.1.3). They were prepared for fMRI in accordance with the rules of use and the study was conducted by two on site ‘authorised’ personnel

Figure 3.8 Schematic of exemplar MRI machine with viewing mirror for experiment presentation. Participants were acclimatised to the scanning environment prior to starting the study. Once briefed and ready to take part, they lay in the scanner and were made comfortable by the researcher. They were given a ‘panic’ button to hold in case they needed the scanning session to stop. The experimenter could talk to the participant over an intercom in the control room and before the session commenced participants were reminded of instructions (to move as little as possible, during Rest conditions to ‘think of nothing in particular,’ and during the presentation of face stimuli they were required to simply observe the faces). Participants could see all the stimuli via a mirror in the head coil.

3 Authorised personnel must undergo the following training and testing prior to attaining this status. 1) Training and operation of the scanner. 2) Training in First Aid (to the level of appointed person). 3) Basic Fire training (internal programme determined by Royal Holloway Safety Officer) 4) Training in removing an unconscious patient from the controlled area. 5) Viewing of current Siemens’s safety video. 6) Attendance at safety lecture given by a suitable qualified person approved by the Management Committee. 7) Reading of the Guidelines for Magnetic Resonance Equipment in Clinical Use. Published by the Medical Devices Agency. 8) Studying all relevant risk assessment forms. 9) Thoroughly reading the local Rules of Operation and successfully completing a written test to be administered by the MRI Safety Officer.
3.3.5.4.1 Resting state protocol

The resting state fMRI scan represented the first step of the experimental phase of data collection.

Before entering the scanner participants were briefed to lay as still as possible in the scanner with their eyes open thinking of ‘nothing in particular’ during the resting scan. Once in the scanner, following set up procedures, participants were reminded of these instructions over the intercom. The resting scan consisted of a cue that the resting period was about to start (the word ‘REST’ written in capital letters in white size 28 in Arial bold font on a black background). This cue was followed by a fixation cross (white cross size 40 Arial font in the centre of a black screen) which was onscreen for a total of six minutes. Once the resting scan had finished, there was a pause before continuing with the next stage of the experimental phase.

3.3.5.4.2 Backwards Masking Protocol

*Task Display*

The face stimuli used in the task blocks are part of the NimStim set of facial expressions (Tottenham et al., 2009). This dataset has been previously rigorously tested for reliability and validity (ibid). Furthermore, when selecting the fear, happy and neutral face stimuli used in this experiment, only those stimuli gaining 70% inter-rater reliability scores or above in the previous study were chosen. In total 240 pictures were selected (40 different subjects, two images for each emotion per subject – two happy, two fearful and two neutral; see Figure 3.9). The face stimuli show an individual from the top of the shoulders upwards, they are in colour and the background has been edited so that it is grey to reduce strain on participant’s eyes where images are flashing up on screen.

Figure 3.9. Examples of the NimStim images selected for use in the study showing from left to right, two fearful faces, two happy faces and two neutral faces.

The resting blocks again consisted of a cue for rest (the word ‘REST’ as in the resting state paradigm) followed by a fixation cross. The task blocks consisted of another cue; ‘FACES’
(written in capital letters in white size 28 Arial bold font on a black background) followed by a block of 16 pairs of an emotional face presented for 33ms (long enough for emotional processing to occur in the brain but short enough for participants to not be consciously aware of this stimulus). The emotional face is rapidly followed by a neutral face image of the same individual which lasts for 1967ms – this is long enough for the volunteer to be consciously aware of it thus acting as a backwards mask (see Figure 3.10). The switch between the face stimulus and the neutral face results in a slight ‘flicker’ of which the volunteer is made explicitly aware being told before they started the study that it was part of the experimental design. This is to deter participants from dwelling on what the flicker may mean. In the neutral face blocks, the two different neutral images were used to mask each other in order to ensure the flicker effect was present for all face stimuli conditions.

Figure 3.10 show an example of the backwards masked stimulus, here a happy face masked with neutral. The image indicates the timings of the presentations (33ms for the masked happy image and 1967ms for the neutral mask, totalling 2 seconds). In the face blocks sixteen of these pairs are presented, each block depicts one type of face stimulus (happy, neutral or fear).

**Procedure**

The backwards masking scan uses a block design. In this experimental phase there were two types of block condition – either resting blocks or task blocks (16 pairs of backwards masked emotional faces). These conditions were presented in 50 second interleaved blocks (in keeping with optimal block design parameters). Participants were reminded to fixate on the crosshair and ‘think of nothing in particular’ during the resting blocks. In the task blocks there were three face image conditions – either fearful masked with neutral, happy masked

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4 This is supported by research which has shown that presentation of 33ms, or less, when participants are subjectively unaware of emotional stimuli, result in unconscious processing (Pessoa, Japee and Ungerleider, 2005).
with neutral or neutral masked with neutral. During task conditions participants were instructed to simply observe the faces focusing on the centre of the screen as much as possible, between each pair of faces there was a cross hair to help participants maintain focus and reduce head movement during scanning (see Figure 3.11). Each participant completed 30 resting blocks and 30 task blocks (10 fearful, 10 happy and 10 neutral). Presentation of the face pairs within the task blocks were presented in a randomly generated order and the order in which the three types of task blocks were presented was counterbalanced. The study was split into three runs (later referred to as sessions in following study chapters) with cued onscreen breaks one third and two thirds of the way through.

![Figure 3.11](image-url)

Figure 3.11. Stimuli sequence schematic showing presentation of stimuli and timings using the backwards masking scan. As shown blocks are cued with either the word ‘FACE’ or ‘REST’. In the Face block the cue is then followed with presentation of masked faces (16 pairs of either fear, happy or neutral masked faces, ten blocks of each). In Rest blocks a crosshair is presented in the centre of the screen for the duration of the block. Each block lasted 50 seconds.

### 3.3.5.5 MRI Acquisition

MRI images for the experimental phase were acquired at the CUBIC MRI unit (http://www.pc.rhul.ac.uk/sites/CUBIC/) on a 3T scanner (Trio, Siemens, Erlangen, Germany) with a 32 channel array head coil.
3.3.5.5.1 Structural scan
High resolution 3D brain MRI images were acquired using a T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) pulse sequence (TR 1830ms, TE 3.03ms, Inversion Time 1100ms, 11° flip angle, FOV 256mm, 160 slices, voxel size 1 x 1 x 1mm voxel size, in-plane matrix 256 x 256).

3.3.5.5.2 resting state scan
For the resting scan a Blood Oxygen Level Dependent (BOLD)-sensitive EPI sequence (TR 1750 ms, TE 30ms, 85° flip angle) was used to collect thirty axial slices (FOV 192 x 192mm, 64 x 64 matrix, 4mm thickness, no gap, 3 x 3 x 4 mm voxel size, IPAT parallel acquisition).

3.3.5.5.3 Functional scan
The backwards masking scan used a BOLD-sensitive EPI sequence (TR 1750 ms, TE 30ms, 85° flip angle). Thirty axial slices (FOV 192 x 192mm, 64 x 64 matrix, 4 mm thickness, no gap, 3 x 3 x 4 mm voxel size, IPAT parallel acquisition) were collected.

3.3.5.6 Imaging preprocessing
Imaging pre-processing was carried out using FEAT (fMRI Expert Analysis Tool) version 5.0, part of FSL (FMRIB, Oxford, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/ (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012)). Pre-processing consisted of motion correction using MCFLIRT (Jenkinson, Bannister, Brady, & Smith, 2002); removal of non-brain structures using BET (S. M. Smith, 2002); reduction of random noise using spatial smoothing of a Guassian kernel of FWHM 5mm; mean-based intensity normalisation; high-pass temporal filtering (Gaussian-weighted least squares straight line filtering, with sigma=50.0s). Spatial registration was performed with FLIRT (FMRIB’s Linear Image Registration Tool; Jenkinson et al., 2002; Jenkinson & Smith, 2001), which uses 12 degrees of freedom affine transformation to transform the functional data into standard MNI space, via their individual T1-weighted structural images.
For first-level time series analysis, FSL uses a version of the General Linear Model (GLM) called FILM (FMRIBs Improved Linear Model), which calculates voxel-wise pre-whitening matrices to improve estimation efficiency (Woolrich, Ripley, Brady, & Smith, 2001).

The resting state MRI data were pre-processed and analysed using Matlab R2013a and SPM8 (software package available at http://www.fil.ion.ucl.ac.uk/spm/). Functional runs were realigned to the first volume to correct for motion artefacts and the mean image was then co-registered to the T1 weighted structural image to ensure that it accurately reflects the anatomical details of each individual's brain in terms of areas of activation detected during the study. They were then normalised to Montreal Neurological Institute (MNI) standard space. To reduce random noise effects this data were then spatially smoothed using a Gaussian kernel of 5mm full width half maximum. See chapter 9 for further details on analysis.
Chapter 4: Study 1
Mood Online Experience Survey (MOOX)

4.1 Chapter overview
The present chapter investigates whether willingness to participate in research, in particular research using functional neuroimaging or performance measurement, is modulated by individual differences in anxiety or gender. Previous research suggests that there may be a self-selection bias in other types of studies, and this chapter particularly focuses on self-selection biases in anxiety and gender in order to elucidate whether the main study cohort in this thesis is a wholly representative sample. An online survey, collecting key demographics, and anxiety measures as well as indication whether participants would be willing to take part in 5 different study types, was available for completion for one month between April 2014 and May 2014. There was an interaction between anxiety and gender such that highly anxious male participants were less likely to want to take part in a neuroimaging study where performance was being measured compared to low anxiety male participants. In addition, native speaking (English as first language) highly anxious individuals were also less likely to take part in these kinds of studies compared to low anxiety native speakers. This research confirms previous research that highly anxious individuals are less likely to take part in perceived 'high stress' research. Furthermore, only the combination of neuroimaging and performance measure (not each in isolation) was aversive for these anxious individuals. In more detail, it is native speaking anxious individuals, in particular males, that are less willing to take part, and this gender imbalance in particular needs to be taken into consideration when looking at the findings in the main study cohort. An understanding of the characteristics of those less likely to volunteer in research will hopefully allow researchers to tailor or improve recruitment strategies, take precautions and measures when collecting, analysing and interpreting group data, and to consider external and internal validity within their samples.

4.2 Introduction
Investigation of willingness to participate informs many different topic areas looking at both what factors affect willingness to participate, and how to increase participation within certain groups. For example, research has looked into participation in organisational change (V. D. Miller, Johnson, & Grau, 1994), farmer participation in agri-environmental measures
participation in mindfulness-based stress reduction (Stainken, Garland, & Mao, 2014) and how racial differences influence participation in medical research (Shavers, Lynch, & Burmeister, 2002).

Of particular interest for this thesis is the examination of characteristics and predictors of willingness to participate. If a certain group or characteristic makes participants less likely to participate, then this leads to a bias in the sample, and less generalisable findings. A key piece of work in this regard is by Rosnow & Rosenthal (1976) which explored those characteristics and predictors that distinguished between volunteers and non-volunteers. Volunteers tended to be more sociable, better educated, more altruistic and more intelligent than non-volunteers. In addition, and more relevant to this thesis, volunteers in studies that involved stress (physical or emotional) tended to be more likely to be male and more likely to have sensation seeking personalities compared to non-volunteers. The gender bias is particularly interesting, as women are more likely to be volunteers across all study types. Parallel work by Zuckerman (1976) proposed a model whereby sensation seeking traits were predictive of aversion towards situations that induce high arousal (e.g. research on altered states of arousal) in individuals who were more sensitive to stress or had recently experienced some kind of trauma or severe stress. Only those individuals who scored lower anxiety were likely to volunteer in these tasks. Further research by Zuckerman (1994) determined that high sensation seekers have less anxiety in risky situations. These findings, in combination with those of Rosnow and Rosenthal, suggest that studies specifically looking to investigate stress and anxiety may be likely to suffer from higher levels of non-participation and a disproportionate gender split. As such, studies in this area are likely to be susceptible to self-selection bias, which as discussed before may influence the generalisability of the findings.

Despite these early studies, very few studies look towards characteristics of non-volunteers, since this data is often unavailable. In addition, although there have been many studies investigating self-selection bias or sensation-seeking behaviours, most have focused on very specific scenarios and participant groups (e.g. characteristics of volunteers in terms of sensation-seeking, gender and substance use (Baker & Yardley, 2002); or the impact of religion and smoking and driving habits (Zuckerman & Neeb, 1980)), with fewer looking at a more generalisable pool of participants. One study that takes a slightly more general approach is that of Pieters, Jennekens-Schinkel, Schoemaker, & Cohen (1992), which looks at research volunteers and self-selection overall in clinical pharmacological research. Of course, this is still a specific domain of research but does take a step towards investigating more general
voluntariness and willingness to participate in research. The researchers found that individuals who volunteered for a study (which did not advertise compensation or the study topic in advance) were seen to have increased levels of extroversion, risk-taking tendencies and sensation-seeking tendencies compared to norms. In addition, they presented with lower than norm levels of state and trait anxiety. These findings in a more general population are very similar to those found by Rosnow & Rosenthal and Zuckerman, and add further evidence that studies focussing on anxiety and stress may be susceptible to skewed recruitment.

As evidenced above, there are emotional barriers that prevent participation in research, regardless of type of study, and these fears and worries need to be addressed in general, but particularly when studying individuals with anxiety. The importance of addressing willingness to participate in individuals with anxiety is demonstrated by a study looking at anxiety (Stein, Simmons, Feinstein, & Paulus (2007)) which screened 3,000 undergraduates to find those who exhibited the target levels of anxiety. Only one in three of these anxious individuals were willing to participate in a follow up MRI study, and only one in two of those could actually be contacted for further study. This resulted in a sample size of thirty-two, out of 3000 undergraduates, just 1% of those screened. The importance of gathering data from healthy populations is paramount in order to convert findings from such psychological and scientific research into clinical practice. Collecting information from healthy sub-clinical populations such as these, and reducing self-selection bias can help in recruitment of large representative samples. Indeed, Rosnow and Rosenthal (1976) made some suggestions aimed at reducing self-selection bias, such as making recruitment as non-threatening as possible to reduce anxiety; explicitly stating the theoretical importance of the research, using snowballing recruitment techniques such as word-of-mouth or two-phase recruitment (low-stress recruitment, then asked to participate in high-stress research) and avoiding research designs that would induce stress. However, even with these suggestions enforced, it is unlikely that self-selection bias will be entirely overcome. Therefore, it is necessary to document and understand the differences in characteristics of those individuals who are willing to take part in research studies and those who are not willing. This will help interpretation and application of results obtained in such studies, with deeper knowledge of the generalisability of the findings in both normative and clinical populations.

A recent study by Oswald, Wand, Zhu, & Selby (2013) looked to compare characteristics of those willing and unwilling to take part in a research study that could be considered 'high-
stress', namely research conducted using positron emission tomography (PET). Furthermore, in keeping with Rosnow and Rosenthal's suggestions, they used a two-phase recruitment process, first collecting questionnaire and behavioural ('low-stress') data from a large group of participants, then later offered them the opportunity to take part in a PET study ('high-stress'). Using this technique, they could compare demographic and personality traits of those willing and unwilling to volunteer in PET studies (in comparison to those willing to take part in questionnaire and behavioural data). Similar findings to previous studies were found, in that males were more likely to volunteer and there were group differences in sensation-seeking tendencies.

Functional magnetic resonance imaging (fMRI) studies could also be considered stress inducing in the same way as PET studies. Indeed, Davidson, Thomas, & Casey (2003) note that it is normal to experience anxiety when entering an fMRI scanner and that this discomfort can escalate when performance is being measured in some way. Furthermore, to this author's knowledge, no research has been conducted into the impact of anxiety on the willingness to participate in different study designs such as those with a performance measure, compared to those involving fMRI (neuroimaging). As a key characteristic of anxiety is risk-avoidance (Lerner & Keltner, 2001; Raghunathan & Pham, 1999), with non-clinical anxiety individuals perceiving situations to be more risky than controls, it seems likely that highly anxious individuals will avoid situations involving performance measures. As such, they may be less willing to participate in studies with performance measures, which are not as typically viewed as stressful compared to MRI. The current study therefore gathers information not only on willingness to participate in fMRI research, but more specifically looks at willingness to participate when performance is measured, both in a neuroimaging (task-based fMRI) and typical setting (task-based computer study), as well as when there is no direct performance measure (neuroimaging; resting-state fMRI; typical: observational or interview studies). In line with Rosnow and Rosenthal's (1976) recommendations for improving generalisability, two-phase recruitment was used, with participants initially recruited to complete an online survey (which would be considered low impact/low ‘stress’), then invited to participate in neuroimaging studies (fMRI, 'high-stress'). This two-phase recruitment strategy allows the analysis of the characteristic of those willing and unwilling to participate in different design types in a similar way to the study on PET imaging by Onslow and colleagues (2013). However, this design also enables investigation of those who suggest they would be willing to take part in the fMRI study, and those who actually volunteered.
4.3 Aims

This chapter aims to look at whether sub-clinical anxiety or gender modulates willingness to volunteer in studies which could be considered 'high stress'; those involving performance measures, as well as those using neuroimaging. In addition, demographics and characteristics of those unwilling to participate are investigated in order to inform interpretation of the findings in this study sample in the light of any possible self-selection bias.

It is predicted that high anxiety participants will be less willing to participate in research designs where performance is measured, as well research designs involving the typically 'high-stress' fMRI environment. Furthermore, based on previous research detailing that women are less likely (or males more likely) to volunteer for research which may be emotionally stressful, and bearing in mind the high prevalence of anxiety in female populations (Solomon & Herman, 2009), it is predicted that gender will interact with willingness and anxiety in study designs that are considered more stressful.

4.4 Method

4.4.1 Study design and recruitment

Participants were recruited using internal e-mail at the University of Surrey, word of mouth and the social media platform Facebook, and were initially asked to fill out a cross-sectional survey. Participant recruitment e-mails were distributed to University of Surrey staff and student e-mail lists and information placed on Facebook on 11th April 2014, and was available for completion until 12th May 2014 (see appendices for copies of the recruitment materials). The online survey was programmed using Qualtrics Research Suite. This online survey tool allows researchers to build, distribute and perform basic analysis on surveys (see Appendices for the full survey).

The purpose of the survey was two-fold; to collect information on the impact of individual differences and how they modulate willingness to participate in research, and to potentially recruit anxious participants for an fMRI study. The survey contained items on participant demographics, anxiety measures (Hospital Anxiety and Depression Scale [HADS] and the short version of the State Trait Anxiety Inventory [STAI-6]), and questions on willingness to participate. Participants who showed an interest in participating in neuroscience research were shown an additional set of slides containing information on the fMRI and resting state...
studies described elsewhere in this thesis (see Chapters 5-9), and given the opportunity to provide their e-mail address to be contacted further, should they wish to find out more information.

Participants received no financial or other incentives to participate, were assured of anonymity (except in those cases where they wanted to be contacted further) and were informed they could withdraw at any time. Before filling in the questionnaire participants were also provided with a brief summary of the study aims and how long the questionnaire should take to complete. Their informed consent was obtained by ticking a box if they agreed to continue and participate in the study. The study received favourable ethical opinion from the University of Surrey ethics committee in April 2014.

4.4.2 Participants
In total, 395 participants visited the survey and consented to take part in the survey, of those 348 (aged 20-45; \( \bar{x} = 29.56 \pm 6.42 \)) completed the survey resulting in a completion rate of 88.10%. Of the forty-seven who consented to take part but did not complete the survey twenty-four provided consent and then withdrew; the remaining twenty-three (aged 21-45, \( \bar{x} = 28.52 \pm 6.80 \), 12 male, 11 female) filled in basic demographics before stopping.

Of those that completed the online survey (n=348), 135 participants were male (39%, aged 21-45; \( \bar{x} = 29.42 \pm 6.57 \)), 213 female (61%, aged 20-45; \( \bar{x} = 29.65 \pm 6.33 \)). All subsequent analysis was carried out on the participants that completed the survey.

4.4.3 Measures
The anxiety measures used in this survey have been described in detail elsewhere, please refer to section 3.3.4 for further information. In brief, after completing questions on demographic information, participants completed the short version of the state-trait anxiety inventory (STAI-6) which is a six-point measure of anxiety that has been shown to be sensitive to fluctuations in state anxiety as well as reliable and accurate measure of trait anxiety (Marteau & Bekker, 1992). Participants also completed the Hospital Anxiety and Depression Scale (HADS), which is another self-report measure of anxiety consisting of 14 questions, seven of which pertain to anxiety and seven to depression. A total HADS score can
be observed as well as the depression and anxiety sub-scales (HADS_D and HADS_A), both of which have been shown to be reliable and valid in their own right.

The final part of the online survey related to a set of questions where participants were asked whether they would be willing to take part in different types of research. There were five polar ‘yes/no’ questions as follows:

1. A study where you have to complete a task where your performance is being assessed?
2. A study where your brain function is being measured whilst your performance on a task is assessed?
3. A study where you brain function is being measured whilst you are not performing an assessed task (at rest)?
4. A study where your behaviour is observed but your performance is not being measured?
5. An interview study?

The first two questions relate to task-active scenarios with performance measures, the second two questions should be relative controls offering task-negative scenarios with no measure of performance, and the final question offers a control design within the context of the other design types.

4.4.4 Analytic Strategy
All descriptive and inferential statistical analysis of the extracted survey data were conducted using IBM SPSS (version 21.0). Due to the nominal nature of the data of interest, after participants had been categorised as high or low anxiety (see results for description), a series of cross-tabulations (chi-squared tests) were used to analyse the data to determine the relationships between anxiety/gender and willingness to participate. For the overall group analysis, four variables were created to be entered into cross-tabulations:

1. Performance: grouping all studies that involved performance based measures (question 1 and 2 above in "measures")
2. Non-Performance: grouped studies that did not involve measures of performance on tasks (task-neutral: question 3, 4 and 5 above in "measures")
3. Neuroimaging: group studies involving neuroscience techniques (measures of brain activity, question 2 and 3 above in "measures")

4. Non-Neuroimaging: grouped studies that do not use brain based measures (question 1 and 5 above in "measures").

These variables were then entered into two cross-tabulations looking at willingness to participate in studies involving performance measures and willingness to participate in brain based studies. It is not possible to run a loglinear analysis on the data to follow up the overall patterns of willingness by anxiety or gender as the sample size is too small to provide robust results. However, a variable was created that coded the number of studies participants would be willing to take part in (from no studies to all five). Cross-tabulations controlling for anxiety group were calculated for willingness to take part in each of the five different types of research design by gender group, age group and whether English was first language (effectively running 3-way cross tabulations). Finally, participants who indicated that they would be willing to participate in any type of neuroimaging study were shown a unique screen during the online survey offering them the chance to take part in the actual MRI studies described elsewhere in this thesis. Cross tabulations were also run to see whether anxiety/ gender modulated this actual willingness to participate in the MRI studies.

4.5 Results

4.5.1 Descriptive Statistics

Using the HADS_A scores, participants were categorised into the high (a score of 11+) or low anxious (0-10) groups. This resulted in a high anxiety group (87 participants (29 male, 33%) aged 21-45, \( \bar{x} =29.13\pm6.14 \)) and a low anxiety group (261 participants (106 male, 41%) aged 20 to 45, \( \bar{x}=29.71\pm6.51 \); see Table 4.1 for demographic breakdown). Both groups had a similar gender balance (\( \chi^2 (1) = 1.46, p =.23, \phi_c =0.07 \)), handedness (\( \chi^2 (2) = 1.04, p =.60, \phi_c =0.06 \)) and age (\( U=10926.50, p=.60, r=-0.03 \)). Furthermore, there were no differences in distribution of age between high and low anxiety groups after age was re-coded into three groups (21-25, 26-30 and 31+; \( \chi^2 (2) = 1.24, p =.54, \phi_c =0.06 \)).

As previously mentioned of the 348 participants who completed the survey 135 participants were male (39%, aged 21-45; \( \bar{x}=29.42\pm6.57 \)), and 213 female (61%, aged 20-45; \( \bar{x}=29.65\pm6.33 \)). There were no differences in handedness (\( \chi^2 (2) = 0.57, p =.75, \phi_c =0.04 \)) or
age (as a continuous variable; U=13816, p=.54, r=-0.03, or between the three re-coded groups \( \chi^2 (2) = 3.84, p =.15, \varphi_c =0.11 \) between men and women.

Table 4.1 Percentage breakdown of demographic information across all participants, and within each demographic (by anxiety group or gender).

<table>
<thead>
<tr>
<th>Demographic information</th>
<th>Overall in the total sample</th>
<th>Proportion in each group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anxiety group</td>
<td>Gender</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Proportion (%)</td>
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<td>N=87</td>
</tr>
<tr>
<td>Anxiety</td>
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<td>25</td>
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</tr>
<tr>
<td>31+</td>
<td>33.6</td>
<td>78.6</td>
</tr>
<tr>
<td>If current student</td>
<td>Yes</td>
<td>76.7</td>
</tr>
<tr>
<td>No</td>
<td>23.3</td>
<td>76.5</td>
</tr>
<tr>
<td>If English first language</td>
<td>Yes</td>
<td>66.7</td>
</tr>
<tr>
<td>No</td>
<td>33.3</td>
<td>72.4</td>
</tr>
</tbody>
</table>

4.5.2 Overall participation likelihood for performance measure and neuroimaging tasks

Overall, 82% of participants (286/348) were willing to take part in research where performance is measured, whereas 18% (62/348) were not (see Table 4.2). Of those not willing to take part if performance was measured, 52% (32/62) were willing to take part in tasks where performance was not measured, whereas 48% (30/32; 8% of entire sample) would not take part in these task neutral studies (i.e. they were unwilling to take part in any
research). Overall, 85% of participants (296/348) were willing to take part in neuroimaging studies, whereas 15% (52/348) were not (see Table 4.2). Of those not willing to take part in neuroimaging studies, 42% (22/52) were willing to take part in non-neuroimaging studies, whereas 58% (20/52, 8% of entire sample) were unwilling (i.e. unwilling to take part in any research).

Table 4.2. Cross tabulation results (count) for overall group results. The Table shows (a) willingness to participate in either studies where performance is measured compared to studies where performance is not measured, and (b) willingness to take part in studies using neuroimaging techniques compared to studies not using neuroimaging techniques. Each subscript letter denotes a subset of Performance Measured tasks categories whose column proportions do not differ significantly from each other at the .05 level.

<table>
<thead>
<tr>
<th></th>
<th>Studies where performance is measured</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not willing</td>
<td>Willing</td>
</tr>
<tr>
<td>A  Studies where performance is not measured</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Not willing</td>
<td>30a</td>
<td>5b</td>
</tr>
<tr>
<td>Yes willing</td>
<td>32a</td>
<td>281b</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>286</td>
</tr>
<tr>
<td>Non-neuroimaging Studies</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Not willing</td>
<td>30a</td>
<td>22b</td>
</tr>
<tr>
<td>Yes willing</td>
<td>4a</td>
<td>292b</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>314</td>
</tr>
</tbody>
</table>

Eighty-one per cent of the entire sample were willing to participate in both types of research, with only 9% willing to only take part in task neutral studies. Data revealed that there was a significant relationship between willingness to participate in task neutral studies and studies measuring performance ($\chi^2 (1) = 122.52, p <0.001, \varphi_c =0.59$), with participants being 52.7 times more likely to take part in a performance based study if they were also willing to take part in a task-neutral study (see Figure 4.1).
Nine per cent of the entire sample would not take part in any research compared to 6% of the entire sample who would only be willing to take part solely in non-neuroimaging studies. Chi squared analysis revealed a significant relationship between willingness to participate in a study involving non-neuroimaging techniques and neuroimaging techniques ($\chi^2 (1) = 159.26$, $p <0.001$, $\phi_c =0.68$), with participants being 99.5 times more likely to take part in a neuroimaging study if they were also willing to take part in a non-neuroimaging study (see Figure 4.2).
4.5.3 Willingness to participate in specific study designs

4.5.3.1 Does anxiety modulate willingness to participate?

In general, high and low anxiety participants were equally likely to participate in no studies at all, one, two, three, four or five study types, and the relationship between anxiety group and number of studies participants are willing to participate in was not significant ($\chi^2 (5) = 6.85, p =.23, \phi_c =0.14$).

Cross tabulation analysis was also run for each of the five study types separately, to look at whether anxiety influenced willingness to take part in any of the five study types. This analysis revealed no significant differences (Table 4.3).

Table 4.3 Percentage of participants who would not participate in each study by anxiety group. ‘N.S.’ denotes non-significant ($p>0.05$) relationships between high and low anxiety participants.

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Low Anxiety (%)</th>
<th>High Anxiety (%)</th>
<th>$\phi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Task-based study: performance measured</td>
<td>23.0</td>
<td>23.0</td>
<td>N.S.</td>
</tr>
<tr>
<td>Brain-based study: performance measured</td>
<td>19.2</td>
<td>26.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>Brain based study: no performance measure</td>
<td>19.2</td>
<td>23.0</td>
<td>N.S.</td>
</tr>
<tr>
<td>Observational study: no performance measure</td>
<td>24.5</td>
<td>25.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>Interview</td>
<td>21.5</td>
<td>21.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>Actual Willingness (follow-up response)</td>
<td>59.7 (N=253)</td>
<td>63.7 (N=80)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

4.5.3.2 Does gender modulate willingness to participate?

There was no significant relationship between gender and number of studies a participant would be willing to participate in ($\chi^2 (5) = 5.59, p =.35, \phi_c =0.13$). Furthermore, there were
no significant differences between gender groups (Table 4.4) in willingness to participate in each of the five types of research study.

Table 4.4 Percentage of participates who would not participate in each study by gender group. ‘N.S.’ denotes non-significant ($p>0.05$) relationships between male and female participants.

<table>
<thead>
<tr>
<th></th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>IT²</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=135</td>
<td></td>
<td>N=213</td>
<td></td>
</tr>
<tr>
<td>Task-based study: performance measured</td>
<td>22.2</td>
<td>23.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>Brain-based study: performance measured</td>
<td>20.7</td>
<td>21.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>Brain based study: no performance measure</td>
<td>21.5</td>
<td>19.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>Observational study: no performance measure</td>
<td>29.6</td>
<td>21.6</td>
<td>N.S.</td>
</tr>
<tr>
<td>Interview</td>
<td>24.4</td>
<td>19.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>Actual Willingness (follow-up response)</td>
<td>55.1</td>
<td>64.1</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

**4.5.3.3 Is there an interaction between Anxiety and Gender with willingness to participate?**

When looking at willingness to participate in each of the four groups split by gender and anxiety (male high anxiety, male low anxiety, female high anxiety, female low anxiety), there was a significant relationship, in males only, between anxiety and willingness to participate in brain based studies where their performance would be measured (high anxiety males: 17% not willing, low anxiety males: 35% not willing; $\chi^2 (1) = 4.24$, $p = .04$, $\phi_c = .18$; see Figure 4.3). No other significant differences on task participation were observed, and no differences were seen in female participants at all (see Table 4.5, Figure 4.3).
4.5.3.4 Is there an interaction with other factors and willingness to participate?

The other demographic factors of interest taken in the survey were age, if participants were current students and if English was their first language. Interactions between these factors and those of anxiety and gender on willingness to participate were explored. There was a significant relationship, in native English speakers only, between anxiety and willingness to participate in brain based studies where their performance would be measured (high anxiety native speakers: 29% not willing, low anxiety native speakers: 16% not willing; $\chi^2 (1) = 4.33$, $p = 0.04$, $\phi_c = 0.14$; see Table 4.5 and Figure 4.4). There was no interaction between gender, native language and willingness to participate, nor between anxiety, gender and native language and willingness to participate ($\chi^2 (1) = 62$, $p = .43$, $\phi_c = 0.04$). There was no other interaction between age and anxiety or gender on willingness to participate in any study design (see Table 4.5 and Figure 4.4).
Figure 4.4 Graph to show the interaction between English as a first language and anxiety in participants’ willingness to participate in a brain based study where performance is measured. There is a significant difference between high and low anxiety native speakers (blue line), showing highly anxious native speakers are less willing to participate in brain based studies where performance is measured compared to low anxiety native speakers. There are no significant differences between high and low anxiety individuals who do not have English as a first language (orange line).
Table 4.5 Inferential results of the three-way cross tabulations of gender/age/English as a first language*anxiety group*different study design types. Cells in grey represent situations where there are significant differences between high and low anxiety participants. ‘N.S.’ denotes non-significant ($p>0.05$) relationships between high and low anxiety participants.

<table>
<thead>
<tr>
<th></th>
<th>Task based study where performance measured</th>
<th>Brain based study where performance measured</th>
<th>Brain based study no performance measure (rest)</th>
<th>Observational study</th>
<th>Interview</th>
<th>Actual Willingness (follow up response)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Typical N=261</td>
<td>% Atypical N=87</td>
<td>% Typical N=261</td>
<td>% Atypical N=87</td>
<td>% Typical N=261</td>
<td>% Atypical N=87</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22.6 20.7</td>
<td>17.0</td>
<td>34.5 (p=0.039)</td>
<td>18.9</td>
<td>31.0</td>
<td>29.2</td>
</tr>
<tr>
<td>Female</td>
<td>23.2 24.1</td>
<td>20.6</td>
<td>22.4</td>
<td>19.4</td>
<td>19.0</td>
<td>21.3</td>
</tr>
<tr>
<td>Age Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-25</td>
<td>21.0 13.3</td>
<td>16.0</td>
<td>20.0</td>
<td>16.0</td>
<td>20.0</td>
<td>16.0</td>
</tr>
<tr>
<td>26-30</td>
<td>14.8 21.9</td>
<td>15.9</td>
<td>21.9</td>
<td>18.2</td>
<td>18.8</td>
<td>23.9</td>
</tr>
<tr>
<td>31+</td>
<td>32.6 36.0</td>
<td>25.0</td>
<td>40.0</td>
<td>22.8</td>
<td>32.0</td>
<td>32.6</td>
</tr>
<tr>
<td>If English first Lang</td>
<td>Yes</td>
<td>19.8 21.8</td>
<td>16.4</td>
<td>29.1 (p=0.037)</td>
<td>15.3</td>
<td>23.6</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>29.8</td>
<td>25.0</td>
<td>21.9</td>
<td>27.4</td>
<td>21.9</td>
</tr>
</tbody>
</table>
4.5.3.5 Follow up willingness to participate

Three hundred and thirty-three of the 348 participants were offered the chance to participate in a follow up fMRI study, of which 131 (39%) consented to being contacted further about participation in this study (aged 21 to 43, $\bar{x}=28.80\pm5.78$; 57 males [44%]). There were no significant interactions between actual willingness to participate in the follow-up fMRI study and anxiety ($\chi^2(1) = 87.00$, $p <0.001$) or gender ($\chi^2(1) = 17.48$, $p <0.001$). Only twenty-nine of the participants who consented to being further contacted were highly anxious (aged 21 to 42, $\bar{x}=26.90\pm5.30$; 9 males [31%]), and only eleven of these responded when contacted, resulting in a 37.9% follow up response rate within the anxious sample (8% within the total sample). However, none of the remaining eleven were able to take part as they either did not meet the scanning criteria or were not available during the study duration. Therefore, full follow-up was not possible in any of the participants recruited via this method.

4.6 Discussion

This chapter found that sub-clinical anxiety does modulate willingness to volunteer in studies which could be considered 'high-stress', but only in very specific sub-groups. In particular, this modulation was only seen in interactions with gender or first language (English as first language). As predicted, high anxiety participants were less likely to participate in research designs where performance was measured and in 'high-stress' fMRI environments. The predicted interaction with gender was present in these results, but it was highly anxious male (not female) participants who were less likely to participate in 'high-stress' research studies compared to low anxiety males. Finally, the effects observed were only for studies involving both performance measurement and neuroimaging, suggesting that it is only in combination that these study design features become aversive for highly anxious individuals. Therefore, studies using each in isolation may have less of a self-selection bias in anxious individuals. Overall, the results of this analysis support previous research, in that willingness to participate in research was modulated by both anxiety and gender. However, this chapter suggest that these relationships are not as straight-forward as predicted, with interactions between anxiety, gender and study type, as well as language. These factors will be considered in turn.
Anxiety did not modulate willingness to participate in number of studies or any study type when investigated in isolation. This pattern was also observed when looking at gender differences alone. Despite the absence of findings in each factor in isolation, the interaction between anxiety and gender significantly modulated willingness to participate in studies involving both performance measures and neuroimaging. However, results indicate it is highly anxious men who are least willing to participate in such research as opposed to highly anxious women as would be predicted by the literature. One possible explanation for this discrepancy could be that females and males approach the question of theoretical participation in a study differently to actual volunteering. Arch (1991) conducted a study to investigate decision-making differences between male and female participants in willingness to participate in three different theoretical research scenarios. The participants were told that the researcher needed to choose from these different scenarios to put one forward for a grant proposal, and needed the participants help with the decision. Arch (1991) found that task efficacy was an important factor in the decision to take part in a study for both genders. Furthermore, an important factor for female participants to make the decision to participate in stressful task situations was whether they believed that they would be able to cope with or control their anxiety and complete the task. It may be that the highly anxious female participants in our current sample felt confident in their ability to cope with their anxiety in the types of research being put forward, thus minimising the potential differences between high and low anxiety female participants. In contrast, as the ability to cope emotionally is not as important to male participants and it may be that task efficacy had a greater role, with highly anxious male participants perceiving the research design combining task based performance measures and brain imaging techniques to be low in efficacy. Since the current study finding showed reduced willingness to participate in highly anxious male participants is somewhat contradictory to previous literature, it is important that this finding is replicated further. In addition, it seems key that measures of participants perceived affective and task based efficacy are measured in order to determine whether these factors indeed contribute to differences observed in voluntariness in potential research participants.

It should be noted that the survey itself could be deemed to represent participation in research. Although a measure of those unwilling to take part in the survey cannot be taken by its very nature, the proportion of those who did take part warrants some discussion. Greater numbers of female participants took part in the survey (61% of all participants), supporting previous research findings (e.g. Rosnow and Rosenthal (1976)), and fewer high anxiety
participants (25% of the sample). As stated previously, unwillingness to participate cannot be directly gleaned from the survey, but proportions of participants can be compared to national statistics of men and women in academia in 2014/15 (as the majority who took part were in academia). Based on these national statistics, 55% of this population were female, compared to 61% in our sample, indicating that indeed women were more willing to participate in this 'low-stress' survey research. The ramification is that self-selection bias could result in gender skewed participant pools across different types of research. However, once recruited into the survey, there were no direct gender differences in willingness to participate in other studies (only the anxiety by gender interaction). It is unclear why there is a disparity between actual participation in the survey and indication of willingness for participation in other studies, but this may warrant further investigation. There is literature looking at web-based compared to paper-based surveys that indicate the use of online surveys in research somewhat redistributes the gender balance amongst respondents compared to paper survey administration (Sax, Gilmartin, Lee, & Hagedorn, 2008; Underwood, Kim, & Matier, 2000). Within the survey they are no questions relating to survey type designs or use of online responses. It is likely that future replications looking at willingness to participate in research should incorporate questions relating to these research design types.

Another strand of investigation in this chapter was whether study type influences willingness to participate, both at group level, and when modulated by anxiety and gender. The study types were split into those involving performance measures (or not), and those involving taking neuroimaging data (or not). The idea was to look at what anxious individuals would find a 'high-stress' environment and avoid by not volunteering to take part. MRI environments have been reported to cause anxiety in normative populations (Davidson et al., 2003), but performance measures could also be perceived as 'high-stress' by anxious individuals. The group level data seems to suggest that participants find these study designs equally stressful or aversive. Around half (52%) of those unwilling to take part in a study involving performance measures agreed to take part in one where performance was not measured. Similarly, around half (42%) of those unwilling to take part in research where brain activity was measured were willing to take part in research not measuring brain activity. This supports the notion that situations perceived to be more stressful (i.e. tasks involving performance measures or a brain scanning environment) may result in skewed participant demographics. It could be that use of these designs may result in artificially sampling only participants who are higher in sensation-seeking qualities and low in anxiety characteristics,
as suggested by previous literature (e.g. Oswald et al., 2013; Rosnow & Rosenthal, 1976; Zuckerman, 1976, 1994). This chapter finds further support of this notion, as highly anxious individuals (in combination with either male gender, or native English speaking) are less likely to take part in a study involving *both* performance measurement and brain imaging. None of the other four experimental designs found any modulation of willingness to participate by anxiety or gender. Importantly for this thesis, studies which involve brain imaging but no performance measure (passive viewing fMRI, resting state fMRI, structural MRI) had no significant modulation in willingness to participate by anxiety or gender. It is perhaps not surprising that the study design involving both performance measurement and brain imaging was the only one to show differences, as it could be considered the most potentially stressful design. It appears, according to these results, that performance measurement and brain imaging in isolation are not aversive to sub-clinical anxiety participants. Only in their combination, and in the interaction of anxiety with either gender or language, are differences in participation observed.

Finally, as discussed above, there was an interaction between anxiety and language such that highly anxious native speakers were less willing to participate in a study measuring performance using brain measures, compared to low anxiety native speakers. This effect is not driven by an underlying gender difference, as there are no differences in the gender split within the high and low anxiety native speakers (or non-native speakers). Logically, one may expect that non-native speakers would be more likely to show this difference, as they have the added 'language anxiety' (anxiety associated with learning or use of another language; Horwitz (1986)) which may affect willingness to participate in tasks where performance is measured. However, a recent study by Keysar, Hayakawa, & An (2012) demonstrated that when individuals have to think in a foreign tongue, individuals must rely on more systematic processes. That is to say, when thinking in a non-native language, individuals utilise a system of thinking that distances them from their innate intuitive system, resulting in decisions that are less influenced by emotional processes. Therefore, non-native speakers may be less prone to emotional bias when deciding on participation in research. In contrast, highly anxious native speakers may be more influenced by these emotional processes, and therefore more biased for reduced willingness to participate in what could be perceived as a more risky type of research. Alternatively, native English speakers could be less variable in their response to both the demographics and questionnaire data (in particular anxiety measures) as well as the decision making processes underlying willingness to participate in research.
4.6.1 Implications and limitations

This current study supports previous work that there may be self-selection biases in recruitment, particularly for anxious individuals, and particularly in study designs that are perceived as 'high-stress'. There is a potential for such bias to influence study results in terms of internal validity and individual differences in sampling of study cohorts. This could be particularly problematic when a cohort is being used as a control or baseline (normative) group. Furthermore, as highlighted by Ganguli et al. (2015), this could have a huge impact on external validity, whereby studies should not be applied to a wider population due to the potential specificity of data gathered from such cohorts.

