The Role of Moral Cognition and Emotions in Remitted Major Depressive Disorder

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy (PhD) in the Faculty of Medical and Human Sciences

2015

Clifford Ian Workman

School of Medicine
## Contents

List of Tables ................................................................................................................................. 7  
List of Figures ............................................................................................................................... 8  
List of Abbreviations .................................................................................................................... 9  
Abstract ....................................................................................................................................... 11  
Declaration ................................................................................................................................... 12  
Copyright Statement ................................................................................................................... 12  
Acknowledgments ........................................................................................................................ 13  
Introduction .................................................................................................................................. 15  
1.1. Preface ................................................................................................................................... 15  
1.2. Depression and Depression Vulnerability ......................................................................... 17  
1.2.1. Clinical Presentation and Diagnosis ............................................................................. 17  
1.2.2. Neuropsychological Impairments in Current MDD ..................................................... 17  
1.2.3. Vulnerability to Major Depressive Disorder ............................................................... 18  
1.3. Moral Cognition and Emotions ......................................................................................... 20  
1.3.1. The Evolution of Morality ............................................................................................ 20  
1.3.2. Philosophical and Psychological Accounts of Morality ............................................. 22  
1.3.3. The Dual-Process Model of Moral Judgment ............................................................... 23  
1.3.4. Event-Feature-Emotion Complex Model of Moral Cognition ..................................... 25  
1.3.5. Methodological Considerations ..................................................................................... 30  
1.4. Moral Emotions and Cognition, MDD, and their Neurobiological Basis ......................... 31  
1.4.1. Moral Emotions in MDD ............................................................................................... 31  
1.4.2. MDD and Performance on Neuroeconomic Paradigms ............................................. 32  
1.4.3. Neural Correlates of MDD, Moral Cognition and Emotions: Intersections ............... 33  
1.4.4. Resting-State fMRI and the Subgenual Cingulate in MDD .......................................... 35  
1.5. Design of Studies .................................................................................................................. 40  
1.6. Aims ..................................................................................................................................... 42  
Subgenual cingulate-amygdala functional disconnection and vulnerability to melancholic depression .......................................................................................................................... 45  
2.1. ABSTRACT ......................................................................................................................... 46  
2.2. INTRODUCTION ............................................................................................................... 47  
2.3. MATERIALS and METHODS ............................................................................................ 49  
2.3.1. Participants .................................................................................................................... 49  

Word count: 69,678
4.4.1. Relationship between explicit and implicit choice preference ........................................ 100
4.4.2. Study 1 ..................................................................................................................... 100
4.4.3. Study 2 ..................................................................................................................... 102
4.4.4. Across both studies ................................................................................................. 104
4.5. DISCUSSION ............................................................................................................. 104
4.5.1. Study 1: Impaired Prosociality and Vulnerability to MDD ...................................... 105
4.5.2. Study 2: Self-Blame Modulates Pure Altruism Choice Preference ........................... 107
4.5.3. Future Directions and Limitations ......................................................................... 107
4.5.4. Conclusions .......................................................................................................... 109

Resting-State Connectivity within a Social Agency Network is Associated with Implicit Prosociality ................................................................. 111

5.1. ABSTRACT ............................................................................................................... 112
5.2. INTRODUCTION ..................................................................................................... 113
5.3. METHODS .............................................................................................................. 116
5.3.1. Participants .......................................................................................................... 116
5.3.2. Altruistic Choices Task (ACT) ............................................................................ 118
5.3.3. ACT: Emotion Priming Paradigm ...................................................................... 119
5.3.4. ACT: Dilemmata and Explicit Choice Preference ............................................... 120
5.3.5. ACT: Implicit Choice Preference ....................................................................... 120
5.3.6. MRI Image Acquisition ...................................................................................... 122
5.3.7. Resting-State fMRI Analysis .............................................................................. 122
5.4. RESULTS ................................................................................................................ 124
5.4.1. Explicit Choice Preference .................................................................................. 124
5.4.2. Implicit Choice Preference ................................................................................ 124
5.4.3. Investigation of Potentially Confounding Variables ............................................ 124
5.5. DISCUSSION .......................................................................................................... 127
5.5.1. Conclusions ........................................................................................................ 130

Discussion ..................................................................................................................... 133

6.1. Purpose ..................................................................................................................... 133
6.2. Summary of the Findings ....................................................................................... 134
6.3. Theoretical and Clinical Implications ..................................................................... 137
6.3.1. Theoretical Implications ................................................................................... 137
6.3.2. Clinical Implications ......................................................................................... 139
6.4. Limitations and Future Directions .......................................................................... 142
6.4.1. Limitations ........................................................................................................ 142
List of Tables

2.1 Reasons for exclusion of volunteers .............................................................. 50
2.2 Demographic variables in the remitted depressed and healthy participants .... 52
2.3 Clinical characteristics of the remitted depressed patients by subtype .......... 54
2.4 Functionally disconnected regions in the melancholic patients ................... 58
S2.1 Inter-rater reliabilities for the SCID-I and MADRS ..................................... 64
3.1 Demographic variables in the remitted depressed and healthy participants .... 71
3.2 Clinical characteristics of the remitted depressed patients by recurrence status .... 73
3.3 Functionally disconnected regions in the resilient depressed patients .......... 77
S3.1 Inter-rater reliabilities for the SCID-I, MADRS, and PSR ............................... 83
4.1 Demographic variables in the remitted depressed and healthy participants .... 94
4.2 Example dilemmas used in the Altruistic Choices Task ................................. 98
4.3 Example BIAT stimuli used in the Altruistic Choices Task .............................. 98
5.1 Reasons for exclusion of volunteers ............................................................ 117
5.2 Demographic variables in the remitted depressed and healthy participants .... 119
5.3 Subgenual cingulate networks correlated with implicit choice preference ...... 127
List of Figures

1.1 The Event-Feature-Emotion Complex................................................................. 26
1.2 Brain regions implicated in depression and in moral cognition and emotion ...... 35
2.1 Subgenual cingulate-amygdala disconnection in melancholia.............................. 57
3.1 Interhemispheric subgenual cingulate disconnection in resilience to depression . 76
4.1 Hypothesized relationship between self-blame and altruistic choice preference .. 91
4.2 Relationships between primed emotions, dilemma types, and choice preference103
5.1 Subgenual cingulate networks correlated with implicit choice preference........ 125
5.2 Scatterplots of the relationship between connectivity and choice preference..... 126
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>ACT</td>
<td>Altruistic Choices Task</td>
</tr>
<tr>
<td>AMYG</td>
<td>amygdala</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>aOFC</td>
<td>anterior orbitofrontal cortex</td>
</tr>
<tr>
<td>ART</td>
<td>Artifact Detection Tools</td>
</tr>
<tr>
<td>ATL</td>
<td>anterior temporal lobe</td>
</tr>
<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
</tr>
<tr>
<td>BIAT</td>
<td>Brief Implicit Association Test</td>
</tr>
<tr>
<td>BOLD</td>
<td>blood oxygen level-dependent</td>
</tr>
<tr>
<td>CAS</td>
<td>Compassionate Altruism Scale</td>
</tr>
<tr>
<td>CBT</td>
<td>cognitive behavioural therapy</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>cMDD</td>
<td>current major depressive disorder</td>
</tr>
<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DMN</td>
<td>default mode network</td>
</tr>
<tr>
<td>DPARSF</td>
<td>Data Processing Assistant for Resting-State fMRI</td>
</tr>
<tr>
<td>DSM-IV-TR</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>EFEC</td>
<td>Event-Feature-Emotion Complex</td>
</tr>
<tr>
<td>EPI</td>
<td>echo-planar image</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FPC</td>
<td>frontopolar cortex</td>
</tr>
<tr>
<td>FTD</td>
<td>frontotemporal dementia</td>
</tr>
<tr>
<td>FWE</td>
<td>familywise error</td>
</tr>
<tr>
<td>GAF</td>
<td>Global Assessment of Functioning</td>
</tr>
<tr>
<td>HC</td>
<td>healthy control</td>
</tr>
<tr>
<td>IAT</td>
<td>Implicit Association Test</td>
</tr>
<tr>
<td>ICC</td>
<td>intra-class correlation</td>
</tr>
<tr>
<td>ICD-10</td>
<td>International Statistical Classification of Diseases &amp; Related Health Problems</td>
</tr>
<tr>
<td>IGQ</td>
<td>Interpersonal Guilt Questionnaire</td>
</tr>
<tr>
<td>L</td>
<td>left</td>
</tr>
<tr>
<td>LIFE</td>
<td>Longitudinal Interval Follow-up Evaluation</td>
</tr>
</tbody>
</table>
M  mean
MADRS  Montgomery-Åsberg Depression Rating Scale
MDD  major depressive disorder
MDE  major depressive episode
mFPC  medial frontopolar cortex
MNI  Montreal Neurological Institute
MPRAGE  magnetization-prepared rapid-acquisition gradient-echo
MRI  magnetic resonance imaging
N  number
NORD  Neurobiology of Resilience to Depression
OFC  orbitofrontal cortex
PET  positron emission tomography
PFC  prefrontal cortex
PHG  parahippocampal gyrus
PPI  psychophysiological interaction analysis
PSR  Psychiatric Status Rating
R  right
rMDD  remitted major depressive disorder
ROI  region of interest
RSATL  right superior anterior temporal lobe
rsfMRI  resting-state functional magnetic resonance imaging
rTPJ  right temporoparietal junction
SCC  subgenual cingulate cortex
SCID-I  Structured Clinical Interview-I for DSM-IV-TR
SCSR  subgenual cingulate and adjacent septal region
SD  standard deviation
SEC  Structured-Event-Complex
SEM  standard error of the mean
SNRI  serotonin norepinephrine reuptake inhibitor
SPM  Statistical Parametric Mapping
SSRI  selective serotonin reuptake inhibitor
STS  superior temporal sulcus
TPJ  temporoparietal junction
UG  Ultimatum Game
VMPFC  ventromedial prefrontal cortex
Abstract

The Role of Moral Cognition and Emotions in Remitted Major Depressive Disorder
Clifford Workman, the University of Manchester
For the degree of Doctor of Philosophy (PhD) 27 January 2016

Background: The aim of this thesis was to investigate the relationship of moral cognition and emotions to the pathophysiology of major depressive disorder (MDD). Patients with MDD may experience excessive guilt or self-blaming biases despite recovery from the depressed state. Since guilt is a moral emotion thought to motivate altruistic behaviours, it has been hypothesized that elevated self-blame in MDD may result in pathological increases to altruism in some patients. The relationship of self-blame to altruistic choices in individuals with remitted MDD (rMDD), however, has not been established. Guilt has been shown to activate the subgenual cingulate and adjacent septal region (SCSR) which is of known importance to the pathophysiology of MDD. Since MDD is thought to arise from network-level dysfunctions, and moral cognition and emotions are hypothesized to emerge from network-level binding, investigating resting-state SCSR functional connectivity in rMDD patients and healthy control (HC) participants could reveal networks of potential relevance both to MDD and to moral cognition and emotions.

Chapter 2: We investigated whether melancholic rMDD patients could be distinguished from non-melancholic and HC groups on the basis of resting-state functional connectivity to an SCSR seed region. Lower SCSR-amygdala connectivity distinguished the melancholic rMDD group from non-melancholic and HC groups.

Chapter 3: We investigated whether patients who remained resilient to recurring depressive episodes were distinguishable from recurring episode MDD and HC groups on the basis of resting-state connectivity to an SCSR seed region. Lower interhemispheric SCSR connectivity distinguished the resilient MDD patients from the recurring episode MDD and HC groups.

Chapter 4: We measured explicit and implicit preferences for social options with and without altruistic motivations relative to selfish options in the rMDD and HC groups during emotion priming to modulate feelings of guilt. The rMDD patients explicitly preferred prosocial options (i.e., social options and altruism directed towards friends or colleagues) less than HC participants. Regardless of group, guilt priming increased explicit and implicit preferences for altruism towards strangers.

Chapter 5: We investigated whether explicit and/or implicit preferences for prosocial options during guilt priming were correlated with resting-state connectivity to an SCSR seed region, and whether this relationship could distinguish the rMDD and HC groups. Across all participants, implicit prosocial choice preference negatively correlated with connectivity between the SCSR and right temporoparietal junction (TPJ). The relationship of SCSR-TPJ connectivity to implicit preferences for social options and for altruism towards friends and colleagues was weaker in the rMDD group compared to the HC group, particularly for implicit altruism.

Conclusions: We identified resting-state SCSR networks associated with vulnerability to melancholia and with resilience to recurring depressive episodes. Patients with rMDD explicitly preferred options entailing social withdrawal, a symptom associated with MDD vulnerability. Irrespective of group, guilt motivated altruism towards strangers but not friends and colleagues. Implicit prosociality was negatively associated with connectivity in a social agency network, and the comparatively weak relationships between connectivity and implicit choice preferences in rMDD patients may reflect a vulnerability factor for MDD.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright Statement

1. The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the “Copyright”) and s/he has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.

2. Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made only in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

3. The ownership of certain Copyright, patents, designs, trademarks and other intellectual property (the “Intellectual Property”) and any reproductions of copyright works in the thesis, for example graphs and tables (“Reproductions”), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

4. Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy (see http://documents.manchester.ac.uk/DocuInfo.aspx?DocID=487), in any relevant Thesis restriction declarations deposited in the University Library, The University Library’s regulations (see http://www.library.manchester.ac.uk/about/regulations) and in The University’s policy on Presentation of Theses.
Acknowledgments

First and foremost, I want to thank Dr. Roland Zahn, Professor Rebecca Elliott, and Dr. Karen Lythe for their supervision the last three years. I owe each of you a huge debt of gratitude, not just for your continued support of my academic goals, but for the time you spent helping me work towards those goals, for challenging me to always do my very best, for the personal support you offered me during some very difficult periods, and for trying to persuade me that taking a holiday once in a while would not be the end of the world. I am proud to have worked under such amazing academics. I next want to thank Professor Gwenn Smith for her mentorship, without which I am certain I would not be here. You are an inspiration to me. I must also thank Robert Deluty, my friend and valued teacher. Thank you, Jenny Gethin, for being a blast to work with. Thanks also to my friends and colleagues from the School of Psychological Sciences, the Institute of Brain, Behaviour and Mental Health, and the Graduate Society. I wish I had space to tell each of you how much you are appreciated. Thank you to my participants, who willingly endured the Altruistic Choices Task. This was no small feat, I assure you.

Before going any further, I must apologise to Mary Rozanski, who is objectively the best mother in the universe, for not thanking her first (sorry, mom!). Thank you so much, mom, for putting up with three years of bad quality Skype calls, for dropping everything to cheer me up whenever I needed it, and for regularly reminding me how proud dad would have been. Thank you, too, dad. I wish you could read this thesis, you would find it really cool. I love you both the most. Thanks also to my grandpa, Roland Workman, for telling me that I cannot let the bastards get me down. Stacey Humphries, I could write an entire chapter devoted to explaining why and how I love you so much. I cannot imagine having reached this point without your unending love and support, and I look forward to our future as an academic power couple. Thank you. Caitlin Rush, I have it on good authority from a braincat that you are and always will be my best friend. Cameron Crook, also my very best friend, you are one of the best people I know and I am incredibly glad you were able to overlook my business cards. Dr. Michelle Hall, you are my newest best friend, but we are closer than skin glued to skin. Trey White, I love you and I miss you. With each person I thank, I think of five more to whom I owe my utmost appreciation. Thank you to my friends from home in Olney and Baltimore, and everyone in Manchester whose name belongs here. I will miss Thursdays the most.
This thesis is dedicated to my dad, Patrick Workman.

The Author
Clifford Workman was raised in Maryland, just outside Washington, DC in the United States. He began his undergraduate degree in Psychology at the University of Maryland Baltimore County in 2003. After university, he worked as a Senior Research Program Coordinator to Professor Gwenn Smith at Johns Hopkins University studying mood disorders with brain imaging. He moved to the United Kingdom in 2012 to pursue his PhD in Medicine at the University of Manchester.

Format
This thesis was approved for submission in the alternative format by the Faculty of Medical and Human Sciences. Each experimental chapter was prepared for publication and will be submitted to high impact peer-reviewed journals. Chapter 1 provides a general introduction to the literature pertaining to topics relevant to the thesis. Chapters 2, 3, 4, and 5 are experimental chapters formatted for publication in peer-reviewed journals. Chapter 6 provides a general discussion of the results presented in the experimental chapters with reference to the material introduced in the general introduction.

Authorship Contributions
This thesis was prepared by Clifford Ian Workman under the supervision of Professor Rebecca Elliott, Dr Karen Lythe, and Dr Roland Zahn. The author conducted 159 telephone screenings and 87 follow-up clinical interviews as part of the research presented in Chapters 2 and 3. The author additionally contacted 282 potential volunteers about taking part in the research described in Chapters 4 and 5 and all 147 eligible participants were interviewed by the author. The author designed the novel task and software used to acquire the behavioural data presented in Chapters 4 and 5. The author designed the interview at which this task as well as others selected by the author were administered. The author performed all analyses presented in Chapters 2 through 5. The author of this thesis was the lead author for each chapter. Drs Lythe and Zahn acquired the neuroimaging data presented in Chapters 2, 3, and 5. The author’s supervisors oversaw the design of the research, the development of methodological and statistical plans, and also provided feedback on each chapter. Full details concerning authorship contributions are provided prior to each experimental chapter.
Chapter 1.

Introduction

1.1. Preface

The primary aim of this thesis was to characterise the relationship of moral emotions and cognition to the pathophysiology of major depressive disorder (MDD). When we feel as though we have failed to meet our own moral standards, we may experience the moral emotion guilt. Guilt is a powerful motivator which can drive us to try and repair our moral transgressions. For example, one might experience feelings of guilt after choosing not to ring an ailing parent and may attempt to assuage these feelings by giving them a call. Excessive or overgeneralised guilt represents a distinctive symptom of MDD and is observed cross-culturally (Sartorius et al., 1980; American Psychiatric Association, 2000). It has been hypothesized that elevated self-blaming moral emotions in MDD may result in pathological manifestations of moral cognition and emotions in some patients (O'Connor et al., 2007). However, in addition to excessive or overgeneralised guilt, patients currently in the depressed state may experience symptoms such as sustained low mood, loss of interest or pleasure in activities normally considered enjoyable, social withdrawal, and others. The presence of these symptoms could obscure attempts to detect abnormal moral cognition and emotion in current MDD patients. Studying patients at elevated risk for developing MDD could lead to the discovery of trait markers of vulnerability to MDD (Bhagwagar and Cowen, 2008). Patients with remitted MDD (rMDD), who are at an increased primary and secondary risk of developing subsequent episodes of depression, have demonstrated increased levels self-blaming moral emotions including altruistic guilt (Green et al., 2013b; Zahn et al., 2015a) but preliminary evidence regarding the relationship of altruistic behaviour to depression vulnerability is inconclusive (Fujiwara, 2009; Pulcu et al., 2015).

Guilt has been shown to activate the subgenual cingulate cortex (SCC; Zahn et al., 2009c)), a region known to play a central role in the pathophysiology of MDD (Mayberg, 2003; Ressler and Mayberg, 2007). We reported increased guilt-related functional connectivity between the subgenual cingulate/septal region (SCSR) and the right superior anterior temporal lobe in rMDD patients who experienced a recurring major depressive episode (MDE) compared to patients who remained resilient (Lythe et
Increased connectivity to the SCC has also been reported in current MDD patients at rest (Greicius et al., 2007; Sheline et al., 2010). Resting-state functional connectivity studies of depression vulnerability are less consistent, with reports of both increased and decreased connectivity between the SCC and a variety of regions (Gaffrey et al., 2012; Herringa et al., 2013; Jacobs et al., 2014; Thomason et al., 2015). As will be discussed in section 1.4.4, these inconsistencies likely stem from methodological and recruitment differences across studies. A carefully controlled resting-state fMRI study designed to characterise resting-state connectivity to SCSR in rMDD patients could improve our understanding of the pathophysiology of MDD and improve stratification based on vulnerability. Furthermore, in view of recent work suggesting the relationship between moral cognition and resting-state functional connectivity is capable of distinguishing psychiatric patients from healthy volunteers (Pujol et al., 2012; Verdejo-Garcia et al., 2014), if abnormal altruistic behaviour were observed in patients vulnerable to MDD this could be associated with underlying dysfunction within networks supporting moral cognition and emotions.