However, investigation of potential sampling biases, as achieved in the present research, allows researchers to understand potential pitfalls and can enable the implementation of controls or checks to ensure that the sample is truly representative. From these findings, and previous research, it would appear that self-selection biases may play an even bigger role in recruitment for studies specifically looking at highly anxious populations, or research in environments which would be considered stressful (e.g. fMRI). Thus, in such research it is important to ensure that the sample meet criteria for participation, with representative samples of high and low anxiety men and women.

In order to reduce bias in recruitment of studies for brain imaging where performance is being measured, it may be necessary to manage participant expectations, with stress-reduction techniques a foremost priority in order to ensure maximal participation. Furthermore, it is also clear that it is vitally important to measure individual differences within sample populations to bolster external validity. Ganguli and colleagues (2015) specifically looked at the potential problem of recruitment biases in an MRI pilot study. The authors demonstrate that through clear characterisation of the participant population and non-participants, results can be corrected for such potential bias allowing a post-hoc method by which we can be sure results are generalizable. This clearly demonstrates the utility of understanding non-participation individual characteristics, which ultimately can be used to contribute to more valid and robust data.

On a research-specific level, current findings suggest that within the field of neuroscience, using research designs using resting state fMRI (Rs-fMRI) may be more viable for ameliorating stress and recruiting highly anxious individuals. This may provide a particularly
useful tool, especially in research into anxiety and stress, as this chapter indicates that highly anxious men/native speakers are equally willing to take part in a passive neuroimaging study despite being less willing to participate in task-based neuroimaging studies. Moreover, a full Rs-fMRI dataset can be collected in as little time as five minutes, and places minimal cognitive burden on the participant, further minimising potential stress. As such, Rs-fMRI may be an ideal, cost-effective tool for gathering data from individuals with anxiety related disorders.

In the context of this thesis, participants were recruited for a study using brain imaging, but no performance measure (passive viewing task, Rs-fMRI and structural MRI). According to this chapter, there is no bias in recruitment for such studies, and as discussed above this protocol (in particular Rs-fMRI) may be an ideal tool for use in anxious cohorts. However, there is an indication that highly anxious males may be under-represented in a study looking at brain imaging and performance measures, and they are perhaps under-represented in the fMRI sample discussed in Chapters 5,6 and 7 (N=50; 11 highly anxious females; 5 highly anxious males; 18 low anxiety females, 16 low anxiety males). Measures of individual differences were recorded in the sample cohort, and in combination with the data gained in this chapter, it is possible to interpret and discuss the findings of the main body of research with confidence.

A number of limitations have been identified during the discussion of the present study. In particular, it is clear that there is very limited research on the characteristics contributing to participants’ willingness to take part in different research designs. Consequently, findings are mixed and replication is necessary to ensure the findings in this chapter are robust. Furthermore, though the present study looks at a relatively large sample of participants, it is still lacking power for some analyses to be conducted. Primarily, it is not possible to look at overall willingness to participate in performance based/ non-performance based research by gender and anxiety, nor the three way interaction. Though the results of looking at each specific measure in turn is valid and has provided useful insight into modulating factors in willingness to participate in specific research designs, it still remains important to investigate this overarching relationship. Future replication studies should therefore collect greater sample sizes to ensure power in such statistical analysis. Furthermore, it is clear from the discussion that questions of participants’ perceived task-efficacy and affect-efficacy are an important measure that was not presently considered and could further help to explain present results.
4.7 Conclusion

In summary, self-selection bias is a risk within research using human participants, and this risk is heightened in studies that could be considered high stress, such as those measuring performance or neuroimaging studies. In particular, individual differences in anxiety, modulated by gender and language play a role in self-selection bias within studies considered high stress. The findings should not be considered to indicate that all research which could be considered 'high-stress', in particular those involving an anxious cohort, are inherently biased. Rather, in such research scenarios precautions must be taken to ameliorate potential bias, such as measurement of individual differences of participants, participant quotas (e.g. proportional numbers of high/low anxiety participants, or men/women) and collection of non-participation data where possible. It is important for researchers to be aware of these modulating factors in order to ensure that the data collected, and the subsequent interpretations of results, are internally and externally valid. Understanding modulating factors involved in willingness to participate are of great value to the field of human emotion research and will help further inform models of human behaviour that can be accounted for when interpreting and applying findings to clinical and non-normative populations.
Chapter 5: Study 2
General Linear Model

5.1 Chapter Overview
The aim of this chapter is to address the controversy surrounding how four specific factors impact amygdala activation during emotion processing. These factors are valence specialisation, lateralisation of function, habituation rates and the modulating effects of gender. In order to do so, a study was conducted which investigated all of these factors together using one cohesive data set. The study focused on how these four factors interact in a sub-clinical group of participants, with the aim of understanding how they could contribute to the underpinning mechanisms in individuals with mood disorders, specifically anxiety disorders. Thus, the study investigated the four key factors, and their interaction with subclinical anxiety in a large dataset with the aim of clarifying the discrepancies observed in previous literature. This is of particular interest in the current climate of accelerating the pace of mental health research to gain better understanding and develop focussed and effective treatments (a key priority of the mental health division of the National Institute of Health, 2015).

5.2 Introduction
Neuroimaging studies show the amygdala plays a key role in emotion processing, commonly being associated with the fear response in particular (e.g. M Davis, 1992; Sarter & Markowitsch, 1985). The fear response and experience of anxiety are considered to be adaptive processes which enable immediate, and appropriate, physiological and behavioural responses to threatening cues. Modern models of emotion processing and regulation are largely grounded in theories of stress and coping. Such stress responses are critical for an organism’s ability to adapt and survive in threatening situations; however as discussed in detail in section 1.6, an inappropriate stress response can lead to maladaptive behaviour such as anxiety disorders and can have harmful psychophysiological effects on the individual (Campos, Fogaca, Aguiar and Guimaraes, 2013; Charmandari, Tsigos, & Chrousos, 2004; Lopez, Akil and Watson, 1999).
A large proportion of affective neuroscience literature investigates the modulating characteristics and impact of individual differences in amygdala reactivity during emotion processing, especially during such stress or fear-related responses. There are four key modulating characteristics of amygdala activation during emotion processing upon which the literature has focussed; valence specification, lateralisation of function, habituation over time and gender (for review see Chochol, & Armony, 2008; Wager, Phan, Liberzon, & Taylor, 2003; Zald, 2003, see section 1.5.3 for detailed discussion of all of these factors). Two theoretical accounts for the function and characteristics of typical amygdala responsivity have been put forward which account for three of these factors; the salience detector theory (focussing on valence specificity of the amygdala) and the dual processing theory (focusing on habituation and lateralisation of the amygdala; Davis & Whalen, 2001; Dolan & Vuilleumier, 2003; LeDoux, 1998b; Sander et al., 2003; Vuilleumier, Armony, Driver, & Dolan, 2003). These accounts emphasise the processes by which an incoming emotion stimulus is received by the amygdala and the cascade of responses that ensue after stimulus detection. As such, these theories currently offer the most complete accounts of the amygdala’s involvement in emotion processing.

The salience detector theory advocates the role of the amygdala in rapidly orienting attention towards threat-related stimuli, particularly in the case of fear, as well as being involved in the emotion processing network within the brain (Davis & Whalen, 2001; Sander, Grafman, & Zalla, 2003). Evidence in favour of the salience detector theory tends to arise from research demonstrating amygdala involvement in processing different emotional valences other than fear, which was previously thought to have been the only emotion processed in the amygdala (e.g. see Adams et al., 2012; Santos, Mier, Kirsch, & Meyer-Lindenberg, 2011; Scharpf, Wendt, Lotze, & Hamm, 2010; Winston, O'Doherty, Kilner, Perrett, & Dolan, 2007; Yang, 2008, see also section 1.5.3 for detail).

The dual processing theory is based on evidence suggesting a fast subcortical thalamoamygdala route for incoming emotional stimuli as well as a slower thalamocortical-amygdala route (e.g. see Ohman, 2005; Romanski & LeDoux, 1992; Shi & Davis, 2001, see section 1.5.2 for detail). In support, there is evidence that the left and right amygdala exhibit differential habituation patterns in response to repeated presentation of threat-related stimuli. Thus there appears to be interplay between hemispheric lateralisation and rate of habituation. Wright and colleagues (2001) presented participants with happy and fearful face stimuli in an fMRI study and observed more sustained left amygdala response, and fast habituation of the
right amygdala in response to the stimulus. This is suggestive of a consistent role in processing threats for the left amygdala, which has been attributed to the slower thalamocortical-amygdala route (Gläscher & Adolphs, 2003; M L Phillips et al., 2001; Plichta et al., 2014).

Despite support for both the dual route model and salience detector model there is still conflicting evidence. In the case of Wright and colleagues 2001 study, only eight participants were used; the use of small samples is a key criticism levelled against such studies into lateralisation and habituation, as highlighted in a review by Baas, Aleman, & Kahn (2004). A more recent review by Sergerie, Chochol, & Armony (2008) observed that evidence for lateralisation and habituation only came from block design studies, a notion further supported by a study looking at lateralisation of emotional prosody by Kotz, Meyer, & Paulmann (2006). These authors found that the use of block design fMRI resulted in right lateralisation valence effects, whereas event-related design resulted in bilateral emotion effects. The conflict in the literature illustrates that further research needs to be conducted to address limitations with design and sample size.

Though theoretical models do not specifically account for gender, there has been an enduring interest in gender differences within affective neuroscience literature; this is likely to have arisen due to the greater prevalence of diagnosed mood related disorders in women compared to men (Hourani et al., 2015; Luxton et al., 2010; Solomon & Herman, 2009). Findings are particularly mixed with some researchers backing the notion that there are sex differences in emotion processing and amygdala activation (e.g. Kring and Gordon, 1998; Hall and Matsumoto, 2004; Hofer et al., 2006; Domes et al, 2010), whilst others assert that there are no sex specific differences (Wager, Phan, Liberzon, and Taylor (2003) for review). As evidenced by the research presented so far, it is perhaps more logical to consider gender interacting with other factors that may impact on amygdala activation. Recent research by Andreano, Dickerson, and Barrett (2013) suggested that the variability of sex-difference interactions and amygdala activation reported in the literature is valence-dependent, endorsing the concept that these individual characteristics may interact.

Similarly to gender, we propose that one of the likely contributors to the variability in the evidence base for amygdala activation in emotion processing is the interaction of individual differences in anxiety. Individuals in clinical cohorts with high trait anxiety exhibit reduced amygdala habituation in response to threat-related stimuli (Etkin & Wager, 2007;
Holzschneider & Mulert, 2011; Richards et al., 2002). This dysregulation of amygdala reactivity is proposed to be a key marker in clinical samples. However, there is a small but growing body of research that has started to look towards levels of anxiety below clinical diagnosis. This body of research has shown similar atypical amygdala activation patterns in sub-clinical highly anxious individuals as well (Barrett & Armony, 2009; Bishop, Duncan, & Lawrence, 2004; Hare et al., 2008; Sehlmeyer et al., 2011). Evidence of differences in amygdala reactivity and emotion processing coming to light from sub-clinical populations bolsters the translational worth of studying these populations as indicators of more serious anxiety disorders (e.g. Stein, Simmons, Feinstein, Paulus, 2007; Etkin, et al. 2004; Bishop, Duncan, Lawrence, 2004; see Taylor and Whalen, 2015 for review).

As apparent from the discussion above, and in section 1.5.3, there is as yet little consensus in the literature on the characteristics of the amygdala in emotion processing. Considering the possibility that some variability in the evidence may arise from these characteristics interacting with the specific design and paradigm used, it is surprising that there is only a very limited body of work that has attempted to tease apart these key characteristics of amygdala reactivity and examine their interactions within the same dataset. Often researchers specifically identify one, or two, factors to study, thus they are potentially unable to show the complexity of the underpinning mechanisms and interactions in emotion processing. This is justifiable as an attempt to reduce the possible confounds related to these additional factors. However, this can be detrimental to the validity of the findings. A logical next step towards understanding the characteristics of the amygdala is to pull together the strands of understanding from meta-analyses and individual studies into one cohesive study.

5.3 Aims
This chapter aims to address the abundance of research relating to discrete factors involved in amygdala reactivity during in emotion processing. Here, one dataset is used to investigate not only the individual impact these factors have on amygdala activation, but also to systematically tease apart the ways in which these factors interact with each other. This will overcome the inherent issues of comparison being made between studies using a range of paradigms, techniques, samples and analysis software. Furthermore, the interplay between these characteristics and measures of state anxiety in this sub-clinical population will be investigated in order to see whether there is a modulating effect of anxiety.
Specific study questions:

- Does the amygdala activate only for fearful faces or for happy and neutral face stimuli as well?
- Is amygdala activation lateralised?
- Does amygdala activation change over time?
  - Is amygdala activation lateralisation modulated by time?
- Is amygdala activation modulated by gender?
- Is amygdala activation modulated by state anxiety?

5.4 Method

The methods involved in data collection for this study are detailed in Chapter 3, the following section contains only a brief description. Please refer back to section 3.3.5 for detail.

5.4.1 Design

A within-subjects design was employed in this study, using block design functional magnetic resonance imaging (fMRI).

5.4.2 Participants

In brief, sixty volunteers (aged 19-45 years, \( \bar{x} =24.72, \ SD= 5.44; \) 28 male, 32 female) took part in an fMRI study based at CUBIC (http://www.pc.rhul.ac.uk/sites/CUBIC/). Ten participants were excluded from data analysis for the following reasons; failure of screening criterion on the day of study (N=1), incomplete paperwork (N=2), and excessive head movement and prior knowledge of the paradigm (N=7), leaving 50 datasets for full analysis with an age range of 19-45 years, \( \bar{x}=24.66 \pm 5.38; \) 21 male, 29 female. Emotion neuro-circuitry has been shown to be modulated by hormone changes across the cycle (see section 1.5.3.4, and Van Wingen, Ossewaarde, Bäckström, Hermans, and Fernández (2011) for detailed review). As such, female participants were only scanned during the first fourteen days of their menstrual cycle to account for possible hormonal variability.

All participants completed the Hospital anxiety and depression questionnaire (HADS; Zigmond & Snaith, 1983) prior to scanning. Based on the anxiety subscale of the HADS
(HADS-A) participants were categorised into the high (a score of 11+) or low anxious (0-10) individuals, resulting in 16 sixteen participants in the high anxious group (mean age 24.63 ±3.98; 5 male, 11 female) and 34 participants in the low anxious group (mean age 24.68 ±5.98; 16 male, 18 female). The study was approved by the University of Surrey Ethics Committee, and written informed consent was obtained prior to participation. All participants met strict screening criteria, including absence of claustrophobia or neurological disorders, and normal or corrected-to-normal vision.

5.4.3 Procedure
Whilst in the scanner participants were presented with a backwards masking paradigm using different emotional valence (fear, happy or neutral) face stimuli from the NimStim set of facial expressions (Tottenham et al., 2009 available at www.macbrain.org). Cued task and rest periods were presented in interleaved blocks with a total of sixty 50 second blocks (comprising of ten blocks per emotion condition and thirty rest blocks). Participants were clearly instructed to passively observe the stimuli during the task blocks and ‘think of nothing in particular’ during the rest blocks.

5.4.4 fMRI Acquisition and pre-processing
MRI images were acquired on a 3T scanner (Trio, Siemens, Erlangen, Germany) with a 32 channel array head coil using a Blood Oxygen Level Dependent (BOLD)-sensitive EPI sequence (TR 1750 ms, TE 20ms, 90° flip angle). Imaging data pre-processing was conducted in FSL v5.0 (available for download at http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). For details on pre-processing parameters please see Chapter 3, section 3.3.5.6.

Multiple regression analyses were performed on each participant’s data using FSL’s FEAT. A regression model (GLM) was created which included seven regressors. These were: fear face blocks (F), happy face blocks (H), neutral face blocks (N), three rest blocks (one following each emotional block) and instruction screens. Key contrasts were the main effects of fear faces, happy faces and neutral faces against baseline (all rest periods; see Table 5.1). Parameter estimates (β) were extracted for the four ROI masks (see ROI analysis below) for each individual participant using FSL’s FEATQUERY.
Table 5.1 Showing different Contrasts that were considered in the second level regression.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fear faces &gt; Baseline</td>
<td>F-Base</td>
</tr>
<tr>
<td>Happy faces &gt; Baseline</td>
<td>H-Base</td>
</tr>
<tr>
<td>Neutral faces &gt; Baseline</td>
<td>N-Base</td>
</tr>
</tbody>
</table>

This analysis produced individual subject-level maps showing statistically different areas of difference in whole brain activations. Second level analysis was conducted using FSL’s FLAME (mixed effects modelling). The z-statistic images were generated through cluster based corrections for multiple comparisons using Gaussian random field theory (Z > 2.3; cluster significance: p < 0.05, corrected, minimum cluster size 10).

5.4.5. ROI Extraction

WFU PickAtlas (available online at http://fmri.wfubmc.edu/cms/software) was used to create undilated masks of the left and right amygdala, and left and right fusiform gyrus. The amygdala ROIs were selected to allow further analysis of different characteristics affecting amygdala activation (valence, habituation, lateralisation, gender, anxiety). The fusiform ROIs were included as a control to ensure results obtained were specific to the amygdala, and not affected by lower level visual processing. ROI masks were generated using FSLmaths by multiplying the standard undilated WFU PickAtlas masks by second level main effect of task versus baseline activation.

Statistical analysis of the extracted beta values was conducted using IBM SPSS (version 21.0). Two four-way mixed ANOVAs with between groups factors of either anxiety group (high, low) or gender (male, female) were run for both bilateral amygdala and bilateral fusiform. Within subject factors were session (three runs based on cued breaks during the scan to represent habituation), valence (fearful, happy, neutral) and hemisphere (left, right). Follow up one-way ANOVAs and simple effects analysis post-hoc tests (Bonferroni corrected) were run to investigate the interactions and main effects of the different characteristics on amygdala activation during emotion processing.
5.5 Results

5.5.1 Demographic Data Analysis

By gender group

There were no significant outliers for age for either gender (converted to Z-scores, cut-off 3 standard deviations from the mean; see Table 5.2 for details) and gender groups did not differ in age (U=279.00, p=.61, r=-0.07). In addition, there were no differences in handedness between the two groups ($\chi^2 (2) = 1.83$, $p = .40$, $\varphi = 0.19$). Looking at the association between gender group and anxiety group there were no statistically significant differences in a two by two chi-squared test ($\chi^2 (1) = 1.12$, $p = .29$, $\varphi = 0.15$). There are no statistically significant differences in the distribution of HADS_D scores or HADS_T scores in either gender group (U=260.50, $p=0.38$, r=0.12; U=235.00, $p=0.17$, r= -0.19 respectively).

Table 5.2 Overview of descriptive results for the male and female participants and the age/ hospital anxiety and depression ranges, mean scores and standard deviations.

<table>
<thead>
<tr>
<th>Gender Group</th>
<th>range</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Age</td>
<td>19-32</td>
<td>19-45</td>
</tr>
<tr>
<td>HADS-A</td>
<td>3-15</td>
<td>3-20</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0-11</td>
<td>0-14</td>
</tr>
<tr>
<td>HADS-T</td>
<td>3-24</td>
<td>4-34</td>
</tr>
</tbody>
</table>

By anxiety group

HADS-A scores ranged from 3-10 (mean 6.03±2.22) for the low anxious group and from 11-20 (mean 14.00±2.88) in the high anxious group. As stated above, both groups had a similar gender balance ($\chi^2 (1) = 1.12$, $p = .29$, $\varphi = 0.15$). They also had similar handedness ($\chi^2 (2) = 2.05$, $p = .36$, $\varphi = 0.20$) and age (U=253.00, $p=.69$, r=-0.006). In addition, as expected, there were significant differences between state anxiety in the two groups ($t(48) = -6.00$, $p < .001$, low: $\bar{x} = 5.74±4.70$; high $\bar{x} = 14.88±5.68$). There was also a significant difference in the
depression scale of the HADS (HADS-D) scores between the two groups (U=39.00, p<.001, r=-0.69) with the high anxious group presenting with higher HADS-D scores (mean rank 40.06) compared to the low anxiety group (mean rank 18.65). This is not unexpected since anxiety and depression are known to be highly co-morbid (Rivas-Vazquez, Saffa-Biller, Ruiz, Blais, & Rivas-Vazquez, 2004). There was also a significant difference in the HADS_T scores between the two groups (U=5.50, p<.001, r=-0.79), again since this is an aggregate score of both the anxiety and depression subscales this difference is not unexpected.

5.5.2 Functional imaging data

5.5.2.1 Group level GLM analysis

The main effect of faces (all tasks: fear, happy, neutral) against baseline (all rest blocks) across all 50 participants shows activation in the key emotion processing areas in the brain. These areas include the insula, hippocampus, thalamus, ventromedial and dorsolateral prefrontal cortex, precuneus, the fusiform gyrus, and amygdala (Shin & Liberzon, 2010). These areas were also seen when looking at each emotion in isolation (fear vs baseline, happy vs baseline, neutral vs baseline, see Figure 5.1). The differences in activation in the amygdala during these emotionally valenced blocks were investigated further using ROI analysis.

![Functional imaging data](image)

Figure 5.1 showing A) whole brain activation for main effect of faces across all participants, B) activation of fear against baseline, C) activation of happy against baseline and D) activation of neutral against baseline.
5.5.2.2 Amygdala Region of Interest (ROI) analysis

Group level

Across all 50 participants the three-way ANOVA revealed no significant main effect of valence ($F(2,87) = 1.31, p=.28, \eta^2_p=.03$), hemisphere ($F(1.49) = .78, p=.38, \eta^2_p=.02$), or any significant two way interactions (valence x lateralisation ($F(2,90) = 0.50, p=.59, \eta^2_p=.01$); valence x session ($F(3,151) = 0.48, p=.70, \eta^2_p=.01$); lateralisation x session ($F(2,82) = 0.83, p=.42, \eta^2_p=.02$) or three-way interaction (valence x lateralisation x session ($F(3,171) =.14, p=.95, \eta^2_p=.003$).

There was however a significant main effect of session ($F(2,86) = 6.99, p=.002, \eta^2_p=.13$). Bonferroni post-hoc tests showed that there was a significant decline in activation from session 1 to 2 ($p=.005$), and session 1 to 3 ($p=.002$) but not between session 2 and 3 ($p=1$; see Figure 5.2).

Figure 5.2 (a) Graph showing significant differences in amygdala activation over time sessions showing a pattern of habituation, (b) graph representing group level amygdala activation to difference valences, (c) graph showing left and right amygdala activation during presentation of face stimuli. Bars represent mean±SE *p<0.05, ** p<0.01, ***p<0.001
**Gender interaction**

Comparison of amygdala activation across emotional blocks revealed that women exhibited significantly higher amygdala activation ($\bar{X} = 1.74$, $SD = 0.90$) compared to male participants ($\bar{X} = 1.16$, $SD = 1.18$; $t(48) = -1.96$, $p = 0.028$, $d = 0.49$).

Results from the mixed ANOVA with gender as a between subjects’ factor revealed a similar pattern of main effects as the group level interaction. However, it was the interactions with gender that are of interest. There was a trend towards a significant interaction between gender and valence ($F(2,96) = 2.84$, $p = .063$, $\eta^2_p = .06$). When this was investigated further it was revealed that there was a significant main effect of valence in the female participants ($F(2,56) = .81$, $p = .005$, $\eta^2_p = 0.17$), but not in male participants ($F(2,40) = .11$, $p = .90$, $\eta^2_p = 0.005$; see Figure 5.3). Post-hoc Bonferroni tests on the female participants revealed that there is a significant difference between the fear condition and neutral condition ($p = .01$), a non-significant trend between the fear and happy condition ($p = .06$) and no significant difference between the happy and neutral condition ($p = 1$) in women.

![Graph showing the gender by valence interaction in the amygdala](image)

*Figure 5.3. Graph showing the interaction of gender by valence in amygdala activation. As seen in the green bars, there are no significant differences in amygdala activation to different valences in male participants. In contrast there are significant differences in amygdala activation in women (red bars) between fear and neutral, and a trend towards a significant difference between fear and happy conditions. Bars represent mean±SE *p<0.05, **p<0.01, ***p<0.001.*

There were no other significant interactions (gender x hemisphere ($F(1.48) = 0.03$, $p = .87$, $\eta^2_p = 0.01$); gender x session ($F(2.85) = 2.29$, $p = .11$, $\eta^2_p = 0.05$); gender x session x hemisphere ($F(2.81) = 0.07$, $p = .90$, $\eta^2_p = 0.002$); gender x valence x hemisphere ($F(2.96) = 1.08$, $p = .34$, $\eta^2_p$
= 0.02); gender x session x valence ($F(3,150)=1.12, p=.35, \eta^2_p = 0.02$); gender x session x hemisphere x valence ($F(4,192)=0.48, p=.75, \eta^2_p = 0.01$).

**Anxiety interaction**

Overall amygdala activation was significantly greater in the high anxiety group ($\bar{x}=1.87\pm0.81$) than the low anxiety group ($\bar{x}=1.32\pm1.12$; $t(48) = -1.73, p = 0.05, d = 0.63$).

Again the mixed ANOVA with anxiety as a between groups variable revealed a similar pattern of main effects as the group level interaction. In addition, there was a significant three way interaction of session x hemisphere x anxiety group ($F(2,82) = 3.87, p = .031, \eta^2_p = .08$). Post-hoc tests adjusting for multiple comparisons on the three-way interaction revealed that in the low anxiety group both the right and left amygdala showed a significant reduction in activation from session 1 to 2 (Right: $p=.005$; Left: $p=.023$) and session 1 to 3 (Right: $p=.003$; Left: $p<.001$) but not session 2 and 3 (Right: $p=1$; Left: $p=.43$). In contrast activation levels were stable across sessions for the right and left amygdala in the high anxiety group (session 1 to 2 Right: $p=.38$, Left: $p=.97$; session 1 to 3 Right: $p=.86$, Left: $p=.86$; session 2 to 3 Right: $p=1$, Left: $p=1$), suggesting an absence of habituation within this group (see Figure 5.4).

![Graphs representing amygdala activation change over time showing habituation patterns in the left amygdala (a) and right amygdala (b) amygdala in the high anxiety (orange bars) and low anxiety (purple) groups. Bars represent mean±SE *p<0.05, ** p<0.01, ***p<0.001](image)

No other significant interactions with anxiety were observed (anxiety x hemisphere ($F(1,48)=0.98, p = .33, \eta^2_p = 0.02$); anxiety x session ($F(2,83)=2.18, p=.13, \eta^2_p = 0.04$); anxiety x valence ($F(2,86)=0.25, p=.76, \eta^2_p = 0.005$); anxiety x valence x hemisphere
\[(F(2,96)=0.20, p= .82, \eta^2_p = 0.004)\]; anxiety x session x valence \[(F(3,148)=0.83, p= .48, \eta^2_p = 0.02)\]; anxiety x session x hemisphere x valence \[(F(3,166)=0.60, p= .64, \eta^2_p = 0.01)\]).

### 5.5.2.3 Control Region of Interest: Fusiform Gyrus

#### Group Level

The three-way ANOVA across all 50 participants revealed no significant main effect of valence \[(F(2,98)=.53, p= .59, \eta^2_p = 0.01)\], but significant main effects of session \[(F(2,83)=19.91, p<.001, \eta^2_p = 0.29)\] and hemisphere \[(F(1,49)=9.08, p= .004, \eta^2_p = 0.16)\]. Follow up post-hoc analysis of the main effect of session showed a significant reduction in activation from session 1 to 2 \((p<0.001)\) and session 1 to 3 \((p<.001)\) but not session 2 and 3 \((p=1)\). The main effect of hemisphere was revealed to be caused by greater activation in the right fusiform gyrus \((\bar{x}=2.45\pm 1.32)\) compared to the left \((\bar{x}=2.10\pm 1.17; t(49)=-3.01, p=.004, d=0.89)\) via simple effects analysis controlling for multiple comparisons.

There was also a significant interaction of session by hemisphere \[(F(2,98)=6.82, p= .002, \eta^2_p = 0.12)\]. Following up this interaction revealed significantly greater activation in right fusiform gyrus compared to left in session 1 \(t(49)=-3.57, p=.001, d=0.33; \) Right: \(\bar{x}=0.99\pm 0.48\), Left: \(\bar{x}=0.83\pm 0.44\) and session 3 \(t(49)=-2.68, p=.010, d=0.22; \) Right: \(\bar{x}=0.75\pm 0.49\), Left: \(\bar{x}=0.65\pm 0.43\). However, this difference did not pass bonferroni correction threshold in session 2 \(t(49)=-2.33, p=.024, d=0.19; \) Right: \(\bar{x}=0.71\pm 0.45\), Left: \(\bar{x}=0.62\pm 0.41\), see Figure 5.5.
Fix 5.5. Graph showing mean fusiform activation over sessions showing habituation in the left (solid blue) and right (patterned blue) hemisphere across all participants. Bars represent mean±SE *p<0.05, ** p<0.01, ***p<0.001

No other significant interactions were observed (valence x session \(F(3,164)=0.16, p=.94, \eta_p^2=0.003\); valence x hemisphere \(F(2,98)=1.83, p=.17, \eta_p^2=0.04\); valence x session x hemisphere \(F(4,196)=.35, p=.85, \eta_p^2=0.01\)).

**Gender Interaction**

In the mixed ANOVA with gender as a between subjects factor there was a similar pattern of main effects as the group level analysis. However, there was only a significant interaction of gender with session \(F(2,83)=5.58, p=.008, \eta_p^2=0.10\). Following this up revealed that female participants showed a significant decline from session 1 to 2 \((p=.001)\), and 1 to 3 \((p<.001)\) with no differences between session 2 and 3 \((p=.523)\) as seen at group level. However, male participants only demonstrated a significant decline from session 1 to 2 \((p<.001)\), with no other session effects passing significance bonferroni correction threshold (session 1-3 \(p=.058\); session 2-3 \(p=.102\); see Figure 5.6).
Gender did not interact with any other factors in the fusiform gyrus (gender x valence ($F(2,96)=1.18, p = .31, \eta^2_p = 0.02$); gender x hemisphere ($F(1,48)=1.62, p = .21, \eta^2_p = 0.03$); gender x valence x session ($F(3,160)=.34, p = .82, \eta^2_p = 0.01$); gender x valence x hemisphere ($F(2,96)=0.34, p = .71, \eta^2_p = 0.01$); gender x session x hemisphere ($F(2,96)=0.28, p = .75, \eta^2_p = 0.01$); gender x valence x session x hemisphere ($F(4,192)=0.25, p = .91, \eta^2_p = 0.01$)).

**Anxiety Interaction**

Results from the mixed ANOVA with anxiety group as between subjects factors revealed a similar pattern of main effects to the group level analysis. However, there was no significant interaction of anxiety group with any aspect of fusiform gyrus activation (anxiety x valence ($F(2,96)=.46, p = .64, \eta^2_p = 0.01$); anxiety x hemisphere ($F(1,48)=.06, p = .81, \eta^2_p = 0.001$); anxiety x session ($F(2,78)=2.62, p = .08, \eta^2_p = 0.05$); anxiety x valence x session ($F(3,160)=0.87, p = .47, \eta^2_p = 0.02$); anxiety x valence x hemisphere ($F(2,96)=.21, p = .81, \eta^2_p = 0.004$); anxiety x session x hemisphere ($F(2,96)=1.22, p = .30, \eta^2_p = 0.03$); anxiety x valence x session x hemisphere ($F(4,192)=0.06, p = .99, \eta^2_p = 0.001$)).
5.6 Summary of Results

The passive mood induction used in this study induces amygdala activation, along with activation in other key emotional processing areas (insula, hippocampus, thalamus, ventromedial and dorsolateral prefrontal cortex, the fusiform gyrus, and amygdala). In relation to amygdala activation, there were no group level (n=50) valence or hemispheric (lateralisation) differences, with only a significant effect of session observed. This effect of session demonstrated habituation over time to repeated presentation of backwards masked emotion stimuli. However, interactions were observed when participants were split by gender or anxiety group.

When accounting for gender, women were found to exhibit greater amygdala activation to emotional faces than men. Furthermore, there was an interaction of valence and gender, with a valence effect only apparent in women. Specifically, women showed greater sensitivity to fearful faces over neutral faces, and a trend towards significant differences between the fearful and happy condition.

When considering state anxiety differences in this subclinical population, highly anxious individuals exhibited greater overall amygdala activation compared to low anxiety individuals. There was also an interaction of anxiety group by hemisphere over time (session) such that low anxiety individuals showed typical habituation patterns, whereas high anxiety individuals showed no habituation across the duration of the study.

The fusiform gyrus control region showed habituation to repeated exposure and greater right hemisphere activation, in keeping with the previous literature. Of note, there was no main effect or interactions with valence. However, there was a difference in habituation between the genders, such that females showed typical group level habituation, whereas male activation increased in session 3 such that there was no longer a significant difference with session 1.

It is evident from results observed here that gender and anxiety are very important when looking at characteristics of amygdala activation in emotion processing. Indeed, without consideration of these two factors there is little evidence of the impact of session (habituation), lateralisation or valence.


5.7 Discussion

The present study expands on the existing literature, helping to clarify previous contradictory results by using a large dataset to provide a more holistic representation of the modulators of amygdala activation in emotion processing. A key finding is that individual differences in gender and anxiety levels, even at a sub-clinical level, can have a major impact on results, and inferences drawn from such data. First, the main aims of this study will be addressed, followed by a more general discussion and consideration of implications, limitations and future directions.

Is the amygdala a valence/salience detector?

The group level results provide no evidence of an impact of valence on amygdala activation during emotion processing. This finding seems to contradict the literature which advocates that the amygdala is specialised as a salience detector, as we would expect to see differences between the more salient emotional stimuli (fearful and happy faces) in comparison to the neutral faces. However, there are two possible alternative explanations for the results found in this study which both have some evidence base within current literature.

Firstly, it may be that the neutral condition is not being perceived as neutral by this cohort. Holland and Gallagher (1999) highlighted the importance of emotion in orienting attention and stressed that the direction of attention strongly influences emotion processing. In a review of the amygdala’s involvement in vigilance and emotion David and Whalen (2001; see also Whalen, 1998) explain that in the case of ambiguous (e.g. neutral) stimuli, the amygdala’s role in modulating an organism’s vigilance as a salience detector would allow the brain to gather more information in order to assess whether to approach or avoid the stimuli. Neuroimaging evidence supports this notion with studies showing that the amygdala is particularly sensitive to ambiguous information (e.g. Hsu, Bhatt, Adolphs, Tranel, & Camerer, 2005; Quiroga, Kraskov, Mormann, Fried, & Koch, 2014). It could be said that a neutral facial expression lacks any clear social cues and increases the unpredictability and the possibility of potential threat. The stimuli used in this study were selected from a previously validated standard set of emotional face stimuli (Tottenham et al., 2009). However, post-scan interviews held with participants revealed a certain level of ambiguity relating to the neutral stimuli:

‘They looked like they were from a ‘wanted’ poster. Not very comforting’ (sub10);
‘Some looked gormless, some looked sinister’ (sub02);
‘Some looked judgemental, some looked mean, most of them didn’t look very friendly’ (sub03).

The level of activation in response to the neutral stimuli may be elevated due to this ambiguity. Furthermore, all emotional stimuli were masked with the neutral stimuli, meaning this ambiguity may have also had an impact on the amygdala response to the emotional conditions. This would need to be addressed in future studies using an alternative mask for the images.

An alternative explanation for lack of a valence effect at group level may relate to the stimuli being perceived to be equally salient by participants within this paradigm. Using a visual search paradigm of emotion and non-emotional face stimuli, Santos, Mier, Kirsch and Meyer-Lindenberg (2011) found evidence to suggest that the amygdala acts as a general face salience detector responding to the behavioural relevance of the target. Irrespective of holding emotional or non-emotional value, they found participants’ amygdala showed more rapid activation to target stimuli over non-targets. In the present study participants were instructed to ‘observe all the faces’, thus effectively labelling all face stimuli as targets. If this is the case it may be that the observation of equal amygdala response regardless of valence results from the pop-out effect of the stimuli regardless of the masked emotional content like in Santos and colleagues study (2011). This alternative explanation undermines the suggestion of the amygdala as a salience detector. Clearly further investigation needs to be carried out in order to address the possibility that the neutral stimuli are not reflecting a truly neutral condition before conclusions can be drawn regarding the salience detector theory.

Is there evidence for the Dual Processing Theory of amygdala activation?

Data collected from all fifty participants did not lend support to the notion that the amygdala activation in emotion processing is lateralised, nor was there an interaction of lateralisation and session such that the left and right amygdalae show different habituation patterns over the course of the study. As such, the whole group results do not support the dual processing theory of amygdala activation. This finding supports the suggestion put forward by a previous comprehensive review of 54 articles (Baas, Aleman, and Kahn, 2004) that concluded there was no evidence to support lateralisation. The authors suggested that those studies which supported lateralisation arose from small sample sizes and may have been statistically underpowered. In support of Bass et al. (2004) a number of others studies have reported no lateralisation effects, or at best mixed results in terms of amygdala reactivity in emotion.
processing (e.g. see Garavan, Pendergrass, Ross, Stein, & Risinger, 2001; Tabert et al., 2001; Yang et al., 2002).

There was an overall effect of habituation across both amygdalae from session 1 to 2, but not 2 to 3 indicating that habituation occurs in the first block. This concurs with previous research showing that in response to repeated presentation of an emotion stimulus, activation declines over time (Wright et al., 2001). Such patterns have been assumed to be an adaptive response (allostatic process) to prevent psychophysiological damage resulting from long term over production, or flooding, of circulating stress related hormones in the brain (López et al., 1999). In addition, this habituation lends some support to the salience detector theory in that it is biologically beneficial to orient attention to salient information initially (high activation), but not beneficial to continue to respond in the same way to a repeated stimulus (habituation after session 1).

Is amygdala activation modulated by gender?

Findings on gender interactions have been mixed, with some researchers continuing to back the notion that there are sex differences in emotion processing and amygdala activation (e.g. Kring and Gordon, 1998; Hall and Matsumoto, 2004; Hofer et al., 2006; Domes et al, 2010), whilst others assert that there are no sex specific differences (Wager, Phan, Liberzon, and Taylor (2003) for review). This study found that gender interacted with valence to modulate amygdala activation, which supports recent research suggesting that the variability of sex-difference interactions on amygdala activation reported in the literature is valence-dependent (Andreano, Dickerson and Barrett, 2013). Andreano and colleagues ascertained in their study that women showed sustained bilateral amygdala response compared to men, but only for negative stimuli which were familiar; novel stimuli produced equivalent activation in men and women. In the present study, there was no interaction of gender by session by valence which would be expected from the findings of Andreano and colleagues. There was however a gender difference such that female participants presented with greater bilateral amygdala activation to faces compared to males, and an interaction with valence. Whereas male participants exhibited no effect of valence, females revealed significantly higher BOLD signal in the fear condition compared to the neutral condition and a trend towards greater activation for fearful stimuli above happy stimuli. This suggests that the amygdala may act as a salience detector, but only in females.
Nevertheless, the lack of a valence effect in male participants in this study, with equivalent activation to fearful, happy and neutral stimuli, does not support the literature suggesting the amygdala plays a role as a salience detector. A previous study also found that females had significantly elevated bilateral amygdala activation in response to negative stimuli (over neutral), compared to male participants (Domes et al, 2010). However, despite these sex differences the authors still observed a valence effect in male participants, with elevated amygdala activation to fear stimuli compared to neutral stimuli. The elevated amygdala activation to neutral stimuli in the present sample of male participants could be explained by it representing more ambiguous stimuli, as discussed above for the group level analysis. However, this would suggest that female participants either do not see this ambiguity, or that amygdala activity towards fearful stimuli is much larger than that for ambiguous stimuli for these individuals. Another explanation could be that the salience detector theory is more generalised and does not require emotional content, as suggested by Santos et al., (2011), and discussed above.

A third explanation may clarify this sex difference with regards to the male data. Results show the male amygdala activation is more variable in comparison to the female data; this in turn could be due to variable engagement with the task, or more variable demographics such as anxiety. There is some evidence for variable engagement in the task from the male fusiform gyrus (FFG) activation. The FFG is selectively involved in the perception of faces (Kanwisher, McDermott, & Chun, 1997), and was selected as a control measure for general visual stimulation in the task. Whereas female participants showed typical habituation effects in FFG, male participant’s activation habituated by session 2, but increased again in session 3. As the FFG is involved in orientation towards the face stimuli, this may indicate variable, or altered engagement in the task by male participants. Research published by Britton, Shin, Barrett, Rauch, and Wright in 2008 also found this pattern in both the amygdala and FFG in response to face stimuli and suggest that the reactivation from session 2 to 3 is part of a survival mechanism. There is evidence from animal studies to support the notion of habituation followed by rapid re-activation to repeated stimuli (e.g. Wilson & Rolls, 1993). However, Britton et al., (2008) found this pattern in both male and female participants, whereas it was only observed in the male participants in this study. There is clearly scope to investigate this gender difference further.

Previous research shows a clear association between anxiety and heightened, sustained amygdala activation (e.g. Etkin and Wager, 2007; Holzschneider and Mulert, 2011).
However, there is no difference in proportion of highly anxious individuals in male and female groups ($p=0.29$). Nevertheless, it could be that an interaction between gender and anxiety is causing the difference in amygdala activation between the male and female groups. This interaction may also be the reason why no gender by session by valence effect, as predicted by Andreano, Dickerson, and Barrett (2013) was found in this study. Unfortunately, further analysis cannot be conducted into this interaction since the proportion of male participants classed as highly anxious is too small to yield valid statistical power.