With one exception identified in section 1.5, the research presented in this thesis was carried out as part of a larger longitudinal investigation of depression vulnerability. The longitudinal study followed a cohort of medication-free rMDD patients, who as mentioned previously are at an elevated lifetime risk for future MDEs, over a period of 14 months. A demographically matched control group was also recruited. After enrolment, participants were invited to complete an MRI scanning session, at which resting-state fMRI and structural sequences were acquired, and to undergo testing with a novel measure of altruistic choice preference. The design of this study has consequently allowed for both cross-sectional and longitudinal analyses of the resting-state fMRI and behavioural data.

This thesis starts with a selective review of the literature on MDD and vulnerability to MDD, moral cognition and emotions, and the neurobiological intersection between these topics (Chapter 1). Methodological concerns are also detailed throughout Chapter 1 and each experimental chapter includes a thorough overview of the methods used, thus a general methods chapter is not provided. Next, four experimental chapters are presented which aim to address the gaps of knowledge identified above. The last chapter provides a brief integrative overview of the results from the experimental chapters, links these findings to the literature described in Chapter 1, and discusses implications for future research.
1.2. Depression and Depression Vulnerability

1.2.1. Clinical Presentation and Diagnosis

Depression is the leading cause of disability and fourth leading cause of disease burden (World Health Organization, 2001), with approximately 350 million people suffering from depression worldwide (World Health Organization), and the six-month prevalence of depression estimated at 17% in a large European sample (Tylee, 2000). Major depression is an episodic disorder with individual major depressive episodes (MDEs) characterised by the presence of at least five symptoms, one of which must be low mood or loss of interest or pleasure in activities ordinarily found enjoyable (World Health Organization, 1992; American Psychiatric Association, 2000). Additional symptoms may include feelings of worthlessness and excessive or inappropriate guilt, and other cognitive, emotional, physical, and vegetative symptoms (American Psychiatric Association, 2000). To meet criteria for a MDE, these symptoms must be present for a period of two weeks or longer for most of the day, nearly every day, and must cause clinically significant distress or functional impairment (American Psychiatric Association, 2000). Patients who have had one or more depressive episodes may receive a diagnosis of “major depressive disorder” (Diagnostic and Statistical Manual, 4th Edition, Text Revision [DSM-IV-TR]; (American Psychiatric Association, 2000)) or “recurrent depressive disorder” (ICD-10: International statistical classification of diseases and related health problems, 10th Edition; (World Health Organization, 1992)). The following review of the literature will focus on MDD without accompanying manic episodes, often referred to as “unipolar depression”.

The DSM-IV-TR provides additional classifications for subtypes of MDD. Symptoms associated with the melancholic subtype include excessive or inappropriate guilt with poor reactivity to pleasurable stimuli and additional physical symptoms (American Psychiatric Association, 2000). The atypical subtype is more closely associated with symptoms of emotional reactivity and interpersonal rejection sensitivity (American Psychiatric Association, 2000). Additional stratification is necessary to improve understanding of the pathophysiology of MDD thereby allowing for the development of tailored approaches to clinical management.

1.2.2. Neuropsychological Impairments in Current MDD

Neuropsychological impairments in current MDD have been reviewed in detail elsewhere (Clark, Chamberlain and Sahakian, 2009; Elliott et al., 2011; Roiser, Elliott and Sahakian, 2012). To summarise briefly, the literature in current MDD describes
abnormal performance on tasks of executive functioning (Elliott et al., 1996; Elliott et al., 1997), episodic memory (Okada et al., 2003; Rogers et al., 2004), autobiographical recall for positive and negative events (Van Vreeswijk and De Wilde, 2004), and facial emotion recognition (Gur et al., 1992; Gilboa-Schechtman, Erhard-Weiss and Jeczemien, 2002; Surguladze et al., 2004). Patients with current MDD may also experience abnormal sensitivity to negative feedback regarding performance on cognitive tasks (Elliott et al., 1996), and attentional and memory biases for negative information (Dunbar and Lishman, 1984; Bradley, Mogg and Williams, 1995; Segal et al., 1995; Bradley, Mogg and Millar, 1996). The impairments described in the literature may be nonspecific to current MDD, for example with impairments to executive functioning and memory observed in other psychiatric disorders. Impairments which are distinctive to MDD may be best observed in studies which do not treat cognition and emotion as separable entities (Clark, Chamberlain and Sahakian, 2009). Novel neuropsychological markers sensitive to cognitive/emotional impairments may improve categorization of MDD and individualization of treatments, and improve detection of vulnerability to recurrence. Measures of moral cognition and emotions may prove particularly valuable to this end. Moral emotions, such as guilt and shame, are “cognitive emotions” (Elliott et al., 2011) hypothesised to emerge from the binding of undirected emotional and motivational states to perceptual, conceptual, and event knowledge, as described in section 1.3.4 of this review. As has been mentioned, excessive or overgeneralised guilt is a symptom which is distinctive of MDD and is observed cross-culturally (Sartorius et al., 1980; American Psychiatric Association, 2000). Using a retrospective symptom assessment, one study revealed that over 80% of rMDD patients had experienced self-blaming moral emotions during a previous MDE (Zahn et al., 2015b). Little is known, however, about the relationship of moral emotions, such as guilt, and cognition in current MDD and less still is known about this relationship in vulnerability to MDD.

1.2.3. Vulnerability to Major Depressive Disorder

Patients who have previously suffered with MDEs are at a high lifetime risk for recurrence, with half of patients who achieve remission with pharmacologic treatment experiencing at least one additional MDE, and with the rate of recurrence increasing to 70% following a second MDE and to 90% following a third MDE (Kupfer, 1991). Several cognitive theories have been proposed to explain vulnerability both for the initial onset of MDD and for experiencing recurring MDEs (Burcusa and Iacono, 2007).
Beck and colleagues’ (1979) cognitive schema theory argues that dysfunctional attitudes observed in MDD are rooted in stable cognitive structures called “schema” which negatively bias information processing. These biases lead to negative beliefs and interpretations about oneself, one’s experiences, and one’s future, referred to by Beck and colleagues (1979) as the “cognitive triad”. Depressogenic schemata may be present without being activated outside of MDEs, may become activated by relevant stimuli, and may become increasingly independent of relevant stimuli for activation with increasing depression severity (Beck et al., 1979). Beck and colleagues suggest that negative schemata and the cognitive triad are maintained by cognitive distortions, such as exaggerated self-blame and overgeneralized sense of duty (Beck, 1963; Beck et al., 1979).

Abramson and colleagues (1978) have proposed an attributional framework which holds that certain attributional styles may increase vulnerability to developing MDD. This framework is a reformulation of Seligman’s learned helplessness model, which draws upon work in non-human animals to suggest that exposure to uncontrollable situations may impair subsequent adaptive responding (1972). According to the model proposed by Abramson and colleagues (1978), helplessness in humans is linked to causal attributions made along three orthogonal, continuous dimensions: globality-specificity, stability-instability, and internality-externality. Proneness to developing MDD is thought to be associated with an increased tendency to make global, stable, and internal attributions of blameworthiness (Abramson, Seligman and Teasdale, 1978). Global factors impact upon a range of situations, stable factors are unchanging, and internal factors relate to the self. An example of a global, stable, and internal attribution might be ascribing poor performance on an exam to “stupidity.” This attribution is global in that low intelligence is likely to influence a multitude of situations, it is stable in that one’s intelligence may be difficult to change, and it is internal in that the blame is inwardly-focussed.

The observation that risk for recurrence increases with successive depressive episodes has led some authors to suggest that depressive episodes produce cognitive “scarring” (additional scar theories have been proposed and are reviewed by (Burcusa and Iacono, 2007)). Teasdale’s (1983) differential activation hypothesis proposes that depressive episodes are precipitated by reciprocal increases in negative emotions and depressive cognitions, and that this association grows stronger and more accessible with subsequent MDEs. Teasdale (1983) formulates this account within the context of associative network theory, which describes emotions and conceptual and event-specific
knowledge as interconnected nodes comprising networks. Activation at one node may spread to other nodes, increasing associations between these nodes (Teasdale, 1983). Thus, MDEs may strengthen associations between negative emotions and depressive cognitions, increasing the likelihood that the experience of subsequent negative emotions and depressive cognitions will trigger each other.

Each of the three models discussed above provides useful heuristic information about vulnerability to developing MDD. However, none of these models provides a complete mechanistic account of vulnerability. For example, it is unclear how the depressive cognitions outlined in Beck’s cognitive framework and in cognitive attribution theory give rise to the negative emotional states associated with MDD (Bebbington, 1985). Additional research is needed to develop innovative neuropsychological markers of vulnerability to MDD. As was mentioned in section 1.2.2, little is known about the relationship between moral emotions and cognition in MDD, and investigating this relationship in rMDD could reveal markers of vulnerability to MDD (Bhagwagar and Cowen, 2008).

1.3. Moral Cognition and Emotions

Morality can be defined either descriptively (i.e., describing an ethical code held by a person or group of people) or normatively (i.e., asserting that, under certain circumstances, a particular ethical code should be adopted by all rational people; (Gert, 2012)). For the purposes of this review, the author will adopt a descriptive definition of moral behaviour as “… the sets of customs and values that are embraced by a cultural group to guide social conduct” (Moll et al., 2005).

1.3.1. The Evolution of Morality

The ability to observe the range of customs and values adopted by one’s social group (i.e., to act “morally”) depends upon cognitive/motivational mechanisms supporting, for example, helping behaviours. Analogues of human moral behaviour in non-human animals suggest that such mechanisms are a consequence of the pressures of natural selection. For example, some non-human primates demonstrate a sense of justice and a willingness to help others at personal cost (de Waal, 2002; Warneken et al., 2007). The extent to which such mechanisms can be considered innate and adaptive, however, remains an active subject of philosophical and scientific debate. This is due in part to disagreement about the extent to which fitness-sacrificing behaviour, defined here as actions that reduce one’s evolutionary fitness in favour of another’s evolutionary fitness (Joyce, 2006), is plausible within an evolutionary context. Although fitness
sacrificing behaviour directed at individuals with a high degree of genetic relatedness is generally considered evolutionarily plausible since this may lead to the improved success of shared genetic material (Hamilton, 1964), it is more difficult to explain fitness-sacrificing behaviour directed at non-relatives.

Theories of direct and indirect reciprocity have been developed to provide an evolutionary account for self-sacrificing behaviour directed at individuals of low genetic relatedness. Direct reciprocity, sometimes called reciprocal altruism, refers to cooperative social exchanges in which an individual engages in fitness-sacrificing behaviour that benefits another individual who later reciprocates with fitness-sacrificing behaviour (Trivers, 1971). An example of direct reciprocity in humans might be giving a friend a lift to the airport with the expectation that one is then owed a lift at a later date. Unlike with direct reciprocity, indirect reciprocal exchanges need not result in reciprocation from the beneficiary of one’s fitness-sacrificing behaviour. In this case, the self-sacrificing agent instead gains the indirect benefit of an improved social reputation (Alexander, 1987). An example of an indirect reciprocal exchange in humans might be refusing to lend money to a friend after learning they have outstanding debts to several mutual friends. In this example, the short-term benefits derived from not paying one’s debts may have long-term repercussions for one’s reputation as “trustworthy”, thereby reducing one’s ability to secure loans from friends in the future.

Despite providing valuable insight into how human morality may have evolved, theories of direct and indirect reciprocity are not suited to explain the full range of helping behaviours of which humans are capable. Consider, for example, the case of Specialist Ross Andrew McGinnis, a soldier in the United States Army during the Iraq War who sacrificed his life to save four soldiers by throwing himself atop a live grenade (Hohmann, 2008). Such behaviours need not benefit lifetime fitness via subsequent reciprocation of fitness-sacrificing behaviour. This is an example of a behaviour grouped under the label “pure altruism”, intended here to mean “[a]cting with the intention of benefitting another individual where this is motivated by a non-instrumental concern for his or her welfare” (Joyce, 2006). Wilson and Wilson (2007) and others have defended several controversial theoretical models, including “gene-culture coevolution” and “group selection”, as explaining how humans may have evolved the capacity for pure altruism (Gintis et al., 2008). Gene-culture coevolution theory extends the view that evolution is concerned with individual fitness, adding that individual fitness depends in part upon the values and structure of one’s social group (Gintis et al., 2008). Individuals then contribute to the overall fitness of their social groups relative to
competing social groups, according to group selection theory (Wilson and Wilson, 2007). Richard Dawkins has objected to this line of reasoning on the grounds that these theories do not address the problem of “free riders” (1989). Dawkins (1989) argues that pure altruists would be exploited by selfish members of a common social group, resulting in enhanced fitness relative to pure altruists which will over time lead to increased representation of genes in the gene pool that support selfish behaviour. “Altruistic punishment” may resolve this problem. Altruistic punishment occurs in cooperative exchanges when a cooperator punishes a free rider although doing so comes at a cost to the cooperator, and it has been demonstrated that altruistic punishment improves group performance on a cooperative gambling task (Fehr and Gächter, 2002).

Although a consensus has not been reached on the tenability of “group selection” and related theories, this section presents a number of theoretical models which are compatible with the view that natural selection has favoured the types of behaviours underlying human morality.

1.3.2. Philosophical and Psychological Accounts of Morality

The nature of human morality has long been a subject of philosophical inquiry, with the significance of reasoning and emotion, respectively, to moral behaviour hotly debated. Historically, rationalist approaches to moral philosophy have enjoyed a great deal of popularity, with prominent philosophers such as Plato maintaining that virtuous behaviour requires that reason exercise control over emotions (Zeyl, 2014). In contrast, sentimentalist philosophers of the Scottish Enlightenment period, including Francis Hutcheson, Adam Smith, and David Hume, argued throughout the 1700’s for the importance of moral sentiments and motivations in shaping moral behaviour (Lamb, 1974; Bishop, 1996), with Hume stating that “[r]eason is, and ought only to be, the slave of the passions, and can never pretend to any other office than to serve and obey them” (Hume, 1978). In the late 1700’s, Kant distinguished between the capacity to make moral judgments and the motivation to act on those judgments and argued that true moral behaviour requires adherence to a priori duties (Durant, 1967; Zahn, de Oliveira-Souza and Moll, 2015).

Following its split from philosophy into an independent academic discipline, psychology has proven fertile ground for continuing this debate. In an extension to the Piagetian approach to describing moral development (Piaget, 1965), Lawrence Kohlberg (1969) put forth a theory postulating that moral development occurs across six stages, with the latter-most stage culminating in a distinctively Kantian appreciation for
universal moral rules. More recently, Jonathan Haidt (2001) has advocated a contrasting view, the “Social Intuitionist” model of moral behaviour, which suggests that moral judgments rely heavily on automatic, spontaneous processing that integrates social and cultural knowledge. In a similar vein, Antonio Damasio and colleagues’ (1994) “somatic marker hypothesis” suggests a critical role for emotions in moral decision making. The somatic marker hypothesis was developed to model erratic behaviour following damage to the ventromedial prefrontal cortex (VMPFC; (Eslinger and Damasio, 1985)). Despite having intact knowledge about socially appropriate behaviour, these patients have difficulty translating this knowledge into action. Damasio and colleagues have argued that VMPFC damage results in an inability to represent bodily states used to tag options in response to situations with emotional significance (“somatic markers”; (Eslinger and Damasio, 1985; Damasio, 1994; Bechara et al., 1996)).

1.3.3. The Dual-Process Model of Moral Judgment

With the advent of brain imaging techniques allowing for in vivo investigations of brain functioning and organization in humans, the last decade has witnessed a boom of research projects aimed at describing the neural correlates of moral cognition. In 2001, Joshua Greene and colleagues conducted a study in which participants underwent fMRI while responding to moral dilemmas. Moral dilemmas are descriptions of situations which invoke competing ethical concerns. One widely-cited example is the “Trolley Dilemma” proposed by Thomson (1986). Imagine seeing a trolley barrelling down a set of tracks towards five people who will surely be killed upon impact. Imagine, too, that the trolley is soon to reach a fork in the tracks, and that pulling a lever could divert its course towards only one person. The dilemma arises when one is asked whether it is morally appropriate to pull the lever to save five people at the expense of one life, and most participants endorse this action (Greene et al., 2001). The related “Footbridge Dilemma” is yet more difficult, asking whether it would be morally appropriate to push a person in front of trolley if one had a priori knowledge that doing so would prevent the trolley from hitting the five others. Strikingly, most participants reject this action despite the fact it produces a similar outcome to that of the Trolley Dilemma. This observation led Greene and colleagues (2001) to classify dilemmas on the basis of agency, grouping dilemmas of the former type as “impersonal moral dilemmas” and those of the latter type as “personal moral dilemmas.”
Greene and colleagues (2001) compared brain activity elicited while choosing whether a given action was “appropriate” or “inappropriate” in response to personal, impersonal, and non-moral dilemmata (for example, whether it is appropriate to buy the generic version of a product rather than its brand-name competitor; see supplemental material in (Greene et al., 2004)). Relative to impersonal and non-moral dilemmata, the investigators observed increased activity in regions claimed to be associated with emotion (medial frontal and posterior cingulate gyri, superior temporal sulcus [STS]) and decreases in regions typically associated with working memory (dorsolateral prefrontal cortex [DLPFC] and parietal regions). Furthermore, reaction times were significantly slower in participants who judged responses to personal moral dilemmas as “appropriate” rather than “inappropriate.” These findings provide the foundation for the “dual-process” model of moral judgment proposed by Greene and colleagues. This model draws upon a previous proposal by Miller and Cohen (2001) that the prefrontal cortex underlies cognitive control to suggest that prepotent emotional responses to high-conflict scenarios (i.e., “personal moral dilemmas”) compete with and are regulated by cognitive control mechanisms (Greene et al., 2001; Greene and Haidt, 2002; Greene et al., 2004).

The results of several imaging and lesion studies have been interpreted as supporting the dual-process model. Greene and colleagues (2004) have demonstrated increased activity in the DLPFC and anterior cingulate cortex (ACC) in response to difficult personal moral dilemmas, regions which they suggest play an active role in resolving conflict arising from incompatible response options. In another study performed by the same group, increased cognitive load was shown to increase reaction time in participants who made utilitarian judgments, or judgments favouring the maximization of overall welfare (Mill, 1998), in response to high-conflict moral dilemmas (Greene et al., 2008). The authors interpret this as an interference with cognitive control mechanisms (Greene et al., 2008). Conversely, in a study by Koenigs et al. (2007), patients with bilateral damage to the VMPFC were more likely than controls to make utilitarian moral judgments in response to high-conflict moral dilemmas. These findings are taken to suggest that VMPFC patients experience affective blunting, reducing competition with cognitive control mechanisms and thereby allowing for overall increases in utilitarian judgments (Greene, 2007; Koenigs et al., 2007).

Despite its popularity, the dual-process model has come under criticism for a number of theoretical and methodological issues (see section 1.3.5 for an overview of
methodological concerns). A re-analysis performed by McGuire and colleagues (2009) of the reaction time data from Greene and colleagues’ (2001) study indicated the previously reported interaction between dilemma type (personal or impersonal) and participants’ choices (appropriate or inappropriate) was driven by a handful of stimuli. These findings cast doubt on the validity of the personal-impersonal distinction used by Greene and colleagues to group moral dilemmas presented to participants during the investigators’ imaging studies. Furthermore, the viewpoint that VMPFC patients demonstrate a utilitarian bias due to affective blunting is not consistent with data in the same patient population demonstrating increased emotionality during a cooperative gambling task as evidenced by an increased likelihood of rejecting unfair offers (Pillutla and Murnighan, 1996; Koenigs and Tranel, 2007; Moll and de Oliveira-Souza, 2007). Another concern in interpreting these data is the anatomical distribution of lesions in the VMPFC patient group, with lesions in some patients encompassing sections of the frontopolar cortex (FPC) and anterior DLPFC (Moll and de Oliveira-Souza, 2007). Presumably, the dual-process model would predict damage to the VMPFC and anterior DLPFC to produce deficits both in emotional processing and in utilitarian reasoning, which was not observed. As a final point, the deliberative processes purportedly underlying utilitarian reasoning have not been clearly defined, and the impact of cultural milieu on what is deemed utilitarian is unknown (Moll et al., 2005).

1.3.4. Event-Feature-Emotion Complex Model of Moral Cognition

Our group has developed an alternative theoretical model of moral cognition and emotion, the event-feature-emotion complex (EFEC), which avoids several of the theoretical limitations inherent in the dual-process model. In contrast to the hierarchical view espoused in the dual-process model, the EFEC model suggests that emotional, reason-based, and other cognitive processes are integrated to give rise to moral judgments (Moll et al., 2005). The EFEC model proposes that moral cognition and emotions emerges from the binding of 1) representations of event sequences and their context, 2) perceptual features and conceptual knowledge of social behaviour, and 3) emotional and motivational states (Moll et al., 2005). These three components are thought to map onto a fronto-temporo-subcortical network (Figure 1.1a), a view which joins recent neuroimaging findings with two earlier conceptual frameworks – the structured-event-complex (SEC) framework, and the moral sensitivity hypothesis (Moll et al., 2002b; Wood and Grafman, 2003; Moll et al., 2005).
Figure 1.1 a) The EFEC model proposes that moral cognition emerges from the binding of 1) representations of event sequences and their context, 2) perceptual features and conceptual knowledge, and 3) emotional and motivational states. These three components are thought to map onto the fronto-temporo-subcortical network pictured above. b) The emergence of the moral emotion “compassion” is suggested to occur through the integration of activations within the fronto-temporo-subcortical network proposed by the EFEC model. Figure from (Moll et al., 2005). EFEC, event-feature-emotion complex.

The SEC framework provides the basis for the first component of the EFEC model. According to the SEC framework, sequential representations of goal-oriented series of events which integrate situational knowledge form the basis of the executive control capacities exercised by the frontal cortex (Wood and Grafman, 2003; Moll et al., 2005). Different types of event knowledge are predicted to be localized to specific regions of the frontal cortex. According to the SEC framework, for example, the VMPFC represents social and emotional event knowledge, including information about social rules, the DLPFC represents non-social event knowledge, including planning and action, and the anterior prefrontal cortex (PFC) represents complex social knowledge, including long-term plans (Wood and Grafman, 2003). These representations, though distributed, are ultimately encoded as a single SEC (Wood and Grafman, 2003).

Initial evidence for the second and third components of the EFEC model is found in the moral sensitivity hypothesis, which integrates findings from several neuroimaging studies to suggest that a network of brain regions is consistently recruited to imbue social situations with moral significance (Moll, Eslinger and de Oliveira-
Souza, 2001; Moll et al., 2002a; Moll et al., 2002b). Moral judgments of sentences, presented both verbally and visually, and passive viewing of pictures with moral content reliably elicited activation of the anterior PFC and orbitofrontal cortex (OFC), as well as the anterior temporal lobe (ATL; excluding passive visual stimuli), posterior STS (excluding auditory stimuli), and subcortical structures (moral and basic emotional visual stimuli) as measured with fMRI (Moll, Eslinger and de Oliveira-Souza, 2001; Moll et al., 2002a; Moll et al., 2002b). Furthermore, functional connectivity measured during passive viewing of moral relative to unpleasant pictures demonstrated increased coupling between several of these regions (i.e., medial OFC, anterior PFC, STS, and precuneus; (Moll et al., 2002b)).

The second component of the EFEC model proposes that the activation of the ATL and STS observed in neuroimaging studies of moral sensitivity is associated with the processing of conceptual knowledge of social behaviour and perceptual information. Zahn and colleagues (2007) have used fMRI to demonstrate that processing social concepts relative to non-social concepts recruits the right superior ATL, with activation occurring independently of emotional valence and correlating positively with the level of social conceptual detail. This suggests that social conceptual knowledge is represented in the superior ATLs, a view generally in agreement with the “hub-and-spoke” model of conceptual processing which conceives of the ATLs as modality-invariant hubs upon which information from modality-specific “spokes” converge (e.g., sensory and motor regions; (Pobric, Jefferies and Lambon Ralph, 2010)). Atrophy of the ATL in patients with semantic dementia, a subtype of frontotemporal dementia (FTD), is associated with cross-modality impairments in representing conceptual information (Bozeat et al., 2000; Hodges et al., 2000), and dysfunction specific to the right ATL has been associated with disturbances to social behaviour (Mendez et al., 2000; Rankin et al., 2006). The posterior STS, a region contiguous with posterior regions of the ATL, is implicated in processing socially-relevant perceptual information. Neuroimaging studies indicate the STS is involved in the perception of biological motion, including eye gaze, hand actions, and other body movements (reviewed by (Allison, Puce and McCarthy, 2000)). Indeed, damage to the STS has been associated with impairments in perceiving eye gaze (Campbell et al., 1990; Akiyama et al., 2006), and STS dysfunction is thought to play a role in the social impairments observed in autism (reviewed by (Pelphrey, Adolphs and Morris, 2004)).

The third component of the EFEC model suggests that subcortical regions (limbic, paralimbic, and brainstem) underlie “undirected” emotional and motivational
states (e.g., sadness, social attachment, sexual arousal), which are bound to contextual and conceptual information stored elsewhere in the brain to give rise to basic and moral emotions (Moll et al., 2005). Moral emotions are distinguishable from “basic” emotions in that they “are linked to the interests or welfare either of society as a whole or at least of persons other than the judge or agent” (Haidt, 2003). Moral emotions are discussed in greater detail in section 1.4.1. Regions associated with undirected emotional and motivational states include the hypothalamus, amygdalae, septal nuclei, ventral striatum, medial forebrain bundle, ventral tegmentum, and paralimbic cortex (reviewed by (Moll et al., 2005)), and damage to these regions has been associated with abnormal emotional and motivational functioning (e.g., the amygdala [(Zola-Morgan et al., 1991)] and hypothalamus [(Stellar, 1994)]).

In summary, the EFEC model predicts that binding of knowledge about event sequences, perceptual information, conceptual representations, and emotional and motivational states across a distributed network of frontal, temporal, and subcortical regions gives rise to moral cognition and emotions. Figure 1.1b provides an example of activations predicted by the EFEC model to underpin the emergence of the moral emotion “compassion” after meeting an orphaned child who is unlikely to be adopted. In this example, the prefrontal cortex represents predictions about the child’s future (e.g., likely to remain in the orphanage), the superior ATL represents concepts relevant to child’s situation (e.g., helplessness), the posterior STS represents perceptual features of the child’s expression (e.g., sadness), and subcortical (limbic, paralimbic, and brainstem) regions give rise to undirected emotionality (e.g., attachment, anxiety, and sadness). The integration of these components results in feelings of compassion directed at the child (Moll et al., 2005).

Several more recent studies have provided support for the EFEC model’s integrative view of moral cognition and emotion. Zahn and colleagues (2009c) have proposed that social values, or principles that direct judgments about the behaviour of the self and others, arise from the coactivation of neural representations of social conceptual knowledge (e.g., generosity) and moral emotions (e.g., guilt). Participants in a study conducted by Zahn and colleagues (2009c) underwent fMRI scanning while judging the pleasantness of sentences describing actions embodying social concepts, such as generosity, effected by the participant or their best friend towards each other. After scanning, participants chose labels to describe their emotions related to each sentence (i.e., shame/embarrassment, guilt, indignation/anger, pride, and gratitude; see supplemental material in (Zahn et al., 2009c)). The authors demonstrated an association
between activation of the right superior ATL and the sentences’ level of social conceptual detail, replicating a previous finding by the same group (Zahn et al., 2007; Zahn et al., 2009c). The authors also demonstrated an association between distinct emotional states and fronto-mesolimbic regions (Zahn et al., 2009c). Moral emotions related to one’s self (i.e., guilt and pride) correlated with activation of the VMPFC, and guilt was selectively correlated with activation of the SCSR (Zahn et al., 2009c). Other-critical moral emotions (i.e., indignation/anger) activated the lateral orbitofrontal-insular cortices (Zahn et al., 2009c). Activations not specific to agency were observed for pride and gratitude in mesolimbic and basal forebrain regions (Zahn et al., 2009c). Green and colleagues (2010) performed psychophysiological interaction analyses (PPI) on these data and demonstrated functional integration between the right superior ATL and the lateral OFC and SCSR during experiences of indignation and guilt, respectively. These data suggest that distinct moral sentiments emerge from the functional integration of a fronto-temporo-subcortical network, as predicted by the EFEC model.

Support for the causal role played by regions within this network in generating moral emotions has been provided by recent studies in patients with FTD (Zahn et al., 2009b; Moll et al., 2011). A hallmark clinical characteristic of FTD is an increased propensity to engage in inappropriate social behaviour relative to the premorbid state (Snowden, Neary and Mann, 2002). Positron emission tomography (PET) studies of resting-state cerebral glucose metabolism in FTD patients have demonstrated hypometabolism in the right superior ATL coupled with impaired social concept discrimination (Zahn et al., 2009b) and hypometabolism in the dorsomedial PFC and amygdala coupled with impairment of other-critical moral emotions (anger, disgust; (Moll et al., 2011)). Although correlational, these data corroborate the EFEC model’s predictions concerning the contributions of frontal, temporal, and subcortical regions to the emergence moral cognition and emotion.

The EFEC model is a robust neurobiological account of moral cognition and emotion which provides explanatory power above and beyond other contemporary accounts. For example, as was mentioned in section 1.3.3, the dual-process model fails to provide an adequate explanation for the observation that patients with VMPFC lesions demonstrate reduced emotionality during a moral judgment task, but increased emotionality when responding to unfair offers in an ultimatum game. This seemingly contradictory behaviour is explained by the EFEC model, which predicts that the damage to the VMPFC in these patients produces deficits in prosocial moral emotions, which is supported by the apparent emotional blunting demonstrated by these patients.
while responding to moral dilemmas (Koenigs et al., 2007). The model further predicts that the VMPFC damage in these patients would leave other-critical moral emotions intact, which is supported by the emotional reactions to unfair offers observed in the patients during the ultimatum game (Koenigs and Tranel, 2007). The EFEC model represents the most comprehensive framework presently available to researchers for understanding moral behaviour.

1.3.5. Methodological Considerations

Neuroscientific studies using moral dilemmata have suffered from a number of methodological limitations stemming from poorly controlled experimental parameters. The discussion below focuses on limitations originating from the design of moral dilemma stimuli and the methods used in measuring participants’ responses to the stimuli. This is not a comprehensive overview, however, and additional methodological concerns have been outlined in detail elsewhere (Kahane and Shackel, 2010; Christensen and Gomila, 2012).

Moral dilemmas have proved a valuable tool in revealing the cognitive architecture of the moral mind, demonstrating, for example, a tendency to evoke seemingly contradictory moral judgments by manipulating parameters such as whether personal force is required in sacrificing one life to save several others (Greene et al., 2009). The dilemmas used in these studies, however, typically describe extreme situations with which participants may have difficulty identifying. As Moll and colleagues (2005) have pointed out, “[m]orality is a real-world business” with moral cognition operating rapidly in everyday life (Haidt, 2001). When participants respond to moral vignettes lacking ecological validity, they may engage deliberative reasoning processes to a greater extent than is required during every day moral reasoning, and they may have difficulty accurately predicting how they would respond to unfamiliar scenarios (Casebeer, 2003; Knutson et al., 2010). This may result in a disassociation of hypothetical reasoning about moral behaviour from actual moral behaviour, which is problematic considering the forces of natural selection outlined in section 2.2.1 are concerned with real-world behaviour (Casebeer, 2003). Future studies should employ ecologically valid dilemmata that are more closely associated with real-life experiences.

Interpreting judgments about whether or not one would perform a certain action poses another problem due to the confounding effect of social desirability. It is unclear to what extent participants’ explicit evaluations are influenced by a conscious desire to provide socially acceptable responses (Moll et al., 2005). One potential solution to this
problem is to measure implicit, as opposed to explicit, moral evaluations, though this represents a methodological challenge. The Implicit Association Test (IAT), initially published by Greenwald, McGhee, and Schwartz (1998), has been used to measure implicit associations between concepts inferred from response latencies (for example, implicit racial and gender biases have been shown in healthy adults who demonstrated increased reaction times when associating concepts such as “black” with “pleasant” and “female” with “strong”; (Knutson et al., 2007)). The IAT has been applied to measure implicit moral attitudes (Luo et al., 2006). In this study, participants were slower to associate “illegal” with “good” and “legal” with “bad.” However, the IAT has not yet been adapted for use with moral dilemma tasks.

1.4. Moral Emotions and Cognition, MDD, and their Neurobiological Basis

1.4.1. Moral Emotions in MDD

As described in section 1.3.4, moral emotions play a critical role in motivating behaviour aimed at improving the welfare of others (Haidt, 2003; Zahn, de Oliveira-Souza and Moll, 2013). Moral emotions include awe/admiration, gratitude, pride, pity/sympathy/compassion, indignation/anger, contempt/disgust, guilt, shame, embarrassment, and self-hate (Zahn, de Oliveira-Souza and Moll, 2013). Moral emotions and the neural networks underlying moral cognition are disturbed in a number of psychiatric disorders, including psychopathy (Cleckley, 1988), autism (Schneider et al., 2012), obsessive-compulsive disorder (Harrison et al., 2012), alcohol dependence (Khemiri et al., 2012), and bipolar disorder (Benedetti et al., 2007). These disturbances may manifest as reductions in moral emotions (Zahn, de Oliveira-Souza and Moll, 2013). Psychopathy, for example, is characterized by symptoms including lack of remorse and shame, callousness, and violent, aggressive, and antisocial behaviour (Cleckley, 1988), and psychopathy is associated with alterations in brain regions implicated in moral cognition and emotion (e.g., grey matter reductions in the fronto-temporo-subcortical network described in section 1.3.4; (de Oliveira-Souza et al., 2008)).

Alternatively, exaggerated moral emotions may be present in some psychiatric disorders (Zahn, de Oliveira-Souza and Moll, 2013). For example, guilt is thought to promote reparative behaviour in response to perceived moral transgressions (Tangney, Wagner and Gramzow, 1992), and excessive or inappropriate guilt represents a diagnostic criterion for MDEs (American Psychiatric Association, 2000). Survivor guilt, defined by O’Connor and colleagues (2002) as “guilt over surviving the death of a loved
one … or guilt about being better off than others”, has been shown to be elevated in current MDD (Blacher, 2000). Elevated empathic concern has been demonstrated in children at genetic risk for developing MDD (Klimes-Dougan and Bolger, 1998), and levels of guilt and empathy in healthy volunteers have been shown to correlate positively with self-reported altruistic behaviour (Yi et al., 2005). O’Connor and colleagues ((2007), (2011)) have drawn upon these findings to advance the viewpoint that overgeneral self-blame in MDD derives from survivor and empathy-based guilt, which can manifest as increased altruistic behaviour.

Preliminary evidence suggests a self-blaming bias may be associated with depression vulnerability. As described in section 1.2.3, the differential activation hypothesis suggests that vulnerability to MDD is associated with a proneness to experiencing negative emotions (Teasdale, 1983). A synthesis of this theory with causal attribution theory may improve understanding of vulnerability to MDD given that emotional experiences are influenced by perceptions of causality (Weiner, 1985). In line with this view, Green and colleagues (2013b) demonstrated a self-blaming bias in rMDD patients coupled with a reduction in other-blaming emotions, a finding which our group recently confirmed with a larger sample of rMDD and healthy control (HC) participants (Zahn et al., 2015a).

1.4.2. MDD and Performance on Neuroeconomic Paradigms

Studies have reported differences between MDD patients and HC participants regarding performance on the ultimatum game, a neuroeconomic paradigm in which one player proposes splitting a sum of money with a second player who can either accept or reject this proposal, with rejection resulting in neither player receiving the money (Güth, Schmittberger and Schwarze, 1982). In a study by Harlé and colleagues (2010), patients with current MDD rejected fewer unfair offers during an ultimatum game than controls despite reporting stronger negative emotional reactions to these offers. In contrast, Destoop and colleagues (2012) did not find a difference between current MDD patients and HC participants with respect to rejection rates for unfair offers. This discrepancy may be attributable to differences in criteria for inclusion into the MDD participant groups. Destoop and colleagues (2012) included only participants with current MDD into the depression group, whereas four of the 15 patients included into the depression group in the study by Harlé and colleagues (2010) were diagnosed with subthreshold MDD.
Destoop and colleagues (2012) have shown that, relative to controls, current MDD patients made larger offers while in the role of the proposer. The authors suggest this tendency is underpinned by a desire to avoid rejection. It is also plausible, however, that the tendency to make larger offers in patients with current MDD is a manifestation of pathological altruism. Indeed, O’Connor and colleagues ((2007), (2011)) have argued that increased altruism may be a feature of MDD, and recent data suggests altruistic behaviour may represent a vulnerability factor for MDD (Fujiwara, 2009). Since these data were collected as part of a large survey study, however, it is difficult to know whether the responses provided by the participants were influenced by a desire to provide socially acceptable responses (Fujiwara, 2009). Furthermore, Pulcu and colleagues (2015) recently reported no differences between rMDD patients and healthy volunteers on several neuroeconomical paradigms designed to probe altruistic behaviours. Additional research is therefore necessary to clarify the relationship between altruistic behaviour and depression vulnerability.

1.4.3. Neural Correlates of MDD, Moral Cognition and Emotions: Intersections

Moll and colleagues (2007) used emotionally-evocative statements to investigate the neural correlates of self-directed prosocial moral emotions, namely guilt, embarrassment, and compassion, relative to other-critical moral emotions, namely disgust and indignation. Prosocial moral emotions were associated with activation of the anterior PFC and medial OFC, and other-critical moral emotions were associated with activation of the lateral OFC, dorsal ACC, and limbic structures (Moll et al., 2007). This suggests that distinct moral emotions are associated with dissociable networks of brain activity.

The neurobiological substrates of guilt may be particularly relevant to understanding the pathophysiology of MDD, and a number of studies in healthy volunteers have attempted to define the neural correlates of guilt. Unfortunately, findings are not generally consistent across these studies, which may be attributable to methodological differences relating to sample characteristics and sizes, measures of guilt, and comparison conditions. Several regions, however, are reported with remarkable consistency, including the FPC, SCSR, and temporal regions (Shin et al., 2000; Takahashi et al., 2004; Kédia et al., 2008; Basile et al., 2011; Wagner et al., 2011; Michl et al., 2012; Morey et al., 2012). Overall, the results of these studies overlap with findings reviewed in section 1.3.4 suggesting the experience of guilt is associated with activation of the SCSR and superior ATL (Zahn et al., 2009c; Green et
al., 2010), and that damage to the FPC and septal region is associated with impaired prosocial moral emotions (Moll et al., 2011). It is important to note that recruitment of the SCSR observed during experiences of guilt may be more broadly associated with social attachment. Moll and colleagues (2006) have shown that the SCSR is activated while donating during an anonymous charitable donations task, and Zahn and colleagues (2009a) have demonstrated an association between empathic concern and activation of the SCSR in response to sentences evocative of guilt and compassion.