This study advances understanding on the interaction between gender and the characteristics of amygdala activation during emotion processing. It is now clear that without considering the modulatory effect of gender, results relating to differences in amygdala specificity and valence could be overlooked. However, it is evident that further investigation is needed to untangle some of the interactions that this study has revealed.

**Is amygdala activation modulated by state anxiety?**

Non-transient sustained amygdala activation has been strongly associated with clinical anxiety disorders (e.g. see Etkin and Wager, 2007; Holzschneider and Mulert, 2011), and present results indicate that this pattern extends into sub-clinical populations as demonstrated by the three-way anxiety by lateralisation by habituation interaction. This supports a growing body of research looking into the impact of sub-clinical anxiety in emotion processing (e.g. Barrett & Armony, 2009; Bishop, Duncan, & Lawrence, 2004; Hare et al., 2008; Sehlmeyer et al., 2011). In detail, whilst data from participants in the low anxiety group reflect the group level data with a significant decrease over time to masked emotional faces, high anxiety participants show no evidence of habituation over the task in either hemisphere.

Importantly, the results from this study showed no difference in habituation between the hemispheres, either at the whole group level, or when split by anxiety group. Sergerie and colleagues (2008) proposed that in event-related designs the right amygdala would not be able to rapidly habituate, and so the altered habituation between the amygdalae supporting the dual processing theory is only observed in block-design studies (Baas, Aleman and Kahn, 2004). However, the results of this study show no lateralisation of activation either across the whole group, or when split into low and high anxiety participants despite differences in habituation in these groups. Therefore, evidence in favour of lateralisation of function cannot
be accounted for as a result of design insensitivity or inappropriateness, as suggested by Segerie et al. (2008) and Bass et al. (2004).

Along with no lateralisation effect, there was no effect of valence across the whole cohort, or when split by anxiety group. This suggests that valence may not be a key modulatory characteristic for amygdala reactivity, although there was a gender by valence effect, as discussed above. Results from this study suggest that the salience detector theory may most closely fit individuals who have low state anxiety levels. These individual’s amygdala orients, and then habituates, to the stimulus as expected in response to repeated exposure, whereas high state anxiety individuals have sustained amygdala activation suggestive of a heightened state of vigilance. It is possible that applying the salience detector theory across individuals in a sub-clinical range may lead to the unclear results seen in the literature.

**Fusiform Gyrus overview**

Activation in the FFG was predominantly as would be predicted by the literature, with bilateral activation but clearly greater activation in the right FFG. This is in line with literature showing right FFG dominance in face processing (McCarthy, Puce, Gore, & Allison, 1997). In addition, there was a strong habituation pattern in the FFG, as would be expected over time. An interaction between gender and session/habituation in fusiform activation has been discussed above which is perhaps indicative of differential engagement on the task between the genders resulting in variable amygdala activation. The results presented here demonstrate that the use of the FFG as a control region of interest was suitable since differences seen in amygdala activation were either unique, or overlap with the FFG in such a way that helps to explain amygdala activity presently observed.

**Overall**

The findings presented here indicate that there is a clear need to integrate future studies such that they account for the multiple different factors that can modulate observed amygdala activation during emotion processing. It is clear that gender and anxiety have a large amount of influence on the results; exclusion of these variables would have led to the conclusion at group level that the only interacting factor in amygdala activation in emotion processing is
habituation. Failure to account for these key individual differences could undermine the worth of any conclusions being drawn.

5.8. Implications

Though the results are not clear cut, findings presented here attempt to address the complexity of these different interacting factors. The large sample size studied compensates for the low statistical power which is often seen in such neuroscience research (Button et al., 2013) and should not only provide robust findings, but also enables us to work towards consolidating understanding of the interplay between these different factors. Furthermore, the present evidence provides little support for the amygdala as a salience detector theory. Of notable importance, these results show even sub-clinical high anxiety levels results in sustained amygdala hyper-responsivity in response to emotional face stimuli, echoing findings from clinical populations. It is clear that there is a real need to account for all interacting factors in order to find consistent and reliable results within the field.

In order to tease apart the effects of gender and anxiety which appear to be key modulators of amygdala reactivity in emotion processing, it is suggested that a follow-up study using matched participants, with equal distribution of high/low anxiety men and women is conducted. Social anxiety has been shown to exist on a continuum in epidemiological studies (e.g. Stein, Torgrud, & Walker, 2000) and thus ensuring a distribution of sub-clinical anxious participants are accounted for will enable researchers to get a clearer picture of typical amygdala reactivity in response to emotion processing. The use of high anxious participants would effectively represent the transitional level of anxiety prior to development of anxiety disorders and allow researchers to really understand what is underpinning maladaptive clinical anxiety disorders.

Neural biomarkers for propensity towards mood disorders

The observed interaction of anxiety group, habituation and lateralisation not only extends understanding of the impact of sub-clinical anxiety levels on emotion processing, but could also indicate a potential neural biomarker of individuals at risk of developing clinical anxiety disorders. There is evidence to support this suggestion from previous research. For example, clinical models of Post-Traumatic Stress Disorder (PTSD) implicate hyper-responsivity in the amygdala in such individuals, with characteristic exaggerated fear responses and persistent
re-living of traumatic memories in such individuals (Rauch, Shin, & Phelps, 2006; Rauch, Shin, Whalen, & Pitman, 1998; Shin & Liberzon, 2010b). The sustained amygdala response in the current sub-clinical cohort is suggestive that such amygdala reactivity, even in non-clinical populations, is a key characteristic of susceptibility to maladaptive emotion processing.

There have been two recent studies conducted by independent research groups which have found similar results to this study, with a relationship in sub-clinical populations between trait-anxiety scores and amygdala habituation as indicated by fear conditioning paradigms (J. Barrett & Armony, 2009; Sehlmeyer et al., 2011). In both studies the authors conclude that the correlation between increasing trait anxiety scores and reduced amygdala habituation in response to threat-related stimuli are indicative of a vulnerability of such individuals towards developing clinical anxiety disorders. Current findings extend beyond these studies showing that even the state anxiety scores in sub-clinical populations show a significant relationship with reduced amygdala habituation.

This notion could point towards a mechanism by which individuals in sub-clinical populations could develop chronic anxiety. A key characteristic of clinical anxiety disorders is an inappropriate stress response (Campos et al., 2013). The neuroendocrine circuit associated with the stress response; the hypothalamic-pituitary-adrenal (HPA) axis is an allostatic system modulated by amygdala (excitatory) and hippocampal (inhibitory) activity (for more discussion see section 1.6.2.1, Bratt et al., 2001; Laryea, Arnett, & Muglia, 2012; Yoshida, Takayanagi, & Onaka, 2014). In chronic stress, this axis changes as cortisol causes the hippocampus to atrophy and thus inhibition is reduced leading to mis-regulation of the amygdala during a stress response (Lee et al., 2009; Magariños et al., 1996). Though state anxiety is a transient condition, repeated high anxious state in sub-clinical participants characterised by an absence of amygdala habituation to a repeated stimulus could result in a de-regulation of the HPA axis over time. Results here raise the prospect of a mechanism whereby such a repeated state could surpass a threshold by which amygdala reactivity becomes chronic as hippocampal activity diminishes. In support of this notion, there is evidence to show reduced hippocampal volume in neuroimaging studies both in patients with clinical anxiety conditions (e.g. PTSD; Douglas, 1995; Gurvits et al., 1996), and in relation to increasing levels of anxiety in non-clinical participants (e.g. Levita et al., 2014, this is discussed further in Chapter 8).
5.9 Limitations

The current study set out to present a more holistic picture of the ways in which different key factors interact with regards to amygdala activation in emotion processing. Due to the complexity of investigating five interacting factors, interpretation of the results can be difficult. Despite this challenge, this study makes the first steps towards clarifying the role of the amygdala and the variation in results present in the literature. That being said, there are some limitations which effect the interpretation of the results, and could be improved upon in future studies.

Criticism could be levelled against the use of a backwards masking paradigm. Though studies have shown amygdala activation in response to backward-masked stimuli (e.g. Morris, Ohman, & Dolan, 1998; Whalen et al., 1998), it has been argued that a reduction in attentional resources will hinder effective emotion processing within the amygdala (Pessoa, Kastner, & Ungerleider, 2002). Previous research has shown that low state anxious individuals only show significant amygdala response to attended, but not unattended, threat-related stimuli, whereas high state anxious individual’s amygdala orients towards attended and unattended stimuli (S. J. Bishop, Duncan, & Lawrence, 2004). Through the use of the backwards masking paradigm a bias against the low anxious group may have been introduced in this study which could have undermined, or masked, the extent of observable amygdala reactivity and the lateralised differences. The dual processing model suggests that the right amygdala would attenuate faster to emotional stimuli and the left amygdala would show a slow reduction in activation over time, reflecting their roles in immediate threat detection and more top down processing respectively. No difference was found between the two amygdala in this study, although a three-way interaction of session x hemisphere x anxiety group demonstrates that lateralisation differences are in part driving the results. Further investigation is required to understand whether the use of a passive backwards masking paradigm has negated any hemispheric differences which would lend support for the dual processing hypothesis.

In addition, in this backwards masking paradigm the same interval between a stimulus and mask was used for each participant, so there is a possibility that individual differences in perceptual speed could have affected the result whereby some participants consciously processed the visual stimuli. It is of note that participants were not directly tested to see if
they recognised masked images as an indicator of conscious processing of the stimuli. However, they were questioned exhaustively after the scanning procedure with regards to the stimuli they had seen. Any participant who revealed they had seen emotional faces, i.e. a smiling face, were removed from any further analysis. Future analysis may alleviate this possible confound by finding each individual’s ‘perception threshold’ using a staircase procedure prior to testing.

It is important to consider the ways in which contrasts are built, i.e. contrasting against baseline, or a different condition (i.e. neutral condition). The suggested nature of resting state is said to be where salient information from experiences (emotional, cognitive etc.) are processed, understood and stored in a system of adaptive learning (Albert, Robertson, & Miall, 2009; Lewis, Baldassarre, Committieri, Romani, & Corbetta, 2009). In light of the suggested function of resting state, it is possible that there will be some ‘carry over’ effect into the resting baseline of the preceding emotion conditions, which would mean that by contrasting against baseline, the baseline and emotional regressors are correlated to some extent. The possibility of the influence of individual emotion conditions was overcome presently by using an average baseline from all resting conditions. However, it is of note that there may still be some influence of emotional state in general on baseline. Future studies could look to characterising and comparing the baseline and preceding emotion condition to see to what extent these possible ‘carry over’ effects could influence resting activation.

There could be some debate as to whether focal ROI analysis, or whole brain activation compared to averaging across a ROI, would be the most appropriate method(s) to study activation presented in this study. Whole brain analysis allows observation of a complete picture of activity across the brain and is useful as a first look at the data. Conversely, ROI analysis reveals subtle differences from more intricate patterns of activity and interactions within the brain. Though ROI analysis could be considered a simplification of whole brain activation, it is a complementary approach and can be necessary to clarify findings. Due to this ‘simplification’ areas identified for ROI analysis should be driven by previous research and theoretically driven (as is the case in this thesis). Focal point ROI, whilst sampling the area with the greatest BOLD response overall, could overlook participants’ whose peak is not in the average data. Therefore, looking at activity across the cluster is a more representative sample. The use of both whole brain and analysis averaging across ROIs presently provide a clear and informed picture of the patterns of activation within the data.
Unexpectedly, activation for the neutral block of faces was not different from the emotionally valenced faces in the fearful and happy conditions across all participants. This seems to be due to higher than expected activation for neutral faces. Explanations for this elevated activation have been given above with regards to variable levels of trait anxiety of participants or the ambiguity of the stimulus. However, as indicated in Section 5.8, further research is needed to confirm whether gender and anxiety do interact with valence differences seen in amygdala activation. In addition, although the stimuli have been assessed as reliable in previous validity research, it would be conducive for future research using backwards masking of such stimuli to alleviate this possible confound by measuring participants own ratings of each stimulus after the backwards masking procedure in order to ensure that the normative categorisation of stimuli are correlated with the participants’ individual perceptions of the images.

5.10 Conclusions
This study suggests that gender and anxiety play particularly key roles in amygdala activation during emotion processing. When looking at amygdala activation discounting these factors, there was only a typical habituation effect, and no interactions between any of the other factors. However, when including gender as a between subjects factor, there is an interaction with valence that appears to support the salience detector theory in females, as well as an interaction with habituation that suggests that the genders may habituate to repeated stimuli in different ways. In addition, when including anxiety as a between subjects factor, there is an anxiety by lateralisation by habituation interaction that suggests that the salience detector theory of amygdala activity most closely applies to low anxiety participants. In those with high state anxiety, there is evidence of the sustained amygdala hyper-activation seen in clinical anxiety populations, whereas those with low anxiety show a typical habituation over time to repeated exposure. This variance in amygdala activation in a sub-clinical population based on state anxiety scores highlights a possible neuro-marker of individuals with a propensity towards developing anxiety disorders.

Overall, the present study highlights the utility of investigating all of the modulating factors when looking at the role of the amygdala in emotion processing, and supports a particular role for demographic factors such as gender and anxiety. Furthermore, by accounting for such
individual differences this study provides an explanation for some of the current discord within the literature. The importance of accounting for demographic and behavioural individual differences when designing future studies should be a primary concern, especially when such research can not only advance understanding of emotion processing in healthy populations but ultimately inform future therapies and treatments in clinical populations.
Chapter 6: Study 3
PsychoPhysiological Interaction

6.1 Chapter Overview
The aim of this chapter is to explore the psychophysiological interactions between bilateral amygdala and frontal regions in the brain (specifically the prefrontal cortex and anterior cingulate cortex) during emotion processing. As mentioned in Chapter 1 (section 1.5.1) there has been a shift in research from focusing on individual sites in the brain being responsible for discrete emotions, to multiple regions working together in a network, with patterns of activity across regions relating to subjective emotion. By looking at the connectivity across regions it is possible to clarify how disruption in emotional processing may lead to maladaptive behaviours such as anxiety.

In this study, particular attention will be paid to the interplay between this connectivity and individual differences in sub-clinical anxiety and gender. This will illuminate the maladaptive processes underlying anxiety in a sub-clinical population, and also explore why there is a higher prevalence of these disorders in women. In addition, this chapter will look at lateralisation of fronto-amygdala connectivity, which is often overlooked, but is nevertheless important in emotion processing.

6.2 Introduction
Though the amygdala plays a pivotal role in emotion processing, it does not work in isolation. Rather, it is part of a network within the brain responsible for detecting, appraising and responding to emotional stimuli (Lindquist et al., 2012; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008); see section 4.2 for more detail). Through the use of highly replicable paradigms such as fear conditioning procedures, researchers have been able to reliably study the underlying neural networks involved in these behavioural responses (e.g. Janak & Tye, 2015; LeDoux, 2003). Such studies of emotion have allowed identification of specific areas within the brain associated with emotion processing; these include areas such as the hippocampus, hypothalamus, thalamus, insula and prefrontal regions (specifically: anterior cingulate cortex, medial prefrontal cortex and orbitofrontal cortex (Banks, Eddy, Angstadt, Nathan, & Phan, 2007a; Etkin et al., 2011; Etkin & Wager, 2007; Lindquist et al.,
2012; Shin & Liberzon, 2010a; Wager, Davidson, et al., 2008). A detailed discussion on specific brain areas implicated in emotion processing is given in section 1.4.

Particular focus has been paid to fronto-amygdala connectivity in studies of emotion processing. Such is the level of connectivity between the amygdala that it has been implicated in a ‘core brain circuit’ constituting of many parts of the brain including areas of prefrontal cortex, and visual cortex (for more details see Modha and Singh, 2010). This functionally central ‘hub’ has been suggested to be a seat for higher cognitive processes involved in aggregation, and distribution, of information (Modha and Singh, 2010; Pessoa and Adolphs, 2010). In a review of theoretical perspectives in affective neuroscience, Pessoa and Adolphs’ (2010) suggested that due to this level of connectivity, the amygdala’s role is not simply automatic, non-conscious processing of affective stimuli, but rather it may be involved in a more complex role in facilitating salience evaluation of an incoming stimulus through cortical networks. As such this would suggest that even in apparent automatic orienting of the amygdala, an increased level of activation would be expected within cortical areas such as the PFC as part of this core brain circuit.

The validity of studying such fronto-amygdala connections has been supported by research such as that of Quirk, Likhtik, Pelletier and Pare (2003), who used extracellular recordings from rat and cat amygdalae to demonstrate that mPFC pre-stimulation decreased responsiveness of the amygdala. Behavioural studies have also established the importance of fronto-amygdala connectivity; Harmer, Thilo, Rothwell and Goodwin (2001) reported that stimulation of the medial frontal cortex using transcranial magnetic stimulation slowed reaction times in an emotional recognition task in response to angry faces, but not happy faces. Both of these studies indicate that the mPFC has an inhibitory role on amygdala function. In addition, reciprocal connections between the amygdala and frontal areas such as the mPFC and the ACC have been shown in animal model studies, these connections have been suggested to be indicative for a sequence in emotion processing from top down areas to the amygdala (Amaral & Price, 1984; Carmichael & Price, 1995; Ghashghaei, Hilgetag, & Barbas, 2007; Porrino, Crane, & Goldman-Rakic, 1981).

Understanding of connectivity between the amygdala and other brain regions involved in emotion processing has advanced with the advent of neuroimaging techniques, allowing in vivo study of emotion neuro-circuitry in healthy humans. In general, neuroimaging studies support the notion that frontal areas perform a top-down, or inhibitory role, with respect to
the amygdala (Banks, Eddy, Angstadt, Nathan, & Phan, 2007b; Harmer, Thilo, Rothwell, & Goodwin, 2001; Motzkin et al., 2014; Ochsner, Bunge, Gross, & Gabrieli, 2002; Quirk et al., 2003; Urry et al., 2006). In a recent review, Etkin, Egner, and Kalisch (2011) suggested a framework for emotion processing in which amygdala connectivity to the dorsal ACC/mPFC is associated with appraisal and expression of negative emotion, and amygdala connectivity with the ventral ACC/mPFC is attributed to generating emotion responses through the limbic system after such appraisal.

Evidence for the modulation of connectivity with the amygdala and these frontal regions has primarily come from research into individuals with emotion disorders, especially anxiety disorders, characterised by emotional dysregulation (Mary L Phillips, Drevets, Rauch, & Lane, 2003). Indeed, in a previous meta-analysis which focused on anxiety disorders, Etkin and Wager (2007) argued that disrupted fronto-amygdala connectivity is a key regulator in clinical mood disorders (Banks et al., 2007b; Eden et al., 2015; Gold, Morey, & McCarthy, 2015; Motzkin et al., 2014; Shin et al., 2001). There is evidence to support this claim from research reporting dysfunctional frontal activation concurrent with the characteristic amygdala hyper-responsivity to negative stimuli in individuals with clinical anxiety (Holzschneider & Mulert, 2011; Rauch et al., 2006, 1998; Shin & Liberzon, 2010b).

Structural changes have also been observed, with greater thickness of pathways between the amygdala and the mPFC/OFC associated with lower trait anxiety in subclinical groups (Eden et al., 2015). In addition, individuals who typically use more reappraisal during emotion regulation (as measured by the German Regulation Questionnaire (Gross & John, 2003)) show greater connectivity between the amygdala and frontal regions.

The study by Eden and colleagues (2015) illustrates that although most of what is currently understood about the influence of amygdala connectivity on emotion processing comes from studies investigating clinical mood disorders, there are individual differences in the healthy sub-clinical population which can also illuminate this relationship. For example, Cremers et al. (2010) found individual differences in level of neuroticism modulated connectivity between the amygdala and ACC/dmPFC. Furthermore, they observed lateralisation of fronto-amygdala connectivity, whereby there was a positive correlation between right amygdala- dmPFC and a negative correlation between left amygdala- ACC connectivity, modulated by neuroticism. This apparent lateralisation of functional coupling appears to support theories of functional lateralisation of the right and left amygdala, or dual processing.
theory (Davis & Whalen, 2001; Dolan & Vuilleumier, 2003; LeDoux, 1998; Sander, Grafman, & Zalla, 2003; Vuilleumier, Armony, Driver, & Dolan, 2003; see section 1.5.2). Many other studies have also observed a lateralisation of fronto-amygdala connectivity, with right amygdala activity to negative stimuli and weaker fronto-amygdala connectivity reported in high anxiety participants or during unpredictable threat exposure (of those presented here Eden et al., (2015), Gold et al., (2015) and Motzkin et al., (2014)). This would suggest that there is an interaction between lateralisation and anxiety on fronto-amygdala connectivity during emotional processing. Such a functional dissociation between the left and right amygdala have received support in previous meta-analyses (Baas et al., 2004; Sergerie et al., 2008; Wager et al., 2003). However, in general, evidence for such lateralisation is mixed at best (see chapter 5, and section 1.5.3.2 of the literature review for detail), with few studies looking specifically at functional lateralisation of amygdala connectivity.

The impact of gender differences on fronto-amygdala connectivity receive even less attention in the literature. Many studies presented here use both male and female participants, however few directly test for gender differences. Women have been shown to be particularly at risk of developing anxiety disorders (Somers, Goldner, Waraich, & Hsu, 2006; Solomon and Herman, 2009; Hourani, Williams, Bray and Kandell, 2015; Luxton et al., 2010), and Cahill (2006) emphasises that differences between the sexes associated with particular disorders warrant investigation both in healthy and clinical populations in order to best understand and treat disorders. Research has shown that women tend to use more emotion regulation strategies and report higher tendency to ruminate when distressed (Nolen-Hoeksema, 2012; Tamres, Janicki, & Helgeson, 2002), processes associated with appraisal. Accordingly, there is evidence to show that the level of recruitment of the prefrontal areas purported to be involved in appraisal is modulated by gender (Domes et al., 2010; McRae, Ochsner, Mauss, Gabrieli, & Gross, 2008). However, results are mixed, with Domes et al. reporting overall elevated levels of prefrontal activation in men compared to women during emotion appraisal, whereas McRae et al. report that women have greater prefrontal engagement than men during appraisal.

### 6.3 Aims

This study aims to draw together the previous literature relating to individual differences in state anxiety and gender in order to assess the modulating impact both of these factors have
on fronto-amygdala connectivity. In particular, a sub-clinical population will be assessed in line with Cahill’s (2006) assertion about the need to look to healthy populations as well as clinical populations in order to disentangle underlying mechanisms involved in emotion disorders. Furthermore, attention will be paid to the lateralisation of left and right amygdala connectivity to ensure that any hemispheric differences are not overlooked. Bilateral amygdala connectivity with frontal areas (ACC, dmPFC, vmPFC) as well as with the Precuneus will be observed. The mPFC is being divided into the dorsal and ventral portions in line with the growing body of evidence suggesting functional diversification between these two regions. The precuneus is included as a region of interest as a result of the established role it has as a key node in orchestrating the default mode network (DMN; Utevsky, Smith, & Huettel, 2014). In assessing cognitive appraisal and emotion regulation in a passive task, this is a vital control to ensure that attention is being paid to the stimulus being presented. In addition, bilateral FFG will be used as a seed region to investigate connectivity to the same frontal areas and precuneus as a control to ensure the amygdala connectivity results are not simply a reflection visual processing differences.

6.4 Method

The methods involved in data collection for this study are detailed in Chapter 3, and also follow on from analysis in Chapter 4. Please refer back to section 3.3.5 for detail, and section 4.4 for an overview of design, participants, procedure and fMRI acquisition. In brief, 50 datasets for full analysis with an age range of 19-45 years, \( \bar{x} = 24.66 \pm 5.38 \); 21 male, 29 female were used in this analysis. Sixteen were in the high anxiety group (mean age 24.63 ±3.98; 5 male, 11 female), and 34 in the low anxiety group (mean age 24.68 ±5.98; 16 male, 18 female) according to the HADS_A cut-offs detailed in section 5.4.2. The following method section will detail the connectivity analysis performed on the pre-processed fMRI data.

6.4.1 PPI Analysis

In present study, we are specifically interested in fronto-amygdala interactions during emotion processing of backwards masked stimuli. Psychophysiological Interaction (PPI) analysis is a method developed to investigate the potential relationship between regions in the brain (the physiological aspect) in relation to the experimental paradigm enlisted (psychological; Friston, 1997, Rogers, Morgan, Newton and Gore, 2007, O’Reilly, Woolrich,
Behrens, Smith, & Johansen-Berg, 2012). If two regions in the brain exhibit linked activity over the course of an experimental manipulation we can infer that these regions (however distant) have a functional association and thus that they are ‘functionally coupled’. This coupling is not limited to parallel activity increases/decreases (‘positive coupling’) but can also indicate times when a region’s activity (increase/decrease) is coupled with the opposite BOLD response in another area (decrease/increase) (‘negative coupling’). PPI analysis was conducted using FSL’s FEAT. Whole brain PPI analysis was initially conducted (section 6.4.1.3). From this whole brain PPI analysis, beta values were extracted for ROI analysis on six specific regions to inform fronto-amygdala connectivity (and fronto-FFG as control) and its modulation by individual differences (section 6.4.1.4).

6.4.1.1 Seed ROI selection-physiological terms
Four seed regions of interest (ROIs) were selected; two were based on a priori hypothesis (bilateral amygdala) and two were included as a form of control in the study (bilateral fusiform gyrus, FFG). Peak points of activation for these ROIs were identified from the second level contrast for Main Effect of Face, constrained by standard cytoarchitectonic maps for each region (defined by undilated automatic anatomical labelling (aal) templates implemented through WFU PickAtlas). For each participant, these coordinates were used to extract the BOLD time-course (from pre-processed [realigned, co-registered, normalised and smoothed] functional data) from either a five millimetre (bilateral amygdala) or eight millimetre (bilateral FFG) radius sphere.

6.4.1.2 Contrasts – psychological terms
Three PPI analyses were conducted, looking at the hemodynamic-response to either the Fear versus Baseline condition, the Happy versus Baseline condition or Neutral versus Baseline condition (fear, happy, neutral and baseline defined earlier in section 5.4.4).

6.4.1.3 PPI Calculation
A three-step analysis was performed. First level analysis was performed, with physiological eigenvariables calculated for each individual seed ROI (left amygdala, right amygdala, left FFG, right FFG, Section 5.5.2.3) and psychological components modelled individually for
each contrast (fear vs baseline, happy vs baseline, neutral vs baseline, Section 5.5.4). As such, for each of the PPI analyses (one for each seed ROI) the design matrix composed seven regressors; one physiological (seed ROI), three psychological components/contrasts (which were entered as regressors of no interest), and the resulting three interaction variables (seed ROI interaction with each contrast), which were the regressors of interest. Positive and negative PPI coupling was calculated, resulting in six PPI activation maps for each subject (positive and negative coupling for three interaction variables). After first level analysis, second level analysis was carried out, with setup equivalent to the first level analysis, but performed at group level to enable investigation across participants. For both levels of analysis, Z statistics images were thresholded at the whole brain level (clusters where $Z>2.3$) and a family-wise error-corrected cluster significance threshold of $p=0.05$. The results of these analyses are presented in the “Overall PPI analysis” section of the results (section 6.4.2).

The final step in the analysis was to extract beta values from frontal and DMN regions of interest (as detailed in next section) in order to perform statistical analysis on fronto-amygdala connectivity (and fronto-FFG as control) and its modulation by individual differences. The results of these analyses are presented in the “ROI analysis” section of the results (section 6.4.3 for amygdala and section 6.4.4 for FFG).

**6.4.1.4 Individual difference ROI Extraction**

ROI masks for the anterior cingulate cortex (ACC), ventromedial prefrontal cortex (vmPFC), dorsomedial prefrontal cortex (dmPFC) and precuneus were generated using FSLmaths by multiplying standard undilated masks created in WFU PickAtlas by the six group level PPI activation maps for the three different conditions (positive and negative coupling). This was done for the PPI data generated by each seed ROI (left amygdala, right amygdala, left FFG, right FFG). FeatQuery was then used to extract parameter estimates ($\beta$) from the first level PPI maps of each individual participant.

Statistical analysis of the extracted beta values was conducted using IBM SPSS (version 21.0). For bilateral amygdala and bilateral FFG, the extracted beta values for the four ROIs were used as dependent measures. To determine overall group effects a two-way ANOVA comprising the repeated measures factors valence (fearful, happy, neutral) and hemisphere of seed ROI (left, right) was run. Subsequently, two three factor mixed ANOVAs independently
examined the between group factors anxiety group (high, low), and gender (male, female), to look at the impact of individual differences. Follow up one-way ANOVAs and simple effects analysis post-hoc tests (Bonferroni corrected) were run to investigate any interactions or main effects.

6.5 Results

6.5.1 Descriptive statistics
For details on descriptive statistics please refer to section 5.5.1 in which the same cohort was studied. In brief, there were no statistical differences between the male and female groups on age, handedness or scores on the hospital anxiety and depression scale (HADS_T) or its subscales for anxiety (HADS_A) and depression (HADS_D). When splitting the participants by anxiety group as described in section 5.4.2, again there were no differences in age or handedness. However, there were differences in the HADS_A scores (t(48) = -6.00, p <.001, low: \( \bar{x} = 5.74\pm4.70 \); high \( \bar{x} =14.88\pm5.68 \)) as would be expected given that categorisation was based on these scores. There were also differences in the HADS_D (U=39.00, p<.001, r=-0.69) and HADS_T scores (U=5.50, p<.001, r=-0.79) in each group, again this might be expected due to the comorbidity between anxiety and depression.

6.5.2 Overall PPI analysis

6.5.2.1 Amygdala
There were no areas which displayed greater positive coupling with the amygdala during the task compared to baseline, with only negative coupling observed across all participants (n=50). Negative connectivity was observed in some visual processing areas (secondary visual cortex, FFG), but also areas involved in the emotion network (insula, putamen, pallidum, thalamus), as well as regions of interest within this analysis (precuneus, ACC, vmPFC) and other frontal areas (mid frontal gyrus, dIPFC).

Greater negative connectivity during the fear condition compared to baseline was displayed between the left amygdala and the left FFG (-30 -40 -12; z=4.51), left vmPFC (-20,64,6; z=3.02), left putamen (-30 -12 0; z= 4.49) and left precuneus (-14 -56 40; z=3.57), and between the right amygdala and the right ACC (8 38 10; z=5.53) and left mid-frontal gyrus (-26 32 30; z= 5.36; see Figure 6.1).
Emotional processing of happy faces compared to baseline was associated with a stronger negative interaction between the left amygdala and left secondary visual cortex (-14 -76 28, z=4.94), left FFG (-24 -48 -12; z=4.85) and bilateral superior temporal gyrus (52 -8 -12; z=4.85 / -42 -16 -2; z=4.71), as well as between the right amygdala and the right insula (36 14 -10; z=4.60) and left thalamus (-8 -4 0; z=4.67; see Figure 6.2).
Finally, there was greater negative interaction in the neutral condition compared to baseline between the left amygdala and the left postcentral gyrus (-54 -22 28; z=3.8) and left pallidum (-26 -6 0; z=3.76), as well as the right amygdala and the left vmPFC (-12 62 22; z=5.41), right dlPFC (32 54 18; z=5.55), bilateral ACC (8 32 22; z=5.34 /-6 38 6; z=5.11) and the left precuneus (-10 -52 42; z=4.89; see Figure 6.3).
Overall PPI Neutral Condition

Figure 6.3. Results of whole brain connectivity with the left and right amygdala during neutral conditions. Activation represents negative coupling only, no positive coupling was detected.

6.5.2.2 Fusiform

Just as in the amygdala results, there were no areas which displayed greater positive coupling with the fusiform gyrus (FFG) during the task compared to baseline, with only negative coupling observed across all participants (n=50). The coupling that was observed for the fusiform area was predominately with areas associated with visual processing, as would be expected, although some interaction with regions of interest (precuneus, dmPFC) is also observed when comparing passive viewing of happy faces to baseline.

Greater negative interaction was observed during the fear condition compared to baseline in visual areas; left FFG (left secondary visual cortex (-14 -64 -6; z= 3.52); right associative visual cortex (14 -82 36; z=3.69)) and right FFG (left primary visual cortex (-18 -68 12;
z=5.99) and right secondary visual cortex (12 -66 16; z=6.47)). In addition, negative coupling between the left FFG and left primary somatosensory cortex (-40 -24 48; z=4.25), as well as between the right FFG and the left FFG (-18 -40 -12; z=6.01) and the right FFG and left vmPFC (-2 56 2; z=3.40) was observed (see Figure 6.4).

During the happy condition compared to baseline negative coupling was seen between the right FFG and right secondary visual cortex (16 -50 -10; z=6.06) the left FFG (-24 -50 -10; z=6.15) and left dmPFC (-32 36 34; z=3.57) and the left FFG and right precuneus (8 -48 50; z=4.75), left insula (-38 16 -14; z=3.64) and right superior temporal gyrus (58 -22 10; z=3.66) (see Figure 6.4)

Figure 6.4: Results of whole brain connectivity with the left and right fusiform gyrus (FFG). (A) Whole brain negative coupling with the left and right FFG during the happy conditions. (B) Whole brain coupling with the left and right FFG during the fear conditions.

Positive coupling was observed in the neutral condition compared to baseline between the Left FFG and the left primary visual cortex (-10 -102 6, z=3.16) and right secondary visual cortex (22 -92 -16; z=3.75). Negative coupling was observed between the right fusiform and
right primary visual cortex (16 -64 10; z= 5.25) and the left secondary visual cortex (-12 -60 0; z=5.46) (see Figure 6.5).

Figure 6.5. Results of whole brain connectivity with the left and right fusiform gyrus (FFG) during neutral condition. (A) displays whole brain connectivity with the left FFG, only positive coupling was seen with the Left FFG during neutral conditions. (B) shows negative coupling with the right FFG during the neutral condition. There was no positive coupling with the right FFG during neutral conditions.

6.5.3. Amygdala ROI analysis

6.5.3.1 Group Level

The two-way ANOVA revealed no significant main effect of valence in connectivity between the amygdala and any of the ROIs across all participants (n=50, see Table 6.1 for details).
There was a main effect of hemisphere in the connectivity between the amygdala and ACC ($F(1,49) = 23.97, p<.001, \eta^2_p=.33$) and a significant interaction of valence by hemisphere in connectivity with the dmPFC ($F(2,81) = 5.93, p=.006, \eta^2_p=.11$). Post-hoc tests of the main effect of hemisphere in ACC-Amygdala connectivity revealed that this was caused by there being no connectivity between left amygdala and ACC ($\bar{x}=0.00\pm 0$), whilst there was significant negative connectivity between the right amygdala and the ACC ($\bar{x}=-.36\pm0.52; t(49)= 4.90, p<0.001, d=.69$).

Table 6.1 Main effect and interaction results of the two-way ANOVA looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral amygdala as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala). Cells shown in grey represent significant interactions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Valence</th>
<th>Hemisphere</th>
<th>Valence*Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>$F(2,74) = .27$</td>
<td>$F(1,49) = 23.97$</td>
<td>$F(2,74) = .27$</td>
</tr>
<tr>
<td></td>
<td>$p=.70$</td>
<td>$p&lt;.001$</td>
<td>$p=.76$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p=.005$</td>
<td>$\eta^2_p=.33$</td>
<td>$\eta^2_p=.005$</td>
</tr>
<tr>
<td>dmPFC</td>
<td>$F(1,59) = 1.18$</td>
<td>$F(1,49) = .41$</td>
<td>$F(2,81) = 5.93$</td>
</tr>
<tr>
<td></td>
<td>$p=.29$</td>
<td>$p=.52$</td>
<td>$p=.006$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p=.02$</td>
<td>$\eta^2_p=.008$</td>
<td>$\eta^2_p=.11$</td>
</tr>
<tr>
<td>vmPFC</td>
<td>$F(1,62) = .32$</td>
<td>$F(1,49) = .40$</td>
<td>$F(1,62) = .78$</td>
</tr>
<tr>
<td></td>
<td>$p=.62$</td>
<td>$p=.53$</td>
<td>$p=.46$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p=.007$</td>
<td>$\eta^2_p=.008$</td>
<td>$\eta^2_p=.02$</td>
</tr>
<tr>
<td>Precuneus</td>
<td>$F(2,86) = .43$</td>
<td>$F(1,49) = 2.67$</td>
<td>$F(1,70) = .90$</td>
</tr>
<tr>
<td></td>
<td>$p=.63$</td>
<td>$p=.11$</td>
<td>$p=.38$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p=.009$</td>
<td>$\eta^2_p=.05$</td>
<td>$\eta^2_p=.02$</td>
</tr>
</tbody>
</table>

Post-hoc tests on the valence by hemisphere interaction between the amygdala and dmPFC, adjusting for multiple comparisons, revealed overall there was not a significant difference in left ($\bar{x}=-.21\pm64$) and right amygdala ($\bar{x}=-.06\pm1.12$) connectivity with the dmPFC ($t(49)= 4.90, p=.52, d=.13$). However, there was a discernible valence-dependent shift in negative connectivity between the left and right amygdala and dmPFC (Figure 6.6). There was no coupling between the left amygdala and dmPFC for fear and neutral conditions, but there was negative coupling between for the happy condition ($\bar{x}=-.21\pm.09SE$, trending towards
significantly different from fear and neutral conditions $p=0.08$). Conversely, the right amygdala showed no coupling with the dmPFC during happy, but a slight negative coupling during fear ($\bar{x}=-0.030\pm0.08SE$) and neutral ($\bar{x}=-0.033\pm0.10SE$), though these were not significantly different from the happy condition.

![Graph showing mean coupling between left amygdala and dmPFC (blue), and right amygdala and dmPFC (orange) in different valence conditions (fear, happy and neutral). LA = Left amygdala, RA= right amygdala and dmPFC = dorsomedial prefrontal cortex.](image)

6.5.3.2. Gender Interaction
In the mixed ANOVA with gender as a between subjects factor, the overall group effects persisted with no significant main effects of valence, a significant main effect of hemisphere in amygdala-ACC connectivity ($F(1,48) = 24.75$, $p<.001$, $\eta^2_p = .34$), and a significant valence by hemisphere interaction between the amygdala and dmPFC ($F(2,80) = 5.16$, $p=.01$, $\eta^2_p = .10$).

There was a significant interaction of valence by gender in the amygdala-dmPFC connectivity ($F(1,58) = 5.01$, $p=.02$, $\eta^2_p = .10$) and a significant hemisphere by gender interaction between the amygdala –dmPFC ($F(1,48) = 4.98$, $p=.03$, $\eta^2_p = .09$) (see Table 6.2). There were no other regions of interest where gender interacted with valence or hemisphere to modulate amygdala connectivity, although a hemisphere by gender interaction approached significance for amygdala-vmPFC connectivity.
Table 6.2 Main effect and interaction results of the mixed ANOVA with gender as a between groups variable looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral amygdala as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala). Cells shown in grey represent significant interactions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Valence*Gender</th>
<th>Hemisphere*Gender</th>
<th>Valence*Hemisphere</th>
<th>Valence*Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Gender)</td>
<td></td>
<td>(Gender)</td>
</tr>
<tr>
<td>ACC</td>
<td>$F(2,73) = 1.91$</td>
<td>$F(1,48) = .87$</td>
<td>$F(2,73) = 1.91$</td>
<td>$p=.15$</td>
</tr>
<tr>
<td></td>
<td>$p=.17$</td>
<td>$p=.36$</td>
<td>$p=.17$</td>
<td>$p=.04$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p = .04$</td>
<td>$\eta^2_p = .02$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dmPFC</td>
<td>$F(1,58) = 5.01$</td>
<td>$F(1,48) = 4.98$</td>
<td>$F(2,80) = .82$</td>
<td>$p=.42$</td>
</tr>
<tr>
<td></td>
<td>$p=.02$</td>
<td>$p=.03$</td>
<td>$p=.02$</td>
<td>$\eta^2_p = .02$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p = .10$</td>
<td>$\eta^2_p = .09$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vmPFC</td>
<td>$F(1,60) = .53$</td>
<td>$F(1,48) = 3.77$</td>
<td>$F(1,61) = 1.59$</td>
<td>$p=.22$</td>
</tr>
<tr>
<td></td>
<td>$p=.51$</td>
<td>$p=.06$</td>
<td>$p=.51$</td>
<td>$\eta^2_p = .03$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p = .01$</td>
<td>$\eta^2_p = .07$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precuneus</td>
<td>$F(2,84) = .87$</td>
<td>$F(1,48) = 3.10$</td>
<td>$F(1,67) = 2.28$</td>
<td>$p=.13$</td>
</tr>
<tr>
<td></td>
<td>$p=.41$</td>
<td>$p=.09$</td>
<td>$p=.41$</td>
<td>$\eta^2_p = .05$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p = .02$</td>
<td>$\eta^2_p = .06$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Investigation of the valence by gender interaction revealed no significant valence interaction in the dmPFC for male participants ($F(1,23) = 1.10, p=.32, \eta^2_p = .05$), but there was for female participants ($F(1,35) = 4.91, p=.03, \eta^2_p = .15$). As expected, post-hoc tests showed no significant difference in amygdala-dmPFC connectivity between fear and happy ($p=.89$), fear and neutral ($p=1$) or happy and neutral ($p=.89$) in dmPFC-amygdala connectivity in male participants (see Figure 6.7). However, in female participants there was a significant difference between fear and happy conditions ($p=.05$), but not between fear and neutral ($p=1$) or happy and neutral ($p=.11$).
Figure 6.7 Graph showing the gender by valence interaction in amygdala-dmPFC coupling. As can be seen there are no significant differences in coupling between the different valence conditions in male participants, however in females there are clear differences with significant differences between Fear and happy conditions but not between fear and neutral or happy and neutral. Bars represent mean±SE *p<0.05, ** p<0.01, ***p<0.001

For the gender by hemisphere interaction, simple effects analysis revealed no significant difference in connectivity with the dmPFC for left or right amygdala in either men (left amygdala: $\bar{x}=-.04\pm.72$; right amygdala: $\bar{x}=-.38\pm.59$; $t(20)=1.54, p=.14, d=.47$) or women (left amygdala: $\bar{x}=-.39\pm.51$; right amygdala: $\bar{x}=.16\pm1.35$; $t(28)=-1.19, p=.09, d=.17$). Despite no overall difference, there appears to be a reversal of connectivity patterns in male and female participants between the left or right amygdala with dmPFC (see Figure 6.8). There was numerically more negative right amygdala connectivity in men, and more negative left amygdala connectivity in women, and to an extent, this trend is seen for the vmPFC and precuneus (see Figure 6.8).
6.5.3.3. Anxiety Interaction

In the mixed ANOVA with anxiety group as a between subjects factor, the overall group effects persisted with no significant main effects of valence, a significant main effect of hemisphere in amygdala-ACC connectivity ($F(1,48) = 19.91, p<.001, \eta^2_p = .29$), and a significant valence by hemisphere interaction between the amygdala and dmPFC ($F(2,80) = 6.54, p=.004, \eta^2_p = .12$).