Green and colleagues (2012) reported functional decoupling between the SCSR, right superior ATL, medial FPC, and limbic regions during the experience of guilt relative to indignation in a cross-sectional study of rMDD patients. Furthermore, magnitude of functional coupling was correlated with scores on a measure of self-blame (Green et al., 2012). A subsequent longitudinal investigation conducted by the same group reported higher SCSR-ATL functional coupling in MDD patients who experienced a recurrent MDE over a 14 month follow up period compared to resilient MDD patients and a HC group (Lythe et al., 2015). This finding led to the hypothesis that functional decoupling between the SCSR and ATL in MDD patients observed in the cross-sectional study could reflect a compensatory mechanism which enhances resilience to recurrent MDEs. Taken together, these results suggest the fronto-temporo-subcortical network described in section 1.3.4 is compromised in patients vulnerable to MDD, and that disturbances in this network are associated with feelings of guilt and overgeneral self-blame. Furthermore, these findings suggest potential neural correlates of the elevated empathic concern and self-blame suggested by O’Connor and colleagues ((2007), (2011)) to drive hyperaltruistic behaviour in MDD.

Although a thorough review of the neuroimaging literature in MDD is beyond the scope of this chapter, it bears mentioning that there is substantial overlap in the brain regions implicated in current and remitted MDD and in moral cognition and emotion. As is described in section 1.3.4, moral cognition and emotion recruit regions including the anterior PFC, OFC, posterior STS, right superior ATL, precuneus, ACC, and additional limbic regions (Figure 1.2; (Moll et al., 2005)). Similar frontal (OFC, VMPFC, and DLPFC), temporal (ATL), and limbic regions (ACC) have been implicated in the pathophysiology of MDD (Figure 1.2; (Fitzgerald et al., 2008; Koenigs and Grafman, 2009; Green et al., 2012; Lythe et al., 2015)).
Figure 1.2 A number of brain regions are routinely implicated in moral cognition, including the anterior PFC, OFC, posterior STS, right superior ATL, precuneus, ACC, and additional limbic regions. This network of regions show substantial overlap with regions implicated in the pathophysiology of major depressive disorder. Figure on left from (Moll et al., 2005). Figure on right from (Elliott et al., 2011), adapted from (Mayberg, 2009). PFC, prefrontal cortex; OFC, orbitofrontal cortex; STS, superior temporal sulcus; ATL, anterior temporal lobe; ACC, anterior cingulate cortex.

1.4.4. Resting-State fMRI and the Subgenual Cingulate in MDD

Perhaps the best replicated neuropathological finding in MDD is abnormal functioning of the SCC (Drevets, Ongür and Price, 1998; Mayberg, 2003; Ressler and Mayberg, 2007), a region described previously as being linked to experiences of guilt and to social attachment (Moll et al., 2006; Zahn et al., 2009a). Prior to the advent of effective antidepressant medications, psychosurgical interventions such as the subcaudate tractotomy and anterior cingulotomy which disrupt the white matter in areas including the SCC were moderately successful in treating chronic MDD (Schoene-Bake et al., 2010). More recently, deep brain stimulation targeting the SCC has been used as an effective intervention for treatment resistant MDD (Mayberg et al., 2005). The decision to target the SCC was informed by a large literature demonstrating elevated resting-state cerebral glucose metabolism and perfusion in this region in current MDD patients (Drevets et al., 1997; Drevets, Bogers and Raichle, 2002; Ressler and Mayberg, 2007). Furthermore, treatment of MDD with antidepressant medications has been
shown to normalize metabolic abnormalities in the SCC (Mayberg et al., 1999; Mayberg et al., 2000; Drevets, Bogers and Raichle, 2002).

Interactions between the SCC and other regions implicated in MDD may be particularly informative for understanding its pathophysiology. Seminowicz and colleagues (2004), for example, have developed a model of dysfunction with a cortico-limbic network in MDD using structural equation modelling which includes the SCC, ACC, FPC, OFC, DLPFC, and limbic regions. Resting-state fMRI, which is often used to identify networks of distributed brain regions which demonstrate synchronous fluctuations in blood oxygenation level dependent (BOLD) signal (Fox and Raichle, 2007), is well suited for identifying functional networks which include the SCC and may be disrupted in MDD for several reasons. From a technical point of view, the ability to acquire resting-state fMRI scans quickly (< 10 minutes) and without having to implement and interpret complex activation paradigms is advantageous. Further to this point, since resting-state fMRI is not constrained by the hypotheses inherent in task-based activation paradigms, resting-state fMRI data can be used to explore a variety of networks without having to design different experimental paradigms. From a neurobiological point of view, whereas task-based fMRI studies produce small changes to neuronal metabolism (< 5%), most of the brain’s energy is consumed at rest which underscores the importance of characterising the functioning of the resting brain (Fox and Raichle, 2007).

Resting-state fMRI is not without disadvantages, however. Perhaps the biggest disadvantage to resting-state fMRI is that it is not possible to access participants’ cognitions whilst they undergo scanning. As a consequence, investigators must exercise caution in interpreting resting-state data since inferences about underlying cognitive processes may be unsupported by the available data. Although directly ascertaining the functional significance of resting-state connectivity is problematic, one way to partially address this issue is to investigate the relationship of clinical outcomes or psychological and clinical measures acquired outside of the scanner to resting-state functional networks. Subsequent interpretation nevertheless should be approached with caution since one can only speculate about the causal nature of such relationships. Resting-state fMRI data are also susceptible to methodological issues primarily centred around the influence of physiological and motion-related artefacts on resting-state network connectivity (Power et al., 2012; Van Dijk, Sabuncu and Buckner, 2012; Power et al., 2014). This issue was underscored by Van Dijk and colleagues (2012) who showed that group differences in seed-based connectivity within the default mode network, a
network of brain regions repeatedly demonstrated to be highly connected at rest, could be observed when comparing healthy volunteers grouped according to whether they exhibited high or low motion whilst undergoing scanning. This suggests that spurious group differences in resting-state functional connectivity may be observed when comparing patient groups, who may be more prone to head motion whilst in the scanner, to healthy control participants. Recent studies have provided a number of post hoc image processing strategies that greatly reduce the influence of motion on resting-state fMRI data (see for example (Power et al., 2012; Satterthwaite et al., 2013; Power et al., 2014). In particular, nuisance regression (e.g., cerebrospinal fluid and white matter signals, expanded sets of motion parameters derived via Volterra expansion) and the elimination of high motion volumes from individual resting-state scans have been shown to reduce the influence of physiological noise and head motion on resting-state fMRI data (Power et al., 2012; Power et al., 2014). Global signal regression is another increasingly popular method used to remove head motion from resting-state fMRI data, although this is a contentious processing step given that it may impose a negative bias that can reduce positive correlations (Fox et al., 2009) and may also distort group differences (Saad et al., 2012).

A variety of techniques are available to analyse processed resting-state fMRI data, each of which is associated with distinct strengths and limitations (Margulies et al., 2010). A full description of these techniques is beyond the scope of the current review, which will instead focus on methods commonly employed in studies of MDD (reviewed in (Dutta, McKie and Deakin, 2014)). Independent components analysis (ICA) is a popular technique which decomposes individual resting-state fMRI scans into a pre-specified number of maximally independent component networks. These data can then be used to identify component networks that differ between groups. ICA requires less processing of resting-state fMRI data than comparable techniques and components representing head motion and physiological noise can be visually identified and removed (Margulies et al., 2010), although not fully (Power, Schlaggar and Petersen, 2015). Limitations to ICA include that it requires both a priori assumptions about the number of components that should be identified as well as post hoc assumptions about which components are of interest (Margulies et al., 2010). Several approaches also allow for the investigation of “local” resting-state activity. Regional homogeneity (ReHo), for example, provides a measure of the similarity of resting-state signals in voxels with their nearest neighbours which is thought to reflect synchronisation of local metabolic activity (Margulies et al., 2010). Although the results
of ReHo analyses benefit from a lack of *a priori* assumptions, they are influenced by the size of smoothing kernel used when processing the data and by the number of neighbouring voxels analysed (Margulies *et al.*, 2010). Alternatively, amplitude of low-frequency fluctuations (ALFF) and fractional ALFF (fALFF) are used to describe the magnitude of low frequency fluctuations in resting-state BOLD data (Margulies *et al.*, 2010). Compared to ALFF, fALFF has the advantage of being less susceptible to the influence of physiological noise (Margulies *et al.*, 2010). However, ALFF has higher test-retest reliability than fALFF and consequently may be more appropriate for comparisons between groups (Zuo *et al.*, 2010).

The analyses presented in this thesis used a seed-based approach, which is perhaps the most commonly used technique for identifying resting-state networks (Margulies *et al.*, 2010). Seed-based analysis first requires the selection of an *a priori* region of interest (ROI). For each resting-state fMRI scan, the time course is extracted from this ROI and correlated with the time courses of all other voxels in the brain. Resulting connectivity maps can then be compared between groups. The primary disadvantage typically cited for seed-based analysis is that it restricts the identification of resting-state networks to those which include the chosen seed ROI (Margulies *et al.*, 2010). By constraining the results of our resting-state analyses with a SCSR seed ROI, however, we were able to focus on networks relevant to a large body of research which has implicated subgenual cingulate networks in both the experience of self-blame and in MDD and vulnerability to MDD, as discussed previously and in more detail below.

Although our group has developed a task-based fMRI paradigm that has been used to directly investigate functional connectivity during the experience of self-blame in patients vulnerable to MDD (Green *et al.*, 2012), it is not possible to examine SCSR connectivity directly using this approach due to methodological constraints that are discussed in *Chapter 2*. Seed-based analysis of SCSR connectivity, in contrast, does not suffer from the same methodological limitations and is therefore one of the best available techniques for identifying functional networks that may be of relevance both to the experience of self-blame and to depression vulnerability. Relatedly, from an exploratory standpoint, I was interested in investigating whether the EFEC model’s predictions concerning task-based connectivity to regions including the SCSR, as described in section 1.3.4, could be used to inform predictions about resting-state connectivity within similar networks.

Consistent with previously described reports of elevated SCC metabolism, current MDD patients demonstrated higher resting-state functional connectivity to the
SCC (Greicius et al., 2007; Sheline et al., 2010; Dutta, McKie and Deakin, 2014) which normalises with treatment (Dichter, Gibbs and Smoski, 2014; Liston et al., 2014). Studies reporting abnormal functional connectivity of the SCC in populations vulnerable to MDD have provided conflicting results. Herringa and colleagues (2013) reported an association between lower SCC-hippocampus connectivity and experiences of childhood maltreatment in a study of adolescents. The same study reported lower SCC-amygdala connectivity in the adolescent females only (Herringa et al., 2013). Thomason and colleagues (2015) observed less negative connectivity between the SCC and amygdala in adolescents with early life trauma compared to healthy youths. By contrast, Jacobs and colleagues (Jacobs et al., 2014) reported increased connectivity between the SCC and the parahippocampal gyrus and other frontal, temporal, and subcortical regions in rMDD patients compared to healthy volunteers. Similarly, Gaffrey and colleagues (2012) found increased connectivity between the SCC and posterior cingulate cortex in preschool children with a history of preschool onset depression relative to HC participants. Discrepancies concerning which regions were abnormally functionally connected to the SCC and whether connectivity was abnormally increased or decreased are likely the consequence of several factors. First, different approaches to assessing vulnerability to MDD were used across studies. Two studies recruited participants with a history of childhood trauma which is known to confer risk for MDD (Herringa et al., 2013; Thomason et al., 2015), whereas two others recruited participants using versions of the DSM criteria for diagnosing a past MDE (Gaffrey et al., 2012; Jacobs et al., 2014). Second, two of the studies investigated connectivity in children aged 15 and under (Gaffrey et al., 2012; Thomason et al., 2015) whereas the other two studies recruited adult participants aged 18 and older (Herringa et al., 2013; Jacobs et al., 2014). Lastly, although each study investigated functional connectivity using a seed-based approach, whereby the timecourse is extracted from an a priori ROI and correlated with the timecourse of each other voxel in the brain, each study used different seed regions (e.g., hippocampus, amygdala, posterior cingulate cortex, SCC). Taken together, the variability present in reports of abnormal resting-state SCC connectivity in populations vulnerable to MDD could be a consequence of differences in sample characteristics or of methodological differences.

Taken together, preliminary data suggest resting-state connectivity to the SCC is abnormal in people vulnerable to MDD, although carefully controlled studies are needed to further characterise which SCC networks are affected and in which direction they are affected. One approach to refine future studies would be to design hypotheses
and select ROIs for seed-based resting-state fMRI analyses with reference to cognitive-anatomical models of MDD which link symptoms of MDD to abnormalities within neuronal networks (Zahn, 2009). The network described by the EFEC model, for example, could be used to generate specific predictions about the relationship of altruistic behaviour to network-level disruptions in MDD patients (Moll et al., 2005; O'Connor et al., 2007; O'Connor et al., 2011). Several studies have investigated the relationship of moral cognition to resting-state functional connectivity in healthy and psychiatric populations. In healthy volunteers, the areas of the brain that are active at rest demonstrate a high degree of anatomical consistency with areas recruited during moral cognition (Harrison et al., 2008). Psychopathic individuals demonstrated reduced connectivity compared to control participants between the dorsomedial and posterior cingulate cortices, regions which were less active in the patient group during moral judgment (Pujol et al., 2012). Using the same approach, cocaine-dependent participants demonstrated lower connectivity compared to healthy volunteers between the anterior cingulate cortex, insula, and other regions (Verdejo-Garcia et al., 2014). Interestingly, Pulcu and colleagues (2014b) used task-based fMRI to demonstrate an enhanced SCSR response associated with altruistic decisions in rMDD patients compared to HC participants despite a lack of behavioural group differences. To my knowledge, however, no studies have investigated the relationship of moral cognition or preference for altruistic behaviours to resting-state functional connectivity in MDD, nor in individuals vulnerable to MDD.

1.5. Design of Studies

The research described in this thesis was conducted as part of two independent Medical Research Council (MRC) funded studies: 1) “Development of Cognitive and Imaging Biomarkers Predicting Risk of Self-Blaming Bias and Recurrence in Major Depression” (Ref No. 07/H1003/194; hereon the “Blame Bias” study, and 2) “Neurobiology of Resilience to Depression” (Ref No. 10/H1014/8; hereon the “NORD” study). Both the Blame Bias and NORD studies investigated patients in remission from MDEs as well as healthy participants using clinical and psychological assessments and brain imaging, as described below. The data presented in Chapters 2, 3, 4 (Study 1) and 5 were collected as part of the Blame Bias study. The data presented in Chapter 4 (Study 2) were collected as part of the NORD study.

The full study protocols for the Blame Bias and NORD studies are provided in Appendices A and B, respectively. Briefly, volunteers for the Blame Bias study were
first assessed for eligibility via telephone screening. Eligible volunteers were invited to complete a clinical interview at which they underwent assessment with the Structured Clinical Interview-I for DSM-IV-TR (SCID; (First et al., 2002)) to diagnose current and past Axis I disorders. Eligible volunteers were also assessed with the Beck Depression Inventory (BDI; (Beck, Steer and Brown, 1996)) to measure current depressive symptoms, the Montgomery-Åsberg Depression Rating Scale (MADRS; (Montgomery and Åsberg, 1979)) to determine the severity of depressive symptoms (current and, if applicable, from the last and most severe MDE), the Global Assessment of Functioning (GAF; (American Psychiatric Association, 2000)) and Life Base to measure psychosocial functioning, a shortened version of the Weissman Family History screen to assess family history of psychiatric disorders, and the Life Events Questionnaire to record any history of traumatic life events. Participants were also tested with computerised versions of the Positive and Negative Affect Scale, Rosenberg Self-Esteem Scale, Interpersonal Guilt Questionnaire (O'Connor et al., 1997), Test of Self Conscious Emotion, Self-Stigma Questionnaire and Group Identification Measure, Personal Style Inventory, and Moral Sentiments Test (Zahn et al., 2009c). The following neuropsychological assessments were also conducted: verbal fluency, trail making test, National Adult Reading Test, and the Addenbrooke's Cognitive Exam (if over 50 years of age; (Mioshi et al., 2006)). Participants who remained eligible following the clinical interview were invited to complete additional visits at which MRI scans, including resting-state fMRI, and electroencephalogram recordings were obtained. Eligible participants were also asked to return for an additional visit to complete the Altruistic Choices Task described in Chapters 4 (Study 1) and 5. At this visit, which I designed in collaboration with my supervisory team and conducted myself, participants were also assessed with the Positive and Negative Affect Scale, Rosenberg Self-Esteem Scale, and Compassionate Altruism Scale (Vaux, Riedel and Stewart, 1987; Berry et al., 2009). Patients with MDD were then followed longitudinally for a period of 14 months and completed follow up visits at 3 (via telephone), 6, and 14 months. At these visits, participants underwent a psychiatric exam as well as assessment with the Longitudinal Interval Follow-up Evaluation, MADRS, BDI, Life Events Questionnaire, Positive and Negative Affect Scale, Rosenberg Self-Esteem Scale, and Moral Sentiments Test.

Volunteers for the NORD study first completed an online assessment of eligibility before being invited for an in person clinical interview. At this interview, volunteers underwent assessment with the SCID, MADRS, and Life Events and
Difficulties Schedule. Participants who remained eligible following the clinical interview were invited to return to complete additional study visits at which MRI scans were obtained and neuropsychological testing was conducted. The neuropsychological test battery included: a self-referential words task, the faces task, the Wisconsin Card Sorting Test, the Stop Task, the Wechsler Test of Adult Reading, the Moral Sentiments Test, the Cambridge Neuropsychological Test Automated Battery, the Profile of Mood States, an anagrams test, and the Sociotropy Autonomy Scale. Eligible participants were also asked to return for an additional visit to complete the Altruistic Choices Task described in Chapter 4 (Study 2). At this visit, which I designed in collaboration with my supervisory team and conducted myself, participants were also assessed with the Life Base, MADRS, Life Events Questionnaire, GAF, Social and Occupational Functioning Assessment Scale (American Psychiatric Association, 2000), Positive and Negative Affect Scale, Rosenberg Self-Esteem Scale, Interpersonal Guilt Questionnaire, and Compassionate Altruism Scale.

The inclusion and exclusion criteria for the Blame Bias and NORD studies are described in detail in Chapter 4. It bears noting that the inclusion and exclusion criteria for both studies, while largely overlapping, differed in the following ways: the Blame Bias study required English as the native language whereas the NORD study required English as the preferred language, the Blame Bias study included all participants over 18 years of age whereas the NORD study only included participants between 29 and 51 years of age, and lastly the Blame Bias study required five symptoms to diagnose a past MDE whereas the NORD study required at least seven symptoms.

1.6. Aims

In Chapter 2, I investigated whether remitted MDD patients with a history of melancholic MDEs can be distinguished from non-melancholic MDD and control participants on the basis of resting-state connectivity. The melancholic subtype of MDD has been associated with personality traits which are present even outside the depressed state (Hecht et al., 1998) such as elevated feelings of dutifulness and concern for others (Sato et al., 1996). I therefore hypothesised that the melancholic rMDD patients would demonstrate lower connectivity compared to the non-melancholic and HC groups between the SCSR and medial prefrontal cortical areas which are critical for social actions and motivations (Moll et al., 2005). A seed-based analysis was carried out using a SCSR ROI previously identified as demonstrating abnormal guilt-related functional connectivity in rMDD patients (Green et al., 2012; Lythe et al., 2015). Group
differences in connectivity were then explored over the whole brain and with closely-connected *a priori* ROIs implicated in the pathophysiology of MDD and in social cognitive and motivational processes.

In *Chapter 3*, I investigated whether resting-state connectivity to the SCSR could distinguish MDD patients who remained resilient over the 14 month follow up period from patients who experienced a recurring MDE and healthy volunteers. I hypothesised that lower connectivity between the SCSR and fronto-subcortical regions would distinguish resilient MDD patients from the recurring episode MDD and HC groups. Again, a seed-based analysis was carried out using the same guilt-related SCSR ROI used in *Chapter 2*. As before, group differences in connectivity were explored over the whole brain and with the same *a priori* ROIs used in *Chapter 2*.

In *Chapter 4*, I investigated preferences for altruistic and social relative to selfish options in rMDD patients and healthy volunteers using a novel behavioural paradigm designed to probe the influence of modulating guilt on implicit and explicit choice preference. As was mentioned earlier, O’Connor and colleagues ((2007), (2011)) have hypothesised that elevated self-blame in MDD gives rise to pathological increases to altruism in some patients. If true, rMDD patients would be expected to prefer altruistic choices more than HC participants since self-blaming emotions including guilt have been shown to be elevated in rMDD (Green *et al.*, 2013b; Zahn *et al.*, 2015a). Alternatively, I have proposed a hypothesis which states that the relationship between self-blame and altruistic choice preference can be modelled as an inverted U-shaped curve, with the peak representing the point after which self-blame is no longer adaptive and consequently reduces altruistic choice preference. This hypothesis predicts that the HC and rMDD groups would be situated on either side of this curve, and increasing or decreasing self-blame with emotion priming would reciprocally increase or decrease preference for altruistic options in healthy volunteers while producing the inverse pattern of changes to choice preference in rMDD patients. Statistical models were then used to test the competing predictions made by O’Connor and colleagues and by my hypothesis. This chapter reports results from data collected across two larger studies, the first of which was described in section 1.1, and the second was a larger longitudinal study of resilience to MDD.

In *Chapter 5*, I investigated whether explicit or implicit choice preferences as measured with the novel behavioural paradigm described above were associated with patterns of resting-state functional connectivity to the SCSR. I hypothesized that, across all participants, choice preference would be correlated with connectivity between the
SCSR and regions implicated in social attachment and reward (Zahn, de Oliveira-Souza and Moll, 2015). I further hypothesized that nature of the relationship between connectivity to the SCSR and choice preference would distinguish the rMDD patients from the HC group. As in Chapters 2 and 3, a seed-based analysis was carried out using the SCSR ROI described previously. The resulting connectivity maps for all participants were then correlated with explicit and implicit preference for altruistic options, specifically altruism directed towards friends and colleagues, and with preference for social options which do not involve an altruistic component. Correlations were carried out over the whole brain and with subcortical a priori ROIs implicated in affiliative emotion and reward. Average correlation coefficients were then extracted from all significant regions and their association with choice preference was compared between the rMDD patients and HC participants.
Chapter 2.

Subgenual cingulate-amygdala functional disconnection and vulnerability to melancholic depression

Authorship Contributions
Clifford I. Workman conducted the data analysis and interpretation, drafted the article, and approved the final version. Karen E. Lythe, Jorge Moll, Jennifer A. Gethin, and John F. W. Deakin aided in the study’s conception and design and provided critical revisions and final approval for the article. Shane McKie aided in the design of the statistical analyses and provided critical revisions and final approval for the article. Rebecca Elliott aided in the data interpretation and provided critical revisions and final approval for the article. Roland Zahn conceived of and designed the study, aided in data analysis and interpretation, and provided critical revisions and final approval for the article.

Submission
This manuscript was prepared for submission to Neuropsychopharmacology (http://www.nature.com/npp/index.html). The reference style, however, was chosen for consistency with the other chapters presented in this thesis. The journal’s preferred referencing style will be adopted prior to submission.
Subgenual cingulate-amygdala functional disconnection and vulnerability to melancholic depression

Clifford I. Workman, B.S.¹,², Karen E. Lythe, Ph.D.², Shane McKie, Ph.D.¹, Jorge Moll, M.D., Ph.D.³, Jennifer A. Gethin, M.Res.³, John F. W. Deakin, F.R.C.Psych., Ph.D.¹, Rebecca Elliott, Ph.D.¹, Roland Zahn, M.D.⁴,²

¹University of Manchester, Institute of Brain, Behaviour and Mental Health, Neuroscience & Psychiatry Unit, UK
²University of Manchester, School of Psychological Sciences, Neuroscience and Aphasia Research Unit, UK
³Cognitive and Behavioral Neuroscience Unit, D’Or Institute for Research and Education, Brazil
⁴Institute of Psychiatry, Psychology, and Neuroscience, Department of Psychological Medicine, Centre for Affective Disorders, King’s College London, UK

2.1. ABSTRACT

The syndromic heterogeneity of major depressive disorder (MDD) hinders understanding of the aetiology of predisposing vulnerability traits and underscores the importance of identifying neurobiologically valid phenotypes. Distinctive fMRI biomarkers of vulnerability to MDD subtypes are currently lacking. This study investigated whether remitted melancholic MDD patients, who are at an elevated lifetime risk for depressive episodes, demonstrate distinctive patterns of resting-state connectivity with the subgenual cingulate/septal region (SCSR), known to be of core pathophysiological importance for severe and familial forms of MDD. We hypothesized that patterns of disrupted SCSR connectivity would be a distinguishing feature of melancholia. Sixty-three medication-free remitted MDD (rMDD) patients (33 melancholic, 30 non-melancholic) and 39 never-depressed healthy controls (HC) underwent resting-state fMRI scanning. SCSR connectivity was investigated with closely connected bilateral a priori regions of interest (ROI) relevant to MDD (anterior temporal, ventromedial prefrontal, dorsomedial prefrontal cortices, amygdala, hippocampus, septal region, and hypothalamus). Lower SCSR connectivity with the right parahippocampal gyrus and left amygdala distinguished melancholic rMDD patients from the non-melancholic rMDD and HC groups (cluster-based familywise error-corrected $p ≤ 0.007$ over individual a priori ROIs corresponding to approximate Bonferroni-corrected $p ≤ 0.05$ across all seven a priori ROIs). No areas demonstrating higher connectivity were observed. Abnormally low connectivity of the SCSR with the amygdala and parahippocampal gyrus distinguished melancholic from non-melancholic rMDD. These results provide the first resting-state neural signature distinctive of melancholic rMDD and may reflect a subtype-specific primary vulnerability factor given a lack of association with the number of previous episodes.
2.2. INTRODUCTION

Major depressive disorder (MDD) is an inherently heterogeneous condition due to its polythetic diagnostic criteria. A diagnosis of MDD requires a combination of only five of nine possible symptoms, meaning two patients may share only one common symptom. This heterogeneity decreases the likelihood of identifying the underlying neurobiological mechanisms underpinning MDD and underscores the importance of identifying neurobiologically valid depressive phenotypes. The melancholic subtype of MDD, characterized by unvarying low mood and drive as well as psychomotor and vegetative symptoms, has been associated with stable personality features which are present outside the depressed state and increase vulnerability to depression (Hecht et al., 1998). Identifying a distinct neural signature for vulnerability to melancholic MDD would provide important evidence of its neurobiological validity and point to an intermediate phenotype (Meyer-Lindenberg and Weinberger, 2006). Neuroimaging studies in patients with current melancholic MDD have provided initial evidence of distinct brain abnormalities including dysfunction in the subgenual cingulate and adjacent septal region (SCSR; (Pizzagalli et al., 2004)). However, attempts at identifying markers capable of distinguishing melancholic MDD from other depressive subtypes have been largely unsuccessful. It is furthermore unclear whether the distinctive abnormalities described were correlates of the depressed state rather than vulnerability traits.

Resting-state functional MRI (rsfMRI), which can be used to measure spontaneous low-frequency fluctuations in blood oxygen level-dependent (BOLD) signal in the resting brain (Fox and Raichle, 2007), has become an increasingly popular imaging method for characterizing network-level disruptions associated with psychiatric disorders. There are strong theoretical and practical motivations for acquiring rsfMRI in psychiatric studies: 1) large metabolic demands of the resting brain suggest a critical role in overall brain functioning (Fox and Raichle, 2007), and 2) rsfMRI scans entail short acquisition times and do not require complex cognitive paradigms. The literature describing resting-state network abnormalities in current MDD has grown substantially over the past decade (reviewed in (Dutta, McKie and Deakin, 2014)). Importantly, a recent rsfMRI study of patients with current MDD revealed lower effective connectivity in attention and interoception networks in melancholic relative to non-melancholic patients and a healthy control (HC) group (Hyett et al., 2015). It is elusive, however, whether these effects reflected differences in symptoms experienced during the depressive episode or neural differences irrespective of current symptom profile. It was
further difficult to control for antidepressant effects. Therefore, it remains unknown whether rsfMRI can be used to reveal distinctive signatures of vulnerability traits predisposing to different MDD subtypes.

Studies of brain functioning in MDD highlight the SCSR as a key region in the pathophysiology of depression (see (Mayberg, 2003; Ressler and Mayberg, 2007)). In HC participants, SCSR BOLD response was associated with guilt proneness (Zahn et al., 2009c), which is often excessive and overgeneralized in current MDD, particularly in the melancholic subtype. Resting-state glucose metabolism in the SCSR has previously been shown to be elevated in current MDD and to normalize upon remission from the depressed state (reviewed in (Ressler and Mayberg, 2007)). Furthermore, current MDD patients demonstrated greater resting-state connectivity of the SCSR with fronto-parieto-limbic regions relative to control participants (Greicius et al., 2007; Sheline et al., 2010). Green and colleagues (2012) recently demonstrated task-related SCSR decoupling in remitted MDD (rMDD) patients during the experience of guilt. Since rMDD patients are at a highly elevated lifetime risk for major depressive episodes (MDE) relative to HC participants (Kupfer, 1991), this suggests guilt-related SCSR decoupling may represent a trait vulnerability factor for MDD rather than a correlate of the depressed state. Additionally, although the literature on resting-state connectivity in depression vulnerability is limited, disrupted SCSR connectivity has been observed in young rMDD patients (Gaffrey et al., 2012) and in at-risk adolescents (Herringa et al., 2013).

The present study employed rsfMRI to investigate functional connectivity of the SCSR in fully remitted, medication-free MDD patients with or without a history of melancholic MDEs. By investigating patients with rMDD, the present study is well-suited to identify stable vulnerability traits for experiencing MDEs (Burcusa and Iacono, 2007). The current study used a seed-based analysis approach as this method has been commonly used in rsfMRI studies of current MDD (Dutta, McKie and Deakin, 2014). We used the SCSR seed region identified in Green and colleagues’ (2012) study which previously demonstrated decoupling with medial frontal and medial and anterior temporal cortices during the experience of guilt in a rMDD patient group that included a high proportion of melancholic rMDD patients. We hypothesized that melancholic rMDD patients would demonstrate a distinctive pattern of disrupted SCSR connectivity when compared with the non-melancholic rMDD patients and HC participants. We further predicted that lower medial prefrontal connectivity with the SCSR would distinguish melancholic from non-melancholic rMDD patients. This prediction was
based on evidence that the medial prefrontal cortex is necessary for social actions and motivations (Moll et al., 2005) which are classically impaired in melancholia (Ebert, Martus and Lungershausen, 1995), as well as its known direct anatomical connections to the SCSR (Vogt and Pandya, 1987; Carmichael and Price, 1996) and previous work in which it demonstrated guilt-related functional disconnection with the SCSR (Green et al., 2012).

2.3. MATERIALS and METHODS

2.3.1. Participants

The present study was approved by the South Manchester National Health Service Research Ethics Committee. After the study procedures were explained in full, participants gave informed consent (verbal consent for a telephone pre-screening and written consent at the outset of each study visit). Participant recruitment was conducted using online and print advertisements as part of the UK Medical Research Council-funded “Development of Cognitive and Imaging Biomarkers Predicting Risk of Self-Blaming Bias and Recurrence in Major Depression” project (Zahn et al., 2015a). Participants received compensation for their time and travel expenses. Prior to taking part in the study, 707 volunteers completed a telephone pre-screening to preliminarily assess eligibility (the pre-screening document is available at http://www.translational-cognitive-neuroscience.org/start/test-materials). Volunteers who passed the initial screening were then invited to complete a clinical interview overseen by a senior psychiatrist (RZ) at which psychiatric, clinical, and family histories were recorded. The Structured Clinical Interview-I for DSM-IV-TR was used to diagnose past MDEs with or without melancholic features (First et al., 2002) with moderate to perfect inter-rater reliability (see Table S2.1). Exclusion criteria were: current Axis I disorders, history of substance abuse or major medical or neurological disorders, exposure to psychotropic medications within 4 weeks (8 weeks for fluoxetine), and contraindications for MRI scanning. Additionally, the HC group had no history of Axis-I disorders and no first-degree family history of mood disorders or schizophrenia. Following the initial clinical interview, 96 rMDD patients and 48 HC participants were eligible for enrollment into the current study (see Table 2.1 for a detailed overview of reasons for exclusion). Of these, 63 medication-free patients with rMDD (33 melancholic, 30 non-melancholic) and 39 never-depressed HC participants underwent MRI scanning. One HC participant’s data were excluded due to the presence of a pituitary abnormality.
<table>
<thead>
<tr>
<th>Reasons for Telephone Pre-Screening Exclusions</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI contraindications</td>
<td>77</td>
</tr>
<tr>
<td>Psychiatric disorders other than MDD</td>
<td>54</td>
</tr>
<tr>
<td>Current antidepressants or other centrally active medications</td>
<td>52</td>
</tr>
<tr>
<td>Withdrawal after telephone pre-screening</td>
<td>33</td>
</tr>
<tr>
<td>Not meeting full screening criteria for MDD</td>
<td>30</td>
</tr>
<tr>
<td>Family history of MDD/bipolar/schizophrenia (HC group)</td>
<td>26</td>
</tr>
<tr>
<td>Substance or alcohol abuse</td>
<td>23</td>
</tr>
<tr>
<td>Current antihypertensive or statin medications</td>
<td>20</td>
</tr>
<tr>
<td>Left-handed</td>
<td>20</td>
</tr>
<tr>
<td>Non-native English speaker</td>
<td>19</td>
</tr>
<tr>
<td>Thyroid function problems</td>
<td>19</td>
</tr>
<tr>
<td>Fulfilling criteria for current MDD</td>
<td>13</td>
</tr>
<tr>
<td>History of cancer</td>
<td>7</td>
</tr>
<tr>
<td>Not remitted for long enough (&gt;6 months)</td>
<td>7</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>5</td>
</tr>
<tr>
<td>No reason recorded</td>
<td>5</td>
</tr>
<tr>
<td>Other general medical conditions</td>
<td>5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4</td>
</tr>
<tr>
<td>Out of age range (18 – 65 years)</td>
<td>4</td>
</tr>
<tr>
<td>Excluded because of age-matching (HC group)</td>
<td>3</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>3</td>
</tr>
<tr>
<td>History of stroke</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>1</td>
</tr>
<tr>
<td>Total excluded after the telephone pre-screening</td>
<td>431 / 707</td>
</tr>
<tr>
<td>Reasons for Clinical Interview Exclusions (remitted MDD patients)</td>
<td>N</td>
</tr>
<tr>
<td>Unable to schedule for additional visits</td>
<td>10</td>
</tr>
<tr>
<td>Fulfilling criteria for a bipolar disorder</td>
<td>6</td>
</tr>
<tr>
<td>Fulfilling criteria for current social anxiety disorder</td>
<td>6</td>
</tr>
<tr>
<td>Not meeting full criteria for MDD</td>
<td>5</td>
</tr>
<tr>
<td>Fulfilling criteria for past substance abuse</td>
<td>4</td>
</tr>
<tr>
<td>Not remitted for long enough (&gt;6 months)</td>
<td>3</td>
</tr>
<tr>
<td>Residual symptoms of post-traumatic stress disorder</td>
<td>3</td>
</tr>
<tr>
<td>Probable personality disorders</td>
<td>2</td>
</tr>
<tr>
<td>Fulfilling criteria for current generalized anxiety disorder</td>
<td>1</td>
</tr>
<tr>
<td>MRI contraindications</td>
<td>1</td>
</tr>
<tr>
<td>Withdrawal after the clinical interview</td>
<td>1</td>
</tr>
<tr>
<td>Total number of remitted MDD patients excluded after the clinical interview</td>
<td>42 / 138</td>
</tr>
<tr>
<td>Reasons for Clinical Interview Exclusions (HC group)</td>
<td>N</td>
</tr>
<tr>
<td>Unable to schedule for additional visits</td>
<td>6</td>
</tr>
<tr>
<td>Probable or definite positive first degree family history of MDD</td>
<td>4</td>
</tr>
<tr>
<td>Fulfilling criteria for a past MDE lasting less than two months</td>
<td>1</td>
</tr>
<tr>
<td>Fulfilling criteria for current adjustment disorder</td>
<td>1</td>
</tr>
<tr>
<td>Fulfilling criteria for current MDD</td>
<td>1</td>
</tr>
<tr>
<td>Fulfilling criteria for current social anxiety disorder</td>
<td>1</td>
</tr>
<tr>
<td>Non-native English speaker</td>
<td>1</td>
</tr>
<tr>
<td>Past depressive episode not fulfilling criteria for a past MDE</td>
<td>1</td>
</tr>
<tr>
<td>Total number of HC participants excluded after the clinical interview</td>
<td>16 / 64</td>
</tr>
</tbody>
</table>

Table 2.1 Reasons for exclusion of volunteers from the current study. Of the 707
volunteers who completed the telephone pre-screening, 276 were eligible (184 remitted MDD patients, 92 HC participants). Of these, 202 participants agreed to complete the clinical interview after having reviewed the study’s participant information sheet (138 remitted MDD patients, 64 HC participants). Following the clinical interview, 144 participants were eligible to complete the remaining study visits (96 remitted MDD patients, 48 HC participants). Of these, 102 participants underwent resting-state fMRI scanning (63 remitted MDD patients, 39 HC participants). fMRI, functional magnetic resonance imaging; HC, healthy control; MDD, major depressive disorder; MDE, major depressive episode.

The rMDD patients (both overall and melancholic) and HC group were closely matched on demographic variables (Table 2.2), as were the melancholic and non-melancholic rMDD patients (Tables 2.2–2.3). The rMDD patients (both overall and melancholic) did not differ from HC group with respect to age, years of education, or sex. Scores on the Beck Depression Inventory (BDI; (Beck, Steer and Brown, 1996)) were slightly elevated in the rMDD patients and differed from the HC group ($t(99)=3.71, p<0.0001$). This was also true when comparing only melancholic rMDD patients to the HC group ($t(69)=4.06, p<0.0001$). Despite this, the mean scores for both the rMDD patients (both overall and melancholic) were within the range of mild subthreshold depressive symptoms (Beck, Steer and Carbin, 1988). Furthermore, the groups did not differ with respect to current Montgomery-Åsberg Depression Rating Scale (MADRS) scores. The rMDD patient group had higher scores of empathic guilt (i.e., omnipotent responsibility guilt [ORG], or feeling responsible for the wellbeing of others, and survivor guilt [SuG], or feeling badly about being better off than others) compared to the HC group (ORG: $t(97)=3.14, p=0.002$; SuG: $t(97)=3.91, p<0.0002$) as measured with the Interpersonal Guilt Questionnaire (IGQ; (O'Connor et al., 1997)). This was also true when comparing only melancholic rMDD patients to the HC group (ORG: $t(67)=2.02, p=0.05$; SuG: $t(67)=3.14, p=0.002$). Interestingly, neither ORG nor SuG were higher in the melancholic rMDD patients compared to the non-melancholic patients. This may reflect that the melancholic subtype of MDD is not defined by symptoms of excessive or inappropriate guilt alone (American Psychiatric Association, 2000). The melancholic and non-melancholic rMDD patients also did not differ with respect to age, sex, number of MDEs, months since remission, months since last psychototropic use, number of patients previously treated, number of suicide attempts, or
### Table 2.2
Demographic variables in the remitted MDD and HC groups. With the exception of BDI and guilt scores, the remitted MDD patients and HC group did not significantly differ on the demographic variables (Contingency Coefficient $<0.008$, $p > 0.94$; $t < 0.79$, $p > 0.43$). Also with the exception of BDI and guilt scores, the remitted melancholic MDD patients and HC group did not significantly differ on the demographic variables (Contingency Coefficient $<0.08$, $p > 0.52$; $t < 1.30$, $p > 0.20$). With the exception of Years of Education, the remitted melancholic and non-melancholic MDD patients did not significantly differ on the demographic variables (Contingency Coefficient $<0.17$, $p > 0.18$; $t < 0.89$, $p > 0.38$). BDI, Beck Depression Inventory; HC, healthy control; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder; SD, standard deviations.