Table 6.3 Main effect and interaction results of the mixed ANOVA with anxiety as a between groups variable looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral amygdala as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala).

<table>
<thead>
<tr>
<th>Region</th>
<th>Valence*Anxiety</th>
<th>Hemisphere*Anxiety</th>
<th>Valence*Hemisphere *Anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>$F(2,72) = 1.14, p=.31, \eta^2_p = .02$</td>
<td>$F(1,48) = .03, p=.87, \eta^2_p = .001$</td>
<td>$F(2,72) = 1.14, p=.31, \eta^2_p = .02$</td>
</tr>
<tr>
<td>dmPFC</td>
<td>$F(1,57) = .16, p=.73, \eta^2_p = .003$</td>
<td>$F(1,48) = .00, p=.01, \eta^2_p = .00$</td>
<td>$F(2,80) = .98, p=.37, \eta^2_p = .02$</td>
</tr>
<tr>
<td>vmPFC</td>
<td>$F(1,60) = .70, p=.50, \eta^2_p = .01$</td>
<td>$F(1,48) = .19, p=.67, \eta^2_p = .004$</td>
<td>$F(1,61) = 2.54, p=.11, \eta^2_p = .05$</td>
</tr>
<tr>
<td>Precuneus</td>
<td>$F(2,84) = .28, p=.73, \eta^2_p = .006$</td>
<td>$F(1,48) = .22, p=.64, \eta^2_p = .005$</td>
<td>$F(1,69) = .89, p=.39, \eta^2_p = .02$</td>
</tr>
</tbody>
</table>
There were no significant interactions between anxiety group and valence or lateralisaton for any of the four regions (Table 6.3). A numerical trend for greater negative coupling during emotionally valenced (fear and happy) blocks compared to neutral was observed across all regions in the high anxiety group of participants however, this was not seen in the statistical analysis.

6.5.4. Fusiform Gyrus ROI analysis

6.5.4.1. Group Level

The two-way ANOVA revealed no significant main effect of valence in connectivity between the FFG and the ROIs across all participants (n=50, see Table 6.4 for details). There was a main effect of hemisphere in the connectivity between the FFG and ACC \( (F(1,49) = 7.20, p=.01, \eta^2_p = .13) \), vmPFC \( (F(1,49) = 5.05, p=.03, \eta^2_p = .09) \) and precuneus \( (F(1,49) = 11.57, p=.001, \eta^2_p = .19) \). In addition there was an interaction of valence by hemisphere in with the precuneus \( (F(2,83) = 5.67, p=.005, \eta^2_p = .10) \).

Table 6.4 Main effect and interaction results of the two-ANOVA looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral fusiform gyrus as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala). Cells shown in grey represent significant interactions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Valence</th>
<th>Hemisphere</th>
<th>Valence*Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>( F(1,69) = .01, p=.96, \eta^2_p = .001 )</td>
<td>( F(1,49) = 7.20, p=.01, \eta^2_p = .13 )</td>
<td>( F(2,74) = .67, p=.52, \eta^2_p = .01 )</td>
</tr>
<tr>
<td>dmPFC</td>
<td>( F(1,63) = .81, p=.40, \eta^2_p = .02 )</td>
<td>( F(1,49) = .90, p=.35, \eta^2_p = .02 )</td>
<td>( F(1,63) = .81, p=.40, \eta^2_p = .02 )</td>
</tr>
<tr>
<td>vmPFC</td>
<td>( F(1,73) = .83, p=.44, \eta^2_p = .02 )</td>
<td>( F(1,49) = 5.05, p=.03, \eta^2_p = .09 )</td>
<td>( F(1,73) = .83, p=.41, \eta^2_p = .02 )</td>
</tr>
<tr>
<td>Precuneus</td>
<td>( F(2,86) = .42, p=.66, \eta^2_p = .01 )</td>
<td>( F(1,49) = 11.57, p=.001, \eta^2_p = .19 )</td>
<td>( F(2,83) = 5.67, p=.005, \eta^2_p = .10 )</td>
</tr>
</tbody>
</table>

Post-hoc tests of the main effect of hemisphere in FFG connectivity (see Figure 6.9) revealed that there was greater negative connectivity between the right FFG and the ACC \( (\bar{x}=-.57\pm 1.40) \), and precuneus \( (\bar{x}=-.63\pm .89) \) than for the left FFG these areas (ACC: \( \bar{x}=-.03\pm .10, t(49)= 2.68, p=.01, d=.38 \); Precuneus: \( \bar{x}=-.22\pm .67, t(49)= 3.40, p=.001, d=.45 \). The left FFG showed no connectivity with the vmPFC \( (\bar{x}=0.00\pm 0) \) compared to the right FFG connectivity.
with the vmPFC (\(\bar{x}=-.82\pm 2.57\)) resulting in a significant difference (\(t(49)=2.25, p=.03, d=.32\)).

![Graph showing the significant interaction of seed ROI hemisphere (left and right fusiform gyrus; FFG) and connectivity between three secondary regions of interest: the anterior cingulate cortex (ACC), the vmPFC (ventromedial prefrontal cortex) and the Precuneus. In all cases it is clear there is greater negative coupling between these regions and the right FFG. Bars represent mean±SE *p<0.05, ** p<0.01, ***p<0.001](image)

Post-hoc tests on the valence by hemisphere interaction between the FFG and precuneus, adjusting for multiple comparisons, revealed a significant difference for left FFG connectivity between happy and neutral conditions (\(p=.04\)) with the left FF showing no connectivity (\(\bar{x}=.00\pm 0\)) in the neutral condition, but no other significant differences for either left FFG (fear and happy: \(p=.17\); fear and neutral: \(p=1\)) or right FFG (happy and neutral: \(p=.53\); fear and happy: \(p=1\); fear and neutral: \(p=.61\)). Bearing in mind the significant hemispheric effect with precuneus activity described earlier, it seems this interaction may be caused by high right FFG connectivity with precuneus with no valence difference, and lower left FFG connectivity with valence differences suggesting greater connectivity during emotionally valent tasks, particularly happy (Figure 6.10).
Figure 6.10. Graphs showing the valence by hemisphere interaction between the left and right fusiform gyrus and Precuneus in different emotion conditions (fear, happy and neutral). As can be seen there are no differences between the different valence conditions in the right FFG connectivity with the Precuneus. In contrast there was a significant difference between the happy and fear conditions negative coupling between the left FFG and Precuneus with the neutral condition revealing no connectivity ($\bar{x}=0.00\pm0$). Bars represent mean±SE *p<0.05, ** p<0.01, ***p<0.001

6.5.4.2. Gender Interaction

In the mixed ANOVA with gender as a between subjects factor, the overall group effects were maintained, with no significant main effects of valence, a significant main effect of hemisphere in FFG coupling with ACC ($F(1,48)=6.10$, $p=.02$, $\eta^2_p=.11$), vmPFC ($F(1,48)=4.12$, $p=.05$, $\eta^2_p=.08$) and precuneus ($F(1,48)=10.07$, $p=.003$, $\eta^2_p=.17$), as well as a significant valence by hemisphere interaction between FFG and precuneus ($F(2,82)=4.74$, $p=.01$, $\eta^2_p=.09$). There were no significant interactions between gender and valence or lateralisation for any of the four regions (Table 6.5).
Table 6.5 Interaction results of the mixed ANOVA with gender as a between groups factor looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral fusiform gyrus as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala) and gender.

<table>
<thead>
<tr>
<th>Region</th>
<th>Valence*Gender</th>
<th>Hemisphere*Gender</th>
<th>Valence<em>Hemisphere</em>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>$F(1,68) = .15, p=.78, \eta^2_p=.003$</td>
<td>$F(1,48) = 1.41, p=.24, \eta^2_p=.03$</td>
<td>$F(2,73) = .17, p=.78, \eta^2_p=.004$</td>
</tr>
<tr>
<td>dmPFC</td>
<td>$F(1,62) = .44, p=.56, \eta^2_p=.009$</td>
<td>$F(1,48) = 1.13, p=.72, \eta^2_p=.003$</td>
<td>$F(1,62) = .44, p=.56, \eta^2_p=.009$</td>
</tr>
<tr>
<td>vmPFC</td>
<td>$F(1,72) = .39, p=.62, \eta^2_p=.008$</td>
<td>$F(1,48) = 1.60, p=.21, \eta^2_p=.03$</td>
<td>$F(1,72) = .39, p=.62, \eta^2_p=.008$</td>
</tr>
<tr>
<td>Precuneus</td>
<td>$F(2,85) = .96, p=.38, \eta^2_p=.02$</td>
<td>$F(1,48) = 1.71, p=.20, \eta^2_p=.03$</td>
<td>$F(2,82) = 1.57, p=.21, \eta^2_p=.03$</td>
</tr>
</tbody>
</table>

However, there was a main effect of gender on FFG-precuneus connectivity ($F(1,48) = 5.54, p=.02, \eta^2_p=.10$). Follow up simple effects analysis (see Figure 6.11) showed that women exhibited significantly greater negative coupling with the precuneus ($\bar{x}=-1.21\pm 1.41$) than men ($\bar{x}=-.35\pm 1.05$; $t(48)= 2.35, p=.02, d=.61$).

![Main effect of gender on Fusiform gyrus-Precuneus Connectivity](image)

Figure 6.11. Graph showing the main effect of gender on Fusiform gyrus – Precuneus connectivity. As seen the female participants (red) showed greater negative coupling between the fusiform gyrus and the precuneus compared to male (blue) participants. Bars represent mean±SE *p<0.05, ** p<0.01, ***p<0.001
6.5.4.3. Anxiety Interaction

Running the mixed ANOVA with anxiety group as the between subjects factor again maintained the overall group effects, with no significant main effects of valence, a significant main effect of hemisphere in FFG coupling with ACC ($F(1,48) = 9.64, p = .003, \eta^2_p = .17$), vmPFC ($F(1,48) = 7.54, p = .008, \eta^2_p = .14$) and precuneus ($F(1,48) = 10.18, p = .003, \eta^2_p = .18$), as well as a significant valence by hemisphere interaction between FFG and precuneus ($F(2,81) = 4.51, p = .02, \eta^2_p = .09$).

There was a significant main effect of valence by anxiety group for FFG-ACC connectivity ($F(1,69) = 3.71, p = .04, \eta^2_p = .07$), but no other interaction with anxiety group for any of the regions of interest (Table 6.6).

Table 6.6. Interaction results of the mixed ANOVA with anxiety as a between groups factor looking at connectivity between four regions of interest (ACC, dmPFC, vmPFC and Precuneus) and bilateral fusiform gyrus as modulated by the valence condition and the seed ROI hemisphere (left or right amygdala) and gender. Cells in grey show significant interactions

<table>
<thead>
<tr>
<th>Region</th>
<th>Valence*Anxiety</th>
<th>Hemisphere*Anxiety</th>
<th>Valence<em>Hemisphere</em>Anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>$F(1,69) = 3.71$</td>
<td>$F(1,48) = 2.46$</td>
<td>$F(2,74) = 2.03$</td>
</tr>
<tr>
<td></td>
<td>$p = .04$</td>
<td>$p = .12$</td>
<td>$p = .15$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p = .07$</td>
<td>$\eta^2_p = .05$</td>
<td>$\eta^2_p = .04$</td>
</tr>
<tr>
<td>dmPFC</td>
<td>$F(1,63) = 1.55$</td>
<td>$F(1,48) = 1.11$</td>
<td>$F(1,63) = 1.55$</td>
</tr>
<tr>
<td></td>
<td>$p = .22$</td>
<td>$p = .30$</td>
<td>$p = .22$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p = .03$</td>
<td>$\eta^2_p = .02$</td>
<td>$\eta^2_p = .03$</td>
</tr>
<tr>
<td>vmPFC</td>
<td>$F(2,72) = 1.18$</td>
<td>$F(1,48) = 2.86$</td>
<td>$F(2,72) = 1.18$</td>
</tr>
<tr>
<td></td>
<td>$p = .30$</td>
<td>$p = .10$</td>
<td>$p = .30$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p = .02$</td>
<td>$\eta^2_p = .06$</td>
<td>$\eta^2_p = .02$</td>
</tr>
<tr>
<td>Precuneus</td>
<td>$F(2,85) = .20$</td>
<td>$F(1,48) = .02$</td>
<td>$F(2,81) = .18$</td>
</tr>
<tr>
<td></td>
<td>$p = .79$</td>
<td>$p = .89$</td>
<td>$p = .80$</td>
</tr>
<tr>
<td></td>
<td>$\eta^2_p = .004$</td>
<td>$\eta^2_p = .00$</td>
<td>$\eta^2_p = .004$</td>
</tr>
</tbody>
</table>
Follow up of the valence by anxiety group interaction in FFG-ACC connectivity revealed that there was no significant valence effect for either the high anxiety ($F(2,51) = 2.08, p = .13, \eta_p^2 = .06$) or low anxiety ($F(1,18) = 1.27, p = .30, \eta_p^2 = .08$) groups. However, the high anxiety group exhibited numerically greater negative coupling between the FFG and the ACC (Figure 6.12), especially for the fear condition (low anxiety: $\bar{x} = -.08 \pm .08$; high anxiety: $\bar{x} = -.47 \pm .31$). There was no significant difference between the groups in FFG-ACC connectivity (main effect of anxiety group on FFG-ACC connectivity: $F(1,48) = 1.69, p = .20, \eta_p^2 = .03$).

![Graph showing numerical differences](image)

Figure 6.12. Graph to show numerical differences between the high (orange) and low (green) anxiety groups in fusiform gyrus – Anterior cingulate cortex connectivity. The high anxiety group appear to show slightly more (though more variable) negative coupling between these two regions compared to the low anxiety group.

### 6.5.5 Summary

Typical PPI analysis using the left and right amygdala as seeds reveals negative frontal connectivity with the right amygdala alone during processing for fear and neutral stimuli. There was also connectivity for both amygdalae with other areas associated with emotion processing (see chapter 1, section 1.4), such as the insula and basal ganglia (putamen, pallidum), areas associated with sensory processing (thalamus, FFG, secondary visual areas), and the precuneus (associated with attention). In contrast, the left and right FFG primarily show patterns of negative connectivity with visual cortex (along with contralateral FFG and precuneus), with minimal connectivity to frontal areas (only connectivity with dMPFC from right FFG during happy blocks).
Looking more specifically at the four regions of interest (ACC, vmPFC, dmPFC, Precuneus), although there was no overall valence effect in fronto-amygdala activity, there was evidence that the amygdala-dmPFC connectivity is important during emotion processing. Amygdala-dmPFC connectivity was modulated by interactions between valence and hemisphere, gender and valence, and gender and hemisphere. Of most interest, females exhibited a valence effect for amygdala-dmPFC connectivity (greater connectivity in happy compared to fear), whereas males did not. There was also greater left amygdala-dmPFC connectivity in general for females, compared to greater right amygdala-dmPFC connectivity in males. In addition to this, left amygdala exhibited a numerically (but not statistically) different pattern of connectivity across valence (greater negative coupling during happy) compared to right amygdala (greater during fear and neutral). Interestingly, there was no interaction between state anxiety group and fronto-amygdala connectivity, despite numerically greater negative connectivity in the high anxiety group, particularly during fear blocks. When accounting for anxiety, an overall hemispheric effect was found for amygdala-ACC connectivity, with no observed left amygdala connectivity.

For the fusiform gyrus, the area used as a control for general visual processing, there was also no overall valence effect on fronto-FFG connectivity, and no interaction with gender. There was however a tentative interaction between anxiety and valence for FFG-ACC connectivity, with numerically greater negative coupling in the high anxiety participants, particularly for the fear condition. There was predominantly more negative connectivity between the right FFG (with ACC, vmPFC, and precuneus) compared to the left, which often showed no connectivity with the regions of interest. FFG-precuneus connectivity was modulated by hemisphere (as above), valence by hemisphere (primarily driven by hemispheric difference) and gender (greater connectivity in females), suggesting that visual processing was predominately modulated by attention mechanisms.

6.6 Discussion
The current study aimed to elucidate how individual differences in gender and state anxiety in a subclinical population modulate fronto-amygdala connectivity during emotion processing. Amygdala connectivity was analysed for left and right amygdala separately in order to shed light on possible lateralisation differences observed in previous literature. Using typical psychophysiological interaction analysis, then extracting information for four regions of
interest, the data suggests that gender modulates connectivity related to valence specificity and state anxiety impacts on orienting attention and visual processing during an emotion processing task. Furthermore, there is some evidence that the different amygdalae do appear to have different connectivity patterns depending on valence, and that this may also be modulated by gender.

6.6.1 Fronto-amygdala connectivity

![Diagram of Frontoamygdala Connectivity](image)

Figure 6.13. A visual representation of the results presented in section 6.4.2. (a) Shows the negative coupling observed in the three different emotion conditions from the psychophysiological interaction analysis. In the fear (red) condition negative coupling was observed between the anterior cingulate cortex (ACC) and the right amygdala and between the left amygdala, ventromedial prefrontal cortex (vmPFC) and precuneus. In the neutral (green) condition negative coupling between the right amygdala, vmPFC and precuneus. In the happy condition (blue) negative coupling was observed between the thalamus and right amygdala. (b) shows the results of the ROI analysis of the PPI results which found a valence by hemisphere interaction between the amygdala and dorsomedial prefrontal cortex (dmPFC) coupling. Although follow up results were not significant, this diagram shows that there appears to be a valence dependent shift in negative coupling between the left amygdala and dmPFC (in the happy condition) and the right amygdala and dmPFC (in the fear and neutral) conditions. In addition analysis revealed that regardless of valence (black), the right amygdala showed negative connectivity with the ACC, whilst the left amygdala showed no connectivity with the ACC.

6.6.1.1 Lateralisation

Observing both results from the typical PPI analysis and from the ROI analysis, it is evident that the two amygdalae are differentially connected during emotion processing. In particular, the right amygdala shows a distinct pattern of negative connectivity with frontal areas (specifically with the vmPFC and to some extent with the dmPFC), as well as with the ACC, during presentation of fearful and neutral stimuli (see Figure 6.13). As discussed previously, there is evidence that neutral stimuli can be interpreted as a potential threat to the individual requiring immediate response, as they are perceived as more emotionally ambiguous
(Michael Davis & Whalen, 2001), (for further discussion see section 5.7). In this way, fearful and neutral stimuli may activate common threat-related processing pathways. The reduction in connectivity between frontal areas and the ACC is suggestive of a decline in or absence of top down inhibition on right amygdala activity. As a result, increased amygdala activation would be expected in response to fearful and neutral stimuli. This pattern of frontal decoupling with the right amygdala supports the evidence of laterality seen in other recent research on amygdala-frontal coupling which also observed weaker right amygdala-frontal coupling (Eden et al., 2015; Gold et al., 2015; Motzkin et al., 2014).

Conversely, negative coupling was observed between the right amygdala and the thalamus during presentation of happy face stimuli only. This is in line with the dual processing model of emotion processing discussed in detail in section 1.5.2 and Chapter 5, whereby there is a direct pathway to the basolateral nucleus of the amygdala from the thalamus and an indirect pathway to the amygdala via the sensory cortex. The direct pathway is involved in rapid detection of basic information and instinctual, cognition free responses to threat, and is associated with the right amygdala. The data here support this notion, with reduced top-down inhibition during fear or ambiguous threat (neutral) stimuli, and disconnectivity with the thalamus during non-threat stimuli that does not require rapid processing (happy).

In contrast to the right amygdala’s role in immediate threat detection, the left amygdala appears to be more involved in some form of salience detection, as negative coupling between the left amygdala and frontal areas occurs only in fearful and tentatively in happy conditions. This result could suggest reduced top-down inhibition, and greater left amygdala activation in response to fearful and happy stimuli which may be part of a system of re-evaluation suggested by Cunningham, Dunfield, and Stillman (2013). The indirect processing pathway, discussed above, is associated with the left amygdala and is involved in deeper evaluation of stimuli using data from multiple sensory inputs. Indeed, while there is evidence that the amygdalae are involved in rapid orientation to a stimulus with a range of differently valenced stimuli (e.g. section 1.5.3.1. Yang et al., 2002), this immediate response bypasses any more in-depth evaluative processing. There is also a need to assess the social, and biological, value of the incoming stimulus using information from multiple sensory inputs. The data here suggests that the functional roles of the amygdalae are divided hemispherically with the right amygdala primarily responsible for rapid orienting of attention, whilst the left amygdala is involved in a more complex system of feedback with sensory cortices and frontal regions of the brain.
6.6.1.2 Valence
Reduction in top-down frontal and ACC inhibition with the right amygdala during fearful and neutral conditions and with the left amygdala during fear and tentatively happy conditions would suggest that there should be an apparent effect of valence on amygdala activation. Whilst there were lateralisation and lateralisation by valence effects as described above, there was no direct effect of valence on the PPI data reported here. The precuneus activation was seen to decouple with the left amygdala during the fear condition and with the right amygdala during neutral. This pattern of decoupling with the precuneus (a key node in the default mode network) suggests an interaction of valence related to attention to task with possible parallel processing of a stimulus. It is only by investigating the interaction between lateralisation and valence that subtle nuances in amygdala function are detected in relation to possible discrete functions.

6.6.1.3 Specificity within the frontal cortex
Looking into the fronto-amygdala connectivity in more detail to consider the dorsal and ventral medial prefrontal cortex (dmPFC, vmPFC), there emerges a pattern of decoupling which could be explained by the recent framework put forward by Etkin, Egner, and Kalisch (2011). As mentioned previously, the authors suggest that amygdala connectivity to the dmPFC is associated with appraisal and expression of negative emotion, whereas amygdala connectivity with the vmPFC is attributed to generating emotion responses through the limbic system after such appraisal. Furthermore, it has been suggested that the specific mechanism through which the vmPFC generates emotion responses is through resolving emotional ambiguity (Kim et al., 2003; Hye-young Kim et al., 2004; Kim, Loucks, et al., 2011), with greater vmPFC activity (and thus decreased amygdala activation) leading to positive perception of ambiguous stimuli and reduced vmPFC activity leading to negative perception (Kim et al., 2011).

The current data reveals decoupling between right amygdala and vmPFC in neutral conditions and a numerical pattern of decoupling with the dmPFC specific to fear and neutral conditions. If the decoupling is assumed to mean increased frontal activity, this could suggest, from the Etkin et al. (2011) model, that dmPFC was involved in the appraisal of the negative emotion, and vmPFC in a positive response to the negative or ambiguous stimuli.
Alternatively, the decoupling could be due to a lack of frontal appraisal and response mechanisms as an immediate response was necessary from the right amygdala. This supports the previous theories suggesting that right amygdala connectivity is arranged in a system conducive to rapid response to threat stimuli. The reduced right amygdala-precuneus coupling in the neutral condition would suggest the latter may be the case as decoupling with the precuneus is associated with attentional shifts and the default mode network (Utevsky et al., 2014). Frontal connectivity with the left amygdala is more complex, with vmPFC decoupling observed during the fear condition and a pattern of dmPFC decoupling during the happy condition. This suggests there is a bilateral amygdala-frontal decoupling during the potentially threatening fear stimuli, with the right amygdala involved in immediate response and the left in appraisal of the threat stimuli. In this case, the vmPFC decoupling may represent positive perception and emotional response to these faces, as discussed above (Kim and colleagues (2011)). Indeed, the left amygdala is thought to be more involved in evaluative processing (Cunningham, Dunfield, and Stillman (2013)), and as such the tentative dmPFC decoupling during the happy condition suggest either lower frontal involvement in the appraisal of happy stimuli, or, more likely, lower left amygdala (evaluative processing) of the non-ambiguous happy stimuli.
6.6.2 Fronto-Fusiform gyrus connectivity

Bilateral FFG was included as a control region in order to determine that any effects observed are unique to amygdala function and not simply due to the processing of the visual stimulus used. As expected, group level results confirm that the FFG is involved with the mechanics of the task, in particular the interaction with the visual cortex, indicative of their key role in processing facial visual stimuli. In addition, the precuneus decouples with the right FFG, regardless of valence, and the left FFG in the fearful and happy conditions (see Figure 6.14). This suggests that FFG activity increases in response to face stimuli, as expected, but for the left FFG this happens only in the socially salient conditions (fearful and happy). There is also bilateral FFG decoupling with frontal areas specifically with right FFG showing significantly greater negative decoupling, regardless of valence, with vmPFC, dmPFC and ACC. The fact that this is regardless of valence suggests this coupling is related to the role of FFG in face processing (Kanwisher et al., 1997).

As can be seen, there are differences in connectivity between the right and left FFG, with greater negative coupling observed between the right FFG and frontal areas and precuneus. This fits with both previous literature showing right side dominance in face processing (McCarthy et al., 1997) and previous analyses of this dataset which showed greater right FFG
activation compared to left FFG ($t(49) = -3.01, p = .004, d = 0.89$; section 5.5.2.3). In addition to the greater right FFG activation and connectivity, there are also differences in how this connectivity alters across the conditions. Right FFG connectivity is altered with frontal areas and precuneus across all the valence conditions, indicating a basic role in face processing. On the other hand, left FFG connectivity with precuneus is only altered for socially salient (fear and happy) conditions, and connectivity with ACC (there was no disconnectivity with either vmPFC or dmPFC) only for the happy condition. The altered connectivity based on valence suggests a different role for left FFG, especially as it showed negative coupling with the left amygdala for the socially salient conditions (fear and happy). There is evidence that the amygdala and FFG are involved in a system of reciprocal feedback, and it has been shown that the amygdala can influence FFG activation (Fairhall & Ishai, 2007; Herrington, Taylor, Grupe, Curby, & Schultz, 2011) to orient visual processing areas. Herrington and colleagues (ibid.) further suggested this could be a resource allocation mechanism which accelerates the processing of sensory stimuli by allocating the necessary attentional capacity, and also co-ordinates the subsequent emotional response.

An alternative explanation of the apparently different roles of the left and right FFG in emotion processing may simply be due to the stimuli used. Despite the precuneus decoupling with all other key nodes irrespective of valence (right amygdala, left amygdala, and right FFG), it only decouples with the left FFG during the fearful and happy condition. A reduction in precuneus activation suggests a shift from DMN to task related network and thus a necessity for increased attentional capacity. As there is coupling between the right FFG and bilateral amygdala with the precuneus, it could be inferred that the absence specifically for the neutral condition with left FFG activation results from more variable left FFG activation in the neutral condition. If this is the case, then this perhaps also explains the lack of right-left FFG and left FFG-left amygdala decoupling during the neutral condition, with decoupling only observed for happy and neutral. The variable activation is not likely to be due to
variability in the stimuli themselves (all stimuli came from previously validated datasets and used the same actor for each emotion), but may be due to a neutral stimulus being perceived as more ambiguous, as discussed earlier. It may be that the ambiguity leads to variability in the level of attention directed towards the neutral stimulus. Some participants may have similar attention to all valences, while others may show reduced attention to the neutral stimuli resulting in weaker overall PPI results for this particular process.

Overall, the FFG connectivity results mirror its role in visual processing, with some indication that left FFG may be involved in some aspects of salience detection via the left amygdala. However, the focus here was not on FFG connectivity, but rather the use of it as a control region. Further investigation paying specific attention to FFG and top-down, bottom-up processes is required to bring clarity to the evidence emerging here.

### 6.6.3 Emotion processing and functional specialisation

![Figure 6.15. Schematic showing connections observed in the PPI and ROI analysis grouping activation patterns into two parallel roles – the left amygdala is suggested to be involved in salience detection as a result of apparent responsivity during fear (red) and happy (blue) conditions and primary association with fusiform gyrus coupling suggested to implicated in sensory feedback. Note the dotted blue line is a tentative connection discussed in section 6.4.3.1. The right amygdala is suggested to be involved in threat detection systems as a result of key connectivity patterns being observed primarily in fear (red) and neutral (green) conditions.](image)

Overall, the group level results suggest that there is a form of dual processing with both amygdala responding to emotional stimuli but the patterns of connectivity with frontal areas
relating to different functional specialisation; the right amygdala is involved in a rapid system of threat detection with connections to the thalamus and frontal areas, whilst the left amygdala is involved in relevance processing and salience detection which may be modulated to some extent through reciprocal feedback with the sensory cortex (FFG) as well as fronto-amygdala connections (see Figure 6.15).

### 6.6.4 Individual differences

On top of the whole-group analysis of amygdala connectivity during emotion processing, this chapter looked into the effect of individual differences in gender and anxiety on these networks.

#### 6.6.4.1 Gender

The results clearly demonstrate the modulating effect gender has on frontal connectivity with the amygdala during emotion processing. Gender primarily appears to modify dmPFC connectivity. There is a lateralisation of amygdala function, with males having greater negative coupling between the right amygdala and dmPFC, and females exhibiting greater negative coupling between left amygdala and dmPFC. In keeping with this, this negative coupling is observed for neutral and fear conditions in males and for the happy condition in females (see Figure 6.16). However, only females had a significant valence effect, with negative coupling for happy being greater than that for fear.
These results suggest that male participants have reduced dmPFC top-down inhibition and increased right amygdala activation during processing of fear and neutral stimuli, meaning reduced frontal appraisal and rapid processing of threat related stimuli. Conversely, this implies that female participants maintain top-down inhibition and appraisal mechanisms during processing of these threat-related or ambiguous stimuli (fear and neutral) over the course of the experiment. Increased left amygdala-dmPFC negative coupling during the happy condition may suggest that in females this emotion requires less appraisal or attention in the long-term. Indeed, if it is assumed that the left amygdala is involved in salience detection, this implies that it is the salience of the happy condition that leads to less frontal appraisal, with females either finding these images less salient or perhaps more socially salient, needing longer-term processing.

The FFG connectivity results have some relevance to the amygdala results, with evidence of a gender difference in attention during the tasks. Females exhibit greater FFG-precuneus decoupling across all conditions compared to males, suggesting that they were attending to the emotion stimuli, or the task overall. This also fits with the GLM result that females show greater amygdala activation throughout the data collection period in comparison to male participants ($t(48) = -1.96, p = 0.028, d = 0.49$; see section 5.5.2.2 for more detail).

Together, the increased amygdala activation, increased attention across the task, and increased appraisal of threat-related stimuli in female participants reaffirms research showing that female participants enlist more emotion regulation strategies. In particular, females report higher levels of rumination when they are emotionally distressed (Nolen-Hoeksema, 2012; Tamres et al., 2002), which has been linked to frontal appraisal. An alternative explanation of these results may come from McRae and colleagues (2008), who found that female participants showed greater overall activation in frontal areas during reappraisal in comparison to male participants, and suggested that this may be due to men being more efficient at regulating their response to negative affect. This conclusion was based on the observation that male participants had a comparative reduction in amygdala activation in response to negative stimuli compared to female participants. No such reduction in amygdala activation (other than habituation over time seen in the Chapter 4) was observed in this current study. There are methodological differences in the McRae et al (2008) study compared to the current one, as they explicitly asked participants to regulate their emotional responses, whereas the present task was looking at implicit emotion responses using a backwards-masking paradigm. One further conclusion by McRae and colleagues does have
parallels in the current data, as they suggest that male participants are either more equipped to automatically regulate emotion responses to threatening stimuli, or simply have faster disengagement of frontal appraisal regions in response to threat stimuli. There is indeed a greater level of amygdala-frontal disengagement during presentation of threat-related stimuli (fear and neutral) in male participants in our study, but the chronometry of the response cannot be measured in the present paradigm.

An important implication of the conclusion by McRae et al. (2008), and the result in this study that seem to affirm their suggestion, is that women are less efficient at regulating emotion in response to threatening stimuli in comparison to men. There is a growing body of evidence to showing a greater prevalence of mood related disorders in women compared to men (Hourani et al., 2015; Luxton et al., 2010; Solomon & Herman, 2009). Often such disorders are characterised by dysfunctional emotion–regulation systems. Perhaps it is the case that in men there is less room for error as their emotion regulation system utilises a greater proportion of automatic processes, and this may explain the discrepancies in mood disorder prevalence between the genders. This also suggests an avenue for therapy, both as a preventative measure for at risk individuals, and for subsequent treatment. It could be that therapies and treatments can be developed that can train individuals to use more automatic processing in response to emotion stimuli, thus reducing the risk factor for mood disorders. In this way this gender difference in emotion processing may be reduced. Indeed, McRae and colleagues (2008) suggested that through cognitive reappraisal training it may be that the discrepancy between the genders in emotion processing and prevalence for mood disorders can be reduced.

Previous research investigating the effects of gender on emotion processing has predominantly focussed on threat related stimuli (either fear or neutral). However, the present study also observed gender differences in fronto-amygda connectivity in response to a happy stimulus, with female participants showing reduced frontal involvement in processing happy stimuli compared to males. This may indicate that whilst males have a more efficient emotion response system to threat related stimuli (see above), stimuli relaying more socially salient information are better regulated in female participants. There is evidence that females are more emotionally competent than men, showing greater ability to understand and recognise others’ emotions rapidly, especially when attentional resources are limited (Donges, Kersting, & Suslow, 2012; N. Eisenberg & Lennon, 1983; Hall & Matsumoto, 2004; Hoffmann, Kessler, Eppel, Rukavina, & Traue, 2010). This emotional competence may
be caused by a more automatic system related to positive stimuli in female participants, as these results suggest. It is an intriguing possibility that the male emotion regulation system is better tuned to rapidly process and respond to negative stimuli in a way that reduces risk of mood disorders, whereas the female emotion regulation system enables rapid processing of positive emotions, aiding in social contexts.

6.6.4.2 Anxiety

There was no significant interaction with anxiety, nor any differences between the high and low anxiety groups. This seems to contradict previous research in clinical samples that has suggested heightened anxiety is associated with disrupted fronto-amygdala connectivity (Banks et al., 2007b; Eden et al., 2015; Etkin & Wager, 2007; Gold et al., 2015; Motzkin et al., 2014; Shin et al., 2001). However, these data are in a sub-clinical population, and results do show a numerical trend of the high anxiety group exhibiting greater negative coupling in all regions of interest, particularly for fear and neutral conditions (section 6.5.3.3). It may therefore be that the pattern of dysfunctional fronto-amygdala connectivity seen in clinical populations is present in sub-clinical populations, but to a lesser degree.

The global numerical reduction in frontal connectivity (section 6.5.3.3) in the highly anxious group suggests that there may be reduced top-down inhibitory processes acting upon the amygdala within this group. In keeping with this, results from the GLM analysis of this data presented elsewhere (see Chapter 5) indicate that there is an absence of amygdala habituation over the course of this study in the highly anxious group. Taken together, the findings here tentatively support the notion that anxiety can impact emotion regulation processes, and this may be accounted for through some level of dysfunctional fronto-amygdala connectivity.

There is further evidence of the effects of anxiety on connectivity when looking at the FFG data, where anxiety significantly modulates overall ACC-FFG connectivity dependent on valence condition. Numerically greater negative connectivity between FFG and ACC is observed for the high anxiety group, with the greatest differences seen in the fear and neutral condition (see Figure 6.12, section 6.5.4.3.). This could suggest that highly anxious individuals allocate greater attentional resources, with increased FFG processing, when attending to neutral and fear stimuli. Indeed, there is evidence from previous studies which suggest a modulatory role of the FFG in emotional processing in anxious individuals. Patients with anxiety disorders exhibited increased FFG activation in response to negative stimuli.
(e.g. Etkin & Wager, 2007; Stuhrmann, Suslow, & Dannlowski, 2011), and an association between amygdala and anxiety scores in healthy individuals was only observed when FFG activation was controlled for (Pujol et al., 2009).

6.6.4.3 Summary of individual differences
Overall, it seems that individual differences in healthy populations do play a modulatory role in amygdala connectivity and emotion processing. However, it appears that gender has a greater direct influence on top-down frontal processes, whereas sub-clinical anxiety appears to have a weaker overall effect, and perhaps an indirect effect via modulation of attentional processes through FFG activation.

6.7 Limitations and implications
The three conditions in the study (fear, happy, neutral) will share similar processing networks and connectivity, differing perhaps only in the strength of some of the connections dependent on the emotion processed. It is this difference that was investigated in the PPI analysis, by using the main effect of each condition as the psychological component. However, this also means the analysis only indicates connectivity unique to each condition, and may overlook subtle nuances in the data, especially when looking further into individual differences. A further possible issue, highlighted in a review by O’Reilly et al. 2012, is the loss of power to detect an effect when relatively similar regressors are put into the same model. We would however predict significantly different connectivity patterns in fear, happy and neutral emotion processing. These issues may have contributed to the total lack of positive connectivity in this data. Although negative connectivity is expected for fronto-amygdala interactions during emotion processing from previous literature, there are other interactions such as amygdala-FFG reciprocal feedback which would be likely to show positive connectivity. Indeed, Frick, Howner, Fischer, Kristiansson, and Furmark (2013) observed increased positive coupling between amygdala-FFG during processing of fearful faces in patients with seasonal affective disorder, in contrast to the negative coupling observed in controls for these regions. It may be that these positive coupling relationships are characteristic of clinical anxiety or mood disorders only, with the present sub-clinical anxiety population not showing as strong connectivity patterns, or even the negative coupling observed in the control population above. Nonetheless, it may be useful in future to not only
look at the connectivity changes unique to each emotion condition, but also attempt to tease apart any shared variance these conditions may have.

The nature of the connectivity results from PPI analysis means that they are neither causal, nor directional. In a negative coupling, it is not known whether activity in the frontal area is inhibiting activity in the amygdala, or vice versa. Therefore, interpretation is based on a number of assumptions, including the results of previous research using different methods. In this example, it has long since been established that frontal regions in the brain exert top-down influence on amygdala and FFG activation in the brain (Banks et al., 2007b; Motzkin et al., 2014; Ochsner et al., 2002; Urry et al., 2006). As a result, negative coupling between these regions, with concurrent observations of increased activation in such regions, has been used to indicate a reduction in frontal activation. It is noted that this may not be the only interpretation based on the results themselves.

Some criticism has been levelled at PPI analysis in general as there could be chronometric disparity between the psychological measures (in real time) and the physiological aspects being measured (with a lag and temporal blurring) (O’Reilly et al., 2012). PPI attempts to find a haemodynamic response function (HRF) with the best fit to the data, but there is no way to confirm whether it represents the true HRF. This study uses a block design where this criticism is less of an issue, as the psychological measure (passive emotion) is constant over the block, and the physiological measure, after an initial lag, should be relatively constant as well. Bearing in mind PPI’s lack of causal or directional results, along with its possible chronometric problems, a future direction after initial connectivity analyses may be to perform more complex connectivity techniques such as Granger Causality or Dynamic Causal Modelling (DCM), which takes a non-linear approach to brain response (for details see Friston, Harrison, & Penny, 2003). Both of these methods can also be used to elucidate directionality of influence between areas, which may further affirm the notion of involvement of the top-down inhibitory processes which are interpreted in this study.

A final consideration in the present study is that the amygdala is an amalgamation of sub-nuclei, each with different connection patterns (Amunts et al., 2005; Roy et al., 2009), but it is treated as one whole seed region in this, and other, studies. In general, the superficial (SF) and laterobasal (LB) sub-nuclei have been found to be input regions for sensory information, whereas the centromedial (CM) has become established as an output region (Bzdok, Laird,
There is also some evidence of different fronto-amygdala connectivity in the nuclei, with variation in connectivity associated with specific psychopathic traits (Yoder, Porges, and Decety (2015)), suggesting that subtle emotion processing connectivity patterns could be overlooked if the sub-nuclei are not examined separately. An effect of hormone concentration on amygdala sub-nuclei activation in response to emotion stimuli (testosterone concentrations; Bos, van Honk, Ramsey, Stein, & Hermans, 2013) and on top-down frontal connectivity of sub-nuclei as rest (levels of estrogen; Engman, Linnman, Van Dijk, & Milad, 2016) also indicates that the influence of gender might be different across the sub-nuclei. From these recent studies it is clear that though the current study goes some way towards understanding the underpinning mechanisms in emotion processing, it is necessary to take it a step further and observe sub-nuclei connectivity. Parcellation was not carried out in this PPI analysis due to the complexity of the paradigm, although a later chapter in this thesis looks at parcellated amygdala connectivity at rest (Chapter 9). It would be useful for future studies to extend this to investigate sub-nuclei connectivity during emotion processing in more detail, and their relation to individual differences (e.g. gender and anxiety).

6.8 Conclusion

This study confirms a negative (inhibitory) fronto-amygdala connectivity, suggesting top-down inhibitory control, along with evidence of lateralisation in connectivity between the amygdalae for different valences which supports the dual processing model. The right amygdala appears to be primarily involved in threat related signal detection through a system of top-down control with the prefrontal cortex. In comparison the left amygdala appears to be primarily involved in a system of biological and social salience detection interacting with prefrontal cortex and sensory cortices. In addition, individual differences in gender and anxiety levels in a sub-clinical population have been shown to impact fronto-amygdala connectivity during emotion processing. Inclusion of gender differences appears to specifically modulate prefrontal connectivity dependent on valence. In particular, female participants show greater levels of frontal emotion appraisal and potentially rumination which could explain higher prevalence of emotion disorder in women compared to men. Sub-clinical state anxiety appears to modulate attention via sensory cortices (specifically FFG) during emotion processing and could theoretically point towards a key mechanism underpinning the dysregulation of emotion that is characteristic in clinical populations.
Overall, the present study shows that through observation of gender, lateralisation and anxiety differences important distinctions in connectivity patterns during emotion processing have been revealed that would otherwise be overlooked. This knowledge contributes to our understanding of both typical and atypical (subclinical) emotion processing. Furthermore, there is some discussion of how these results, and more in-depth knowledge of the individualised mechanisms of emotion processing, may aid in developing tailored treatments and therapies. The research presented here represents a good basis of understanding upon which further research can be done to look at the questions raised and overcome the limitations inherent to the paradigm and analysis technique used.
Chapter 7: Study 4  
Categorisation Analysis

7.1 Chapter Overview
This chapter uses a relatively new approach to analysing functional magnetic resonance imaging data. Multi-voxel pattern analysis is a multivariate approach, where data from each individual voxel is jointly analysed as distributed, whole-brain patterns of activity. It categorises fMRI data into experimental conditions based on these patterns of activity, producing overall accuracy of classification as well as highlighting individual areas whose activity best distinguishes between experimental conditions. This method can be used to reinforce or expand upon findings from typical univariate analytical methods by highlighting the most important neural areas for each condition, and aiding in characterisation of condition, or indeed diagnosis of clinical state, using fMRI data. Although key characteristics of mood disorders, especially anxiety, and theoretical causes have been identified, there are still no definitive diagnostic indicators, or biomarkers, which can be used to identify individuals at risk of developing mood disorders. This chapter uses data created from the GLM and PPI chapters (5 and 6 respectively) to look at areas specific to emotion processing of fear, happy and neutral in a healthy sub-clinical population. Results from this could be used as a basis for future multi-voxel pattern analysis studies in clinical populations aimed at diagnosis and tailored treatment dependent on neural areas of difference.

7.2 Introduction
A fundamental aim of this thesis is to assess the key characteristics of amygdala activation in response to emotion processing, and furthermore understand the subtle influences of individual differences such as gender and anxiety. These individual differences can then aid our understanding of mechanisms underlying more extreme cases of anxiety, with awareness of where processing differs from the norm contributing to the design of effective treatment. Neuroimaging technology has allowed research into emotion disorders to investigate whether the data collected with animal models and clinical lesion studies could also be observed in healthy sub-clinical cohorts. This in turn allowed a greater number of studies on emotion processing to be undertaken, meaning the field of emotional processing has evolved rapidly.
Functional MRI (fMRI) data offers an incredibly rich source of information; with each single scan containing haemodynamic information at one time point (e.g. every 2s) from approximately one to two hundred thousand voxels within the brain. Current statistical analysis of fMRI data largely relies on univariate models in order to hone in on functional activity in specific regions. Traditional methods of analysis are necessary in order to look at specific brain regions at a more micro-level, yet it is also important to take a more macro-approach looking at whole brain activation in response to stimuli as univariate models do not account for, or take advantage of, the complexities of the fMRI signal. In recent years, analysis models have been developed in order to address this issue by taking a more holistic approach using multi-voxel pattern analysis methods (MVPA; Haxby, 2012; Haxby et al., 2001; Yang, Fang, & Weng, 2012). This approach identifies the whole-brain activation patterns that are most discriminant for specific conditions, patterns which can be used to accurately categorise experimental condition based on underlying brain activity. In other words, algorithms are used to learn, categorise and then predict brain response to a specific stimulus or groups of stimuli, in essence reading the brain (Cox & Savoy, 2003). Indeed, such techniques have been successfully used to decode and categorise specific cognitive states (e.g. Mitchell et al., 2004; Polyn, Natu, Cohen, & Norman, 2005; Wang, Hutchinson, & Mitchell, 2003), but, Norman, Polyn, Detre and Haxby (2006) suggest that this method could also aid in understanding neural information processing. This is particularly important in situations where one particular region in the brain may contain multiple voxels which are involved in bidirectional coupling. Standard analysis may not be sensitive enough to detect such nuances, instead focussing on relationships between psychological variables and single voxels rather than multiple voxels interacting. MVPA methods use all the information contained within scanning data, and as such, are more likely to detect such subtleties emerging within the data.

Recent meta-analysis have demonstrated that emotion processing does not rely on regions working in isolation but rather large scale cortical networks in the brain working in parallel (Kober et al., 2008; Vytal & Hamann, 2010; see Chapter 1 literature review for more information). Research into emotion processing would therefore benefit from the use of MVPA methods, and Kragel and LaBar (2014) have discussed the importance of applying such techniques to advance emotion theory. The authors strongly endorse the notion that univariate methods are simply not good enough, in isolation, to unravel the complexities of emotional experiences. For example, there is clear evidence that the medial prefrontal cortex
(mPFC) is heavily involved in emotion related processes from univariate analysis of fMRI data. However, such studies indicate that the mPFC is involved in a wide range of different processes, from appraisal to orchestrating behavioural responses (see section 1.4.2.4). Recent studies using MVPA techniques have been able to expand upon the univariate data to show discrete neural signatures of different emotions which indicate that the mPFC play a more fine grained role, rather than simply activating in response to all emotions (Kassam, Markey, Cherkassky, Loewenstein, & Just, 2013; Kim et al., 2015; Saarimäki et al., 2015). This MVPA data fits with the functional connectivity analysis of the current dataset (PPI, Chapter 6), which indicated that the mPFC is differentially involved in emotion processing depending on the emotional valence state. Reduced fronto-right amygdala connectivity was observed in more threat related contexts, and reduced fronto-left amygdala connectivity in survival and socially salient contexts (see section 1.4.2.4.2). Studies such as those by Kassam et al (2013), Kim et al (2015) and Saarimäki et al (2015) demonstrate the worth of applying this analytical method to neuroimaging data in order to augment previous univariate analysis to better understand the underlying neural mechanisms of emotion processing.

Common MVPA classifier algorithms include Support Vector Machine (SVM; Boser, Guyon, & Vapnik, 1992; Vapnik, 2013) and maximum uncertainty linear discrimination analysis (MLDA; Sato et al., 2008). These algorithms allow researchers to extract discriminatory maps, which show neural areas with specific activation differences between experimental conditions, and have successfully been applied to fMRI data (Fu et al., 2008; Mourão-Miranda, Bokde, Born, Hampel, & Stetter, 2005; Mourão-Miranda et al., 2012; Z. Wang, Childress, Wang, & Detre, 2007). Of particular interest, Hahn and colleagues (2011) demonstrated that it was possible to use these MVPA methods in order to investigate discriminatory biomarkers of clinical illnesses, in this case depression. Mourão-Miranda and colleagues (2012) extended this further by using a pattern recognition approach to detect group differences between individuals with unipolar depression, bipolar depression and healthy controls, using neuroimaging data acquired when participants viewed happy or neutral faces. The results of these studies confirmed that MVPA techniques can be used to highlight abnormal brain activation in clinical populations which could aid diagnosis, and the techniques have also been successfully applied to other clinical disorders (e.g. to aid diagnosis of schizophrenia (Kasparek et al., 2011), autism (Ecker et al., 2010) and biopolar personality disorder ( Sato et al., 2012)). Furthermore, some studies have started to use SVM techniques to track transitional changes in the brain that relate to disease progression and prognosis, for
example the progression from mild cognitive impairment to Alzheimer’s disease in individuals (Davatzikos, Bhatt, Shaw, Batmanghelich, & Trojanowski, 2011), or the development of psychosis in those individuals identified as vulnerable or at risk (Koutsouleris et al., 2010). Indeed, a recent review on the subject proposed that SVM can be used in aid of diagnosis, treatment and even prevention in a number of clinical disorders (for details see Orrù, Pettersson-Yeo, Marquand, Sartori, & Mechelli, 2012).

7.3 Aims
This study aims to investigate whether emotion condition can be predicted by brain activation maps. SVM and MDLA classification methods will both be applied to fMRI data obtained from a sub-clinical population during a masked emotion paradigm. By using these techniques, we also aim to elucidate the neural areas whose activity is most discriminatory for the valence of the emotion processed. There is strong evidence to show that individuals diagnosed with clinical anxiety show different neural responses to emotional stimuli, especially threat related stimuli, compared to healthy controls. For example those with clinical anxiety have elevated amygdala activation, in particular to threatening faces (Etkin & Wager, 2007), and a negativity bias in response to a threatening stimulus (Mogg, Bradley, & Williams, 1995; Williams et al., 2009). Using a sub-clinical population, this analysis looks to identify activation patterns associated with specifically valenced emotion processing which can be used as the basis for future clinical work into maladaptive neural processing underlying both those clinically diagnosed and those at risk of developing mood disorders.

7.4 Method
The methods involved in data collection for this study are detailed in Chapter 3, and the pre-processing of the GLM and PPI data used here is detailed in chapters 5 and 6. Please refer back to sections 3.3.5, 5.4 and 6.4 for detail on design, participants, procedure, fMRI acquisition and pre-processing.

7.4.1 Data from first level GLM and PPI
Support vector machines (SVM) and maximum uncertainty linear discrimination analysis (MLDA) were conducted on individual (n=50) first-level general linear models (GLM) and
psychophysiological interaction analysis (PPI) contrast maps (for details of participant demographics and pre-processing to create first-level data see chapter 5 and 6, as above). For the PPI data, the connectivity data related to Left Amygdala and Right Amygdala was used. The contrasts used were fear vs baseline (Fear), happy vs baseline (happy) and neutral vs baseline (neutral). These were then used with the MVPA techniques to distinguish activation differences between each emotional state: Fear vs Happy, Fear vs Neutral and Happy vs Neutral. As discussed in the next section, small sample sizes can lead to reduction in the reliability and power of these analyses. It was therefore not possible to use MVPA analysis in this dataset to categorise anxiety groups or gender.

7.4.2 Support vector machines and maximum uncertainty linear discrimination analysis

Though SVM and MLDA methods utilise different mathematical algorithms, essentially both methods attempt to accurately categorise different groups/conditions of data using a hyperplane (classification boundary). SVM primarily attempts to identify the maximal point of difference between two discrete samples without making assumptions about the distribution of the data. To be precise, SVM relies on support vectors, which are the values closest to the hyperplane and thus the most discriminant observations for the classification. In contrast, MLDA tries to maximise the between/within group variance through observation of all of the data regardless of proximity to the hyperplane (for detail see Sato et al., 2011). The classification rule for these two analysis are thus determined orthogonally to the discrimination hyperplanes specified by \( W_{SVM} \) and \( W_{MLDA} \). The coefficients \( W_{SVM} \) and \( W_{MLDA} \) have been demonstrated to reveal the level of discriminative information in each voxel (for more information see Moura˜o-Miranda, Bokde, Born, Hampel and Stetter, 2005). Research has shown that there is no optimal method of analysis (Sato et al., 2009), with each having advantages and disadvantages dependent on the data. It is therefore deemed best to conduct both sets of analysis in order to determine which is most appropriate and accurate in relation to the specific study protocol.

A key concern with running such algorithms on fMRI data that has been previously analysed using GLM or PPI analysis is the issue of non-independence. Running non-independent statistical analysis on previously analysed data has been associated with exaggerated effect sizes and invalid results (Esterman, Tamber-Rosenau, Chiu, & Yantis, 2010; Mahmoudi,
Furthermore, a specific problem with these classifier methods is that in order to identify the most determinant voxels within the brain and then test accuracy of classification, the data must be split into two sets; a training dataset and a test dataset. In limited samples of data this can severely reduce the amount of information on which we can determine our classifier models, and can therefore reduce accuracy and lead to over-fitting of the model. A method which has been devised to overcome the issues of non-independence and over-fitting is leave one subject out cross validation (LOSO-CV; Esterman et al., 2010). One subject, or run, is left out of first level analysis and then used to test the classifier identified in the training stage. This process is repeated iteratively for all subjects (i.e. leaving each subject out one at a time) thus overcoming problems of non-independence and bias in testing the classifier.

### 7.4.3 Classification and Brain Mapping

The classification methods enlisted in this study closely follow those methods described in Sato et al. (2011). The procedure for SVM and MLDA is the same up until point 6, and very similar after this. In brief the following steps were used to analysis and process the data:

1. A gray matter mask was applied to the contrast data from the GLM and PPI first level data thus ensuring only voxels with interpretative gray matter coefficients were selected for further analysis.
2. A feature matrix was built with $X = \text{[Subject (rows) by Voxel (columns) Matrix]}$ and $Y = \text{[condition; one column, 0, 1, 2]}$
3. LOSO-CV method applied removing one subject (row) out as described above.
4. Training Phase: data of each subject [n=49 (as one has been left out in step 3)] are normalised so that the mean is equal to zero and the SD is equal to one.
5. Principle components analysis (PCA) applied to reduce computational load and time
6. SVM / MLDA computed using the feature matrix $X$ and the labels in $Y$
7. Hyperplane coefficients found: $W$ (this step is different for SVM or MLDA as briefly mentioned in section 7.4.2 and discussed in more detail in Sato et al. 2011; Sato et al. 2009)
8. Identified most discriminative voxels by ranking $W$ coefficients and apply feature selection retaining $10\%$ of most discriminative voxels
9. Retrain classifiers (SVM / MLDA) for the selected ‘most discriminant’ voxels
10. Test phase: process the subject left out in Step 3 for normalisation (Step 4) and feature selection (Step 8); predict condition of subject that was left out in the training stage using classifier from training phase (Step 9)
11. Iteratively loop through again from steps 3-10 LOSO-CV for all subjects
12. Classifier accuracy estimated using by ranking the leave-one-out values of each classifier to build receiver-operator-characteristic (ROC) curves
13. SVM / MLDA analysis applied with all subjects
14. Most discriminative voxels identified.

This procedure outputs the classification accuracy (%) by which the procedure can determine the category (e.g. is the activation related to ‘fear’ or to ‘happy’), along with a map indicating the top 10% most discriminatory voxels (the voxels whose activity most determines the experimental category). Accuracy above 59% in our SVM and MLDA models is deemed to be significant (p<0.05) when using the 10% most discriminant voxels.

7.5 Results
Table 7.1 shows the estimated classification rates using SVM and MLDA on the GLM and PPI data. The MLDA classifier accuracy was not above chance (>59%) for any of the categorisation analyses for either GLM or PPI. However, the SVM classifier achieved 60% accuracy in the fear versus happy condition for both the GLM analysis and the left amygdala PPI connectivity analysis. No other categorisation accuracy was above chance for SVM, and neither classifier method achieved greater than chance accuracies in relation to PPI data for the right amygdala.
Table 7.1: Classification accuracy for data from (a) GLM analysis and (b) PPI analysis. Results from both Support Vector Machine (SVM, top row) and Maximum uncertainty Linear Discriminant Analysis (MLDA, bottom row) classifier algorithms are shown. All results show the accuracies for the top 10% most discriminant voxels. * p<0.05

a. GLM Analysis

<table>
<thead>
<tr>
<th></th>
<th>Fear vs Happy</th>
<th>Fear vs Neutral</th>
<th>Happy vs Neutral</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVM</td>
<td>60%*</td>
<td>50%</td>
<td>59%</td>
</tr>
<tr>
<td>MLDA</td>
<td>56%</td>
<td>54%</td>
<td>55%</td>
</tr>
</tbody>
</table>

b. PPI Analysis

<table>
<thead>
<tr>
<th></th>
<th>Left Amygdala</th>
<th>Right Amygdala</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fear vs Happy</td>
<td>Fear vs Neutral</td>
</tr>
<tr>
<td>SVM</td>
<td>60%*</td>
<td>49%</td>
</tr>
<tr>
<td>MLDA</td>
<td>52%</td>
<td>51%</td>
</tr>
</tbody>
</table>

The 10% most discriminant voxels for classifying the fear condition compared to the happy condition are seen in Figure 7.1, for the left amygdala PPI data, these areas overlap with those identified in the GLM data described in section 7.5. The areas identified include key areas involved in emotion processing such as right amygdala (21 -6 -16); bilateral vmPFC (-23 62 9/ 21 64 12); vIPFC (-41 46 0) and bilateral insula (-41, 9, -12/ 27, 12, -5). There are also a number of other areas distributed across the brain including visual processing areas/ visual cortex (BA17/18/19 -19, -65 6/-19, -60, 4) and bilateral FFG (29, -33, -19/ -30, 33, -19); as well as areas involved in somatosensory processing – BA 5 19, -56, 66/ proprioception (BA7 -32, -56, 60); and Precuneus (Right, 12, -45, 43). This variability that is most evident in Figure 7.1 in the data may be due to the fact that the SVM analysis was only just above chance (60%).
Figure 7.1. Most discriminant (top 10%) voxels for distinguishing between fear and happy conditions, using the PPI results from the left amygdala. Note these areas overlap with those identified in the GLM data, key areas are described in section 7.5.

### 7.6 Discussion

This chapter sought to apply SVM and MLDA classification methods to whole-brain GLM and PPI connectivity data obtained from a sub-clinical anxiety population of participants who underwent a passive backward masked emotion paradigm. The possibility that this data could be used to predict the emotion condition participants were viewing, and to highlight the neural areas whose activity most discriminates between conditions, was explored. Initial results indicate that such prediction is not possible with the current dataset and paradigm, with only one SVM comparison (fear vs happy) showing above chance accuracy (60%, just above 59% threshold) for the GLM and PPI data. Although containing voxels in key areas to do with emotion processing, the Figure showing the 10% most discriminatory voxels for these comparisons from the PPI results (Figure 7.1), shows a diffuse pattern across the whole brain in keeping with minimal prediction accuracy. This demonstrates a level of sensitivity of the analysis to detect voxels within the ROIs, but with voxels also observed outside of the regions of interest it calls into question the specificity of the analysis when applied to this particular data. The other seven SVM comparisons were below chance, along with all MLDA comparisons.
The previous GLM and PPI chapters (chapters 5 and 6) have indicated a major modulatory role of individual factors such as gender and anxiety level, such that underlying differences may not be apparent without accounting for these factors. Valence effects were only apparent in women in the GLM analysis, specifically for the fear condition, with greater amygdala activation seen overall. Following on from this, the PPI connectivity results revealed that gender modulated fronto(dmFPFC)-amygdala connectivity with respect to valence, again with only female participants showing a valence effect on connectivity. Of particular note with respect to this chapter, this connectivity significantly differed between the fear and happy conditions, and the negative connectivity was most apparent in the left amygdala for females. Anxiety also exerted an influence, with higher anxiety linked to lower amygdala habituation over time in the GLM analysis, and numerically greater fronto-amygdala connectivity during the fear and happy conditions. Furthermore, there was a possible effect of attention such that fusiform face area (FFA) connectivity with precuneus was modulated by gender (greater in females) and connectivity with ACC modulated by anxiety (numerically greater coupling in the high anxiety group for the fear condition).

In considering other studies of similar scope to the present study, two recent studies have managed to produce high degrees of classifier accuracy for neural processing of discrete emotions using either movie clips and mental imagery (6 emotions; Saarimäki et al, 2015) or fractal images previously associated with emotions (5 emotions; Kim et al, 2015). Both of these studies demonstrate the possibilities of these techniques, and that with different emotion processing paradigms it is possible to predict the emotion condition a participant is viewing from their neural data. Furthermore, these studies were able to highlight neural areas whose activity discriminates between processing of the different emotions. Despite the low level of accuracy achieved by the classifiers in the present study, there is overlap between the most discriminatory areas in the previous studies (Saarimäki et al, 2015; Kim et al, 2015) and the current dataset. Areas of overlap include the medial prefrontal cortex, and precuneus with the amygdala and FFG overlapping to a lesser extent. However, in keeping with the low classifier accuracy in this study, there are discriminatory areas diffuse throughout the brain, not just in areas which overlap with previous studies. There could be a few reasons for the weaker results found here compared to previous studies, such as differences in paradigm design, stimuli used, individual variations in gender and anxiety influencing emotional processing, and sample size. These will be discussed in turn below.
As mentioned, one reason for the lack of convincing results may be the paradigm used. Previous studies have focused on using these techniques to categorise fMRI data during explicit perception of emotion (overt stimuli), rather than passively induced implicit emotions (using the backwards masking paradigm here) (e.g. Ethofer, Van De Ville, Scherer, & Vuilleumier, 2009; Kassam, Markey, Cherkassky, Loewenstein, & Just, 2013; Pessoa & Padmala, 2007; Sitaram et al., 2011; see Kragel & LaBar, 2014 for more detail). Furthermore it could be that the underlying neural processing of the emotions being observed (fear, happy and neutral) is similar enough, or that the activity differences are sufficiently minor that it is difficult to distinguish between them using these techniques. However, as mentioned, previous studies have been able to accurately distinguish between up to six emotion states (Saarimäki et al, 2015; Kim et al, 2015). The use of the backwards masking paradigm relying on passive, implicit emotion processing, may therefore elicit more subtle processing differences and may also be open to modulation by individual differences in processing. As such, the data used in this study may contain more individual variance, especially for ambiguous stimuli such as the neutral condition. This impression is substantiated by the fact that when this study used the top 5% most discriminant voxels for feature selection, as is more commonly used, significant prediction was no longer possible. We therefore opted to use the top 10% of voxels, but this means that the predictive power of the model may have been reduced as some redundant features/attributes may not have been removed.

Another consideration for the weaker results found presently compared to other literature is the type of stimulus used. From previous GLM and PPI analysis of the data it is apparent that observed valence effects are caused by different processing during the fear condition. Thus suggesting that only fear processing has sufficiently unique underlying features allowing for accurate classification with this dataset. Conversely it could be that emotional processing of fear is more consistent across participants, and over the length of the study, when compared to happy or neutral processing, which may be prone to individual differences or changes in attention across time. Saarimäki et al, (2015) were able to distinguish discrete patterns between a number of different emotions, albeit using a different paradigm and stimuli, endorsing the notion that it may be the consistency in processing across participants over time that is driving the variability in the results presented here. Indeed, inherent ambiguity of neutral stimuli has previously been discussed (see section 5.7), and there is evidence that this ambiguity may lead to highly individualised perception and processing of the stimulus. For example, highly anxious individuals tend to show a negativity bias, perceiving such stimuli as
more threatening than individuals who show lower levels of anxiety (Constans, Penn, Ihen, & Hope, 1999; Winton, Clark, & Edelmann, 1995). Only the comparison that did not use the neutral condition (fear vs happy) revealed accuracy above chance, so it could be that the use of neutral condition in the other comparisons (fear vs neutral; happy vs neutral) is causing variability in the results due to individual differences (such as anxiety) in the processing of the neutral stimuli. The neutral condition was included as a baseline in order to determine the effects of ‘fear’ or ‘happiness’ whilst controlling for general non-emotionally valenced face processing. However, it may be that this condition is not perceived as consistently emotionally void by some participants (especially those who are highly anxious), thus sharing some emotional processing activity with the fear and happy conditions and introducing variability. To counteract this possible variability in future studies an additional baseline of scrambled faces could be used to mitigate any effect of anxiety on the face processing control condition, also allowing further investigation of neutral face processing.

Extending this point previous GLM (chapter 5) and PPI (chapter 6) analysis of the data on which this chapter is based, do suggest that individual factors such as gender and anxiety may be creating variability in the data. Unfortunately, as previously discussed, the sample size means that SVM or MLDA analysis on groups split by gender or anxiety would be prone to problems of reduced power and over-fitting, reducing reliability. However, the previous analyses do have some bearing on the data in this chapter. Classification was only achieved above chance when attempting to distinguish between the fear and happy conditions, and for the PPI, only for the left amygdala. PPI analysis also revealed that females had a fronto-amygdala connectivity difference between fear and happy conditions only, and that this coupling was strongest in the left amygdala (for females; see section 6.6.4.1). This is in keeping with the stronger results seen for this comparison in the SVM analysis, but suggests analysis where groups could be split by gender may be more revealing. Gender has been used as a category in previous MVPA analyses (e.g. Ahrens, Hasan, Giordano, & Belin, 2014; Huf et al., 2014; L. Wang, Shen, Tang, Zang, & Hu, 2012). In particular, Wang and colleagues (2012) found sex differences in resting state regional homogeneity (ReHo; a measure of local synchronisation used in resting state studies, see Zang, Jiang, Lu, He, & Tian, 2004); male participants revealed greater resting ReHo in the right hemisphere and females had greater resting ReHo in the left hemisphere, after controlling for brain volume differences. Since gender differences are evident in the GLM and PPI there may be a gender effect in the present study. The discrepancies between current results and those of previous studies may
arise due to group composition. In a review article looking at applications of MVPA methods to emotion research, Kragel and Lebar (2014) identify key emotion related studies; of the eight studies reviewed only three show equal groups of male and female participants (Ethofer et al., 2009; Kotz, Kalberlah, Bahlmann, Friederici, & Haynes, 2013; Rolls, Grabenhorst, & Franco, 2009). Within the remaining five studies one omits information on participant gender (Sitaram et al., 2011), and the rest have far greater numbers of female participants compared to male participants (Baucom, Wedell, Wang, Blitzer, & Shinkareva, 2012; Kassam et al., 2013; Pessoa & Padmala, 2007; Said, Moore, Engell, Todorov, & Haxby, 2010). In our study gender groups are relatively balanced (21M, 29F) however without a larger sample possible gender effects can not be extrapolated.

A final factor which may account for inconsistencies between the present results and literature in the sample size used. The current dataset is much larger (n=50, 21M, 29F) than the average sample size of previous studies (average: 15; Range: 9-22; (Kragel & LeBar, 2014)). As previously discussed, use of such small samples in studies using MVPA and machine learning leads to elevated risk of bias such as over-fitting the model due to non-independence of the test and training dataset. Although techniques such as LOSO-CV have been devised to help alleviate the inherent problem of non-independence, when study samples are so small, data are still likely to be relatively homogenous and not as representative of the wider population. If the aim is a wider application such as identification of neural biomarkers of risk of emotion disorders, then the larger the sample the greater the test accuracy, reliability and generalisability. In consideration of this point, Huf et al. (2014) found that test accuracy dropped to 65% or below when a classifier model was applied to an external, more representative sample, even in studies which yielded classifiers of up to 80% accuracy within their study population. It may therefore be that the relatively variable results observed in the current dataset reflect the larger sample used, which may more accurately represent the inherent variation within the general population. Perhaps one conclusion of this study is that it is difficult to apply classification methods to categorise cognitive processes such as emotions which are susceptible to individual differences within the general population. A clear line of future research would be to look further into these individual differences in a larger sample or in a meta-analysis, to investigate whether these methods can be used to classify emotion processing in high and low anxiety groups or in males and females. In the present sample it is unfortunately not possible to split groups down into these categories to investigate further, in order to maintain statistical integrity as discussed above.
7.7 Limitations and implications

Possible reasons for the weaker categorisation seen in this study compared to previous studies have already been discussed including differences in paradigm design, stimuli used, sample size, and individual variations in gender and anxiety influencing emotional processing. This study was designed to elicit innate emotion processing void of top down control through use of the backwards masking paradigm. It is a more complicated design than the simpler stimuli used to overtly elicit emotion. All stimuli are backwards masked with a neutral face stimulus, and it could be that the overtly presented neutral stimuli interfere with the underlying processing of the covertly presented fear and happy conditions. However, previous GLM and PPI results reveal altered processing for the fear condition in particular, and the results here also demonstrate significant classification of the fear condition over and above happy. This suggests that even if interference has occurred, it has not entirely masked differences in emotional neural signatures. Using scrambled faces as the backwards mask has been suggested as a possible solution to this confound in this manuscript. In addition, varying the amount of time the masked stimulus is presented (33ms in this study) to occasionally capture overt, explicit processing of the emotional face stimulus could enable further analysis to discount the possible confound of the mask. In a similar vein, it may be more methodologically straightforward, and perhaps more meaningful, to simply classify between fear versus neutral and happy versus neutral category. By including the fear versus happy category it could be argued that some of the same signal is in more than one of the contrasts thus reducing the unique variance that can be observed and classified. Furthermore in considering future directions of research, it may be of interest to focus on a specific ROI (i.e. the amygdala) and see whether classification methods such as MVPA or SVM could be applied to provide more information about the ways in which this particular region may distinguish between fear and happy conditions.

If research is able to reliably identify discrete activation patterns globally, or in specific ROIs, associated with different emotions in a healthy population regardless of stimuli modality (auditory, visual, imagery etc) as suggested is possible by Saarimäki et al. (2015), then these neural substrates of emotion are likely to be altered in the case of mood disorders. One ramification of such a finding would be that individuals who develop emotion disorders may have a fundamental neural susceptibility to mood based disorders, and that emotional resilience (the ability to bounce back from adverse events) may be predetermined by their
biology. Being able to track transitional changes in the progression of such disorders through classifier models, as has been applied to different disorders previously (Davatzikos et al., 2011; Koutsouleris et al., 2010) will help answer such questions and pave the way for the most appropriate, individualised intervention and treatment methods.

7.8 Conclusion

Present results suggest that it is not possible to predict the induced emotion condition participants were passively viewing using the functional activation or connectivity fMRI data. Minor differences were seen between fear and happy condition using SVM analysis for both the GLM and left amygdala PPI. This is in keeping with the analyses conducted on the data in previous chapters (chapters 5 and 6). However, the accuracy of the models was only just above threshold, and the most discriminatory voxels were diffuse throughout the brain. The lack of results in this study is in contrast to previous studies on emotion processing, which were able to categorise up to six discrete emotions using functional data (Saarimäki et al, 2015; Kim et al, 2015). The discrepancies between this data and previous studies are likely to be due to paradigm design (using passive, implicit processing compared to explicit), stimuli used (backward masking with neutral faces), sample size (much larger sample than previous studies) and individual differences in anxiety and gender in the cohort. These in combination may have increased the variability and overlap between the conditions (in particular the neutral condition). However, the worth of being able to predict the emotion state of a healthy participant from their brain activation patterns remains considerable, especially in light of the current, and increasing, prevalence of mood based disorders globally. If a reliable model or template pattern of healthy brain activation to different emotions can be identified, then this can be used as a complimentary diagnostic tool for practitioners to use alongside clinical assessments. Furthermore, such classification maps can be used to track disease progression and create individualised treatments and interventions.
Chapter 8: Study 5
Cortical Thickness Analysis

8.1 Overview
The aim of this chapter is to determine whether sub-clinical anxiety is related to volumetric differences in regions such as the amygdala, hippocampus and medial prefrontal cortex in a comparable way to that observed in previous research on clinical populations. Evidence of volume reductions in sub-clinical anxious participants would suggest either an innate predisposition to clinical mood disorders in these individuals, or that a continuum of atrophy can occur across the anxiety spectrum, which may then exacerbate the symptoms and lead to longer term difficulties. Determining which it is would help illuminate the mechanisms behind clinical anxiety disorders, and serve as a biomarker for early detection of those with a pre-disposition for the development of such disorders. Preventative therapy can then be targeted towards these individuals, with the aim of reducing the number of individuals developing anxiety disorders, and thus in turn easing the burden on current national health care systems.

8.2 Introduction
There is no doubt that anxiety disorders can arise from environmental triggers; this is most evident in the case of post-traumatic stress disorder (PTSD; characterised by recurring and intrusive recollections relating to a traumatic event that the individual has been exposed to, heightened psychological distress, and physiological reactivity to memories of the event, Bisson, 2007). However, the extent to which an individual’s psychological and biological history can make them predisposed to develop such disorders, or indeed make them more emotionally resilient to similar environmental challenges is still not clearly established. Furthermore, whether such environmental experiences exacerbate an innate neurological abnormality, or whether these experiences themselves can initiate long-term neural plasticity changes within a previously unaffected brain is uncertain.

What is clear is that there is an underlying neurological basis for clinical mood disorders, with a characteristically hyper-responsive amygdala activation to anxiety-provoking stimuli...
Indeed, findings such as this have triggered wider investigation of neuroanatomical studies of anxiety. However, these studies have primarily focused on functional and biochemical evidence, with relatively few studies investigating the structural differences associated with anxiety. Despite their relative sparsity, studies which have explored structural variation in those with clinical anxiety and other related disorders have generally found evidence for structural differences in key fear circuitry areas (medial prefrontal cortex (mPFC), amygdala, insula and anterior cingulate cortex (ACC) (Etkin, 2012; J. E. LeDoux, 2000)) when compared to healthy controls. Structural studies have particularly focused on the PFC, especially the ventral medial prefrontal cortex (vmPFC; sometimes referred to as medial orbitofrontal cortex), whereby volume reduction of the PFC is observed in anxiety related disorders, such as post-traumatic stress disorder (PTSD; Keding & Herringa, 2014; Pitman et al., 2012; Rauch et al., 2003), obsessive compulsive disorder (OCD; Atmaca, Yildirim, Ozdemir, Tezcan, & Poyraz, 2007; Kang et al., 2004; Szeszko et al., 1999), social anxiety disorder (SAD; Syal et al., 2012; Talati, Pantazatos, Schneier, Weissman, & Hirsch, 2013), panic disorder (Roppongi et al., 2010; Sobanski et al., 2010; Vythilingam et al., 2000) and general anxiety disorder (GAD; Cha et al., 2014). The hippocampus has also been studied relatively extensively, as there is evidence that chronic stress responses and increased cortisol levels can cause hippocampal cells to atrophy (Lee, Jarome, Li, Kim, & Helmstetter, 2009; Magariños, McEwen, Flügge, & Fuchs, 1996; see chapter 1, section 1.6.2 for more information). For this reason, reduced hippocampal volume has been speculatively associated with the sustained or chronic stress response in individuals with anxiety related disorders. Structural studies have shown reductions in hippocampal volume in PTSD (Bremner et al., 1995; Gurvits et al., 1996; Pavić et al., 2007), generalised social phobia (Irle et al., 2010) and in relation to anxiety symptoms in patients with major depressive disorder (Campbell, Marriott, Nahmias, & MacQueen, 2014; Videbech & Ravnkilde, 2004; Weniger, Lange, & Irle, 2006). Furthermore, there is evidence that increased hippocampal volume may lead to better inhibition in response to anxiety provoking stimuli, with one study revealing a positive correlation between hippocampal volume and behavioural inhibition scores (Levita et al., 2014).

Current evidence for clinical anxiety related changes in amygdala volume is less compelling, with some studies observing left amygdala volume reduction in those with PTSD (meta-analysis by Karl et al. (2006), and others finding inconclusive volume and morphometry results in those with PTSD (more recent meta analyses by Kühn & Gallinat, 2013; Woon &
Hedges, 2009). Similarly, inconclusive findings have also been found in a small group of studies of healthy sub-clinical populations. Blackmon and colleagues (2011) investigated the correlation between structural volume variations and self-report anxiety scores from the Beck Anxiety Inventory (BAI), and State-Trait Anxiety Inventory (STAI) in a group of healthy participants, and found that amygdala volume was negatively correlated with BAI anxiety scores on both measures. This was also found in an earlier study looking at the relationship between voxel-based morphometry (VBM) differences and self-report STAI (Spampinato, Wood, De Simone, and Grafman (2009). However, other recent studies using similar methods have either found no correlation between amygdala volume and STAI (Kühn, Schubert, and Gallinat (2011) or a positive correlation between amygdala volume and STAI (Baur, Hänggi, and Jäncke (2012). Baur and colleagues suggest that this contradictory finding is due to differences in sampling (in particular, age differences) and analytical method used compared to other studies, and highlights the need for further research in order to disentangle these inconsistencies. Indeed, the mixed pattern of results regarding amygdala volume is somewhat perplexing in light of the robust evidence of amygdala involvement, and characteristic hyper-responsivity, in emotion disorders noted previously (Etkin & Wager, 2007). This could be due to the paucity of studies looking at structural changes, an inequality in comparison to the numerous functional investigations into models of anxiety highlighted by Blackmon et al., (2011). These authors further highlighted that very few studies have investigated such structural changes in sub-clinical populations, an area which merits more attention due to utility in scrutinising models of anxiety.

8.3 Aims

The current study aims to build on the literature from clinical populations by investigating prefrontal and hippocampal structural alteration in a subclinical population. Furthermore, it aims to address the discrepancies within the literature with regard to alterations in amygdala volume in relation to anxiety using a self-report scale in a large sub-clinical population. It is hoped that this work will not only bring some clarity to the literature about structural differences related to anxiety, but that it will aid in understanding the mechanisms involved in individuals at risk of transitioning from sub-clinical to clinical anxiety.
8.4 Method

The methods involved in data collection for this study are detailed in Chapter 3. The following methodology section will only briefly touch on acquisition, with more detail on the specific analysis and demographics used in this chapter. For more detail on design and acquisition, please refer back to section 3.3.5.

8.4.1 Participants

In brief, morphological data were collected from 57 individuals. One participant was subsequently excluded from volumetric analysis due to being an outlier in age. Although they were just within the cut-off for significant outliers of three standard deviations from the mean (z= 3.24), exclusion was warranted due to clear evidence of structural changes over time in healthy aging (e.g. see Fjell & Walhovd, 2010). As such, it was decided to be conservative in the use of age outliers in this study in order to reduce possible confounds. This resulted in 56 participants (aged 19-39 years, \( \bar{x} = 24.43, \pm 4.87; \) 25 male, 31 female), who completed the Hospital Anxiety and Depression Scale (HADS). Participants were categorised into high and low anxious groups using the anxiety subscale (HADS_A), such that there were 18 in the high anxious group (score ≥11; aged 19-30, \( \bar{x} = 24.78\pm3.78, \) 6 male, 12 female) and 38 in the low anxious group (score: 0-10; aged 19-39, \( \bar{x} = 24.26\pm5.35, \) 19 male, 19 female). In addition, a subset of the initial sample (N=40; aged 24.8±3.9, 9 male, 31 female) also completed the short version of the State-Trait Anxiety Inventory (STAI-6; scores ranged from 18-66.66, \( \bar{x} = 39.03\pm13.24). \) As discussed in the methods chapter (chapter 3), HADS is used as a screening tool for clinically significant anxiety, whereas STAI-6 used here is a measure of anxiety. As HADS_A is the typical measure used in screening for sub-clinical anxiety, it was the clear choice to split participants into high and low anxious groups in the majority of this thesis. However, this structural chapter is looking at correlations between anxiety and brain morphology, therefore the widely used STAI-6 was also utilised as an alternative measure of anxiety. Participants were also categorised into high (n=9; aged 20-32, \( \bar{x} = 26.89\pm4.17, \) 2 male, 7 female) and low (n=31; aged 19-39, \( \bar{x} = 24.74\pm5.26, \) 7 male, 24 female) groups using the STAI-6. Cut-offs to determine these groups come from the Spielberger STAI manual (Spielberger, 1983; Spielberger, 2010). Normative data for different age groups is given and scores above the 90th percentiles for the age group observed presently is taken to indicate very high levels of anxiety. The STAI (and STAI-6) measures both state and trait anxiety with cut-off raw scores ranging between 49 and 52 depending on...
gender and state/trait (see Table 8.1). A conservative cut-off of 49 and above for state and trait anxiety was taken for ease of grouping participants. The STAI-6 is used to identify the presence and severity of current anxiety symptomology and propensity towards anxiety and for the purposes of this chapter total STAI-6 scores were observed (see section 3.3.3.3 for more details on calculating STAI-6 scores) representing a combination of trait and state anxiety.

Table 8.1. Showing scores for the 90th percentile state and trait anxiety scores from normative data in the Spielberger STAI manual. The Table shows both state and trait cut-offs for men and women. In this study a conservative cut-off of 49 for all participants was taken to group them into ‘high’ or ‘low’ anxiety.

<table>
<thead>
<tr>
<th>Normal adults aged 19-39</th>
<th>State</th>
<th>Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>51</td>
<td>50</td>
</tr>
<tr>
<td>Female</td>
<td>52</td>
<td>49</td>
</tr>
</tbody>
</table>

8.4.2 MRI Acquisition
Images were acquired on a 3T scanner (Trio, Siemens, Erlangen, Germany) with a 32 channel array head coil. High resolution 3D brain MRI images were acquired using a T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) pulse sequence (TR 1830ms, TE 3.03ms, Inversion Time 1100ms, 11° flip angle, FOV 256mm, 160 slices, voxel size 1 x 1 x 1mm³, in-plane matrix 256 x 256).

8.4.3 Volumetric Data
Volumetric data were calculated using the FreeSurfer (v.5.3.0) software package (http://surfer.nmr.mgh.harvard.edu/). FreeSurfer automatically segments, parcellates, and labels unique structures within the brain using probabilistic mapping based on a labelled training set (for full methodological description see Fischl et al., 2002, 2004). In brief, pre-processing of the T1-weighted images involved non-uniform intensity correction, affine registration using the Talairach transform to map the images into the Montreal Neurological Institute (MNI) space, white matter intensity normalization and skull-stripping to remove non-brain tissue. Final segmentation is based on labelling voxels, determined using algorithms based on collected probabilistic atlases of image intensities and
structure location (subject-independent probabilistic atlases were constructed from a training data set previously labelled by hand and normalised). Labels for a given voxel in each subject’s brain image were calculated using a Bayesian prior (the subject-independent probabilistic atlases) and based on the maximum \textit{a posteriori} probability (Fischl et al., 2002, 2004; Oscar-Berman & Song, 2011).

\textbf{8.4.4 Statistical Analysis: Freesurfer}

Whole-brain volumetric analysis was performed on each voxel using the general linear model (GLM) embedded in the QDEC (Query, Design, Estimate, Contrast) interface in order to produce volumetric maps illustrating any group differences. On account of reliable prior evidence of correlations between age and subcortical volumes (Walhovd et al., 2011), age of participant was included as a covariate in statistical analysis of the structural data. In addition, partial correlations between HADS_A scores, STAI-6 scores and volumetric data were calculated across participants. The results were multiple comparison corrected using False Discovery Rate (FDR) at a threshold of \( p<0.05 \).