<table>
<thead>
<tr>
<th>Demographic Variables</th>
<th>HC (N=38)</th>
<th>rMDD (N=63)</th>
<th>Melancholic rMDD (N=33)</th>
<th>Non-Melancholic rMDD (N=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age</td>
<td>36.2</td>
<td>13.8</td>
<td>36.4</td>
<td>12.3</td>
</tr>
<tr>
<td>Years of Education¹</td>
<td>16.8</td>
<td>2.3</td>
<td>16.8</td>
<td>2.4</td>
</tr>
<tr>
<td>BDI Score²</td>
<td>0.9</td>
<td>1.7</td>
<td>3.6</td>
<td>4.1</td>
</tr>
<tr>
<td>MADRS Score</td>
<td>0.7</td>
<td>1.3</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Omnipotent Responsibility Guilt³</td>
<td>41.9</td>
<td>7.8</td>
<td>46.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Survivor Guilt⁴</td>
<td>59.9</td>
<td>10.3</td>
<td>68.6</td>
<td>11.0</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>13/25</td>
<td>22/41</td>
<td>9/24</td>
<td>13/17</td>
</tr>
<tr>
<td>Framewise Displacement (mm)</td>
<td>0.24</td>
<td>0.15</td>
<td>0.27</td>
<td>0.25</td>
</tr>
</tbody>
</table>

¹Significantly different between the remitted melancholic and non-melancholic MDD groups ($t(61)=2.61$, $p=0.01$).
²Significantly different between the remitted MDD and HC groups ($t(99)=3.71$, $p<0.0001$), and between the remitted melancholic MDD and HC groups ($t(69)=4.06$, $p<0.0001$).
³Significantly different between the remitted MDD and HC groups ($t(97)=3.14$, $p=0.002$), and between the remitted melancholic MDD and HC groups ($t(67)=2.02$, $p=0.05$).
⁴Significantly different between the remitted MDD and HC groups ($t(97)=3.91$, $p<0.0002$), and between the remitted melancholic MDD and HC groups ($t(67)=3.14$, $p=0.002$).
family history of MDD. However, relative to the non-melancholic rMDD group, melancholic patients had more years of education ($t(61)=2.61, p=0.01$) and their last and most severe MDE was longer in duration ($t(61)=2.41, p=0.02$) with MADRS scores indicating greater severity ($t(61)=3.28, p=0.002$). These potential confounders were controlled for in subsequent analyses (see below).

2.3.2. Image Acquisition

Resting-state echo-planar images (EPI) were acquired on a 3T Philips Achieva MRI scanner (Philips Medical Systems, Best, the Netherlands) with an 8-channel coil and were optimized for the detection of ventral frontal signal (240 volumes; 40 axial slices; 3mm slice thickness; ascending sequential acquisition; repetition time: 2000ms; echo time: 22ms; field of view: 240x240x120mm; acquisition matrix: 80x80 voxels; reconstructed voxel size: 3mm$^3$; flip angle: 90°). Participants were instructed to lie still with eyes open and to remain awake and were debriefed after scanning to ensure adherence to the instructions. A 3-dimensional T1-weighted magnetization-prepared rapid-acquisition gradient-echo (MPRAGE) structural image was also obtained for each participant (160 axial slices; 0.9mm slice thickness; repetition time: 8.4ms; echo time: 3.9ms; field of view: 240x191x144mm; acquisition matrix: 256x163 voxels; reconstructed voxel size: 0.94x0.94x0.9mm; flip angle: 8°). For further clinical assessment, T2-weighted structural images were also acquired.

2.3.3. Seed Region Selection

The seed-based rsfMRI analyses were conducted using an *a priori* SCSR region of interest (ROI; Montreal Neurological Institute [MNI] coordinates: -4, 23, -5; 6mm sphere) which demonstrated functional decoupling during a task evoking feelings of guilt in patients vulnerable to experiencing MDEs (Green et al., 2012). The seed region is close in proximity to an SCSR region which demonstrated resting-state functional disconnection in patients vulnerable to MDD (MNI coordinates: 2, 23, -6; (Herringa et al., 2013)). Taken together, the seed region used in this study was selected because of its association with depression vulnerability. Furthermore, although located more medially, the seed region’s coordinates are close to subgenual cingulate regions that demonstrated greater connectivity in current MDD patients relative to HC participants (Greicius et al., 2007; Sheline et al., 2010).
<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Melancholic MDD (N=33)</th>
<th>Non-Melancholic MDD (N=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of previous MDEs</strong></td>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
</tr>
<tr>
<td>1</td>
<td>8/33</td>
<td>10/30</td>
</tr>
<tr>
<td>2</td>
<td>9/33</td>
<td>7/30</td>
</tr>
<tr>
<td>3</td>
<td>8/33</td>
<td>6/30</td>
</tr>
<tr>
<td>4</td>
<td>3/33</td>
<td>3/30</td>
</tr>
<tr>
<td>5</td>
<td>4/33</td>
<td>2/30</td>
</tr>
<tr>
<td>6 or more</td>
<td>1/33</td>
<td>2/30</td>
</tr>
<tr>
<td><strong>Average number of previous MDEs</strong></td>
<td>2.8</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Last and most severe MDE details</strong></td>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
</tr>
<tr>
<td>Average length of MDE (months)$^1$</td>
<td>20.6</td>
<td>23.5</td>
</tr>
<tr>
<td>Average time in remission (months)</td>
<td>30.9</td>
<td>28.6</td>
</tr>
<tr>
<td>Average MADRS score for MDE$^2$</td>
<td>37.3</td>
<td>5.1</td>
</tr>
<tr>
<td>No psychotropic medication since (months)</td>
<td>53.1</td>
<td>73.6</td>
</tr>
<tr>
<td><strong>Previous treatment</strong></td>
<td><strong>N</strong></td>
<td><strong>N</strong></td>
</tr>
<tr>
<td>SSRI antidepressant</td>
<td>29/33</td>
<td>22/30</td>
</tr>
<tr>
<td>SNRI antidepressant</td>
<td>1/33</td>
<td>1/30</td>
</tr>
<tr>
<td>Tricyclic antidepressant</td>
<td>4/33</td>
<td>1/30</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>0/33</td>
<td>1/30</td>
</tr>
<tr>
<td>Unknown class of antidepressant</td>
<td>5/33</td>
<td>3/30</td>
</tr>
<tr>
<td>Benzodiazepines only</td>
<td>0/33</td>
<td>1/30</td>
</tr>
<tr>
<td>No antidepressant medication</td>
<td>2/33</td>
<td>2/30</td>
</tr>
<tr>
<td>CBT</td>
<td>10/33</td>
<td>4/30</td>
</tr>
<tr>
<td>Self-guided CBT via internet, books</td>
<td>3/33</td>
<td>1/30</td>
</tr>
<tr>
<td>Hypnotherapy</td>
<td>0/33</td>
<td>1/30</td>
</tr>
<tr>
<td>Counselling</td>
<td>17/33</td>
<td>12/30</td>
</tr>
<tr>
<td><strong>Suicide attempts</strong></td>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Lifetime axis-I comorbidity$^3$</strong></td>
<td><strong>N</strong></td>
<td><strong>N</strong></td>
</tr>
<tr>
<td>Panic disorder with agoraphobia</td>
<td>1/33</td>
<td>0/30</td>
</tr>
<tr>
<td>Bulimia nervosa</td>
<td>1/33</td>
<td>0/30</td>
</tr>
<tr>
<td>Post-traumatic stress disorder</td>
<td>1/33</td>
<td>0/30</td>
</tr>
<tr>
<td>No life-time co-morbidity</td>
<td>30/33</td>
<td>30/30</td>
</tr>
<tr>
<td><strong>Family history</strong></td>
<td><strong>N</strong></td>
<td><strong>N</strong></td>
</tr>
<tr>
<td>First degree relative with MDD</td>
<td>17/33</td>
<td>20/30</td>
</tr>
<tr>
<td>No family member with history of MDD</td>
<td>13/33</td>
<td>9/30</td>
</tr>
<tr>
<td>First degree relative with schizophrenia or bipolar disorder</td>
<td>3/33</td>
<td>1/30</td>
</tr>
</tbody>
</table>
Table 2.3 Clinical characteristics of the remitted melancholic and non-melancholic MDD patients. All MDD participants stopped medication before the required washout phase. Means and standard deviations are reported and/or the number of cases. Remitted melancholic and non-melancholic MDD participants did not significantly differ on number previous episodes, average time in remission, average time since last taking psychotropic medications, previous treatments, number of suicide attempts, lifetime axis-I comorbidity, or family history (Contingency Coefficient < 0.21, \( p > 0.09; t < 1.55, p > 0.13 \)). CBT, cognitive behavioural therapy; MDD, major depressive disorder; MDE, major depressive episode; SD, standard deviations; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin norepinephrine reuptake inhibitor.

1Significantly different between the remitted melancholic and non-melancholic MDD groups (\( t(61)=2.41, p=0.02 \)).
2Significantly different between the remitted melancholic and non-melancholic MDD groups (\( t(61)=3.28, p=0.002 \)).
3All co-morbid disorders were fully remitted at the time of study and none were likely to be the primary cause of the depressive episodes.

2.3.4. Resting-state fMRI Analysis

EPIs and MPRAGE images were preprocessed using the Artifact Detection Tools (ART; http://web.mit.edu/swg/software.htm), DPARSF Advanced Edition (Chao-Gan and Yu-Feng, 2010), and SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) MATLAB (MathWorks) toolboxes. SPM8 was used for preprocessing to ensure compatibility with DPARSF. The first 10 volumes of the EPIs were discarded prior to slice timing and head motion correction. ART was used to produce regressors of high-motion volumes (i.e., framewise signal intensity > three standard deviations from the global mean, framewise head displacement > 1mm). The MPRAGE images were co-registered to the EPIs and segmented. EPIs underwent linear detrending and nuisance covariates regression (24 motion parameters (Friston et al., 1996), white matter signal, cerebrospinal fluid signal, and ART regressors), normalization using nonlinear transformation parameters derived during segmentation, and smoothing using a 6mm full-width at half-maximum Gaussian kernel, prior to band-pass filtering to retain frequencies between 0.01Hz and 0.08Hz. Volume censoring was then performed to remove high-motion volumes identified by ART and resulting segments of uncensored
data comprised of fewer than five contiguous volumes. All resulting EPIs contained ≥ five minutes of data (≥ 150 volumes).

Functional connectivity maps were computed using the resultant EPIs for each participant by correlating the average time course within the seed region with the time course of each voxel within the brain. The functional connectivity maps were then Fisher Z-transformed and entered into an analysis of variance (ANOVA) in SPM12 because it allows for cluster-level familywise error (FWE) correction of F-tests for differences between melancholic rMDD, non-melancholic rMDD, and HC groups. Significance was determined using an uncorrected voxel-level threshold of \( p<0.001 \) and a cluster-level FWE-corrected threshold of \( p<0.05 \) across the whole-brain \( (p<0.05) \) or \textit{a priori} ROIs \( (p<0.007 \) corresponding to approximate Bonferroni-corrected \( p<0.05 \) for the seven ROIs used in our analyses). The following bilateral \textit{a priori} ROIs were defined: ventromedial prefrontal cortex, dorsomedial prefrontal cortex, anterior temporal cortex, amygdala, hippocampus, septal region, and hypothalamus. The definition of these ROIs was described previously (Zahn et al., 2009c) except for the hippocampal ROI which combined the left and right hippocampus masks from the Automatic Anatomical Labeling atlas (Tzourio-Mazoyer et al., 2002). These ROIs were chosen because of their close structural and/or functional connections with the SCSR (Vogt and Pandya, 1987; Carmichael and Price, 1996; Kondo, Saleem and Price, 2003; Johansen-Berg et al., 2008), their role in the pathophysiology of depression (Mayberg, 2003; Elliott et al., 2011; Green et al., 2012), as well as in social cognitive and motivational processes (Moll et al., 2005; Zahn et al., 2009c; Elliott et al., 2011). Mean Fisher Z-transformed correlation coefficients for each group were extracted from all clusters surviving FWE-correction and entered into a one-way ANOVA in SPSS 20 (SPSS Inc., Chicago, Illinois, USA) to probe pairwise post-hoc comparisons between groups at Bonferroni-corrected \( p<.05 \). The results of all statistical tests reported herein are two-tailed.

2.4. RESULTS

A main effect of group was observed across the melancholic rMDD, non-melancholic rMDD, and HC groups for connectivity between the SCSR seed region and the left amygdala and right parahippocampal gyrus (Table 2.4; Figure 2.1). This was confirmed for the extracted cluster averages in both regions (left amygdala: \( F(2,98)=11.2, p<0.0001 \); right parahippocampal gyrus: \( F(2,98)=13.5, p<0.0001 \). Bonferroni-corrected post-hoc pairwise comparisons revealed lower SCSR connectivity
with the left amygdala in the melancholic rMDD patients (M=0.11, SD=0.16) compared to both the non-melancholic rMDD (M=0.25, SD=0.13, \( p=0.001 \), mean difference=-0.14, 95% CI [-0.22,-0.05]) and HC groups (M=0.25, SD=0.13, \( p<0.0001 \), mean difference=-0.14, 95% CI [-0.22,-0.06]). The same pattern of distinctively lower SCSR connectivity in melancholic rMDD emerged for the right parahippocampal gyrus (M=0.13, SD=0.14) compared with the non-melancholic rMDD (M=0.23, SD=0.15, \( p=0.013 \), mean difference=-0.11, 95% CI [-0.20,-0.02]) and HC groups (M=0.31, SD=0.15, \( p<0.0001 \), mean difference=-0.18, 95% CI [-0.26,-0.09]).

SCSR connectivity across the rMDD patients was not associated with the number of previous MDEs (left amygdala: \( r_s = -0.005, p=0.97 \); right parahippocampal gyrus: \( r_s = 0.10, p=0.43 \)). Across the melancholic and non-melancholic rMDD groups, we ruled

---

**Figure 2.1** a) The network of regions demonstrating resting-state functional disconnection with the subgenual cingulate seed region in the remitted melancholic MDD patients when compared to the remitted non-melancholic MDD and HC groups. Whole-brain images were cropped and displayed at an uncorrected voxel-level threshold of \( p<0.001 \). b) Bar plots showing group differences in average Z-transformed correlation coefficients and standard errors for the right parahippocampal gyrus and left amygdala clusters. AMYG, amygdala; HC, healthy control; L, left; MDD, major depressive disorder; PHG, parahippocampal gyrus; R, right; SCSR, subgenual cingulate cortex.
<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Regions</th>
<th>Peak MNI Coordinates</th>
<th>Peak z Score</th>
<th>Cluster Size</th>
<th>FWE-Corrected p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>Parahippocampal gyrus</td>
<td>24 -27 -15</td>
<td>4.22</td>
<td>52</td>
<td>0.045(^1)</td>
</tr>
<tr>
<td>L</td>
<td>Amygdala</td>
<td>-24 -6 -15</td>
<td>3.54</td>
<td>10</td>
<td>0.006(^2)</td>
</tr>
</tbody>
</table>

Table 2.4 Resting-state functional disconnection in the remitted melancholic MDD patients vs the non-melancholic patients and HC group. FWE, familywise error; HC, healthy control; L, left; MDD, major depressive disorder; MNI, Montreal Neurological Institute; R, right; ROI, region of interest.

\(^1\) FWE-corrected at the cluster-level over the whole brain (\(p<0.05\)).

\(^2\) FWE-corrected at the cluster-level over an \textit{a priori} amygdala ROI (\(p<0.007\) corresponding to approximate Bonferroni corrected \(p<0.05/\text{number of ROIs to correct for the number of ROIs used in the study}\)).
out effects of potentially confounding variables unrelated to subtype by showing that SCSR connectivity was neither associated with years of education (left amygdala: \( r_s = -0.005, p = 0.97 \); right parahippocampal gyrus: \( r_s = 0.10, p = 0.43 \)) nor current BDI scores (left amygdala: \( r_s = -0.08, p = 0.55 \); right parahippocampal gyrus: \( r_s = -0.06, p = 0.67 \)). Of note, there were no group differences in mean framewise displacement, a measure of relative head displacement between contiguous volumes (Power et al., 2012)(Table 2.2).

In further analyses, we investigated whether categorical differences between rMDD subtypes in SCSR connectivity could be dissociated from differences in past MDE severity and duration that are closely associated with the definition of melancholic versus non-melancholic MDD. As expected, patients with longer past episode duration showed lower levels of SCSR connectivity with both regions (left amygdala: \( r_s = -0.30, p = 0.02 \); right parahippocampal gyrus: \( r_s = -0.32, p = 0.01 \)) and there was a trend towards patients with more severe past episodes to show lower SCSR connectivity with the left amygdala (\( r_s = -0.23, p = 0.07 \)). Additional analyses, however, showed that categorical differences in SCSR connectivity between subtypes remained when adjusting for the effects of the duration (group difference adjusted for duration: left amygdala: \( t(60) = 2.93, p = 0.005 \); right parahippocampal gyrus: \( t(60) = 2.24, p = 0.03 \)) and severity (group difference adjusted for severity, left amygdala: \( t(60) = 3.16, p = 0.002 \)) of past episodes.

2.5. DISCUSSION

As predicted, patients vulnerable to melancholic MDEs demonstrated distinctive patterns of resting-state functional disconnection with the SCSR when compared with non-melancholic rMDD patients. This extends previous work showing that SCSR metabolism is distinctively altered in current melancholic MDD (Pizzagalli et al., 2004). Intriguingly, contrary to our more specific predictions, we found SCSR disconnection with the amygdala and parahippocampal gyrus rather than medial frontal cortices to be distinctive of melancholic rMDD. These group differences in functional connectivity were not due to potentially confounding effects of education, residual symptoms, or the duration and severity of past depressive episodes. Of note, the melancholic and non-melancholic rMDD patients did not differ with respect to previous treatment for MDD, further strengthening the interpretation of group differences in SCSR connectivity as correlates of subtype-specific vulnerability.
Our finding of SCSR functional disconnection from the amygdala in melancholic rMDD is in agreement with reports of abnormal connectivity and activation of these structures in current MDD and MDD vulnerability. Lower functional connectivity between the SCSR and amygdala was associated with vulnerability to experiencing depression in a healthy female cohort (Herringa et al., 2013). Kruschwitz and colleagues (2014) recently demonstrated aberrant functional connectivity of the amygdala in individuals homozygous for the short allele of the 5-HTTLPR/rs25531 polymorphism, and the 5-HTTLPR polymorphism is known to confer depression risk. Interestingly, a support vector classification model incorporating resting-state connectivity of regions including the SCSR and amygdala has shown promise for distinguishing patients with current MDD from a HC group (Craddock et al., 2009). Relatedly, SCSR and amygdala reactivity to emotional stimuli was predictive of response to cognitive behavioural therapy in a cohort of MDD patients (Siegle, Carter and Thase, 2006). Patients with current MDD also exhibited microstructural white matter abnormalities between the SCSR and amygdala (Cullen et al., 2010). Resting-state blood flow to the amygdala was associated with a negative emotional bias in rMDD patients following acute tryptophan depletion (Roiser et al., 2009). Amygdala activation in response to emotional faces has been widely reported as abnormal in current MDD (reviewed in (Elliott et al., 2011)) and its activation was associated with shame experiences in remitted MDD (Pulcu et al., 2014a).