\textbf{8.4.5 Statistical Analysis: ROI Extraction}

Volumetric information was extracted from seven bilateral regions of interest (ROIs; amygdala, hippocampus, precuneus, fusiform gyrus (FFG), anterior cingulate cortex (ACC), ventromedial prefrontal cortex (vmPFC) and dorsomedial prefrontal cortex (dmPFC)) to be analysed using IBM SPSS (version 21.0). A multivariate analysis of covariance (MANCOVA) was carried out to look at ROI volume differences between high and low anxious participants (categorised by both the HADS_A and the STAI-6), controlling for age and intracranial volume. A number of follow up univariate analysis of covariance (ANCOVAs) were carried out separately for each of the seven ROIs (again, categorised by both the HADS_A and the STAI-6), controlling for age. In addition, partial correlation analysis was performed to look at the relationship between the volumetric data in each ROI and the individual HADS_A (\( n=56 \)) and STAI-6 (\( n=40 \)) scores. Of note, the masks used to extract data from the ROI’s were created using the automated labelled system, which has been shown to be reliable and valid in its determination of anatomical boundaries (Boes et al., 2009; Desikan et al., 2006; Riley, Moore, Cramer, & Lin, 2011). The mask for the dmPFC was generated using the superior frontal cortex label, the ACC mask was a combination of
the rostral ACC and caudal ACC, and the vmPFC mask was generated from a combination of the medial orbitofrontal cortex and the lateral orbitofrontal cortex.

8.5 Results

8.5.1 Descriptive Statistics
HADS-A scores ranged from 0-20 (mean 8.54±4.57) across all participants, with the scores for the high anxiety ranging from 11-20 (mean 14.00±2.79) and ranging from 0-10 (mean 5.95±2.45) for the low anxious group. Both groups had a similar gender balance (χ² (1) = 1.37, p =.24, φc =0.16), handedness (χ² (2) = 0.48, p =.79, φc =0.09) and age (U=300.00, p=.46, r=-.10).

STAI-6 scores ranged from 18-66.66 (mean 39.03±13.24) across all participants, with the scores for the high anxiety ranging from 50.00-66.66 (mean 57.78±6.45) and ranging from 18-46.66 (mean33.59±8.98) for the low anxious group. Both STAI-6 groups had a similar gender balance (χ² (1) = 0.001, p =.98, φc =0.004), handedness (χ² (2) = 0.23, p =.89, φc =0.08) and age (U=99.00, p=.19, r=-.21). Despite the different group composition of high and low anxiety participants based on HADS_A and STAI-6 scores, the raw STAI-6 scores of the sample sub-set (n=40) were highly correlated with their HADS_A scores (r (40) = 0.68, p <0.001).

8.5.3 Volumetric results: Freesurfer
There were no group differences (between gender groups or anxiety groups) when controlling for age. Nor were there any significant partial correlations between HADS_A scores, STAI-6 scores and volumetric data, when correcting for multiple comparisons

8.5.4 Volumetric results: ROI analysis
The MANCOVA revealed no differences in volume between the anxiety groups for any of the seven ROIs, both when using the HADS_A (V=0.27, F(14,40)=1.07, p=.41, ηp²=0.27) and when using the STAI-6 in a sub-group of participants (N=40; V=0.24, F(14,24)=0.55, p=.88, ηp²=0.24).
However, follow up univariate ANCOVA analysis did demonstrate group differences in volume, when using the HADS-A, for the left amygdala ($F(1,53)=4.53, p=.04, \eta^2_p=0.08$), right amygdala ($F(1,53)=4.22, p=.05, \eta^2_p=0.07$) and right vmPFC ($F(1,53)=4.78, p=.03, \eta^2_p=0.08$). In all cases, the ROI volume was smaller in the high anxiety group (left amygdala: 1394.5 ± 44.3mm$^3$, right amygdala: 1408.2 ± 55.4mm$^3$, right vmPFC:12887.8 ± 363.6mm$^3$) compared to the low anxiety group (left amygdala: 1508.9 ± 30.4mm$^3$, right amygdala: 1546.2 ± 38.1mm$^3$, right vmPFC:13853.5 ± 250.1mm$^3$; see Figure 8.1). No other ROIs showed a significant difference between the high and low anxiety groups when using the HADS-A (see Table 1), and there were no significant univariate relationships when using the STAI-6 (see Table 1).

![Figure 8.1](image.png)

**Figure 8.1.** Volume differences between high and low anxiety groups, categorised using the HADS_A. (a) volume differences for left (red) and right (blue) amygdala, (a) volume differences for right vmPFC. Bars represent mean±SE *p<0.05, ** p<0.01. HADS_A: hospital anxiety and depression scale, anxiety subscale; vmPFC: ventromedial prefrontal cortex.
Table 8.2. ANCOVA results showing group differences for every ROI, when using HADS_A (middle column, main sample: n=56) and STAI-6 (right column, sub-sample: n=40). Significant differences are shown in bold type and shaded cells. HADS_A: hospital anxiety and depression scale, anxiety subscale; STAI-6: state-trait anxiety scale.

<table>
<thead>
<tr>
<th>Region</th>
<th>HADS_A (N=56) ANCOVA</th>
<th>STAI-6 (N=40) ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Amygdala</td>
<td>$F(1,53)=4.53, p=.04, \eta^2_p=0.08$</td>
<td>$F(1,37)=0.43, p=.52, \eta^2_p=0.01$</td>
</tr>
<tr>
<td>Right Amygdala</td>
<td>$F(1,53)=4.22, p=.05, \eta^2_p=0.07$</td>
<td>$F(1,37)=0.23, p=.64, \eta^2_p=0.01$</td>
</tr>
<tr>
<td>Left Hippocampus</td>
<td>$F(1,53)=0.05, p=.82, \eta^2_p=0.01$</td>
<td>$F(1,37)=0.15, p=.70, \eta^2_p&lt;0.01$</td>
</tr>
<tr>
<td>Right Hippocampus</td>
<td>$F(1,53)=.79, p=.34, \eta^2_p=0.02$</td>
<td>$F(1,37)=0.45, p=.51, \eta^2_p=0.01$</td>
</tr>
<tr>
<td>Left vmPFC</td>
<td>$F(1,53)=2.13, p=.15, \eta^2_p=0.04$</td>
<td>$F(1,37)=0.27, p=.61, \eta^2_p=0.02$</td>
</tr>
<tr>
<td>Right vmPFC</td>
<td>$F(1,53)=4.78, p=.03, \eta^2_p=0.08$</td>
<td>$F(1,37)=2.09, p=.16, \eta^2_p=0.05$</td>
</tr>
<tr>
<td>Left dmPFC</td>
<td>$F(1,53)=0.53, p=.47, \eta^2_p=0.01$</td>
<td>$F(1,37)=0.03, p=.87, \eta^2_p&lt;0.01$</td>
</tr>
<tr>
<td>Right dmPFC</td>
<td>$F(1,53)=1.21, p=.28, \eta^2_p=0.02$</td>
<td>$F(1,37)=0.02, p=.90, \eta^2_p&lt;0.01$</td>
</tr>
<tr>
<td>Left ACC</td>
<td>$F(1,53)=3.14, p=.08, \eta^2_p=0.06$</td>
<td>$F(1,37)=0.87, p=.36, \eta^2_p=0.02$</td>
</tr>
<tr>
<td>Right ACC</td>
<td>$F(1,53)=0.02, p=.88, \eta^2_p&lt;0.01$</td>
<td>$F(1,37)=0.02, p=.88, \eta^2_p&lt;0.01$</td>
</tr>
<tr>
<td>Left Precuneus</td>
<td>$F(1,53)=2.36, p=.13, \eta^2_p=0.04$</td>
<td>$F(1,37)=0.10, p=.76, \eta^2_p&lt;0.01$</td>
</tr>
<tr>
<td>Right Precuneus</td>
<td>$F(1,53)=1.01, p=.32, \eta^2_p=0.02$</td>
<td>$F(1,37)=0.002, p=.97, \eta^2_p&lt;0.01$</td>
</tr>
<tr>
<td>Left FFG</td>
<td>$F(1,53)=1.65, p=.21, \eta^2_p=0.03$</td>
<td>$F(1,37)=1.30, p=.26, \eta^2_p=0.03$</td>
</tr>
<tr>
<td>Right FFG</td>
<td>$F(1,53)=2.26, p=.14, \eta^2_p=0.04$</td>
<td>$F(1,37)=0.63, p=.43 \eta^2_p=0.02$</td>
</tr>
</tbody>
</table>

Partial correlations with the seven ROI revealed a significant negative correlation between HADS_A scores and left amygdala volume ($r(53) = -0.30, p = 0.03$); bilateral vmPFC (left: $r(53) = -0.32, p=0.02$; right: $r(53) = -0.37, p=0.005$ ) and the right dmPFC ($r(53) = -0.31, p=0.02$; see Figure 8.2), as well as a significant negative correlation between STAI-6 scores and right FFG volume ($r(37) = -0.34, p = 0.03$); see Figure 2). No other ROI volume yielded a significant correlation with either HADS_A or STAI-6 scores.
Figure 8.2. Partial correlation scatterplot showing negative correlation, controlling for effect of age, between HADS_A scores and (a) left amygdala, (b) left vmPFC, (c) right vmPFC and (d) right dmPFC and (e) between STAI-6 scores and right FFG. HADS_A: hospital anxiety and depression scale, anxiety subscale; STAI-6: state-trait anxiety scale; FFG, fusiform gyrus; dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex. The HADS data represents the whole sample, (n=56), the STAI-6 data represents a subset of participants (n=40).

8.6 Discussion

This study looked to address the paucity of research into structural correlates of anxiety, in particular studies which use sub-clinical populations (e.g. Blackmon et al., 2011). It revealed evidence of prefrontal cortex volume reduction (bilateral vmPFC, right dmPFC) related to increased anxiety, which was also observed when the data were categorised into high and low anxious groups (right vmPFC). Furthermore, despite previously mixed results, this study found evidence of a relationship between reduced amygdala volume (left amygdala) and anxiety in a sub-clinical population. Bilateral amygdala volume reduction which has not previously been reported in sub-clinical healthy controls was observed in high anxious groups when the sample was split into groups. No significant results were found for hippocampus volume. In addition, the results as discussed above were only seen when the HADS anxiety subscale was used, with increased anxiety measured by the STAI-6 only correlating with a reduction in right fusiform gyrus volume. Due to the nature of the volumetric changes expected from the literature, and the exploratory nature of such an analysis, it was deemed most insightful to first look at any general differences in brain morphology across ROI (MANCOVA), before investigating individual differences in each ROI (ANCOVA) where
previous literature shows some evidence of individual differences. Thus, although MANCOVA did not yield significant results, individual ANCOVA were still run in order to give insight into this dataset. However, the interpretation of any differences in the ANCOVA must be tempered by the lack of overall difference in the MANCOVA. This is especially true as some of the ANCOVA results would not survive multiple comparison correction. More stringent criteria would be required in future replications perhaps focusing specifically on the key results identified within this study.

8.6.1 Comparison to previous literature using STAI

Previous studies investigating volumetric difference with relation to anxiety in sub-clinical populations used STAI as a measure of anxiety (Baur, Hänggi, & Jäncke, 2012; Blackmon et al., 2011; Kühn et al., 2011; Spampinato et al., 2009), although Blackmon and colleagues (2011) used STAI measures in conjunction with BAI measures. These found varying results in the relationship between anxiety and amygdala volume, with Baur and colleagues (2012) noting that the differences may be due to different age groups studied and analytical tools used. In order to address this, and allow comparison to these previous studies, a sub-set of participants in the current study completed the STAI measure of anxiety (see Table 8.3 for comparison). The present study closely matches Baur and colleagues (2012) study in mean age, STAI and analysis methods, yet does not support their findings. No relationship between STAI anxiety and amygdala or hippocampal volume was found, with only a relationship with right FFG reported.

Indeed, the finding of a correlation between increased STAI anxiety score and reduced right FFG volume is a novel one when compared to any of the previous studies. However, there is some research suggesting that the trait scale of the STAI includes items that more accurately reflect measures of depression as well as ‘pure anxiety’ items (Bieling, Antony, & Swinson, 1998), and reduced FFG volume has been shown in healthy individuals with a cognitive vulnerability to depression (CVD) (Zhang et al., 2012). The possibility that the results in the present study may relate to something other than anxiety differences is further endorsed by the lack of group differences in volume when splitting participants into high and low anxiety groups using the STAI. Therefore, when looking at the STAI results alone, this study has added to the mixed results of previous studies by adding more variety in findings. It could be a general weakness inherent to studies reliant on self-report measures of anxiety. However, the results using HADS are much clearer with regards the relationship between structure and
sub-clinical anxiety suggesting it may be a better measure than STAI for these kinds of studies.

Table 8.3. Overview of the current study in relation to four previous studies looking at anxiety and neural structure in sub-clinical population.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>STAI scores</th>
<th>Analysis method</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>Mean±S.D</td>
<td>Range</td>
<td>Mean±S.D</td>
</tr>
<tr>
<td><strong>Current study</strong></td>
<td>19-39</td>
<td>25.2±5.1</td>
<td>18-66.7</td>
</tr>
<tr>
<td><strong>Spampinato et al. 2009</strong></td>
<td>No range</td>
<td>28±5.6</td>
<td>No range</td>
</tr>
<tr>
<td><strong>Kühn et al. 2011</strong></td>
<td>19-47</td>
<td>30.5 (no S.D.)</td>
<td>No range</td>
</tr>
<tr>
<td><strong>Baur, Hänggi, Langer, et al. 2012</strong></td>
<td>20-37</td>
<td>24.9±4.6</td>
<td>25-55</td>
</tr>
</tbody>
</table>

*(also based on Fischl et al. (2002) methods)*

### 8.6.2 Sub-clinical anxiety related structural differences when using HADS

As stated above, the majority of results from this study come from the use of HADS anxiety subscale (HADS_A) as a measure of anxiety. The relationship between increased anxiety and reduced left amygdala volume was also found by Blackmon et al. (2011) and Spampinato et al. (2009), with neither previous study observing a correlation between anxiety and right amygdala volume. It has been suggested that the left and right amygdala are functionally specialised, with the left amygdala being more involved in salience detection and the right amygdala being involved in more direct, immediate threat response systems (Ohman, 2005; Romanski & LeDoux, 1992; Shi & Davis, 2001, also see chapter 5 & 6). The observation of
higher anxiety being related to reduced volume in the left amygdala may therefore suggest that volumetric reductions in the amygdala may lead to less efficient appraisal and emotional processing, which in turn produces increased anxiety levels. Indeed, this may be what is underlying the characteristic amygdala hyper-responsivity observed in a previous chapter in this thesis (Chapter 5), and in previous studies in clinical populations (Holzschneider & Mulert, 2011; Rauch, Shin, & Phelps, 2006; Rauch, Shin, Whalen, & Pitman, 1998; Shin & Liberzon, 2010b). In contrast, those with low anxiety levels would have a larger amygdala, a larger number of neurons, and more efficient appraisal and emotion processing.

This study also observed that reduced mPFC (bilateral vmPFC, right dmPFC) was related to higher anxiety, as also reported in Spampinato et al. (2009). As discussed previously in the literature review and the PPI chapter (chapters 1 and 6), Etkin, Egner, and Kalisch (2011) put forward a framework of frontal-amygdala connectivity in which they suggested that connectivity with the dmPFC is related to appraisal, whereas connectivity with the vmPFC relates to generating emotion responses following appraisal. Furthermore, frontal areas are known to exert inhibitory top-down control on the amygdala (Banks et al., 2007b; Motzkin et al., 2014; Ochsner et al., 2002; Urry et al., 2006). Yet again, this suggests that a reduction in volume of these frontal executive areas may result in less effective emotion processing and increased anxiety levels. A reduction in dmPFC volume would reduce functional ability to appraise an incoming stimulus, and volume reduction in both dmPFC and vmPFC would diminish the inhibitory function of the amygdalae, decreasing effective and adaptive processing properties and potentially overloading an already inefficient system (in the case of left amygdala). Reduction in top-down appraisal and control from the dmPFC on the left amygdala may also explain the negativity bias in those with increased anxiety (Richards et al., 2002), with the amygdala continuing to respond to stimuli which have not been effectively appraised for salience due to potential ambiguity, resulting in overtly negative behavioural responses (as discussed in chapter 5). In support of this, previous chapters have found a reduced ability to habituate in the amygdalae in those with high anxiety (Chapter 5), and some evidence of altered dmPFC-amygdala connectivity suggesting a lack of top-down control (Chapter 6).

When categorising participants into the high or low anxiety group using the HADS_A, there were similar, but not identical, differences in amygdala and mPFC volume. Highly anxious participants had significantly smaller bilateral amygdala and right vmPFC compared to the low anxious group. These results support the findings and discussion of the correlation
analysis, whereby reduced volume of key structures in highly anxious individuals’ results in an inefficient emotional regulation system. Of note, the areas with reduced volume are similar in the group-wise analysis, but not identical. In particular, the dmPFC and left vmPFC are not significantly different between the high and low anxiety group, whereas there is now a significant difference in right amygdala volume.

Although it should also be noted that group sizes were such that a disproportionate number of participants classed as low anxiety using the HADS_A (39 to the 18 highly anxious individuals), differences in results are more likely due to the analyses themselves and the way volume is distributed across the data. Correlation analysis looks at a continuum of anxiety and its relation with structure, with subtle variations in structure occurring within the population. A further analysis was conducted to look at categorical group (high/low anxiety differences), using the HADS as a cut-off criteria. This analysis is more in keeping with clinical diagnosis, which assumes that those above the cut-off are fundamentally different from those below. It is more useful for finding structural biomarkers of clinical anxiety or those at risk of developing clinical anxiety, but is less likely to find the subtle differences seen in the correlational analysis. Variability in volume of right dmPFC and left vmPFC may be spread over a continuum among participants washing out any significant differences seen between the two groups. Conversely, the differences in right amygdala volume may be masked as only the most highly anxious individuals present with reduced right amygdala. This is an interesting point to consider, since other studies tend to run correlational analysis in isolation, where group-based analysis could provide additional information about categorical structural changes. A reduction in bilateral amygdala volume has not previously been reported in sub-clinical healthy controls, and when linked with mPFC volume reductions they could be potential indicators of those at risk of developing anxiety disorders.

Overall, the analysis with HADS_A anxiety scores suggest a link between heightened anxiety and reduced neuronal count in key emotion processing areas, leading to inefficient appraisal and response mechanisms in both the short and long-term. However, the direction of this effect is unclear. Is the reduction in left amygdala/PFC volume biologically innate, subsequently leading to altered emotional processing, or does the sustained stress response in individuals with higher levels of anxiety causes such reductions? The high genetic heritability observed in these regions (left amygdala:0.80 and bilateral mPFC: 0.83; Peper et al. 2007; Pol et al. 2006) supports the former explanation, with perhaps some minor influence of environmental factors on these regions. Consequently, it is reasonable to speculate that the
reduced volume in these areas was genetically inherited in our sub-clinical population, and could therefore provide early biomarkers of individuals susceptible to high levels of anxiety. It must be noted that although the literature would advocate finding a reduction in hippocampal volume related to anxiety measures, both this study and the majority of previous studies present no evidence of this relationship in subclinical populations. This may suggest that volumetric changes in hippocampal volume are specifically associated with clinical populations, with the chronic stress observed in sub-clinical populations not yet resulting in structural changes in this area. In addition, the hippocampus has only a moderate genetic heritability (40–69%; Peper et al. 2007), suggesting this brain structure is more susceptible to environmental influences than other areas in the brain such as the amygdala and PFC.

8.7 Implications

This study demonstrates a relationship between sub-clinical state anxiety and reduced volume of PFC and amygdala, regions overlapping with previous sub-clinical and clinical research into anxiety. The amygdala and mPFC have long since been considered key regions in emotion regulation, with suggestion that the dysfunctional emotion regulation observed in clinical disorders results from a disrupted fronto-amygadal network in the brain (Etkin and Wager, 2007). Indeed, that a difference in these areas are observed between high and low anxiety groups in our sub-clinical population point towards potential structural biomarkers of individuals who are at risk of developing a clinical mood disorder. Furthermore, the finding highlights the utility and necessity of research into anxiety related structural changes in healthy populations, to give insight into dysfunctional processing that leads to anxiety disorders and enable development neurological biomarkers for early detection of those showing a predisposition for such disorders. It should be noted that previous subclinical research has ordinarily focused on trait-based anxiety measures (e.g. the trait measure from the STAI), whereas this study used both the STAI-6 and state-based HADS. That the main results were observed when using the HADS suggests this may be a better measure to use in the future.

In addition to early detection, these findings may also aid in highlighting the possibility of tailored treatment and prevention programmes. Results support the idea that dysfunctional anxiety emerges from disruption in mediation between the amygdala and mPFC during emotion processing. The association between anxiety disorders and top-down control from frontal areas on structures such as the amygdala has led to focus on therapies such as
cognitive behavioural therapy (CBT), which target cognitive restructuring, and psychoeducation techniques that aim to modulate feelings and behaviours and promote new learning. In a meta-analysis of literature, Porto et al. (2009) found that successful CBT was associated with reduced amygdala activation in patients after treatment. This finding has been confirmed in a more recent systematic review and meta-analysis of various other psychotherapies for anxiety disorders (Newby, McKinnon, Kuyken, Gilbody, & Dalgleish, 2015). There is research emerging showing that psycho-therapeutical treatments such as CBT may enact their effect by causing neural plasticity changes, modifying the neural circuits involved in anxiety disorders (for review see Barsaglini et al., 2014; Etkin, Pittenger, Polan, & Kandel, 2005; Jokić-Begić, 2010; Porto et al., 2009). In brief, the novel and repeated behaviours promoted by these therapies may result in anatomical changes in the brain by increasing demands on particular brain structures, with the most frequently used structures successfully competing for cortical space using neural plasticity (Jones, 2000).

Furthermore, there is some evidence that these therapeutic interventions may be able to reverse the cerebral atrophy underlying the volume reductions in clinical disorders. De Lange et al. (2008) demonstrated some reversal in lateral prefrontal cortex atrophy caused by chronic fatigue syndrome, and other researchers have revealed CBT-related structural changes in hippocampal volume in patients with PTSD (Levy-Gigi, Szabó, Kelemen, & Kéri, 2013). However, these findings are preliminary and must be qualified by research demonstrating no alterations of changes in amygdala or medial prefrontal cortex after therapy (Dickie, Brunet, Akerib, & Armony, 2013; Levy-Gigi et al., 2013). The discrepancies between findings may be explained by evidence showing that pre-treatment volume in such regions can predict successful response and associated changes in such structures in response to therapy (Dickie et al., 2013).

Whether treatment can promote structural changes, or whether pre-treatment structure in some way facilitates successful treatment, it is clear that the identification of regions whose volume is associated with sub-clinical anxiety is an important finding. It would suggest that therapies could potentially be delivered to sub-clinical populations as a preventative measure for those at risk of developing anxiety. This could ultimately reduce the number of individuals who go on to develop clinical anxiety disorders.
8.8 Limitations

As discussed above, the major results from this chapter were found when measuring anxiety using the HADS_A subscale, and the results in the sub-set where the STAI-6 was used were weak and did not match those of previous literature. The STAI-6 scores were normally distributed in our sub-set of participants, but the mean score (39.03, range 18-66.66) is higher than that suggested to be typical from normative data reported in initial validation studies (scores of 34-36; Spielberger, 1983; Spielberger, 2010). It could be that the disproportionately high number of anxious participants in our sub-sample has altered results in comparison to previous literature. However, two of the four previous comparison studies also had elevated STAI scores above this proposed cut-off (Baur, Hänggi, & Jäncke, 2012; Blackmon et al., 2011), indicating that the discrepancies between this study and the previous literature is not likely to be due to this sampling difference. Furthermore, the inclusion of apparently elevated levels of anxious participants in these previous studies suggests that perhaps the STAI cut-off is more of an arbitrary rule of thumb. In addition, as mentioned earlier, STAI scores have been linked to measures of depression (Bieling et al., 1998) which is known to be highly co-morbid with anxiety (Rivas-Vazquez et al., 2004). Although current participants were screened for confounding clinical disorders, it may be that the STAI is more susceptible to depressive traits in participants. As such, the STAI may benefit from being used in conjunction with other measures of anxiety in future studies.

As discussed above, causality and direction of effect between anxiety and structural differences cannot be established with this current dataset, although some causality can be inferred from evidence based on heredity research. Longitudinal studies in healthy individuals would help evidence directionality as well as causality of structural changes in relation to sub-clinical/clinical symptomology, and help establish the preventative treatments discussed in the section above. Such studies would help researchers ascertain whether chronically high levels of anxiety trigger volume reductions, or whether naturally occurring reduction in these regions exacerbates anxiety symptomology. Indeed, there have been tentative steps made towards this goal in clinical populations (e.g. Bonne et al., 2001; De Bellis, Hall, Boring, Frustaci, & Moritz, 2001). However, such studies are scarce due to the clear logistical burden of conducting such research, and to this authors knowledge there are no current sub-clinical longitudinal studies.
There was no evidence for structural differences in the anterior cingulate cortex, hippocampus, or precuneus in this sub-clinical population. These areas were included in the region of interest analysis due to associations with being involved in fear circuitry (Etkin, 2012; LeDoux, 2000) or as attention-related or visual processing controls (FFG and precuneus). However, other regions in the brain have been implicated in anxiety disorders, in particular the orbitofrontal cortex (OFC; Bishop, 2007). In fact, both Kuhn and colleagues (2011) and Blackmon et al. (2011) reported that structural changes in the OFC were associated with anxiety scores in sub-clinical populations. Blackmon and colleagues (2011) also observed an association between increased temporo-parietal cortical thickness and anxiety scores in their participants, with the authors suggesting this region may play a supporting role in emotion processing and could contribute to dysfunctional emotion regulation. The ROI selected in this study were based on previous literature on emotion processing, as well as initial GLM results (Chapter 5), but there is a case to be made that other areas could have been included in the analysis. Future studies could include these areas as ROI, or more likely conduct a whole-brain structural analysis to be sure that no areas of interest are overlooked in sub-clinical populations.

8.9 Conclusions
The present study was aimed at investigating whether there is a relationship between self-reported anxiety and volumetric differences in a sub-clinical population, building on the clinical literature and clarifying the mixed results with regards to the amygdala. It found that increased sub-clinical anxiety was related to volume reductions in the amygdala (left in correlation, bilateral in group-based analysis) and medial prefrontal areas (right dmPFC and bilateral dmPFC in correlation, right vmPFC in group-based analysis). Bilateral amygdala volume reduction is of particular note, as it has not been found previously in subclinical populations. Taken together, these findings support the idea from previous literature and previous chapters that dysfunctional emotion regulation can be attributed to disruption in the fronto-amygdala network within the brain. The discovery of such structural changes in a subclinical population, echoing those in clinical populations, raises the question of whether these changes are hereditary or whether chronic anxiety can induce structural changes in emotion networks. It also suggests that there may be biological markers of those at risk of developing clinical anxiety disorders, which opens the possibility of tailoring therapy to prevent these individuals transitioning from subclinical to clinical anxiety.
Chapter 9: Study 6
Resting state and Parcellation of the amygdala

9.1 Overview
The aims of this chapter are fourfold and methods and results will be presented in two parts. Previously in this thesis amygdala activation has been explored during active processing, here the overall aim is to explore amygdala connectivity at rest in a large sub-clinical population. The first part of this chapter will focus on determining not only what the connectivity of the amygdala is at rest, but furthermore whether any of these connectivity patterns are modulated by levels of anxiety. In addition, these connectivity patterns will be explored through direct comparison of high and low anxiety participants, and male and female participants to investigate the potential impact of individual differences on resting amygdala connectivity. In the second part data will be analysed using methods laid out by Roy et al. (2009) who were able to functionally parcellate amygdala connectivity at rest into three sub-divisions; the centromedial, laterobasal and superficial nuclei. Overall, this chapter considers assessment of amygdala connectivity at rest in a large sub-clinical population and how individual differences in anxiety and gender may modulate such connectivity. The literature on abnormal emotion processing suggests that it arises from dysfunctional connectivity, and this chapter looks to determine whether this is a fundamental characteristic observable at rest, or only manifests during specific emotional processing tasks.

9.2 Introduction
It is clear that despite the amygdala being the primary focus of emotion research, it does not work in isolation, but rather is involved in a complex network of regions within the brain during emotion processing (section 1.5.1). A key focus has been the interplay between frontal areas and the amygdala during emotion processing. It has been suggested that a dysfunctional interaction between these two regions during emotion processing underpins emotion disorders and the resulting maladaptive characteristics in such disorders (Banks et al., 2007b; Eden et al., 2015; Etkin et al., 2011; Etkin & Wager, 2007; Gold et al., 2015; Motzkin et al., 2014). Research continues to investigate this interaction, and data presented in chapter 6 further bolsters the idea that despite the array of brain regions involved in emotion processing, fronto-amygdala interactions are fundamental for typical and atypical emotional responses. This fronto-amygdala interplay is also observable at rest, when no emotional processing is required of participants (e.g. Anticevic et al., 2013).
Resting state functional magnetic resonance (Rs-fMRI) has been introduced in chapter 1 (section 1.3.2.3). In brief, Rs-fMRI allows observation of spontaneous neural activity indicative of underlying functional networks in the absence of task-induced correlations or \textit{a priori} predictions. The technique allows insight into the ‘natural state’ of the brain without external influences and places minimum burden on participants. In study cohorts such as individuals presenting with anxiety disorders who can be particularly sensitive to stress in the unfamiliar scanning environment, a reduction in cognitive burden alongside reduced scanning time is pertinent. Despite the value of using this technique, there are only a few studies that use Rs-fMRI to investigate emotional connectivity of the amygdala at rest. These are also discussed in detail in section 1.5.4 but a few of these studies will be considered briefly here.

Kim, Gee, Loucks, Davis, & Whalen (2011) collected resting state data from 29 healthy participants to investigate the effect of anxiety levels on fronto-amygdala connectivity, in particular connectivity with the medial prefrontal cortex (mPFC). They found that only low anxiety participants exhibited the typical connectivity pattern predicted by the literature (positive amygdala coupling with ventral mPFC, and negative amygdala coupling with dorsal mPFC; Roy et al., 2009), with individuals with high levels of anxiety exhibiting negative amygdala coupling with vmPFC and no effect with dmPFC. These results support the notion that pathological anxiety, or even sub-clinical anxiety, may result from disruptions in fronto-amygdala connectivity. A more recent study by Motzkin et al. (2014) explored the role of the mPFC on amygdala connectivity at rest in a clinical population with bilateral vmPFC lesions. They found that patients had greater amygdala connectivity, particularly between the right amygdala and anterior temporal cortex. Furthermore, these patients exhibited elevated amygdala activity to aversive stimuli in an emotion task, supporting a top-down control mechanism of fronto-amygdala connectivity. However, it should be noted that these patients did not exhibit elevated anxious traits or any difference in perception of aversive stimuli compared to controls, somewhat contradicting the notion that fronto-amygdala connectivity underlies pathological anxiety (Kim, Gee, Loucks, Davis, & Whalen, 2011; Quirk & Gehlert, 2003; Rauch, Shin, & Phelps, 2006). It is clear that despite a growing body of evidence from task-based fMRI studies that fronto-amygdala connections are involved in affective psychopathology, questions still remain regarding the involvement of this connectivity at rest. However, as indicated by Kim and colleagues (2011), understanding this resting connectivity underpins the ‘very baseline upon which task-based investigations of normal and pathological anxiety are conducted’ (Kim, Gee, Loucks, Davis & Whalen, pp.1).
A novel and potentially interesting avenue of research which may help explain the mixed findings of previous research is the investigation of connectivity in sub-regions within the amygdala (Ball et al., (2007); Roy et al., (2009)). Ball and colleagues (2007) used probabilistically defined sub-regions of the amygdala (the laterobasal (LB), centromedial (CM) and superficial (SF) nuclei, as defined in a study by Amunts et al. (2005)) to investigate emotional responses to music. Of greater interest for this chapter, Roy and colleagues (2009) sought to take this line of investigation further by looking at the resting connectivity of these sub-divisions of the amygdala. Within neuroscientific emotion research, the amygdala has principally been treated as one structure, largely as a result of methodological limitations such as spatial resolution in fMRI, as well as ease of comparison of results across studies. However, evidence shows the amygdala is not a body of analogous structures, but rather a group of heterogeneous structures that have been arbitrarily grouped together for ease of study. In observing these distinct sub regions of the amygdala, Roy and colleagues (2009) found that not only were the three regions structurally different, but also they appear to be functionally independent, with distinctly different patterns of resting connectivity with the rest of the brain. Their findings also provide further evidence for the importance of fronto-amygdala connectivity, with connectivity observed between frontal areas and the LB amygdala subdivision. The CM and SF amygdala had much lower frontal connectivity at rest, with CM nuclei involved in generating behavioural responses and SF nuclei primarily involved in affective processes. It is therefore clear studying these structures independently, where possible, is vital in order to tease apart what role the ‘amygdala’ plays in emotion processing.

The relatively recent development of Rs-fMRI means that there is a dearth of information on amygdala connectivity at rest in comparison to networks of connectivity in the brain associated with active emotion processing. This gap in the literature is slowly being addressed, although questions still remain which are important to address in order to understand these underlying networks, as they are the basis on which task-related research is conducted (Kim et al., 2011). It could be that amygdala connectivity in particular, fronto-amygdala connectivity, is impaired at rest, and it is this that contributes to the changes seen during task-based studies. Alternatively, the connectivity may only be impaired during emotion processing, or a weakness at rest may be exposed further by increased processing load during tasks. Elucidating which of these scenarios underlies atypical emotion processing is important as it feeds into intervention and therapy of these individuals. This chapter
focuses on the key characteristics of resting amygdala connectivity in a sub-clinical anxiety population to try to tease apart any modulating factors influencing fronto-amygdala connectivity in this cohort. In addition, in replicating Roy and colleagues (2009) parcellation methods it is hoped to bolster the notion that such parcellation of amygdala activity at rest is possible. This will hopefully further our understanding of the intricacies of amygdala activation and involvement in emotion processing.

9.3 Aims

Study A: Whole amygdala resting state

1. To determine the resting state connectivity pattern of the amygdala across participants
2. To determine whether this connectivity pattern is modulated by anxiety
3. To determine whether there are group differences in fronto-amygdala connectivity between anxiety and gender groups

Study B: Parcellated amygdala resting state.

1. To replicate Roy et al.’s (2009) parcellation methods and determine resting state connectivity of the amygdala subdivisions.

9.4 Methods

The methods involved in data collection are described in detail in Chapter 3 section 3.3.5, the following is a brief summary of the methods for reference.

9.4.1 Participants

In brief, resting state functional resonance imaging (Rs-fMRI) data were collected from 57 volunteers (aged 19-45 years, \( \bar{x} = 24.72, \pm 5.54 \); 25 male, 32 female); all participants met strict screening criteria. Participants completed the Hospital Anxiety Depression Scale Anxiety sub-scale (HADS_A). Participants were categorised into the high anxiety group (18
participants, aged 19-30, $\bar{x} = 24.78 \pm 3.78$; 6 male, 12 female) or low anxiety group (39 participants, aged 19-45, $\bar{x} = 24.79 \pm 6.23$; 19 male, 20 female).

9.4.2 Procedure
Participants were instructed to lay as still as possible in the scanner with their eyes open thinking of nothing in particular. On screen in the scanner a cross hair was displayed centrally in order to give participants something to focus on and to minimise the possibility of motion artefacts. The resting scan lasted for six minutes, acquisition parameters were: thirty axial slices (FOV 192 x 192mm, 64 x 64 matrix, 4mm thickness, no gap, 3 x 3 x 4 mm voxel size, IPAT parallel acquisition), TR: 1750 ms, TE 30ms, 85° flip angle). A high resolution 3D brain MRI images was also acquired using a T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) pulse sequence (TR 1830ms, TE 3.03ms, Inversion Time 1100ms, 11° flip angle, FOV 256mm, 160 slices, voxel size 1 x 1 x 1mm voxel size, in-plane matrix 256 x 256).

9.4.3 Pre-processing
Imaging data were pre-processed and analysed using Matlab R2013a and SPM8 software package available at (http://www.fil.ion.ucl.ac.uk/spm/). Functional runs were realigned to the first volume to correct for motion artefacts and the mean image was then co-registered to the T1 weighted structural image to ensure that it accurately reflects the anatomical details of each individuals’ brain in terms of areas of activation detected during the study. They were then normalised to Montreal Neurological Institute (MNI) standard space. To reduce random noise effects this data was then spatially smoothed using a Guassian kernel of 5mm full width half maximum.

9.4.4 Study A: Whole amygdala resting state
The resting state data were analysed using the REST toolkit (V1.8, available from http://www.restfmri.net/forum/REST_V1.8). Additional pre-processing steps were applied: the time-course for each voxel was band-pass filtered (0.01-0.08 Hz band) and the data were de-trended. Such band-pass filtering is supported by literature showing that it is the low frequency fluctuations of the resting state that are of physiological importance (Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995; Chao-gan & Yu-feng, 2010) and reflect
‘spontaneous neuronal activity’ (Lu et al., 2007). De-trending accounts for any physiological shifts present in the data after a continuous scanning session (this may be excess noise related to movement after realignment or instrumental instability). This was done using the MATLAB function ‘detrend’ which simply removed a linear trend (the mean) from the data in order to allow observation of the fluctuations in the data about the trend. Pre-processing methods used in this study follow recommended pre-processing pipeline for resting state fMRI data (Cho-Gang and Yu-Feng, 2010).

### 9.4.4.1 Functional Connectivity Analysis

Amygdala seed regions were based on co-ordinates from the main effect of face in this group of participants, constrained by standard cytoarchitectonic maps as defined by undilated automatic anatomical labelling (aal) templates implemented through WFU PickAtlas (Right amygdala: 22, -4, -12; Left amygdala: -20, -4, -14). These coordinates were previously determined from the GLM results, and used in the PPI chapter (for details see section 5.4.5). ROIs were determined by a 5mm sphere around these coordinates, and time series were extracted for each ROI. The correlation analysis was performed in a voxel-wise manner, allowing comparison of the time series of each ROI with each voxel in the rest of the brain, thus producing the functional connectivity maps. Note, global signal was not entered as a covariate as the benefits of this has been widely debated within resting-state research with some evidence suggesting that doing so can actually result in significant anti-correlations between the default-mode network and attention networks (e.g. Chao-gan & Yu-feng, 2010; Fox, Henderson, Marshall, Nichols, & Ghera, 2005; Greicius, Krasnow, Reiss, & Menon, 2003). This 3D correlation coefficient image was then converted using Fisher’s z transformation in order to improve normality (Rosner, 2006; cited in Chao-gan & Yu-feng, 2010). For the overall functional connectivity analysis the Fisher’s Z-maps were entered into a one-sample t-test within the REST toolbox to detect regions showing significant functional connectivity with the left and right amygdala. In addition, Fisher’s Z-maps were then split by either anxiety group or gender group and entered into two, two-sample t-tests within the REST toolbox in order to determine whether there is a difference in connectivity between these groups.
9.4.4.2 Correlation Analysis
The individual Fisher’s Z-maps generated from the functional connectivity analysis were then entered into two second level correlation analyses for the right and left amygdala implemented using the REST toolbox. In these correlations the group data were entered as the dependent variable and the independent variable was based on participants’ absolute HADS_A score.

9.4.4.3 Statistical Analysis
The statistical result of each method used (one-sample t-test, two sample t-tests, and correlations) were all corrected for multiple comparisons using the AlphaSim option in the REST toolbox. AlphaSim is based on Monte Carlo simulations. For the t-tests, combination threshold of voxels' $p<0.001$ and cluster size $>443\text{mm}^3$ were considered significant, which corresponded to a corrected $p<0.001$. For the correlations, a combination threshold of voxels' $p<0.001$ and cluster size $>263\text{ mm}^3$ were considered significant, which corresponded to a corrected $p<0.05$. Statistical maps generated were overlaid onto the anatomical template (ch2better.nii) in MRICroN software (available at [http://www.mccauslandcenter.sc.edu/mricro/mricron/](http://www.mccauslandcenter.sc.edu/mricro/mricron/) for presentation purposes and in order to explore results. Tables of results were generated through a combination of cluster reports generated using the ‘slice viewer’ in the REST toolbox and also through exploring superimposed results in MRICroN.

9.4.5 Study B: Parcellated amygdala resting state
9.4.5.1 Method
Resting state connectivity analysis of the parcellated amygdala replicated the methods detailed by Roy and colleagues (2009), although here the analysis was run in SPM8, wheres the initial analysis by Roy and colleagues was run in FSL. This necessitates some alterations which are highlighted later in the methods. For detailed methodology please refer to Roy et al, 2009.

9.4.5.2 Functional Connectivity: Time Series Extraction
In line with Roy and colleagues methods, time series were extracted from three key subdivisions of the amygdala; laterobasal (LB), centromedial (CM) and superficial (SF) nuclei.
These sub-divisions were based on probabilistic maps developed by and available for
download from Amunts and colleagues (Amunts et al., 2005), these probabilistic maps
account for interindividual anatomic variability. In order to make sure that time series signal
extracted was unique to each sub-division, the probabilistic maps were thresholded at 50%
probability of inclusion in each sub-division, and overlap was assigned to the region of
highest probability to avoid duplication. For each individual subject, a personal time course
was then generated for each of these three regions in the left and right hemisphere by
multiplying each voxels time course by the weighted probability of inclusion and then
extracting the mean time series for each subject from within these three regions.

9.4.5.3 Statistical Analysis
Multiple regression analysis were performed for each subject and for each hemisphere
(left/right). The regression model included the three amygdala predictors (LB, CM and SF)
and nine nuisance variables (global signal, white matter, cerebral spinal fluid and six motion
parameters). Note that SPM automatically orthogonalises these predictors as part of the GLM
analysis to ensure only unique variance is observed; whereas Roy and colleagues had to
manually implement orthogonalisation of the data in FSL.