Our finding of SCSR disconnection from the parahippocampal gyrus is in keeping with the known importance of medial temporal lobe structures in MDD. Situated in close proximity to both the amygdala and hippocampus, the parahippocampal gyrus is anatomically connected both to medial temporal structures (Amaral and Price, 1984) and the SCSR (via the rostral cingulate (Vogt and Pandya, 1987)). In agreement with our findings, lower resting-state SCSR-hippocampal connectivity was described in a study of adolescents vulnerable to MDD (Herringa et al., 2013). Aberrant functional connectivity of the parahippocampal gyrus was also reported in rMDD patients during a sad mood induction paradigm (Zamoscik et al., 2014). Studies employing local measures of connectivity such as regional homogeneity consistently describe reductions in these measures in the parahippocampal gyrus in current MDD (reviewed in (Dutta, McKie and Deakin, 2014)). In healthy volunteers, the parahippocampal gyrus has been shown to play a role in representing visual imagery (Downing et al., 2006) and in the retrieval of episodic, including autobiographical, memories (Gardini et al., 2006).
The network of lower subgenual-amygdala-parahippocampal connectivity emerging from this study corresponds well to the limbic compartment of Mayberg’s (2003) limbic-cortical model of MDD. Mayberg suggests dysfunction within a limbic-cortical network is crucial in understanding the heterogeneity of MDD. In demonstrating disconnection within the limbic compartment associated with vulnerability to a specific MDD subtype, our findings support and extend this view. Notably, the limbic compartment in Mayberg’s (2003) model is associated with somatic and vegetative symptoms, which are commonly present in melancholic MDD. Alternatively, the subgenual-amygdala-parahippocampal disconnection we observed in the melancholic rMDD patients may be associated with guilt proneness. Although empathic guilt was not elevated in the melancholic compared to the non-melancholic rMDD patients, individual differences in empathic guilt correlated positively with connectivity to the SCSR seed region in the melancholic patients alone (see Supplementary Results). These results are consistent with previous imaging studies that implicated both the SCSR and amygdala in the experience of self-blaming emotions in rMDD patients (Green et al., 2012; Pulcu et al., 2014a; Lythe et al., 2015). Interestingly, guilt was positively correlated with connectivity to the SCSR, such that lower connectivity was associated with less empathic guilt. The scale we used to measure empathic guilt may capture, not only depressiogenic components of guilt, but also resilience enhancing components that are associated with the subgenual-amygdala-parahippocampal disconnection we observed in the melancholic patients. Future studies should seek to clarify the relationship of self-blaming moral emotions to connectivity within this network in melancholic MDD patients.

Our finding that melancholic rMDD patients demonstrate lower SCSR connectivity runs counter to rsfMRI studies in current MDD reporting normalization with treatment of initially elevated SCSR connectivity (Greicius et al., 2007; Sheline et al., 2010; Dutta, McKie and Deakin, 2014; Liston et al., 2014). This discrepancy may reflect methodological differences across studies. For example, our seed region is located medially to the subgenual areas captured in rsfMRI studies in current MDD (Greicius et al., 2007; Sheline et al., 2010). This is an unlikely source of discrepancy, however, given that elevated resting-state activation of subgenual cingulate/septal areas is reported across studies in current MDD using a variety of imaging modalities and analysis techniques (reviewed in (Drevets, Savitz and Trimble, 2008)). A more probable explanation for the lower SCSR connectivity in the melancholic rMDD patients is that studies reporting normalization of SCSR connectivity with treatment have been
conducted in recently recovered patients instead of patients in full remission for several months as studied here (Liston et al., 2014). Indeed, the lower connectivity we report in the melancholic rMDD patients is in keeping with a previous study in a cohort also vulnerable to depression which reported resting-state disconnection of the SCSR (Herringa et al., 2013). Our results are also in keeping with a recent study which demonstrated lower effective connectivity at rest in current melancholic MDD patients (Hyett et al., 2015). Furthermore, these results are in agreement with previous work showing guilt-selective decreases in functional connectivity in a rMDD group which included a large proportion of melancholic patients (Green et al., 2012). Given that MDD represents a lifetime diagnosis, this suggests abnormal SCSR connectivity is a trait marker for melancholic MDD where the direction of connectivity is state-dependent.

The following limitations of our study need to be discussed: It could be argued that use of a seed-based approach to analyze the rsfMRI data represents a limitation of the current study given the a priori assumptions required for seed selection (e.g., see (Dutta, McKie and Deakin, 2014)). However, SCSR dysfunction in MDD is well-established and using a SCSR seed provides a direct link to the neuroimaging literature in MDD, including the study in rMDD from which the seed region was selected (Green et al., 2012). Another potential limitation of the current study is that we cannot firmly conclude whether lower connectivity is associated with primary vulnerability which precedes the initial onset of MDD or secondary vulnerability where “scarring” increases vulnerability to subsequent MDEs (Burcusa and Iacono, 2007). The lack of correlation between connectivity and number of previous MDEs, however, renders an association with primary vulnerability more likely.

Taken together, the present study successfully used a SCSR seed-based rsfMRI approach to identify patterns of lower connectivity distinctive of melancholic rMDD. Melancholic rMDD patients demonstrated lower connectivity of the SCSR with the amygdala and parahippocampal gyrus when compared with the non-melancholic rMDD and HC groups. These results provide the first resting-state neural signature distinctive for melancholic rMDD and may reflect a subtype-specific primary vulnerability factor for MDD. Longitudinal investigations of patients with a positive family history prior to their first MDE could be used to further validate these results as a biomarker of primary vulnerability to melancholic MDD.
2.6. Supplementary Information

2.6.1. Supplementary Results

Empathic guilt, measured with the survivor guilt and omnipotent responsibility guilt scales of the Interpersonal Guilt Questionnaire (O'Connor et al., 1997), was not elevated in the melancholic remitted major depressive disorder (rMDD) patients compared with the non-melancholic rMDD patients (Table 1). Here, we sought to investigate whether connectivity to the subgenual cingulate/septal region (SCSR) seed region was associated with individual differences in empathic guilt, and whether such a relationship was capable of distinguishing the melancholic and non-melancholic rMDD patients despite a lack of group differences in empathic guilt. Across all remitted major depressive disorder (rMDD) patients, survivor guilt was positively associated with connectivity between the seed region and the left amygdala ($r_s=0.28, p=0.03$). Survivor guilt was not, however, associated with connectivity between the seed region and the right parahippocampal gyrus ($r_s=0.20, p=0.13$), nor was connectivity to the seed region associated with omnipotent responsibility guilt (left amygdala: $r_s=0.20, p=0.13$; right parahippocampal gyrus: $r_s=0.11, p=0.38$). In the melancholic rMDD patients, survivor guilt was positively associated with connectivity to the seed region (left amygdala: $r_s=0.38, p=0.03$; right parahippocampal gyrus: $r_s=0.42, p=0.02$), whereas omnipotent responsibility guilt was not (left amygdala: $r_s=0.14, p=0.44$; right parahippocampal gyrus: $r_s=0.16, p=0.40$). Connectivity to the seed region in the non-melancholic rMDD patients, however, was neither associated with survivor guilt (left amygdala: $r_s=-0.03, p=0.88$; right parahippocampal gyrus: $r_s=-0.08, p=0.67$) nor with omnipotent responsibility guilt (left amygdala: $r_s=0.13, p=0.50$; right parahippocampal gyrus: $r_s=0.01, p=0.96$). Taken together, the positive relationship between individual differences in survivor guilt and resting-state connectivity between the SCSR and left amygdala we observed appears specific to the melancholic subtype of MDD.
Table S2.1 Inter-rater reliability for SCID-I subtype diagnoses and for MADRS scores.

Reliability for the SCID-I mood disorders module subtype diagnosis is given as a kappa value. Reliability for the MADRS is given as an intra-class correlation (ICC) value (two-way mixed with absolute agreement). RZ, KL, and JG completed the recommended training for the SCID-I for DSM-IV-TR. The SCID-I was modified to allow lifetime diagnoses of MDD subtypes, including melancholic and atypical specifiers. The MADRS was used to assess depression severity at the time of the clinical interview, and was modified to allow for retrospective assessment of the last and most severe MDE. The Kappa values for the SCID-I subtype diagnoses reflect moderate to perfect agreement (Landis and Koch, 1977), and ICC values for the MADRS (both current and previous MDE) reflect moderate to excellent agreement (Fleiss, 1986). ICC, intra-class correlation; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder; MDE, major depressive episode; SCID-I, Structured Clinical Interview-I.
Chapter 3.

A novel resting-state functional MRI signature of resilience to recurrent depression

Authorship Contributions

Clifford I. Workman conducted the data analysis and interpretation, drafted the article, and approved the final version. Karen E. Lythe, Jorge Moll, Jennifer A. Gethin, and John F. W. Deakin aided in the study’s conception and design and provided critical revisions and final approval for the article. Shane McKie aided in the design of the statistical analyses and provided critical revisions and final approval for the article. Rebecca Elliott aided in the data interpretation and provided critical revisions and final approval for the article. Roland Zahn conceived of and designed the study, aided in data analysis and interpretation, and provided critical revisions and final approval for the article.

Submission

This manuscript was prepared for submission to Psychological Medicine (http://journals.cambridge.org/action/displayJournal?jid=PSM). The reference style, however, was chosen for consistency with the other chapters presented in this thesis. The journal’s preferred referencing style will be adopted prior to submission.
A novel resting-state functional MRI signature of resilience to recurrent depression

Clifford I. Workman, B.S.¹,², Karen E. Lythe, Ph.D.², Shane McKie, Ph.D.¹, Jorge Moll, M.D., Ph.D.³, Jennifer A. Gethin, M.Res.², John F. W. Deakin, F.R.C.Psych., Ph.D.¹, Rebecca Elliott, Ph.D.¹, Roland Zahn, M.D.⁴,²

¹University of Manchester, Institute of Brain, Behaviour and Mental Health, Neuroscience & Psychiatry Unit, UK
²University of Manchester, School of Psychological Sciences, Neuroscience and Aphasia Research Unit, UK
³Cognitive and Behavioral Neuroscience Unit, D’Or Institute for Research and Education, Brazil
⁴Institute of Psychiatry, Psychology, and Neuroscience, Department of Psychological Medicine, Centre for Affective Disorders, King’s College London, UK

3.1. ABSTRACT

BACKGROUND: A high proportion of patients with remitted major depressive disorder (MDD) will experience recurring episodes, whilst some develop resilience and remain in recovery. The neural mechanisms of resilience to recurrence are elusive. Abnormal resting-state connectivity of the subgenual cingulate/septal region (SCSR) was previously found in cross-sectional studies of MDD suggesting its potential pathophysiological importance. The current study aimed to investigate whether resting-state connectivity to a left SCSR seed region distinguishes resilient patients from those developing recurring episodes. METHODS: Forty-seven medication-free remitted MDD patients and 38 healthy controls underwent resting-state fMRI at baseline. Over 14 months, 30 patients remained resilient whilst 17 experienced a recurring episode. RESULTS: Attenuated interhemispheric left-to-right SCSR connectivity distinguished the resilient from the recurring episode and control groups and was not correlated with residual depressive symptoms. CONCLUSIONS: The current study revealed a neural signature of resilience to recurrence in MDD and reflects a novel compensatory mechanism.
3.2. INTRODUCTION

Major depressive disorder (MDD) is recurrent in a large proportion of patients, whilst some patients develop resilience after recovering from a major depressive episode (MDE; (American Psychiatric Association, 2000)). The neural mechanisms mediating resilience to recurrent MDEs are poorly understood. There is therefore an urgent need to characterize the neural bases of resilience and, relatedly, vulnerability to recurrence to improve stratification of patients and to identify novel targets for therapeutic interventions. Resting-state fMRI, frequently used to measure low frequency fluctuations in blood-oxygen-level dependent (BOLD) signal (Fox and Raichle, 2007), is particularly promising for understanding the neural mechanisms of resilience from the perspective of network models of MDD (Seminowicz et al., 2004; Price and Drevets, 2010).

Abnormal functional connectivity within subgenual cingulate cortex networks has been demonstrated repeatedly in cross-sectional studies of MDD (Greicius et al., 2007; Sheline et al., 2010; Gaffrey et al., 2012; Herringa et al., 2013; Dutta, McKie and Deakin, 2014) and this region is thought to play a central role in its pathophysiology (Dunlop and Mayberg, 2014). In a cross-sectional activation fMRI study, our group reported lower functional connectivity between an anterior temporal lobe (ATL) seed region and a subgenual cingulate/septal region (SCSR) during the experience of guilt (self-blame) relative to indignation (other-blame) in remitted MDD (rMDD) patients compared to a HC group (Green et al., 2012). In a subsequent prospective activation fMRI study by our group, functional connectivity between these regions was higher during self-blame in rMDD patients who subsequently developed a recurring episode (Lythe et al., 2015) compared with those who remained stable and with a HC group. Taken together, this led to the hypothesis that the lower self-blame-selective ATL connectivity in rMDD patients seen in the first study (Green et al., 2012) reflected a signature of resilience rather than vulnerability as was initially thought (Lythe et al., 2015). This was based on the observation that the cross-sectional study included a large proportion of MDD patients in full recovery for more than one year as well as a large proportion of first episode patients (Green et al., 2012). When investigating ATL-SCSR functional connectivity irrespective of psychological condition (i.e., self-blame vs. other-blame), however, there was no evidence of abnormalities in either the resilient or the recurring episode MDD groups (Lythe et al., 2015). These activation fMRI data precluded a more systematic investigation of SCSR network connectivity that included
regions other than the ATL because activation fMRI-based connectivity requires the selection of seed regions that show equal levels of average activation during the psychological conditions of interest in order to avoid confounding co-activation and connectivity (Friston et al., 1997). Since the SCSR region displays higher activation in guilt-prone individuals during self-blame relative to other-blame (Zahn et al., 2009a; Zahn et al., 2009c; Green et al., 2012), it could not be used as a seed region in our previous activation fMRI-based connectivity studies. In contrast, resting state fMRI-based connectivity does not suffer from this limitation and is therefore well-suited to mapping subgenual cingulate networks underpinning resilience more systematically. Furthermore, the acquisition of resting-state fMRI has some important advantages for clinical neuroimaging investigations since scans can be acquired relatively quickly (less than 10 minutes) and without needing to implement and interpret complex psychological paradigms.

Higher resting-state functional connectivity between the subgenual and posterior cingulate cortices distinguished vulnerable adolescents remitted from preschool onset MDD from a HC group (Gaffrey et al., 2012). Treatment studies using resting-state fMRI in MDD have revealed a relationship between treatment response and pre-treatment connectivity to the subgenual cingulate cortex (reviewed in (Dichter, Gibbs and Smoski, 2014)). Whether patterns of subgenual cingulate resting-state functional connectivity, however, are distinctly altered in rMDD patients who will remain resilient compared with those who will go on to experience a recurrent MDE remains unknown.

We aimed to address this question by investigating whether resting-state functional connectivity to the SCSR could distinguish rMDD patients who would remain resilient over a 14 month follow up period from patients who would go on to experience a recurrent MDE and from a HC group. This was accomplished using a seed-based approach to analyze resting-state fMRI data acquired at the outset of study participation. The left anterior SCSR seed region was placed using coordinates described by Green and colleagues (2012) and was chosen for its close proximity to subgenual regions implicated in vulnerability to MDD (Green et al., 2012; Herringa et al., 2013; Workman et al., in press). We predicted that abnormal connectivity of the SCSR with a fronto-subcortical network would distinguish resilient from recurring episode MDD patients. More specifically, we predicted that lower connectivity of the SCSR would be observed in the resilient MDD patients compared to both the recurring episode MDD and HC groups. In other words, we predicted that the direction of connectivity in the resilient MDD patients would be the opposite to that reported in
currently depressed patients, previously found to demonstrate hyperconnectivity of the subgenual cingulate cortex (reviewed in (Dutta, McKie and Deakin, 2014)).

3.3. METHOD

3.3.1. Participants

This study received approval from the South Manchester National Health Service Research Ethics Committee (Ref No: 07/H1003/194) and all participants gave informed after the study procedures were explained in full (verbal consent for the telephone-based screening and 3 month follow-up interviews and written consent at the start of each study visit). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Participants were recruited with online and print advertisements and received compensation for their time and travel expenses as part of the UK Medical Research Council-funded “Development of Cognitive and Imaging Biomarkers Predicting Risk of Self-Blaming Bias and Recurrence in Major Depression” project (Lythe et al., 2015; Zahn et al., 2015a). A preliminary assessment of eligibility was conducted via telephone for 707 volunteers (a copy of the screening form is available at http://www.translational-cognitive-neuroscience.org/start/test-materials). The 276 eligible volunteers following the telephone screening were invited to complete a clinical interview overseen by a senior psychiatrist (RZ). The 202 participants who agreed to the interview provided clinical and family histories, a urine sample for toxicology screening, and were assessed with the Structured Clinical Interview-I for DSM-IV-TR (SCID-I) to diagnose past MDEs and to detect current Axis 1 disorders (moderate to perfect inter-rater reliability; Table S3.1; (American Psychiatric Association, 2000; First et al., 2002)). Of these, 48 HC participants and 96 rMDD patients were eligible to take part in the present study following the clinical interview. Thirty-nine HC participants subsequently underwent MRI scanning, though imaging data were excluded for one HC participant due to a pituitary abnormality, resulting in a final HC sample of N=38. Sixty-three rMDD patients underwent MRI scanning, though imaging data were excluded for 6 patients who did not complete the longitudinal study visits described below, resulting in a final patient sample of N=57.

A detailed overview of the reasons for which participants were excluded is provided in Table S2.1. Inclusion criteria were: aged 18 or older, right handed, English spoken as the native language, and normal or corrected-to-normal vision and hearing.
Additional inclusion criteria for the rMDD group were: past MDE and MDD diagnosed by a senior psychiatrist (RZ) according to DSM-IV-TR criteria (American Psychiatric Association, 2000), International Classification of Diseases 10th Revision-diagnosed past moderate or severe MDE (World Health Organization, 1992), and remission of symptoms at least 6 months prior to enrolment. Exclusion criteria were: current or relevant past Axis I disorders (e.g., history of substance abuse), psychotropic medication use within 4 weeks of enrolment (8 weeks for fluoxetine), acute suicidality/self-harming behaviours, impaired psychosocial functioning measured with the Global Assessment of Functioning scale (American Psychiatric Association, 2000), a Montgomery-Åsberg Depression Rating Scale (MADRS) score >10 (Montgomery and Åsberg, 1979; Zimmerman, Posternak and Chelminski, 2004), history of neurological or medical disorders affecting brain functioning, developmental disorders or learning disabilities, an Addenbrooke’s Cognitive Exam score >88 (conducted in participants aged over 50; (Mioshi et al., 2006)), and contraindications for MRI scanning. Additional exclusion criteria for the HC group were: history of Axis-I disorders, first-degree family history of mood disorders or schizophrenia.

The rMDD patients completed follow up interviews via telephone or in person at 3, 6, and 14 months after enrolment using the MDD module and psychosocial functioning assessment from the Longitudinal Interval Follow-up Evaluation interview for DSM-IV (LIFE-IV; (Keller et al., 1987)). The LIFE interview includes a 6-point Psychiatric Status Rating (PSR): 1) no residual symptoms, 2) one or more mild symptoms causing no relevant distress or impairment, 3) mild symptoms causing no more than moderate distress or impairment, 4) major symptoms not meeting full criteria for an MDE, and 5-6) major symptoms meeting criteria for an MDE. The raters were trained by the creators of the LIFE interview and inter-rater reliability was excellent (Table S3.1). Of the 57 rMDD patients who completed the study, 30 remained in stable remission (resilient MDD group), 17 experienced a recurrent MDE (recurring episode MDD group), and 10 developed symptoms not meeting full criteria for an MDE (i.e., a PSR of 3 requiring treatment or a PSR of 4; subthreshold symptom group). The analyses presented below include the resilient and recurring episode MDD groups, but exclude the subthreshold symptom group.

The resilient MDD, recurring episode MDD, and HC groups were well-matched on demographic variables (Tables 3.1-3.2). The resilient and recurring episode MDD groups did not differ from the HC group on age, sex, or years of education. Compared to the HC group, however, scores on the Beck Depression Inventory (BDI; (Beck, Steer...
and Brown, 1996)) were higher in both the resilient ($t(66)=2.96, p=0.004$) and recurring episode MDD groups ($t(53)=4.72, p<0.0001$). BDI scores were also higher for the recurring episode MDD group compared to the resilient MDD group ($t(45)=2.22$, $p=0.03$).