Group level analysis was conducted, controlling for age and gender, and corrected for
multiple comparisons (Z>2.3, p<0.05). This resulted in six main effects z-score maps
indicating which voxels were correlated with the particular amygdala subdivision of interest
(Left LB, Left CM, Left SF, Right LB, Right CM, Right SF). Further comparisons were made
between each region to explore differences in functional connectivity associated with each
sub-division (CM vs SF/LB, LB vs SF/CM, SF vs CM/LB; done for both left and right
hemisphere). Finally, conjunction analysis was conducted in order to determine whether any
areas were functionally connected with all three regions in each hemisphere. The resulting
maps for the right and left amygdala subdivisions were each overlaid onto a standard high
resolution spatially normalised single subject structural T1-weighted image from SPM8
templates to explore the areas exhibiting significant positive or negative connectivity.
Thresholded SPM maps were exported to MRICroN and overlaid onto the anatomical
template (ch2better.nii) for presentation purposes.
9.5 Results

9.5.1 Study A: Whole amygdala resting state

9.5.1.1 Functional Connectivity
Analysis of the overall resting functional connectivity pattern across participants revealed significant positive connectivity between the left amygdala and frontal areas, including the right medial orbitofrontal cortex (BA11; often referred as vmPFC), bilateral inferior orbitofrontal cortex (BA11/47) and superior frontal cortex (BA6) (see Figure 9.1, Table 9.1). In addition, the left amygdala showed positive association with ventral anterior cingulate (vACC, BA24), insula, striatum (caudate, nucleus accumbens), left hippocampus, thalamus, precuneus, fusiform gyrus, precentral and left postcentral gyrus.

The right amygdala did not show positive connectivity with the same frontal areas seen for left amygdala, but did exhibit significant positive connectivity with bilateral superior temporal pole, bilateral amygdala and superior parietal gyrus along with structures also observed for left amygdala (ventral anterior cingulate (vACC, BA24), striatum [caudate, extending to nearby putamen], right hippocampus, thalamus, precuneus and right postcentral gyrus).

Of note, the resting functional connectivity results did not reveal any areas with significant negative connectivity with either amygdala.
Figure 9.1. Whole amygdala functional connectivity across participants (N=57). Thresholded patterns of significantly positive (red) and negative (blue*) connectivity are shown. Sagittal (x=-10), coronal (y=-9), axial (z=-13) views presented in standard MNI space, radiological convention, Z>2.3, Clusters>10 significance: $p<0.001$, corrected. (* no negative functional connectivity was found).
Table 9.1 List of brain regions showing significant positive relationship with the left and right amygdala (Z>2.3, Clusters>10 significance: \( p<0.001 \), corrected). Co-ordinates given in standard MNI space. There were no areas with significant negative connectivity with either left or right amygdala.

<table>
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<th>Peak intensity</th>
</tr>
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</tr>
<tr>
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<td>-4</td>
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</tr>
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<td>39</td>
</tr>
<tr>
<td></td>
<td>Right Frontal inf. orb</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td></td>
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<td>31</td>
</tr>
<tr>
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<td>Left Insula</td>
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</tr>
<tr>
<td></td>
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<td>14</td>
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<td>Left Caudate</td>
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<td>Right &quot;</td>
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<td>-19</td>
</tr>
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</tr>
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<td>-2</td>
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<td>Right Pallidum</td>
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<td>Left Parietal. Sup</td>
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<td>-49</td>
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</table>
9.5.1.2 Correlation with anxiety

Analysis of brain regions where resting state connectivity with the amygdala (left/right) correlated with anxiety measures (HADS_A) revealed that again primarily frontal areas were associated with the left amygdala, whereas more parietal areas were associated with the right amygdala (see Figure 9.2, Table 9.2). In detail, the resting state connectivity between the left amygdala and right frontal regions (including right middle frontal gyrus, right superior frontal gyrus and right inferior orbitofrontal gyrus) was negatively associated with the HADS_A anxiety measure. In addition, left amygdala connectivity with right insula, left fusiform, left inferior temporal gyrus and right angular gyrus was negatively correlated with HADS_A anxiety scores. This means that for those individuals with greater anxiety, there was less connectivity between the left amygdala and frontal regions at rest, as well as other areas involved in emotional processing.

On the other hand, right amygdala resting connectivity with the bilateral precuneus was positively correlated with HADS_A anxiety scores, suggesting that those individuals with greater anxiety also have greater connectivity between these areas at rest.
Figure 9.2. Areas where resting functional connectivity correlates with anxiety. Patterns of significantly positive (red) and negative (blue) correlations for the left and right amygdala. Sagittal (x=-10), coronal (y=-9), axial (z=-13) views presented in standard MNI space, radiological convention. Z>2.3, Clusters>10 significance: p<0.05, corrected.
Table 9.2 List of brain regions whose connectivity with the left or right amygdala is significantly (positive or negative) correlated with HADS_A (a measure of anxiety). (Z>2.3, Clusters>10 significance: p<0.05, corrected). Co-ordinates given in standard MNI space.

<table>
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<tr>
<th>Hemisphere</th>
<th>Structure</th>
<th>MNI Coordinates</th>
<th>R</th>
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<td></td>
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</tr>
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</tr>
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</tr>
<tr>
<td></td>
<td>Right</td>
<td>27</td>
<td>-53</td>
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</tbody>
</table>

9.5.1.3 Group Comparisons

Anxiety

The two sample t-test between high (N=18) and low (N=39) anxiety groups revealed that high anxiety individuals exhibited reduced connectivity between the right amygdala and one frontal region (left inferior orbitofrontal gyrus), as well as between right and left amygdala compared to low anxiety participants (see Figure 9.3, Table 9.3 for results).

In addition, high anxiety participants showed greater resting connectivity between the cerebellum and both amygdalae, and reduced functional connectivity between temporal regions and both amygdalae (Left: including right hippocampus, right inferior temporal gyrus, left middle temporal gyrus, bilateral fusiform; Right: left middle temporal gyrus and inferior temporal gyrus) compared to low anxiety participants (see Figure 9.3, Table 9.3).
Figure 9.3. Differences in resting functional connectivity of the amygdalae between high (N=18) and low (N=39) anxiety participants. Figures show areas where connectivity was greater in high anxiety participants (red), and areas where connectivity was greater in low anxiety participants (blue). Sagittal (x=-10), coronal (y=-9), axial (z=-13) views presented in standard MNI space, radiological convention, Z>2.3, Clusters>10 significance: p<0.05, corrected.
Table 9.3 List of brain regions whose resting functional connectivity with the amygdalae (left/right) is significantly different between high and low anxiety groups. Positive T value: greater connectivity in high anxiety participants; negative T value: greater connectivity in low anxiety participants. (Z>2.3, Clusters>10 significance: p<0.05, corrected). Co-ordinates given in standard MNI space.

<table>
<thead>
<tr>
<th>Anxiety Group differences (2-sample t-test)</th>
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<th>Structure</th>
<th>MNI Coordinates</th>
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<td>Left</td>
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<td></td>
<td>Right</td>
<td></td>
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<tr>
<td></td>
<td>Right</td>
<td>Inf. temporal</td>
<td>54 -6 -26</td>
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</tr>
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<td></td>
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<tr>
<td></td>
<td>Left</td>
<td>Fusiform</td>
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<td>-3.19</td>
</tr>
<tr>
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<tr>
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<tr>
<td></td>
<td>Right</td>
<td>Angular</td>
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<td></td>
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<td>Inf. frontal orb</td>
<td>-32 -8 16</td>
<td>-2.08</td>
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</table>

**Gender**

The two sample t-test between male (N=25) and female (N=32) participants revealed that female participants exhibited reduced connectivity between frontal areas and both amygdalae (Left: right superior frontal gyrus, left superior medial frontal gyrus, right inferior orbitofrontal gyrus and left middle orbitofrontal gyrus; Right: right medial orbitofrontal gyrus), as well as increased connectivity between the left cerebellum and both amygdalae compared to male participants (See Figure 9.4, Table 9.4).
In addition, female participants exhibited reduced connectivity between both amygdalae and temporal areas (Left: right hippocampus, bilateral fusiform; Right: right hippocampus, left middle temporal gyrus), as well as bilateral precuneus, compared to male participants. Finally, female participants demonstrated reduced connectivity between bilateral insula and the left amygdala, as well as right anterior cingulum and right amygdala, compared to male participants.

Figure 9.4. Differences in resting functional connectivity of the amygdalae between male (N=25) and female (N=32) participants. Figures show areas where connectivity was greater in male participants (red), and areas where connectivity was greater in female participants (blue). Sagittal (x=10), coronal (y=9), axial (z=13) views presented in standard MNI space, radiological convention, Z>2.3, Clusters>10 significance: p<0.05, corrected.
Table 9.4 List of brain regions whose resting functional connectivity with the amygdala(s) is significantly different between male and female groups. Positive T value: greater connectivity in male participants; negative T value: greater connectivity in female participants. (Z>2.3, Clusters>10 significance: p<0.05, corrected). Co-ordinates given in standard MNI space.

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Structure</th>
<th>MNI Coordinates</th>
<th>T value</th>
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<td>&quot;</td>
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</tbody>
</table>
9.5.2 Study B: Parcellated amygdala resting state

9.5.2.1 Amygdala Subdivisions

This section will first look at the resting functional connectivity for each subdivision, reporting both the basic functional connectivity as well as areas where connectivity was significantly different for this sub-division compared to the other two. Then it will look at areas of overlap (conjunction analysis) in the functional connectivity of these three sub-divisions.

9.5.2.2 Laterobasal

The laterobasal (LB) subdivision of the amygdala exhibited negative resting functional connectivity with some frontal areas (middle frontal gyrus (BA 10, 46), orbitofrontal gyrus) as well as the precuneus, parietal lobe, supplementary motor area, precentral gyrus, cerebellum and caudate (see Figure 9.5 Column 1, Table 9.5). These results were similar to those of Roy and colleagues (2009), with activity in dorsal frontal and posterior regions negatively associated with resting activity.

In addition, positive functional connectivity was observed between the LB subdivision and temporal regions, including the hippocampus and right inferior temporal gyrus, as well as the insula. It should be noted that there were some differences in right and left LB functional connectivity, with right LB demonstrating positive connectivity with the right superior frontal gyrus and left inferior orbitofrontal cortex, as well as bilateral rectus.
Figure 9.5. Functional connectivity of amygdala regions of interest at rest. Patterns of significantly positive (red) and negative (blue) relationships for the laterobasal (LB), centromedial (CM) and superficial (SF) sub-regions of the amygdala. Sagittal (x=-10), coronal (y=-9), axial (z=-13) views presented in standard MNI space, radiological convention, Z>2.3, Clusters>10 significance; p<0.05, corrected.
Table 9.5 List of brain regions showing a significant positive or negative relationship with the right and left laterobasal (LB) sub-region of the amygdala. (Z>2.3, Clusters>10 significance: p<0.05, corrected). Co-ordinates given in standard MNI space.

<table>
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<td></td>
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<td>y</td>
</tr>
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<td>-6</td>
</tr>
<tr>
<td></td>
<td>Right Temporal Pole Sup</td>
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<td>14</td>
</tr>
<tr>
<td></td>
<td>Right Thalamus</td>
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</tr>
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<td></td>
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<td></td>
<td>Right Insula</td>
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<td>-8</td>
</tr>
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<td></td>
<td>Right Supp. Motor Area</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Left &quot;</td>
<td>-2</td>
<td>24</td>
</tr>
<tr>
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<td>Right Precuneus</td>
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</tr>
<tr>
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<td>Right Caudate</td>
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<td>Right Supra Marginal</td>
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<td>Left Cerebellum</td>
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<td>Right Frontal Inf. Orb</td>
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<td>Left Inf Parietal</td>
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<td>-54</td>
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<td>Left Supp Motor Area</td>
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<tr>
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<tr>
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<td>Right &quot;</td>
<td>4</td>
<td>26</td>
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<td>Right Occipital Sup</td>
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<td>-74</td>
</tr>
<tr>
<td></td>
<td>Right Frontal Mid</td>
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<td>22</td>
</tr>
<tr>
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<td>Right Precentral</td>
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<td>6</td>
</tr>
<tr>
<td></td>
<td>Left &quot;</td>
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<td>Left &quot;</td>
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</tr>
<tr>
<td></td>
<td>Left Frontal Mid. Orb</td>
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<td>60</td>
</tr>
<tr>
<td></td>
<td>Left Frontal Mid</td>
<td>-32</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Left Cerebellum</td>
<td>-32</td>
<td>-32</td>
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<td></td>
<td>Right &quot;</td>
<td>32</td>
<td>-30</td>
</tr>
<tr>
<td></td>
<td>Vermis</td>
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</tr>
<tr>
<td></td>
<td>Left Pallidum</td>
<td>-16</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>Right Caudate</td>
<td>6</td>
<td>14</td>
</tr>
</tbody>
</table>
In comparison with the centromedial (CM) and superficial (SF) sub-divisions, the LB had greater positive connectivity with temporal areas (hippocampus, parahippocampal, middle temporal, fusiform) and some frontal areas (Right LB only; superior frontal gyrus and rectus (BA 9/11)) (see Figure 9.6 column 1, Table 9.6).

The negative connectivity pattern was less clear, however, left LB had significantly greater negative connectivity with dorsal posterior regions including the right precuneus and left cerebellum, as well as with the right supplementary motor area, pallidum, caudate, putamen and thalamus (see Figure 9.6 column 1, Table 9.6)
Figure 9.6. Direct comparisons of the functional connectivity of each sub-division of the amygdala in comparison to the other two sub-divisions. Red shows where activation in the specified region is significantly more positively predicted by spontaneous activity than by the other two subdivisions. Blue shows where activation in the specified target is significantly more negatively predicted by spontaneous activity in comparison to the other two sub-regions. Sagittal (x=10), coronal (y=9), axial (z=13) views presented in standard MNI space, radiological convention, Z>2.3, Clusters>10 significance: p<0.05, corrected.
Table 9.6 List of brain regions showing a significant positive or negative relationship with the right and left laterobasal (LB) sub-region of the amygdala compared to the centromedial (CM) and superficial (SF) sub-regions. (Z>2.3, Clusters>10 significance: p<0.05, corrected). Co-ordinates given in standard MNI space.

<table>
<thead>
<tr>
<th>Direction</th>
<th>Structure</th>
<th>MNI Coordinates</th>
<th>T value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Left Hippocampus</td>
<td>-20 -6 -20</td>
<td>Inf, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Left Temporal Mid</td>
<td>-50 0 -20</td>
<td>7.33, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Left Temporal Pole Sup</td>
<td>-34 14 -18</td>
<td>7.14, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right Hippocampus</td>
<td>22 -6 -20</td>
<td>Inf, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right Parahippocampal</td>
<td>34 -12 -28</td>
<td>7.24, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right Fusiform</td>
<td>30 -26 -20</td>
<td>6.81, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right Temporal Mid</td>
<td>54 2 -20</td>
<td>5.66, p=0.002</td>
</tr>
<tr>
<td></td>
<td>Left Rectus</td>
<td>-8 38 -20</td>
<td>5.96, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right &quot;</td>
<td>10 40 -22</td>
<td>5.77, p=0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>Right Pallidum</td>
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<td>6.89, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right Caudate</td>
<td>20 -4 22</td>
<td>5.75, p=0.001</td>
</tr>
<tr>
<td></td>
<td>Right Thalamus</td>
<td>8 -4 8</td>
<td>5.55, P=0.004</td>
</tr>
<tr>
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<td>Right Precuneus</td>
<td>4 -74 60</td>
<td>6.34, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right Thalamus</td>
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<td>5.96, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right Supp. Motor Area</td>
<td>0 -64 -14</td>
<td>5.91, p=0.001</td>
</tr>
<tr>
<td></td>
<td>Vermis</td>
<td>0 -56 -8</td>
<td>5.63, p=0.003</td>
</tr>
<tr>
<td></td>
<td>Left Cerebellum</td>
<td>-10 -82 -28</td>
<td>5.66, p=0.002</td>
</tr>
<tr>
<td></td>
<td>Right Putamen</td>
<td>24 20 4</td>
<td>5.39, p=0.010</td>
</tr>
<tr>
<td>Positive</td>
<td>Right Hippocampus</td>
<td>26 -4 -20</td>
<td>Inf, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right Parahippocampal</td>
<td>30 -26 -18</td>
<td>Inf, P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Right Temporal Pole Mid</td>
<td>42 16 -30</td>
<td>Inf, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Left Hippocampus</td>
<td>-24 -4 -22</td>
<td>Inf, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Left Temporal Pole Mid</td>
<td>-36 18 -38</td>
<td>6.14, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Left Frontal Sup</td>
<td>-28 42 48</td>
<td>6.00, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Left Rectus</td>
<td>-8 30 -14</td>
<td>5.80, p=0.001</td>
</tr>
<tr>
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<td>6 40 -16</td>
<td>5.22, p=0.022</td>
</tr>
<tr>
<td>Negative</td>
<td>Right Hippocampus</td>
<td>18 -8 -12</td>
<td>Inf, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Vermis</td>
<td>2 -66 -10</td>
<td>5.63, p=0.003</td>
</tr>
</tbody>
</table>

9.5.2.3 Centromedial
The centromedial (CM) subdivision of the amygdala exhibited negative resting functional connectivity with the hippocampus (Left CM: left hippocampus; Right CM: right hippocampus) and right middle cingulum (left CM only). Positive functional connectivity was only observed for the left CM with right putamen and left insula (See Figure 9.5 column 2, Table 9.7).
Table 9.7 List of brain regions showing a significant positive or negative relationship with the right and left centromedial (CM) sub-region of the amygdala. (Z>2.3, Clusters>10 significance: p<0.05, corrected). Co-ordinates given in standard MNI space.

<table>
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<tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Right Putamen</td>
<td>34 -14 -6</td>
<td>5.56, p=0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left</td>
<td>Insula</td>
<td>-38 -2 0</td>
<td>5.46, p=0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Left Hippocampus</td>
<td>-14 -8 -14</td>
<td>7.16, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right</td>
<td>Mid. Cingulum</td>
<td>10 -22 36</td>
<td>5.54, p=0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right CM</td>
<td>Positive</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Right Hippocampus</td>
<td>18 -8 -12</td>
<td>Inf, p&lt;0.001</td>
</tr>
</tbody>
</table>

In comparison to the LB and SF, the CM primarily exhibits greater negative coupling with bilateral hippocampus, parahippocampus and temporal areas, with the right CM also showing greater decoupling with the left amygdala (See Figure 9.6 column 2, Table 9.8). Greater positive coupling was only observed for the left CM, and was seen for the right superior temporal gyrus and left cuneus and rolandic operculum.
Table 9.8 List of brain regions showing a significant positive or negative relationship with the right and left centromedial (CM) sub-region of the amygdala compared to the laterobasal (LB) and superficial (SF) sub-regions. (Z>2.3, Clusters>10 significance: p<0.05, corrected). Co-ordinates given in standard MNI space.

<table>
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<th>MNI Coordinates</th>
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<td>Cuneus</td>
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</tr>
<tr>
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<td>Temporal Sup</td>
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</tr>
<tr>
<td></td>
<td>Left</td>
<td>Rolandic Oper</td>
<td>-62 2 8</td>
<td>5.58, p=0.004</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>Hippocampus</td>
<td>-18 -6 -18</td>
<td>Inf, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Fusiform</td>
<td>-34 -14 -22</td>
<td>5.97, p&lt;0.001</td>
</tr>
<tr>
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<td>Right</td>
<td>Parahippocampal</td>
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</tr>
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<td>Vermis</td>
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<td>5.65, p=0.002</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Hippocampus</td>
<td>18 -8 -12</td>
<td>Inf, p&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Fusiform</td>
<td>36 -10 -32</td>
<td>5.48, p=0.007</td>
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<td>Left</td>
<td>Amygdala</td>
<td>-20 -4 -16</td>
<td>7.04, p&lt;0.001</td>
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</tr>
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<td>Fusiform</td>
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<td>Paracentral Lobule</td>
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<td>Frontal Mid. Orb</td>
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<td>5.68, p=0.002</td>
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<td>Left</td>
<td>Rectus</td>
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<td>5.63, p=0.003</td>
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<tr>
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<td>Right</td>
<td>Temporal Mid</td>
<td>56 -10 -16</td>
<td>5.55, p=0.004</td>
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</table>

**9.5.2.4 Superficial**

The superficial (SF) subdivision of the amygdala exhibited positive resting functional connectivity with the hippocampus (Left SF: left hippocampus; Right SF: bilateral hippocampus), left middle temporal gyrus (left SF only) and the left amygdala (right SF only) (See Figure 9.5 column 3, Table 9.9). However, negative coupling was also seen between the SF and the hippocampus (Left SF: left hippocampus; Right SF: right hippocampus), along with the right cuneus and supramarginal gyrus for left SF only. The hippocampal results seem
perplexing, as this region contains both positive and negative coupling to the same hemisphere hippocampus, and this will be considered in the discussion section.

Table 9.9 List of brain regions showing a significant positive or negative relationship with the right and left superficial (SF) sub-region of the amygdala. (Z>2.3, Clusters>10 significance: p<0.05, corrected). Co-ordinates given in standard MNI space

<table>
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<tr>
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<td>y</td>
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</tr>
<tr>
<td>Positive</td>
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<td>-26</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>-60</td>
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</tr>
<tr>
<td>Positive</td>
<td>Right</td>
<td>18</td>
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<td>-20</td>
<td>-12</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>-22</td>
<td>-4</td>
</tr>
<tr>
<td></td>
<td>Vermis</td>
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<td>-52</td>
</tr>
<tr>
<td>Negative</td>
<td>Right</td>
<td>28</td>
<td>-6</td>
</tr>
</tbody>
</table>

In comparison with the laterobasal (LB) and centromedial (CM) sub-divisions, the SF had greater positive connectivity between the right SF and the right hippocampus, as well as greater negative connectivity between bilateral SF and bilateral hippocampus (see Figure 9.6 column 3, Table 9.10). Greater negative connectivity was also observed for other temporal (Left SF: right parahippocampal and bilateral middle temporal gyrus; Right SF: left parahippocampal and right fusiform) and occipital areas (Left SF: left middle occipital gyrus).

As in the SF results described above, the significant difference between the SF and other amygdala subdivisions in both positive (right SF only) and more substantial negative coupling with the hippocampus is a little puzzling. Especially as the CM results suggest that it also has greater negative coupling with the bilateral hippocampus in comparison to the other subdivisions. In addition, it should be noted that the LB results indicated that this area
had greater positive coupling with the hippocampus and surrounding tempora areas. These discrepancies will be considered in the discussion.

Table 9.10 List of brain regions showing a significant positive or negative relationship with the right and left superficial (SF) sub-region of the amygdala compared to the laterobasal (LB) and centromedial (CM) sub-regions. (Z>2.3, Clusters>10 significance: p<0.05, corrected).
Co-ordinates given in standard MNI space

<table>
<thead>
<tr>
<th>Direction</th>
<th>Structure</th>
<th>MNI Coordinates</th>
<th>T value</th>
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<td>x</td>
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<tr>
<td>Positive</td>
<td>N/A</td>
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</tr>
<tr>
<td>Negative</td>
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<td>-22</td>
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<tr>
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<td>-18</td>
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<tr>
<td>Left Parahippocampal</td>
<td>-28</td>
<td>-8</td>
<td>-26</td>
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9.5.2.5 Conjunction analysis
Conjunction analysis revealed only a few areas where functional connectivity overlapped significantly between the three subdivisions (See Table 9.11). There was significant convergence for positive functional connectivity between the amygdala and bilateral hippocampus (Left amygdala: left hippocampus; Right amygdala: right hippocampus), as well as between right amygdala and itself (right amygdala). The hippocampal finding is particularly intriguing as two of the four sub-divisions had greater negative coupling with the hippocampus, whereas only the LB had greater positive coupling. This suggests that the perplexing CM and SF results in relation to the hippocampus may be explained by a strong
positive coupling with this area from the LB sub-division of the amygdala. The significance of this finding is considered in the discussion.

Another important note is that there were no regions where negative connectivity pattern significantly overlapped between the subdivisions (See Table 9.11), which was also found for the whole amygdala analysis at the beginning of the results section. The significance of this is also considered in the discussion.

Table 9.11 List of brain regions showing significant positive or negative convergences across all three amygdala sub-divisions. (Z>2.3, Clusters>10 significance; p<0.05, corrected). Co-ordinates given in standard MNI space. Note the co-ordinates for the convergences in right amygdala are associated with the centromedial amygdala.

<table>
<thead>
<tr>
<th>Conjunction Analysis</th>
<th>Direction</th>
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<th>MNI Coordinates</th>
<th>T value</th>
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<td></td>
<td>Right</td>
<td>Positive</td>
<td>Right Hippocampus</td>
<td>24 -10 -12</td>
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<td></td>
<td>Right</td>
<td>Negative</td>
<td>N/A</td>
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9.5.3 Summary
From the whole amygdala resting state analysis, bilateral amygdala activity was seen to positively correlate with areas associated with fear circuitry and emotion processing such as ventral anterior cingulate cortex (ACC), hippocampus, thalamus, fusiform gyrus (FFG) and precuneus (Etkin, 2012; J. E. LeDoux, 2000). In addition, positive connectivity was observed between the left amygdala and frontal areas (medial and inferior orbitofrontal cortex) and the right amygdala and temporal regions (temporal pole). Of particular note, especially for the subsequent parcellation analysis, whole amygdala resting state analysis did not reveal any areas with negative connectivity to either amygdala.

Looking at the modulation of resting state connectivity by anxiety, correlation analysis revealed that greater anxiety score was associated with reduced connectivity between the left amygdala and frontal regions (inferior orbitofrontal, middle frontal, superior frontal), as well
as other areas associated with emotion processing (insula, FFG) and the inferior temporal lobe and angular gyrus. In addition to this, increased anxiety was associated with increased connectivity between the right amygdala and bilateral precuneus. Group-level analysis also revealed that individuals with high anxiety exhibited reduced connectivity between both amygdalae and areas associated with emotion processing (hippocampus, fusiform gyrus) as well other temporal regions (inferior/middle temporal gyrus, angular gyrus). However, group analysis also presented novel findings in relation to the previous correlation analysis, with the high anxiety group exhibiting reduced connectivity between the right amygdala and frontal areas (inferior orbitofrontal) and left amygdala, and increased connectivity between both amygdalae and the cerebellum.

Looking at group-level modulation of resting connectivity by gender, females exhibited similar results to those with high anxiety (correlation, or group-wise). Reduced connectivity was observed between both amygdalae and frontal areas (inferior/middle/medial orbitofrontal, superior frontal, superior medial frontal), as well as areas associated with emotion processing (hippocampus, fusiform gyrus, precuneus). The reduction in fronto-amygdala connectivity in females was mostly seen in the left amygdala, which also exhibited reduced connectivity with the insula (also associated with emotion processing). Females also exhibited greater negative connectivity between the right amygdala and the temporal lobe (middle temporal) and greater positive connectivity between both amygdalae and the cerebellum.

In the parcellated amygdala analysis, laterobasal (LB) amygdala connectivity exhibited a similar pattern to that seen in the paper by Roy and colleagues (2009) (primarily, positive connectivity with temporal and frontal areas (for right LB) and negative connectivity with dorsal and posterior areas). There is also some overlap between current results for the centromedial (CM) and superficial (SF) amygdala and those reported by Roy and colleagues (2009). However, the present results do not provide as extensive connectivity as previously reported (Roy et al., 2009). Resting CM activation is negatively correlated with hippocampal regions and the middle cingulum, and positively correlated with the putamen and insula (left CM only). The SF amygdala exhibits both negative and positive connectivity with the hippocampus, along with left SF negative connectivity with more posterior regions (cuneus, supramarginal gyrus) and positive connectivity with temporal areas (middle temporal). In addition, right SF exhibited positive connectivity with left amygdala. When looking at the areas of convergence in connectivity pattern across these sub-divisions, overlap was found in
positive connectivity with the bilateral hippocampus and right amygdala. In keeping with the whole amygdala connectivity results, conjunction analysis did not reveal any areas with significant negative connectivity.

9.6 Discussion
The present chapter aimed to explore amygdala connectivity at rest in a healthy population of individuals, investigating the modulation of this connectivity by gender and sub-clinical anxiety. Furthermore, it set out to determine whether more reliable information can be gained by parcellating the amygdala than by investigating the structure as one cohesive entity. The results demonstrate that the amygdala exhibits resting connectivity with areas associated with fear and emotion processing, and that the sub-divisions of the amygdala have different connectivity patterns that may be hidden by whole-amygdala analysis. In addition, the data reveal that these connectivity patterns are modulated by sub-clinical anxiety and gender. Resting fronto-amygdala connectivity was reduced in those with higher self-reported anxiety, as well as in females, providing further evidence for the notion that a key characteristic of anxiety pathology is disrupted fronto-amygdala connectivity. Furthermore, these findings suggest that the connectivity is disrupted at rest, and may therefore underlie the changes observed in task-related fMRI connectivity. At the very least, it demonstrates that the baseline on which task-based fMRI connectivity analysis is conducted varies in relation to individual differences, even in a sub-clinical cohort. Finally, these findings validate the use of resting state analysis in affective neuroscience research, as it is able to collect useful data with no a priori hypothesis in relatively short periods of time.

9.6.1 Resting amygdala connectivity
Resting connectivity analysis of the amygdala as a whole revealed patterns of connectivity which are consistent with previous studies in animals and humans (e.g. Amaral & Price, 1984; Kim et al., 2011; Roy et al., 2009). In particular, spontaneous amygdala activity correlated with activity in regions associated with monitoring, assessment and generation of emotional states (including medial orbitofrontal cortex, inferior orbitofrontal cortex, ventral anterior cingulate, insula, striatum, hippocampus, fusiform face area, and thalamus). However, unlike previous studies only positive coupling passed threshold; no negative resting
state connectivity was observed. Furthermore, areas identified in Roy and colleagues (2009) study as being negatively coupled with resting amygdala activity were positively coupled in the present study (including superior frontal cortex, precuneus and pre-central (primary motor cortex) and post-central gyrus (somatosensory cortex)). These areas could be considered, as suggested by Roy and colleagues, to be involved in the more ‘effortful regulation of affect’ (Roy et al., 2009, pp.7). The positive association of such areas in relation to the amygdala suggests that perhaps within the present sample participants were in a state of readiness, monitoring the environment for emotion related stimuli. Resting state data were collected prior to a passive emotion task (Chapters 5, 6, 7), and participants were briefed on the emotion task before resting state acquisitions. It may be that in preparing for the later task, emotion regulation areas were primed, resulting in the positive resting connectivity observed in this study. Furthermore, the instruction to stay still during the 6 minute resting task may have caused participants to actively inhibit movement. There is research to show that prefrontal cortex and insula, as well as some motor areas (including primary motor cortex and superior parietal areas) activate in anticipation of movement and emotion regulation (e.g. Cunnington, Windischberger, Deecke, & Moser, 2002; Denny, Ochsner, Weber, & Wager, 2014). The possible anticipatory emotion effect could be tested by conducting a study where resting scans took place before or after an emotion-related task.

Analysis of resting connectivity of the amygdala, parcellated into three sub-divisions (laterobasal, centromedial and superficial) revealed that although there were some areas of overlapping connectivity, each sub-division had its own unique pattern of connectivity. First the overlapping connectivity will be discussed, as it is most analogous to the whole amygdala analysis presented above. There were only two areas with significant connectivity across the subdivisions, one being a positive connectivity between all three regions and the hippocampus. This result differs from that of Roy and colleagues (2009), but is not unexpected considering that the amygdala and hippocampus are known to be richly connected (Stefanacci, Suzuki, & Amaral, 1996). The hippocampus is implicated in encoding and retrieval of emotional memories (Greenberg et al., 2005; Smith, Stephan, Rugg, & Dolan, 2006). Retrieval of emotional memories during resting state is perhaps not surprising and the possibility that emotional memories are being encoded and stored is in keeping with the suggestion that resting state brain activation enables processing of experiences (both cognitive and emotional) in a system of adaptive learning (Albert, et al., 2009; Lewis, et al., 2009). The second area of convergent connectivity was the amygdala itself, with the right
hemisphere amygdala sub-divisions demonstrating positive connectivity with the right CM amygdala. This region of the amygdala is a key output region involved in not only mediating behavioural response but also orienting attention to salient information (Davis, 1992; Roy et al., 2009). In addition, the right amygdala has been implicated in the more immediate threat response system in the brain (Ohman, 2005; Romanski & LeDoux, 1992; Shi & Davis, 2001); it is reasonable to infer that the CM amygdala is highly connected to the other regions within the right amygdala in order to process incoming and outgoing information quickly and efficiently with feedback systems in place from each sub-region of the amygdala for rapid response. Although the connectivity results here suggest that this may be the case, this is at the moment a tentative explanation of the results, and more research would be necessary on the directionality of the connections between amygdala sub-divisions at rest. Determining such a relationship would further bolster arguments put forward regarding hemispheric specialisation of function and dual processing of emotion stimuli (see section, 1.5.2 and Chapter 5). The detection of such subtle connectivity profiles demonstrates the utility of investigating connectivity of each amygdala sub-division and not treating the structure as one homogenous area. It should be noted that in the conjunction analysis, no negative connectivity was observed with the amygdala, much like the results of the whole amygdala analysis discussed earlier. It could be that whilst the negative connectivity patterns are unique to the sub-divisions, positive connectivity patterns share greater overlap and are thus visible at whole-amygdala or conjunction analysis.

Indeed, the connectivity results of each of the sub-divisions further evidences the utility of a parcellation approach in gaining more nuanced data, albeit with some methodological caveats. Laterobasal connectivity results reflect those of Roy et al. (2009), revealing positive connectivity with primarily temporal, and some frontal regions with the right LB. This is consistent with evidence from the literature suggesting the laterobasal amygdala's involvement in associative learning processes (e.g. Roy et al., 2009; Bzdok, Laird, Zilles, Fox, & Eickhoff, 2013). Furthermore, as indicated by Roy et al, (2009) the present patterns of negative connectivity between LB in more dorsal and posterior regions (including middle frontal gyrus, precuneus, right parietal lobe and cerebellum), as well as the right LB and other frontal regions (orbitofrontal, inferior frontal), bilateral precentral, supplementary motor area and right caudate, suggest a clear role for the LB division (especially the right LB) of the amygdala in overall emotion regulation. Such results are consistent with evidence from task-based studies showing similar emotion circuitry (Guo, Nguyen, Hyett, Parker, & Breakspear, 2013).
2015; Hariri, Bookheimer, & Mazziotta, 2000; Ochsner et al., 2004), and indicate that although no negative connectivity is seen at a whole amygdala or conjunction analysis level, typical emotion-related negative connectivity can be observed by parcellating the amygdala.

The results from the centromedial amygdala also correspond, to some extent, with Roy et al. (2009) findings, with positive connectivity observed between the CM and the insula and basal ganglia (putamen). As mentioned earlier, activation of the CM amygdala is implicated in in orienting attention and preparatory mobilisation of the motor system (Davis, 1992). The insula has been consistently implicated in response to appraisal of distressing stimuli, as well as general salience or emotion processing (Dupont, Bouilleret, Hasboun, Semah, & Baulac, 2003; Phillips et al., 1997). The putamen contains neurons that activate prior to movement (Alexander & Crutcher, 1990), adding to the evidence that CM connections are involved in readying motor responses and attention that will allow rapid appraisal and behavioural responses to emotional stimuli.

The superficial amygdala is thought to be involved in attentional shifts towards incoming somatosensory signals and salience appraisal of these stimuli (Price, 2003, Goossens et al., 2009; Koelsch et al., 2013), and is positively coupled with the hippocampus and middle temporal cortex. Roy and colleagues (2009) did not observe any coupling with the middle temporal regions, but its implication in emotion appraisal processes (Ochsner et al., 2004) supports Roy and colleague's assertion that the SF amygdala plays a key affect role in emotion.

Both the CM and SF regions exhibited negative connectivity with the hippocampal region at rest (CM: hippocampus, and right middle cingulum; SF: hippocampus, cuneus and supramarginal gyrus), and both exhibited significantly greater negative connectivity with hippocampal regions compared to the other sub-divisions (hippocampus, parahippocampus, fusiform, middle temporal gyrus). Furthermore, results indicate that the SF has both positive and negative connectivity with the hippocampus, as well as significantly greater positive and negative connectivity in comparison to other regions. Finally, despite two of the three sub-divisions demonstrating significantly greater negative connectivity with the hippocampus, conjunction analysis reveals an overlap in positive connectivity only. The contradictory nature of these results highlight the complicated nuances of connectivity within these amygdala sub-divisions, but also adds to a growing body of evidence that the hippocampus is also anatomically and functionally heterogeneous, with studies looking to functionally
parcellate the hippocampus itself (Cheng & Fan, 2014; Yushkevich et al., 2010; Zarei et al., 2013). The apparently mixed results in relation to the hippocampus cannot be teased apart with the current dataset, but do suggest that LB, CM and SF are perhaps connected to functionally unique regions of the hippocampus.

Whilst the LB and CM areas appear to be negatively correlated with motor output areas (LB: precentral, supplementary motor area, caudate; CM: middle cingulum), the whole amygdala resting state analysis showed positive connectivity with motor areas (primary motor cortex). This apparently contradicts the notion that participants were actively inhibiting movement, or indeed that the CM is involved in motor-preparedness and response to emotional stimuli (positive connectivity with putamen). This is also an interesting contradiction that cannot be investigated further with the current dataset, but requires more investigation. At the sub-division level, previous studies by Roy and colleagues (2009) and Ball et al. (2007) found evidence of a pattern of reciprocity between the LB and CM activation at rest, and animal models also evidence high level of connectivity (Collins and Pare (1999), Campese, Gonzaga, Moscarello, and LeDoux (2015)), possibly involved in a threat response. Perhaps the mutual relationship between these areas underlies the contradictory findings in relation to motor response at the sub-division and whole amygdala level.

It should also be noted that the size of the sub-divisions may influence the results gained in this study. For example, it is noticeable that fewer areas passed threshold in this study for SF connectivity in comparison to Roy et al. (2009) findings. This sub-region is particularly small, and is made smaller by the rigorous methods enlisted to ensure no overlapping regions were measured. A small voxel sample for the SF perhaps means it is more likely that some of the replication results in this area differ from the original study. In addition, another possible explanation for the apparently contradictory hippocampal connectivity findings is that the LB region is the largest region, with greater voxel sampling and perhaps statistical power. Therefore, the comparison of areas may have been overly-biased towards this area, with greater negative coupling in CM and SF due to the greater positive coupling seen in LB. The positive coupling in the conjunction analysis may also be due to greater statistical power coming from this one area. This is a methodological caveat that must be considered when interpreting the results.

Despite these methodological considerations, it is clear that parcellation of the amygdala enables more subtle analysis of unique patterns of connectivity, and that the findings in the
study are in line with previous literature (Ball, Rahm, Eickhoff, Schulze-Bonhage, et al., 2007; Roy et al., 2009). This confirms the validity, and importance, of parcellating results when studying connectivity of the amygdala in affective neuroscience.

9.6.2 Modulation by anxiety

The correlation results revealed that increasing self-report of (sub-clinical) anxiety was associated with reduced connectivity between the left amygdala and frontal regions, insula, FFG, inferior temporal gyrus and angular gyrus. Group-wise analysis also revealed reduced fronto-amygdala connectivity (orbitofrontal-right amygdala) in those with high anxiety. The reduction in fronto-amygdala connectivity in highly anxious participants in the present study supports the notion that disruption to this connection underlies abnormal or dysfunctional anxiety (Etkin & Wager, 2007). This study finds evidence of this disruption in resting connectivity (not during active emotional processing), and even in a healthy (sub-clinical) population, suggesting that there are possible early indicators or risk factors for propensity towards clinical anxiety. Kim et al. (2011) also looked at modulation of resting state connectivity by self-report measures of anxiety in a healthy female population, specifically looking at amygdala connectivity with medial prefrontal cortex (the dorsal and ventral divisions). They found that the negative connectivity between dmPFC (defined as superior frontal gyrus) and the amygdala was reduced in high anxiety participants compared to low anxiety participants. The current study found a reduction in connectivity between the amygdala and superior frontal gyrus (along with orbitofrontal and middle frontal gyrus) with increasing anxiety scores. However, despite the sub-division analysis revealing some negative fronto-amygdala connectivity (for LB only), the whole amygdala analysis on which this correlation is based only revealed positive connectivity between the left amygdala and frontal regions. This would therefore imply that individuals with higher anxiety have reduced positive connectivity between the amygdala and frontal areas. Reasons for the lack of negative connectivity at the whole amygdala level have been discussed previously. In general, these findings support the notion of the key role of fronto-amygdala connectivity in anxiety, but the nature of this connectivity appears to be different.

Of the other areas whose connectivity was modulated by anxiety, the vlPFC (inferior orbitofrontal cortex) and insula are key parts of the ventrofrontal parietal network (Corbetta,
Patel, & Shulman, 2008), which is implicated in reflexive re-orienting of attention to an unexpected, non-focal external stimulus. In addition, the fusiform gyrus is known to be involved in attentional processes (Kanwisher & Wojciulik, 2000) and the anterior insula has been associated with salience and emotion perception and control of emotional and goal directed behaviour (Bebko et al., 2015). This pattern of intrinsic decoupling in individuals with higher anxiety may allude to pathophysiological processes that may lead to an impaired or absent ability to direct attention to the most pertinent features of an incoming stimulus in order to process salience of such stimuli. Indeed, there is evidence showing individuals high in anxiety show impaired attentional control (Bishop, Duncan, Brett, & Lawrence, 2004; Bishop, Duncan, & Lawrence, 2004, also see Chapters 5 and 6). Bishop, Duncan, & Lawrence (2004) showed that high anxiety participants attended to stimuli that were both within or outside of the current focus of spatial attention for performance of a task, whereas controls only attended to those stimuli within the focus of spatial attention. As whole brain connectivity between the amygdala and these areas was in a positive direction, this decoupling suggests that these areas are less likely to act in unison in those with high anxiety. This perhaps suggests a reduced ability to couple attention to those aspects that are of most salience, resulting in less focused attention and hypervigilance. In general, these results suggest a dysregulation in amygdala-attentional networks at rest as a function of anxiety.

However, it must be mentioned that, contrary to findings here, previous resting connectivity studies have found increased amygdala-insula connectivity both when examining participants with various clinical anxiety diagnoses (Hamm et al., 2014; Prater, Hosanagar, Klumpp, Angstadt, & Phan, 2013; Rabinak et al., 2011; Sripada et al., 2012) and in non-clinical populations investigating state anxiety (Baur, Hänggi, Langer, et al., 2012; Dennis, Gotlib, Thompson, & Thomason, 2011). In this study, reduced resting connectivity was observed between the amygdala and the insula (seen here in both correlation and group-wise analysis (where the orbitofrontal region borders posterior insula)). This type of reduction in connectivity has been shown before in a study looking at behaviourally and emotionally dysregulated youth (including those seeking help for mood related disorders) compared to healthy controls (Bebko et al., 2015), and it should be noted that the sub-clinical studies only reported increased connectivity with the anterior insula. It is clear that further resting state research in healthy anxious populations, as well as other forms of anxiety disorder groups need to be run to confirm current findings.
There was a positive correlation between self-reported anxiety and connectivity between the right amygdala and precuneus. The amygdala exhibited a positive connectivity profile with the precuneus across participants, suggesting that increased anxiety therefore results in greater positive connectivity between these areas. As the precuneus is part of the default mode network, it shows consistently heightened activation (Utevsky et al., 2014) and the highest resting metabolic rate (Gusnard & Raichle, 2001) at rest. This therefore suggests that amygdala activation is more consistently elevated at rest in those with anxiety, and can therefore be taken as evidence for a hyper-responsive amygdala and hyper-vigilance in these individuals (e.g. Barrett & Armony, 2009; Etkin & Wager, 2007; Quirk & Gehlert, 2003).

There was some evidence of anxiety-related modulation of amygdala connectivity with temporal areas (reduced connectivity with inferior temporal gyrus and angular gyrus) in the correlation analysis, and the group-wise analysis also revealed reduced connectivity with temporal areas (inferior/middle temporal, hippocampus and FFG) in those with high anxiety. In addition, as mentioned before, both the correlation and group-wise analysis indicate that there is a reduction in (positive) connectivity between orbitofrontal regions and the amygdala. Temporal regions (specifically the lateral temporal cortex which includes inferior and superior temporal gyri) have been implicated in semantic and conceptual processing in an automatic system of social inference (Crinion, Lambon-Ralph, Warburton, Howard, & Wise, 2003; Rissman, Eliassen, & Blumstein, 2003; Satpute & Lieberman, 2006). The inferior orbitofrontal gyrus (specifically posterior insula region identified here) is highly connected with sensorimotor cortices and is said to be involved in interoceptive and emotion processing and visceral experiences (Bebko et al., 2015; Cauda et al., 2012; Dupont et al., 2003). The reduced coupling between the amygdalae and these regions in highly anxious individuals may therefore indicate a reduced ability to integrate affective, semantic and interoceptive information.

Specific to the group-wise analysis of anxiety, individuals with high anxiety revealed less connectivity between right and left amygdala and greater connectivity between both amygdalae and the cerebellum. The reduced coupling between the left and right amygdala further endorses the idea that highly anxious individuals have impaired feedback and reduced cross-talk between the nodes involved in emotion processing and the nodes underlying response systems. This fits together with the discussion above about the temporal and orbitofrontal differences seen in anxious individuals. The cerebellum has been shown to be involved in emotion processing, in particular in response preparation and anticipation of
negative stimuli (Schraa-Tam et al., 2012). Increased resting connectivity would yet again endorse the idea that highly anxious individuals are in a heightened state of emotional readiness even at rest compared to low anxiety participants. Indeed, research looking at autonomic arousal has indicated that individuals with anxiety disorders have hypersensitive central nervous system, linked specifically to amygdala activity (Bakker, Tijssen, van der Meer, Koelman, & Boer, 2009), and there is a large body of evidence linking anxiety disorders and exaggerated auditory startle response (Bakker et al., 2009; Grillon & Baas, 2003; Ludewig et al., 2005; Morgan, Grillon, Southwick, Davis, & Charney, 1996). This previous research fits well with evidence indicating hypervigilance, altered attention and some level of anticipatory motor response at rest in our sub-clinical anxiety sample.

9.6.3 Modulation by gender
Gender modulations of resting amygdala connectivity were similar to those observed in the anxiety group, with females exhibiting reduced connectivity between the amygdala and frontal regions, the insula, precuneus and temporal regions as well as increased connectivity with the cerebellum. Therefore, similar conclusions can be drawn from the results for the female group as have been discussed above for the high anxiety group. As mentioned in previous chapters, research shows women tend to report with higher prevalence of anxiety related disorders (Solomon & Herman, 2009). It is therefore highly likely that this overlap is not borne of chance, and is at least in some way reflective of the gender composition of the high and low anxiety groups (high anxiety group = 6 male, 12 female; low anxiety group 19 male, 20 female). This may also indicate the likelihood of these different groups taking part in such a study (see chapter 4). However, it is not possible to tease apart the relative effects of gender and anxiety in this sample, this requires a larger sample to allow for robust statistical testing. The similarity of findings in the female and high anxiety groups, if not entirely due to group composition, has some implications for sampling and collection of individual differences in such research. For instance, as has been seen in the rest of the thesis, sampling of only one gender would lead to very different results, and a more representative sample can lead to more nuanced and interesting findings.
9.7 Limitations
A key criticism levelled against resting state fMRI studies is the susceptibility of the spontaneous low-frequency BOLD fluctuations being measured to movement and noise (Duncan & Northoff, 2013; Power, Barnes, Snyder, Schlaggar, & Petersen, 2012). In particular, a study by Power et al., (2012) has demonstrated that head movement can have serious implications for resting state results, introducing false positive correlations. Furthermore, the authors determined that even when following standard movement pre-processing steps, these spurious results remained. Despite reducing the accepted threshold for movement within this study from the standardly accepted 5mm head movement, to a stricter 3mm range, techniques such as data scrubbing (documented by Powers and colleagues (2012)) could be employed in future replications to further ensure the data are reliable and valid.

A second limitation is the use of a seed-based approach, correlating the time series of ROI seeds with voxel across the whole brain to determine functional connectivity at rest. It has been noted that this approach can reduce the validity of observed results depending on how the ROI is defined. This is particularly problematic in studies using predefined anatomical masks from atlases or using a seed regions defined by a sphere around standard co-ordinates published within the literature as these results are not likely to detect the inter-subject variability in a specific sample. This study took steps to overcome this limitation. Peak points of activation for bilateral amygdala were calculated from second level data collected during the task-based study (passive viewing, see chapter 5), and the mask created from this analysis was constrained by standard cyto-architectonic maps defined in WFU PickAtlas to ensure the peak lay within the amygdala. The seed time series with a sphere of radius 5mm were then extracted for each participant around the data-driven peak points. It is hoped that this data driven approach informed by standardised maps overcomes some of the limitations levelled against using a seed-based approach.

As previously discussed the spatial resolution (3x3x4) of the data presently being reported means that parcellation is difficult (see section 9.6.1. for discussion of the size of the different sub-divisions). Future replications should use higher resolution scans which may enable better parcellation and clearer results, particularly of the SF region which were notably smaller than the LB and CM. Increasing spatial resolution would mean lower temporal resolution for connectivity analysis. Within the remit of this study, and that of Roy et al.
2009, the compromise was spatial resolution, but further analysis needs to be conducted in this area.

It must also be noted that the observed functional connectivity between amygdala and the rest of the brain reported here does not allow for inferences about causal relationships or directionality of these relationships. This has been made clear throughout the discussion, and the discussion of other connectivity related chapters (Chapter 6), with alternative perspectives being taken into account and using an evidence base to back up suggestions inferring directionality. Future studies are needed to corroborate the present findings; in addition, studies using dynamic causal analysis will greatly inform the current results.

Finally, criticisms within the field of neuroimaging have been levelled against the use of resting state fMRI at all. There is heavy debate as to how informative ‘resting state’ data actually is. In particular questions have been raised as to whether the brain is actually ‘at rest’; the number of factors that may influence resting state (i.e. even use of different instructions given or the context of study); what default mode means from an empirical standpoint and how we can interpret such data. Since resting state fMRI is still in it’s infancy as a technique, these debate points are valid and worth further consideration. However, a full debate about the role and utility of resting state in neuroimaging research is beyond the scope of this thesis, but can be seen in recent review articles (Lee, Smyser and Shimony, 2013; Murphy, Birn and Bandettini, 2013; van den Heuvel and Pol, 2010).

A particular area of debate, surrounds the issue of negative coupling, or anti-correlated networks, identified within research. In brief, there is not only debate regarding the interpretation of negative coupling, but also debate as to whether an artificial bias towards identification of negative coupling may result from a standard procedure of correcting for global signal (Murphy et al., 2009). Interpretation negative coupling, in particular, is open to debate. There is no causative link between activity in brain areas and negative coupling could be understood in a number of ways, i.e. either that area A is inhibiting area B; area B is inhibiting area A, or even that a third area is influencing both. Interpretation of the direction of negative coupling needs to be informed by strong evidence from within neuroscience literature, without such evidence results should be treated with caution and not assigned causal links. With regards to regressing out global signal (to reduce likelihood of non-neuronal physiological noise confounding results); the possibility that this standard pre-processing step risks artificially inflating negative coupling observed within the data is
concerning. However, there are no clear conclusions with proponents within the field still backing both arguments (to regress it out or not). Importantly the informative potential of functionally negative networks within the brain enhancing understanding of underlying functional networks means that disregarding any observed negative coupling would be detrimental to advancing such understanding (e.g. see Popa et al., 2009). Again, in depth discussion of this issue is not within the remit of the current thesis however, Cole, Smith and Beckman (2010) present a particularly enlightening discussion on the issues surrounding anti-correlated or negative networks. It is of note that within this chapter the analysis pipelines have differed which could account for the differing findings in the whole amygdala and sub-region analysis. Specifically, in light of evidence from the literature surrounding global signal, it was not regressed out during pre-processing of the whole brain resting connectivity analysis. Conversely, within the parcellation analysis, global signal was included as a regressor since this analysis was attempting a partial replication of the study by Roy et al. (2009) who also included global signal as a nuisance variable. Future studies drawing comparison between whole brain and parcellation data should look towards matching analysis strategies more closely in order to rule out any differences in results due to differing analysis pipelines.

9.8 Conclusion
The present chapter describes data from an Rs-fMRI study of a large group of sub-clinical participants, looking at the functional connectivity of the amygdala at rest. Results presented here endorse previous findings in the literature that the sub-nuclei of the amygdala have distinct and unique patterns of connectivity at rest (Ball, Rahm, Eickhoff, Schulze-Bonhage, et al., 2007; Roy et al., 2009). Furthermore, it has been demonstrated that patterns of resting functional connectivity of the whole bilateral amygdala modulates as a function of self-reported measure of anxiety and individual differences in gender also impact upon these patterns of connectivity. Results not only support the claim that a key pathophysiological characteristic of anxiety is disrupted fronto-amygdala connectivity but also shows that this disrupted connectivity is present even at rest, indicating anxiety may relate to a fundamental underlying neurological disruption. Furthermore, it provides evidence to suggest that disruption to connectivity between the amygdala and ventrofrontal parietal network and motor cortices at rest may explain characteristics of anxiety and mood disorders such as hyper-responsivity of the amygdala, hypervigilance and increased auditory startle response.
These results have implications for diagnostic biomarkers of anxiety, potential markers for the progression of anxiety disorders and also have research based ramifications. Future task-based studies of anxiety disorders must consider individual differences in baseline resting activation when interpreting task-based connectivity results.
Chapter 10: Discussion

10.1 Overview of aims
The primary focus of this thesis is to characterise amygdala activation during emotion processing in a healthy population of participants, and examine how this activation is modified by individual factors such as sub-clinical anxiety and gender. In particular, sub-clinical anxiety participants were investigated as an insight into the maladaptive emotional processing underlying clinical affective disorders. Chapters 5, 6 and 9 all explored the interaction between amygdala activation and sub-clinical anxiety (as well as gender), with chapter 5 focusing on typical amygdala responsivity (habituation, lateralisation, valence) during a passive emotion induction task, chapter 6 focusing on the connectivity of the amygdala during this task, and chapter 9 focusing on resting amygdala connectivity (non-task related). These chapters aimed at characterising these interactions, as well as identifying potential underlying neural biomarkers of emotion processing and proclivity towards developing anxiety disorders. Chapter 7 used machine learning methods to attempt to categorise the neural responses generated in chapters 5 and 6 into those underlying processing of fear, happy and neutral emotion. This aim was to identify patterns which distinguish "typical" emotional processing, and inform future analyses by elucidating the most discriminatory regions or connections. Chapter 8 investigated whether there were any structural changes associated with sub-clinical anxiety, similar to those found in clinical affective disorders, which may represent possible markers of increased risk of developing mood disorders. Finally, Chapter 4 was a supporting chapter which investigated whether anxiety or gender influences willingness to volunteer in research, dependent on type of research design (performance measurement/brain scanning). This is an important factor to ensure study cohorts are representative and generalisable, enabling the translation of findings in highly anxious sub-clinical populations to clinically anxious individuals.

10.2 Summary of results by study
Chapter 5 revealed that amygdala activation is not modulated by valence (fear, happy, neutral) in a passive viewing paradigm of emotional faces, nor are there any differences in activation of the left and right amygdala in response to these stimuli. However, there is a clear habituation effect over time to repeated presentation of the backwards masked stimuli.
These results are modified when anxiety and gender are taken into account. Highly anxious individuals’ exhibit greater amygdala activation in response to emotional faces compared to low anxiety participants, and women are also found to exhibit greater amygdala activation in response to emotional faces (in particular fear faces) compared to men. Furthermore, the high anxiety participants also exhibited heightened amygdala activation (hypervigilance) across the duration of the study, compared to the typical patterns of habituation observed in low anxiety participants. In addition to amygdala activation patterns, fusiform gyrus (FFG) was investigated as a control area to account for anxiety and gender influences on general processing of the stimuli. Results from the FFG are suggestive of a gender difference in processing, with male results perhaps suggesting alteration in attention over the course of the task.

Chapter 6 investigated the connectivity of the (right and left) amygdala during the same task used in chapter 5 (backwards masked presentation of emotional faces). The group level psychophysiological interaction (PPI) analysis revealed a pattern of negative coupling between the right amygdala and frontal regions during fear and neutral conditions. Looking specifically at connectivity with the four a priori regions of interest (anterior cingulate cortex (ACC), ventromedial prefrontal cortex (vmPFC), dorsomedial prefrontal cortex (dmPFC) and the precuneus), connectivity with the dmPFC appeared to be particularly important in emotion processing. Anxiety did not significantly interact with fronto-amygdala connectivity, but connectivity between the dmPFC and amygdala was modulated by interactions between valence and hemisphere, gender and valence, and gender and hemisphere. In particular, women showed significant patterns of a valence effect (greater connectivity in happy compared to fear), whereas men exhibited no valence related differences in connectivity. Females also revealed greater left amygdala connectivity with the dmPFC, whereas males exhibited greater right amygdala-dmPFC connectivity. The FFG was again used as a control for general visual processing, and analysis revealed that FFG-ACC connectivity was modulated by an interaction between anxiety and valence. Numerically greater negative FFG-ACC coupling was observed in the high anxiety participants, particularly for the fear condition. Of interest as its role as a control region, no interactions were seen in fronto-FFG connectivity and gender.

Chapter 9 observed the resting, spontaneous connectivity of the (right and left) amygdala. Analysis across all participants revealed positive connectivity between the left amygdala and frontal areas, and the right amygdala and superior temporal regions. Bilateral amygdala
connectivity was also positively coupled with areas associated with fear circuitry and emotion (including ventral ACC, hippocampus, thalamus and precuneus). This resting connectivity was modulated by anxiety, with increasing anxiety associated with reduced connectivity between the left amygdala and frontal regions, the insula, FFG, inferior temporal gyrus and angular gyrus, as well as reduced connectivity between both amygdalae and temporal regions, reduced inter-amygdala connectivity and reduced right amygdala-orbitofrontal connectivity (in group analysis). Increased anxiety was also associated with increased connectivity between the right amygdala and bilateral precuneus, as well as increased connectivity between both amygdalae and the cerebellum (group analysis). Similar modulations in connectivity were observed for gender differences, with women exhibiting decreased connectivity between frontal and temporal regions and greater connectivity between bilateral amygdala and cerebellum. This chapter also applied Roy et al.’s (2009) parcellation techniques to the resting state data, with results showing some overlap with the earlier paper. In particular, this study replicated the patterns of positive connectivity between the laterobasal (LB) amygdala and temporal and frontal regions, and negative coupling between the LB and dorsal and posterior brain regions. However, results did not reveal as extensive connectivity between the centromedial (CM) and superficial (SF) amygdala and the rest of the brain as seen in Roy and colleagues (2009) earlier study.

Chapter 7 sought to use support vector machine (SVM) and maximum uncertainty linear discrimination analysis (MLDA) to classify whole brain activation patterns (GLM, Chapter 5) and connectivity patterns (PPI, Chapter 6). Only one SVM comparison (fear versus happy condition) revealed above chance level accuracy for either GLM and PPI data. The pattern of most discriminatory areas for fear versus happy processing was extremely diffuse (although included areas associated with emotion processing), precluding their use in informing future analyses. All other SVM, and MLDA comparisons, were below chance level accuracy.

Chapter 8 investigated structural correlates of anxiety, and revealed that increased anxiety was associated with reduced prefrontal cortex volume (bilateral vmPFC, right dmPFC). When participants were categorised in high and low anxiety groups, this pattern was preserved in right vmPFC volume only. Furthermore, there was evidence of a correlation between increased anxiety and reduced left amygdala. When split into groups, high anxiety participants exhibited a reduction in volume for both left and right amygdala reduction. No significant structural differences were observed in hippocampal volume. A state-trait anxiety scale was also used in the structural data (in addition to the hospital anxiety and depression
scale (HADS) used throughout the thesis), as enough of the participants had completed this questionnaire to enable analysis. Interestingly, there was a correlation between increased anxiety on this scale and reduced volume in the fusiform gyrus (FFG).

Chapter 4 revealed that willingness to participate in research is not modulated by anxiety or gender alone, but the interaction of these two factors with the type of research being conducted. Highly anxious men were less willing to take part in the most 'high-stress' of all the research designs (combining both performance measurement and a brain scanning environment) compared to low anxious men. In addition, highly anxious native speakers were less likely to be willing to take part in this particular study design compared to low anxiety native speakers. No differences were seen between high and low anxiety females, and no differences were seen on performance measurement tasks and brain scanning tasks in isolation.

### 10.3 Implications of results

This study looked at a number of different aspects of amygdala activation in the context of emotion processing in a sub-clinical anxiety population. Crucially, each analysis was done using the same cohort (with some small changes in numbers for particular analyses; see Figure 3.7), which allows stronger inferences to be drawn from the data across the different analyses. The overall implications for findings discussed within this thesis are three-fold; not only do they make a significant contribution to current theoretical debates and possible models of anxiety, there are methodological implications and perhaps most importantly, practical implications in terms of contributing to diagnostic criteria and potential identification of neural biomarkers of anxiety. These three implications will be discussed in turn.

#### 10.3.1 Theoretical Implications

Three key theoretical models have been discussed at length throughout this thesis; the dual processing model of amygdala activation (LeDoux, 1996; Morgan & LeDoux, 1995; Phillips & LeDoux, 1992; Romanski & LeDoux, 1992), the salience detector theory of amygdala activation (Davis & Whalen, 2001; Sander, Grafman, & Zalla, 2003) and the disrupted fronto-amygdala theory of emotion disorders (Etkin & Wager, 2007). The first two theories
are often treated within the literature as opposing theories; however, the salience detector theory primarily focuses on valence specificity of the amygdala and its role in emotion processing, whilst the dual processing theory focuses on habituation and lateralisation of the amygdala during emotion processing. The evidence presented within this thesis appears to suggest that perhaps the two theories should be integrated. In particular, results from chapter 5 revealed habituation patterns in amygdala activation over the course of emotion presentation. This finding lends support to the salience detector theory, since repeated presentation of a stimulus without meaningful consequences renders it no longer salient and consequently attentional resources can be saved by no longer responding (Wright et al., 2001). Further support for this theory was found when applying PPI analysis to investigate connectivity patterns during the task (chapter 6). Left amygdala connectivity with both frontal regions and sensory processing areas (FFG) was primarily evident for fear and happy conditions. In contrast, the right amygdala revealed a pattern of connectivity with frontal areas and the thalamus, primarily in fear and neutral conditions. These results suggest that the left amygdala is involved in more socially salient emotion processing, whilst the right amygdala may be more responsive in a threat detection role. Though chronological differences in amygdala activation were not observed in the GLM analysis (no lateralisation differences in habituation at group level), this apparent functional specificity in connectivity in the PPI analysis supports the notion that the left and right amygdala process incoming emotionally stimuli in parallel in a synergistic manner. Consequently, not only do these findings endorse the salience detector theory (especially the left amygdala connectivity), but they also provide support for the dual processing theory. The resting connectivity analysis also provides support for this salient parallel processing of emotion, with significant positive coupling converging in the right centromedial amygdala from the other sub-nuclei of the right amygdala (chapter 9). As discussed in chapter 9, research has suggested that the centromedial amygdala plays a role in mediating behavioural responses and orienting attention (Davis, 1992; Roy et al., 2009). By inference, such converging connectivity in the right amygdala would suggest a requirement for efficient appraisal and reaction to incoming emotion stimuli. This fits with the notion that the right amygdala is specialised for rapid response to threat based cues as put forward in chapter 5 and by the dual processing theory. The development of a hybrid theory of emotion processing combining these two theories could not only reduce the level of contention within emotion literature but also enable further progression within the field of emotion research with less restrictive theoretical frameworks in which to decipher such a complex phenomenon.
Evidence has also been provided within this thesis for the concept that maladaptive emotion processing could arise from disruption of fronto-amygdala connectivity, as first put forward by Etkin and Wager (2007), who assessed the data from clinical populations with anxiety disorders. It has long been established that frontal regions, in particular the dmPFC and vmPFC, are involved in a system of top-down inhibitory control. Furthermore, these frontal regions are known to interact with the amygdala in the process of emotion regulation. Therefore, the detection of volumetric reductions in the prefrontal cortex (bilateral vmPFC and right dmPFC) and left amygdala with increasing self-reported anxiety (Chapter 8), is particularly interesting. The patterns of volumetric reduction persisted even when participants were categorised into anxiety groups (reduced right vmPFC and bilateral amygdala volume in high anxiety participants compared to low anxiety). These findings support the idea that fronto-amygdala connectivity is particularly susceptible to anxiety (as suggested by Etkin and Wager, 2007). Furthermore, such results reveal early signs of such disruption in emotion processing in a sub-clinical population of participants, perhaps hinting at latent risk factors for anxiety disorders or the effects of long-term low-level anxiety (e.g. raised cortisol levels) on brain plasticity and structure. To this authors knowledge, such a structural change has not been evidenced before in a sub-clinical population. Structural differences in these fronto-amygdala regions could result in inefficient appraisal and emotion processing from a combination of a lack of top-down control from frontal regions, which then overwhelm a reduced amygdala. This could result in characteristic amygdala hyper-responsivity seen in clinical populations (Etkin and Wager, 2007). The lack of habituation observed during the study, seen only in high anxiety participants (discussed in Chapter 5), along with numerically reduced fronto-amygdala connectivity in high anxiety participants compared to low anxiety participants (Chapter 6), and reduced fronto-amygdala and amygdala-amygdala connectivity at rest in anxious participants (Chapter 9) would endorse this suggestion of inefficient processing arising from structural reductions in key emotion processing regions. In this way, this thesis links structural changes to functional (activation and connectivity) changes in the same population in a way that plausibly explains the data from the sub-clinical anxiety participants.

Within this thesis, the fusiform gyrus has been included as a control region to ensure results obtained were specific to the amygdala, and not affected by lower level visual processing. However, inclusion of this region proved fortunate as it not only served as a control region, but also tentatively revealed that attentional systems may be disrupted in maladaptive
emotion processing. Therefore, maladaptive emotion responses associated with anxiety may not just result from inefficient appraisal in top-down regions, and disruption between these prefrontal regions and the amygdala, but could also result from ineffective orienting to emotional stimuli. In other words, anxiety may be related to inefficient salience detection and appraisal. Research has recognised that enhanced sensory responses are associated with emotional stimuli (Lane, Chua, & Dolan, 1999; Vuilleumier, Armony, & Dolan, 2003; Vuilleumier, Armony, Driver, & Dolan, 2001; Vuilleumier, 2005), with many studies using attention based tasks and finding participants orient to emotional stimuli even when these are not the specific target (Krolak-Salmon, Fischer, Vighetto, & Mauguiere, 2001; Schupp, Junghöfer, Weihe, & Hamm, 2003). Evidence presented in chapter 6 suggested that anxiety modulates ACC-FFG connectivity, particularly in fear and neutral conditions, and structural investigation in chapter 8 revealed reduced volume of the right FFG as anxiety scores increased. Furthermore, resting connectivity analysis (Chapter 9) revealed that as anxiety scores increased, there was a reduction in connectivity between the amygdala and FFG, ventrolateral prefrontal cortex and insula (both part of the ventofrontal parietal network involved in orienting attention (Corbetta et al., 2008)), and increased connectivity between the right amygdala and the precuneus (a key node in orchestrating the default mode network (DMN; Utevsky, Smith, & Huettel, 2014). Taken as a whole, these resting connectivity results also indicate the importance of attentional systems during emotion processing. Across the chapters, there is evidence that attentional systems are overactive in those with high anxiety, perhaps related to the reduced cortical volume observed, with this heightened attention meaning they are unable to selectively attend to or distinguish between salient and non-salient stimuli. This overactive attention, or hypervigilance, is again corroborated by a lack of habituation observed in the same participants during the backwards masked task. It is of note that these observations are weaker than results for the amygdala, with only numerical differences between anxiety groups for FFG activation and connectivity in chapters 5 and 6, and reduced FFG volume only associated with measures of anxiety using the state-trait anxiety inventory (STAI-6), not the HADS (Chapter 8). As a result, further investigation is necessary, specifically looking at the interplay of attentional systems rather than the secondary observations in the current body of work. Nonetheless, the observation that attentional systems may also interact with anxiety could inform future theories, and perhaps when creating a hybrid theoretical model that integrates the dual processing model and salience detector theories, attentional systems should also be included.
10.3.2 Methodological Implications

A reoccurring implication, identified in almost every chapter, is the moderating impact that individual differences in gender and anxiety can have on results. In particular, it is clear in chapter 5, and to some extent chapters 6 and 8, that without accounting for these individual differences, group level results may not be sensitive enough to detect subtle differences in neural substrates of emotion processing. To a certain extent this has theoretical implications, for example in chapter 5, group level results only revealed habituation effects in amygdala response to presentation of a backwards masked emotion stimulus, which only supports the salience detector theory. However, accounting for gender revealed valence and habituation interactions, and accounting for anxiety revealed a lateralisation by habituation interaction, providing further support for salience detector theory and the dual processing theory. Beyond these theoretical implications, this finding clearly indicates that it is imperative that individual differences are measured in emotion research. Even if they are not used for further investigation, they certainly need to be controlled for, as they clearly present potential confounds. Furthermore, current findings arise from a sample of the population who could be considered a control population; all participants had no previous diagnosis of mood-related disorders which is often a screening criteria used in emotion based research. Regardless of the lack of clinically evaluated mood disorders, clear differences in the group composition were seen, with a split of high and low anxiety participants who revealed markedly different brain responsivity, connectivity and volumetry in task based fMRI and at rest. The importance of including measures of individual differences to inform neuroscientific findings was highlighted by Kosslyn et al. (2002), who suggested accounting for individual differences would be a useful complementary approach to bridge the gap between biological understanding and psychological theories. This notion has been highlighted since, with studies demonstrating the scope of individual differences, and the level of variance they can introduce into research implications (e.g. Hamann & Canli, 2004; Hofer et al., 2006; McLean, Asnaani, Litz, & Hofmann, 2011; Ochsner & Gross, 2008; Tian, Wang, Yan, & He, 2011; Wager, Phan, Liberzon, & Taylor, 2003). Despite clear awareness within the field of the importance of incorporating measures of individual differences, these are still overlooked and it is often only through meta-analyses that the importance of accounting for multiple individual differences is discussed. Accounting for multiple individual differences can be time consuming and result in complex models, and even more complex analysis. However,
perhaps increasing levels of collaboration within emotion research, along with the use of standardised research paradigms can boost the scope of emotion research, and allow effective evaluation and clear inferences to be drawn.

A wider methodological implication is emphasised in chapter 9, which demonstrates that these individual differences can have an impact at rest, by modulating spontaneous neural connectivity even in a sub-clinical population. Since rest could be considered the ‘baseline’ condition in many experimental paradigms, this has ramifications for how researchers calculate task-based activity. If this baseline does not represent an equivalent level of activation across participants, then assuming it to be akin to a zero-activity condition could lead to variability, reduced integrity of analysis and erroneous conclusions. This point was clearly demonstrated in a study by Stark and Squire (2001) who systematically investigated the effects of resting connectivity during both a block and event-related design using a memory encoding paradigm designed to facilitate activation in the medial temporal lobe. The researchers observed that results inferred in the typical manner (assuming baseline to be zero) would lead to the conclusion that the parahippocampal cortex is the sole region in the medial temporal lobe to respond to viewing novel pictures, with no medial temporal specialisation to viewing familiar images. However, when considering the baseline condition (rest) it was apparent that there was extensive responsivity throughout the medial temporal lobe to both novel and familiar pictures. Contrasting these task-active and rest periods would therefore lead to an unsuitable conclusion. The current findings demonstrating variations resulting from individual differences again points towards the need to not only account for these individual differences, but also that the baseline condition needs to be treated as another condition (not a zero-activity condition). If an appropriate contrasting condition can be determined, this may prove more informative. For example, Stark and Squire (2001) contrasted medial temporal responsivity during viewing familiar/novel pictures with what was deemed a mindless task of making odd/even judgements, this was found to be a more appropriate comparison condition than baseline, revealing the underlying medial temporal responsivity which was not seen when using typical baseline as the contrast condition.

10.3.3 Practical Implications
Finally, this thesis offers practical implications within the field of emotion research. In particular, results have not only underlined methods by which neural biomarkers of anxiety
can be identified, but also emphasize that it is possible to find these biomarkers within sub-clinical populations. Throughout chapters 5-9 it is evident that there are clear differences in amygdala responsivity, even in this sub-clinical population, which could be indicatory of propensity to developing anxiety disorders. The methods introduced in chapter 7 and chapter 8 enabled discussion of the possibility of identifying neural biomarkers within sub-clinical populations. Results in chapter 7 were inconclusive, however the use of classifier models in emotion research cannot be ruled out without further research and corroboration in larger samples. Results of the volumetric analysis reveal that there were significant structural differences in key structures involved in emotion processing, even in a sub-clinical population. Though beyond the scope of this thesis, this result suggests that it would be a useful extension to apply SVM and MLDA classifier methods to structural data within emotion research. The potential utility of being able to scan a participant and use the resulting brain activation patterns to determine whether they are exhibiting a typical emotion response to stimuli offers a non-invasive method which could supplement traditional diagnostic criteria for diagnosis. Furthermore, such methods could be used to identify individuals with a propensity to developing anxiety disorders, and be used to track and predict progression of these changes. Indeed, machine learning classification techniques such as these have already been successfully applied in tracking and predicting brain changes resulting in the at-risk mental state for psychosis (ARMS; Koutsouleris et al., 2010)) and in the progression from mild cognitive impairment to a diagnosis of Alzheimer’s Disease (Davatzikos et al., 2011). The use of such techniques may enable early identification to take place prior to clinical diagnosis, and may pave the way for preventative measures or coping mechanisms to be put in place pre-emptively, as well as widen the scope for possible therapeutic treatments. As discussed on chapter 8, there is evidence to suggest that therapies which target cognitive restructuring, such as cognitive behavioural therapy (CBT), may facilitate neural plasticity changes which could modify neural circuits involved in anxiety disorders (for review see Barsaglini et al., 2014; Etkin, Pittenger, Polan, & Kandel, 2005; Jokić-Begić, 2010; Porto et al., 2009). If identification of individuals at risk of developing anxiety is possible, then such therapies can be introduced at an earlier stage potentially slowing, or even preventing further changes that may result in clinical anxiety. Whilst the potential utility of this technique is obvious, we must first conduct further research to assess its value and efficacy for affective neuroscience.
In addition, observation of heightened amygdala activation in the high anxiety participants for the duration of the study prompted the suggestion of a possible mechanism of clinical anxiety as described in chapter 5. It was suggested that a repeated state of heightened anxiety could surpass a threshold by which amygdala reactivity transitions from sub-clinical to chronic, as hippocampal activity diminishes. Indeed, it was noted that there is evidence showing reduced hippocampal volume in neuroimaging studies both in patients with clinical anxiety conditions (e.g. PTSD; Douglas, 1995; Gurvits et al., 1996), and in relation to increasing levels of anxiety in non-clinical participants (e.g. Levita et al., 2014). However, there was no evidence of anxiety-related hippocampal volume changes in the chapter investigating structural changes in this cohort (chapter 8). This could be taken either as evidence to negate this mechanistic suggestion, or it may be that within the current cohort levels of heightened state anxiety has not surpassed the point at which hippocampal volume starts to deteriorate. The relatively moderate genetic heritability of the hippocampus (40–69%; Peper et al., 2007) indicates that this structure is particularly susceptible to environmental influences. This would, to some extent support this latter assertion, with environmental effects acting over the course of a lifetime to alter hippocampal volume. Furthermore, the majority of studies illustrating anxiety-related hippocampal reduction come from clinical populations, with only one (to this authors knowledge) looking at a sub-clinical cohort (Levita et al., 2014). In this study, hippocampal volume reduction was associated with anxiety indexed by the Sensitivity to Punishment sub-scale (StP; Sensitivity to Punishment and Sensitivity to Reward questionnaire (Torrubia, Avila, Moltó, & Caseras, 2001)), with no significant relationships were identified between reduced hippocampal volume and the other measures of anxiety enlisted (the STAI and the Beck Depression Inventory (Erbauch, 1961). It is of note that the StP was specifically designed to assess Greys Behavioural Inhibition system. As noted by Levita and colleagues (2014), without corroboration from other measures of anxiety, one cannot be sure that there is not some inherent selectivity in this measure to detect positive relationships with hippocampal volume. Taken together, there does seem to be evidence to support the argument that hippocampal volume reduction may occur slowly over a lifetime of heightened sub-clinical anxiety, with significant volume reductions only evident in those with clinical anxiety.
10.4 Methodological limitations

A key limitation identified in the neuroimaging chapters relates to the sample selected for study. This thesis offers evidence from a relatively large cohort for a single neuroimaging study (50-57 participants dependent on chapter) allowing categorisation of participants by gender or anxiety group. However, after statistical analysis it is apparent that results may often arise from a further interaction of gender by anxiety. In order to adequately tease out inferences from these interactions it would be necessary to divide groups further into anxiety by gender groups. Unfortunately, this was not possible in the current sample, as groups sizes would have fallen below suitable thresholds for statistical analysis and scientific rigour. A clear future direction would be to increase sample size in a replication study. This would not only allow testing of the validity and reliability of current findings, but also enable analysis of these interactions to further clarify current findings.

Another issue touched upon in the earlier chapters was the use of neutral stimuli in the backwards masking paradigm. Though the neutral stimuli have been previously validated (Tottenham et al., 2009), anecdotal evidence from the current sample suggested that participants were perceiving these images as emotionally loaded in some way (often negatively), and not void of emotion as was intended (see chapter 5 for full discussion). These anecdotal assertions are supported by evidence in the literature indicating that neutral facial expressions are often considered emotionally ambiguous (e.g. Adams et al., 2012; Whalen, 1998), and imaging studies showing that the amygdala is particularly sensitive to ambiguous stimuli (Hsu, Bhatt, Adolphs, Tranel, & Camerer, 2005; Quiroga, Kraskov, Mormann, Fried, & Koch, 2014). Furthermore, research has shown that the interpretation of such emotionally ambiguous stimuli is modulated by anxiety, with highly anxious participants perceiving neutral stimuli more negatively than controls (Constans, Penn, Ihen, & Hope, 1999; Winton, Clark, & Edelmann, 1995). Cooney, Atlas, Joormann, Eugène, and Gotlib (2006) investigated neural activation when viewing neutral face stimuli and oval shapes in participants with social anxiety disorder (SAD) and controls. The researchers found that in both SAD participants and controls, neutral stimuli elicited differential amygdala activation in comparison to the oval stimuli, not only suggesting that anxiety modulates processing of emotionally ambiguous stimuli, but also that neutral face stimuli may not actually be neutral. This ambiguity could be the underlying cause of the elevated patterns of amygdala response in relation to neutral stimuli in Chapter 5, and the patterns of connectivity for neutral mimicking those observed during the fear condition in Chapter 6. Furthermore, in
lieu of evidence suggesting that mask-type can influence amygdala response (Kim et al., 2010), the use of neutral stimuli as the masking stimuli in this study may have influenced amygdala response in the other emotion conditions (fear and happy as discussed in Chapter 7). Future studies would need to address these issues in future studies using an alternative mask for the images such as a pattern mask or a simple oval matched on colour, shape and size as used in Cooney et al. (2006) study.

Another potential limitation comes from the evidence contained within the thesis, specifically from the large online survey of willingness to participate in different research studies (Chapter 4). This chapter identified that it is likely that self-selection bias still plays a role within emotion research, in particular in studies investigating anxiety using neuroimaging methods and task performance (which could be considered more stressful design types). Whilst none of the data acquisition combined both neuroimaging and an explicit measurement of performance, the backwards masking, passive viewing task is the closest approximation of this particular design type. Participants were not informed of the exact nature of the task due to the subconscious nature of the paradigm, only told that they would be 'passively viewing faces'. Therefore, the chapters reliant on this data (chapter 5, 6, 7) could have potentially been susceptible to a self-selection bias at the recruitment stage, and contained less high anxiety males, or high anxiety native speakers than the general population. However, sampling criteria were in place during recruitment to ensure a large enough sample, and to provide relatively equal groups of female and male participants and high and low anxiety. We would also argue that such was the passive, low demand, nature of the task-based study it was unlikely to be perceived to be particularly high in performance related stress and is more similar to non-performance related neuroimaging studies like resting state fMRI. The online survey did not reveal any differences as modulated by gender or anxiety in participants’ willingness to take part in neuroimaging studies where task-performance was not measured (i.e. resting state fMRI). As a result, it is less likely that data presented within this thesis was affected by self-selection bias.

A final limitation is the specific population selected for study. A key aim was to investigate the effects of sub-clinical anxiety, as such all participants were recruited from a Caucasian student population. Though it could be argued that this population is not representative of the general population, research has shown that depression and anxiety are highly prevalent mental health problems associated with university students (Zivin, Eisenberg, Gollust, & Golberstein, 2009) and thus makes this an ideal group to study the effects of sub-clinical
anxiety on amygdala activation. Furthermore, the higher proportion of anxious female participants compared to male participants reflects the disproportionate number of women that suffer from anxiety compared to men in the general population. It could be contended that some of the sample may be at clinical levels of anxiety, and evidence suggests students typically show low help-seeking behaviours with regards to mental health (D. Eisenberg, Golberstein, & Gollust, 2007). However, this is not something that can be accounted for presently beyond the steps already in place for recruitment and screening. Future studies looking to recruit sub-clinical samples should ensure that all potential participants are assessed by a qualified health professional in order to rule out the possibility that some would be classed as clinically anxious.

10.5 Conclusion
This thesis sought to investigate the neurobiological mechanisms of emotion processing, specifically in the amygdala, in a healthy sub-clinical cohort. The modulating effect of anxiety on amygdala habituation, fronto-amygdala connectivity (during emotion processing and at rest) and neural structure has been demonstrated in this sub-clinical population, demonstrating the translational worth of studying such groups to inform our understanding of maladaptive emotion processing and clinical anxiety disorders. Furthermore, in addition to evidence for fronto-amygdala disruption in sub-clinical anxiety, this thesis presented evidence that there may be an attentional component to the hypervigilance observed that needs to be incorporated into models of maladaptive anxiety. The thesis also provides evidence for the utility of resting state fMRI as a short, low-cost alternative to task-based fMRI, as well as machine learning within the study of anxiety. Along with the theoretical and practical (diagnostic criteria/tools and treatment) implications for research into anxiety, this thesis also generated implications for affective neuroscience and emotional processing research in general. In particular, it has been suggested that by combining previous theoretical models of emotion (dual processing theory, salience detector theory) into one cohesive model of emotion processing, whilst accounting for the modulating impact of individual differences, it may be possible to reduce the contention and inconsistencies within the emotion literature. The consideration of the impact of individual differences on results within affective neuroscience should become standard practice to enable a clearer understanding of the neural underpinnings in emotion processing. Not only should individual differences be accounted for, but also researchers must pay heed to the possibility of self-
selection bias, and accordingly adjust sampling procedures to ensure representative samples are collected. Finally, further investigation should be conducted into structural differences in sub-clinical populations and the possible merit of applying analytical methods such as machine learning classification techniques to data to develop diagnostic tools that can track disease progression and identify individuals with a propensity towards developing anxiety disorders. The possible identification of neural biomarkers of a predisposition towards disordered anxiety paves the way for research to look for therapeutic treatments and interventions which could prevent individuals from transitioning from sub-clinical anxiety to chronic anxiety disorders.
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295


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303


Appendices

Appendix A
Post Scan Interview

Participant Code:…………………………………….
Date:……………………………….

Opening:

• I would like to thank you for taking part in this study. To finish off I would like to ask you some questions about your experiences and thoughts during the scanning session. I hope to use this information to better inform the results from your brain scans. This should only take a couple of minutes. You do not have to answer any questions you do not wish to and are free to leave at anytime without any consequence to yourself or your employment if you are a member of staff. Are you available to respond to some questions at this time?

Body:

• During the study was there anything that stood out in the faces presented?

• Were they any features or aspects of the faces you would like to comment on?

• Were there any emotional aspects of the faces you would like to comment on?

• Whilst in the rest periods was there anything in particular you were thinking about
  - Any thoughts that you dwelled on for a prolonged time?
•

• Do you have any comments regarding your experiences whilst in the machine?

Closing:

• So to summarize you have said……………………………………
• I appreciate the time you’ve taken to complete this interview. Is there anything you think would be helpful for me to know?
• I should have all the information I need. Thank you again for your time.