<table>
<thead>
<tr>
<th></th>
<th>Recurring Episode MDD (N=17)</th>
<th>Resilient MDD (N=30)</th>
<th>HC (N=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35.9 (12.4)</td>
<td>37.6 (12.7)</td>
<td>36.2 (13.8)</td>
</tr>
<tr>
<td>Years of Education</td>
<td>16.4 (2.6)</td>
<td>17.4 (2.0)</td>
<td>16.8 (2.3)</td>
</tr>
<tr>
<td>BDI Score$^a$</td>
<td>5.2 (5.0)</td>
<td>2.6 (2.9)</td>
<td>0.9 (1.7)</td>
</tr>
<tr>
<td>MADRS Score</td>
<td>0.9 (1.7)</td>
<td>0.8 (1.4)</td>
<td>0.7 (1.3)</td>
</tr>
<tr>
<td>Omnipotent Responsibility Guilt$^b$</td>
<td>48.1 (6.5)</td>
<td>46.2 (6.4)</td>
<td>41.9 (7.8)</td>
</tr>
<tr>
<td>Survivor Guilt</td>
<td>74.7 (8.7)</td>
<td>68.8 (9.8)</td>
<td>59.9 (10.3)</td>
</tr>
<tr>
<td>Sex (Male / Female)</td>
<td>6 / 11</td>
<td>12 / 18</td>
<td>13 / 25</td>
</tr>
<tr>
<td>Framewise Displacement (mm)</td>
<td>0.26 (0.14)</td>
<td>0.24 (0.15)</td>
<td>0.24 (0.15)</td>
</tr>
</tbody>
</table>

Table 3.1 Demographic variables in the recurring episode and resilient MDD patients and HC group. With the exception of BDI and guilt scores, the recurring episode MDD patients and HC group did not significantly differ on the demographic variables (Contingency Coefficient<$0.02, p>0.93; t<0.62, p>0.53$). Also with the exception of BDI and guilt scores, the resilient MDD patients and HC group did not significantly differ on the demographic variables (Contingency Coefficient<$0.06, p>0.62; t<1.05, p>0.30$). Again, with the exception of BDI scores, the recurring episode and resilient MDD patients did not significantly differ on the demographic variables (Contingency Coefficient<$0.05, p>0.74; t<1.41, p>0.16$). BDI, Beck Depression Inventory; HC, healthy control; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder.

$^a$Significantly different between the recurring episode MDD and HC groups ($t(53)=4.72, p<0.0001$), between the resilient MDD and HC groups ($t(66)=2.96, p=0.004$), and between the recurring episode and resilient MDD groups ($t(45)=2.22, p=0.03$).

$^b$Significantly different between the recurring episode MDD and HC groups ($t(52)=2.80, p=0.007$) and between the resilient MDD and HC groups ($t(65)=2.43, p=0.02$).

$^c$Significantly different between the recurring episode MDD and HC groups ($t(52)=5.01, p<0.0001$), between the resilient MDD and HC groups ($t(65)=3.56, p=0.001$), and trending between the recurring episode and resilient MDD groups ($t(43)=2.00, p=0.052$).
Nevertheless, average BDI scores for all groups were below 10 suggesting the presence of only minimal subthreshold depressive symptoms (Beck, Steer and Carbin, 1988). Additionally, no group differences were observed for current scores on the MADRS. Compared to the HC group, empathic guilt measured with the Interpersonal Guilt Questionnaire (i.e., omnipotent responsibility guilt [ORG], or guilt related to feeling responsible for others’ wellbeing, and survivor guilt [SuG], or guilt related to others being worse off than oneself; (O'Connor et al., 1997)) was also higher in the recurring episode (ORG: $t(52)=2.80$, $p=0.007$; SuG: $t(52)=5.01$, $p<0.0001$) and resilient MDD groups (ORG: $t(65)=2.43$, $p=0.02$; SuG: $t(65)=3.56$, $p=0.001$). Furthermore, there was a trend towards higher survivor guilt in the recurring episode MDD group compared with the resilient MDD group ($t(43)=2.00$, $p=0.052$), although no difference in omnipotent responsibility guilt was observed ($t(43)=0.94$, $p=0.35$). The resilient MDD group also did not differ from the recurring episode MDD group on age, sex, education, past MDD subtype, average length of last MDE, months since remission, severity of the last MDE measured with the MADRS, months since last psychotropic use, number of patients previously treated, number of suicide attempts, or family history of MDD. The recurring episode and resilient MDD groups differed, however, with respect to the average number of previous MDEs ($t(45)=3.39$, $p=0.001$).

3.3.2. Image acquisition

MRI data were acquired on a 3T Philips Achieva scanner (Philips Medical Systems, Netherlands) with an 8-channel coil. A resting-state echo-planar image (EPI) was acquired for each participant using a sequence optimized for detecting ventral frontal signal (240 volumes; 40 axial slices; 3mm slice thickness; ascending sequential acquisition; repetition time: 2000ms; echo time: 22ms; field of view: 240x240x120mm; acquisition matrix: 80x80 voxels; reconstructed voxel size: 3mm3; flip angle: 90°). Participants were asked to lie motionless with eyes open during the scan to ensure wakefulness and were debriefing afterwards to confirm the instructions were followed. A 3-dimensional T1-weighted magnetization-prepared rapid-acquisition gradient-echo (MPRAGE) structural image was also acquired for each participant (160 axial slices; 0.9mm slice thickness; repetition time: 8.4ms; echo time: 3.9ms; field of view: 240x191x144mm; acquisition matrix: 256x163 voxels; reconstructed voxel size: 0.94x0.94x0.9mm; flip angle: 8°). In order to rule out clinically significant neurological abnormalities, T2-weighted structural images were also acquired.
<table>
<thead>
<tr>
<th>Past MDD subtype</th>
<th>Recurring Episode MDD (N=17) Mean (SD)</th>
<th>Resilient MDD (N=30) Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With melancholic features</td>
<td>9/17</td>
<td>17/30</td>
</tr>
<tr>
<td>With atypical features</td>
<td>0/17</td>
<td>2/30</td>
</tr>
<tr>
<td>No specific subtype</td>
<td>8/17</td>
<td>11/30</td>
</tr>
<tr>
<td><strong>Number of previous MDEs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1/17</td>
<td>13/30</td>
</tr>
<tr>
<td>2</td>
<td>5/17</td>
<td>5/30</td>
</tr>
<tr>
<td>3</td>
<td>2/17</td>
<td>9/30</td>
</tr>
<tr>
<td>4</td>
<td>4/17</td>
<td>1/30</td>
</tr>
<tr>
<td>5</td>
<td>3/17</td>
<td>2/30</td>
</tr>
<tr>
<td>6 or more</td>
<td>2/17</td>
<td>0/30</td>
</tr>
<tr>
<td>Average number of previous MDEs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 (2.0) (range: 1–9)</td>
<td>2.1 (1.2) (range: 1–5)</td>
</tr>
<tr>
<td><strong>Last and most severe MDE details</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average length of MDE (months)</td>
<td>17.7 (25.2) (range: 1–96)</td>
<td>15.3 (18.8) (range: 1–81)</td>
</tr>
<tr>
<td>Average time in remission (months)</td>
<td>21.5 (20.9) (range: 6–72)</td>
<td>37.9 (53.8) (range: 6–282)</td>
</tr>
<tr>
<td>Average MADRS score for MDE</td>
<td>34.6 (5.2) (range: 24–44)</td>
<td>35.1 (5.7) (range: 20–44)</td>
</tr>
<tr>
<td>No psychotropic medication since (months)</td>
<td>42.7 (54.4) (range: 2–173)</td>
<td>60.0 (86.5) (range: 3–372)</td>
</tr>
<tr>
<td><strong>Previous treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRI antidepressant</td>
<td>15/17</td>
<td>25/30</td>
</tr>
<tr>
<td>SNRI antidepressant</td>
<td>0/17</td>
<td>1/30</td>
</tr>
<tr>
<td>Tricyclic antidepressant</td>
<td>0/17</td>
<td>2/30</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>1/17</td>
<td>0/30</td>
</tr>
<tr>
<td>Unknown class of antidepressant</td>
<td>3/17</td>
<td>3/30</td>
</tr>
<tr>
<td>Benzodiazepines only</td>
<td>0/17</td>
<td>1/30</td>
</tr>
<tr>
<td>No antidepressant medication</td>
<td>1/17</td>
<td>2/30</td>
</tr>
<tr>
<td>CBT</td>
<td>6/17</td>
<td>6/30</td>
</tr>
<tr>
<td>Self-guided CBT via internet, books</td>
<td>0/17</td>
<td>3/30</td>
</tr>
<tr>
<td>Counselling</td>
<td>6/17</td>
<td>14/30</td>
</tr>
<tr>
<td><strong>Suicide attempts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.18 (0.53) (range: 0–2)</td>
<td>0.20 (0.61) (range: 0–3)</td>
</tr>
<tr>
<td><strong>Lifetime axis-I comorbidity</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panic disorder with agoraphobia</td>
<td>1/17</td>
<td>0/30</td>
</tr>
<tr>
<td>Bulimia nervosa</td>
<td>0/17</td>
<td>1/30</td>
</tr>
<tr>
<td>No life-time co-morbidity</td>
<td>16/17</td>
<td>29/30</td>
</tr>
<tr>
<td><strong>Family history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First degree relative with MDD</td>
<td>10/17</td>
<td>16/30</td>
</tr>
<tr>
<td>No family member with history of</td>
<td>6/17</td>
<td>11/30</td>
</tr>
</tbody>
</table>
Table 3.2 Clinical characteristics of the recurring episode and resilient MDD patients. All MDD patients stopped medication before the required washout phase. Means and standard deviations are reported and/or the number of cases. Recurring episode and resilient MDD patients did not significantly differ on past MDD subtype, average length of the last MDE, average time in remission, average MADRS score for the last MDE, average time since last taking psychotropic medications, previous treatments, number of suicide attempts, lifetime axis-I comorbidity, or family history (Contingency Coefficient<0.20, \( p > 0.18 \); \( t < 1.21, \ p > 0.23 \)). CBT, cognitive behavioural therapy; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder; MDE, major depressive episode; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin norepinephrine reuptake inhibitor. 

\(^{a}\)Significantly different between the recurring episode and resilient MDD groups (\( t(45)=3.39, \ p=0.001 \)).

\(^{b}\)All co-morbid disorders were fully remitted at the time of study and none were likely to be the primary cause of the depressive episodes.

3.3.3. Resting-state fMRI analysis

The pre-processing pipeline for the resting-state fMRI data has been described in detail elsewhere (Workman et al., in press). Briefly, pre-processing was performed using SPM8 [http://www.fil.ion.ucl.ac.uk/spm/] for compatibility with the DPARSF Advanced Edition (Chao-Gan and Yu-Feng, 2010) and Artifact Detection Tools (ART; [http://web.mit.edu/swg/software.htm]) MATLAB (MathWorks) toolboxes used in subsequent steps. For each EPI, the first 10 volumes were discarded, then slice timing and head motion correction were performed, and then regressors were created for high-motion volumes using ART (framewise signal intensity >3 standard deviations from the global mean, framewise head displacement >1mm). Next, the MPRAGE images were co-registered to the EPIs and segmented, then linear detrending and nuisance covariates regression were performed on the EPIs (24 motion parameters [(Friston et al., 1996)], white matter and cerebrospinal fluid signal, ART regressors), and then the EPIs were normalized with parameters derived during segmentation. After this, the EPIs were smoothed with a 6mm kernel and band-pass filtered to preserve frequencies between 0.01Hz and 0.08Hz. High motion volumes identified by ART were then removed, as
were sections of data spanning fewer than 5 contiguous volumes. All resulting EPIs contained at least 5 minutes of data (150 volumes).

For each EPI, the average time course within a left anterior SCSR seed region was correlated with the time course of all other brain voxels, resulting in seed-based functional connectivity maps for each participant. The left anterior SCSR was chosen as the seed region because it was previously implicated in connectivity studies of rMDD patients (Montreal Neurological Institute [MNI] coordinates: -4, 23, -5; 6mm sphere; (Green et al., 2012; Lythe et al., 2015; Workman et al., in press)), it is in close proximity to an anterior SCSR region which demonstrated abnormal resting-state functional connectivity in children vulnerable to MDD (MNI coordinates: 2, 23, -6; (Herringa et al., 2013)), and it is close to subgenual cingulate regions which demonstrate hyperconnectivity in current MDD patients (Dutta, McKie and Deakin, 2014). The resulting seed-based functional connectivity maps were then Fisher Z-transformed to improve normality.

Next, we conducted a voxelwise analysis of variance (ANOVA) to compare the seed-based functional connectivity maps from the resilient MDD, recurring episode MDD, and HC groups. Since we sought to identify a main effect of group, the analyses were carried out in SPM12 given that cluster-level familywise error (FWE) correction of F-tests cannot be performed in SPM8. We also used 7 bilateral a priori regions of interest (ROI) with known structural or functional connections to the anterior SCSR (Vogt and Pandya, 1987; Carmichael and Price, 1996; Kondo, Saleem and Price, 2003; Johansen-Berg et al., 2008) and which have been implicated in MDD (Elliott et al., 2011; Green et al., 2012) or social emotional and/or motivational processing (Moll et al., 2005; Zahn et al., 2009c; Elliott et al., 2011): ventromedial prefrontal cortex, anterior temporal cortex, amygdala, hippocampus, septal region, and hypothalamus. A detailed description of the creation of these ROIs has been provided elsewhere (Zahn et al., 2009c; Workman et al., in press).

Results were considered significant at an uncorrected voxel-level threshold of \( p<0.001 \) and a cluster-level familywise error (FWE)-corrected threshold of \( p<0.05 \) across the whole brain and a priori ROIs. Mean correlation coefficients were extracted from each surviving cluster and entered into a one-way ANOVA with post-hoc Bonferroni pairwise comparisons to identify significant group differences in connectivity to the left anterior SCSR, and results were considered significant at \( p<0.05 \) two-tailed.
3.4. RESULTS

3.4.1. Main Effect of Group for Functional Connectivity

Our analyses revealed a main effect of group (resilient MDD, recurring episode MDD, HC group) for connectivity between the left anterior SCSR seed region and the right anterior SCSR and left posterior SCSR (Table 3.3; Figure 3.1). Using the extracted cluster averages from both regions, we confirmed the main effect of group (right anterior SCSR: \( F(2,82) = 14.0, p < 0.0001 \); left posterior SCSR: \( F(2,82) = 8.7, p < 0.0004 \)). Subsequent post-hoc Bonferroni-corrected pairwise comparisons showed lower connectivity between the seed region and the right anterior SCSR in the resilient MDD group (M=0.31, SD=0.14) compared to both the HC group (M=0.48, SD=0.12, \( p < 0.001 \), mean difference=-0.17, 95% CI [-0.25,-0.09]) and the recurring episode MDD group (M=0.42, SD=0.15, \( p = 0.01 \), mean difference=-0.12, 95% CI [-0.22,-0.02]). Connectivity between the seed region and the right anterior SCSR did not differ between the recurring episode MDD group (M=0.42, SD=0.15) and the HC group.

![Figure 3.1](image)

**Figure 3.1** a) The network of regions demonstrating resting-state functional disconnection with the left anterior SCSR seed region in the resilient MDD patients. The solid arrow points to regions demonstrating functional disconnection in the resilient MDD patients compared to both the recurring episode MDD and HC groups. The dashed arrow points to regions demonstrating functional disconnection in the resilient MDD patients compared to the HC group only. Whole-brain images were cropped and displayed at an uncorrected voxel-level threshold of \( p < 0.001 \). b) Bar plots showing group differences in average Z-transformed correlation coefficients and standard errors for the right anterior SCSR cluster. HC, healthy control; L, left; MDD, major depressive disorder; R, right; SCSR, subgenual cingulate/septal region.
Table 3.3 Regions significant for a main effect of group (recurring episode MDD, resilient MDD, HC group) for functional connectivity to the left anterior subgenual cingulate/septal region seed region. FWE, familywise error; HC, healthy control; L, left; MDD, major depressive disorder; MNI, Montreal Neurological Institute; R, right; ROI, region of interest.

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Regions</th>
<th>Peak MNI Coordinates</th>
<th>Peak z Score</th>
<th>Cluster Size</th>
<th>FWE-Corrected p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>Anterior subgenual cingulate/septal region</td>
<td>9 21 -12</td>
<td>4.03</td>
<td>22</td>
<td>0.039&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>L</td>
<td>Posterior subgenual cingulate/septal region</td>
<td>-6 15 -3</td>
<td>3.20</td>
<td>4</td>
<td>0.043&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>FWE-corrected at the cluster-level over an <i>a priori</i> ventromedial prefrontal cortex ROI.

<sup>b</sup>Lower connectivity with the seed region in the resilient MDD patients (M=0.31, SD=0.14) compared to both the recurring episode MDD (M=0.42, SD=0.15, <i>p</i>=0.013, mean difference=-0.12, 95% CI [-0.22,-0.02]) and HC groups (M=0.48, SD=0.12, <i>p</i>&lt;0.0001, mean difference=-0.17, 95% CI -0.25,-0.09]).

<sup>c</sup>FWE-corrected at the cluster-level over an <i>a priori</i> septal region ROI.

<sup>d</sup>Lower connectivity with the seed region in the resilient MDD patients (M=0.61, SD=0.22) compared to the HC group (M=0.81, SD=0.18, <i>p</i>&lt;0.0003, mean difference=-0.20, 95% CI [-0.31,-0.08]) but not the recurring episode MDD group (M=0.71, SD=0.19, <i>p</i>=0.29, mean difference=-0.10, 95% CI [-0.24,0.04]).
(M=0.48, SD=0.12, \( p = 0.55 \), mean difference=-0.05, 95% CI [-0.15,0.04]). Furthermore, lower connectivity was observed between the seed region and the left posterior SCSR in the resilient MDD group (M=0.61, SD=0.22) compared to the HC group (M=0.81, SD=0.18, \( p < 0.003 \), mean difference=-0.20, 95% CI [-0.31,-0.08]) but not compared to the recurring episode MDD group (M=0.71, SD=0.19, \( p = 0.22 \), mean difference=-0.10, 95% CI [-0.24, 0.04]). Again, connectivity between the seed region and the left posterior SCSR did not differ between the recurring episode MDD group (M=0.71, SD=0.19) and the HC group (M=0.81, SD=0.18, \( p = 0.20 \), mean difference=-0.10, 95% CI [-0.23,0.04]). Therefore, resting-state functional disconnection between the left and right anterior SCSRs, but not between the left anterior and posterior SCSRs, is an abnormality which distinguished the resilient MDD patients from the recurring episode patients.

3.4.2. Investigation of Potential Confounding Variables

Next, we investigated whether connectivity between the left and right anterior SCSRs was associated with BDI scores or number of previous MDEs, both of which were elevated in the recurring episode MDD patients relative to the resilient patients. Across the rMDD patients, however, connectivity between the left and right anterior SCSRs was not associated with BDI scores (\( r_s = -0.11, p = 0.47 \)) or number of previous MDEs (\( r_s = 0.13, p = 0.39 \)). Furthermore, group differences in connectivity between the left and right anterior SCSRs remained significant for the resilient and recurring episode MDD patients after controlling for the effects of BDI scores (group difference adjusted for BDI scores: \( t(44)=3.44, p=0.001 \)) and number of previous MDEs (group difference adjusted for number of previous MDEs: \( t(44)=2.61, p=0.01 \)). Importantly, no group differences were observed in framewise displacement, a metric of relative head displacement between volumes (Power et al., 2012), suggesting the groups were well-matched for head motion (Table 3.1).

3.5. DISCUSSION

3.5.1. Main Findings and Interpretation

Consistent with our hypothesis, lower connectivity of the left anterior SCSR distinguished resilient from recurring episode MDD patients. Interestingly, the resilient MDD group showed abnormally low connectivity whilst the recurring episode MDD patients displayed no difference from the HC group. More specifically, we found lower interhemispheric SCSR connectivity to be distinctive of the resilient MDD patients.
This pattern of lower functional connectivity was not explained by residual depressive symptoms. Furthermore, the recurring episode MDD patients had more previous MDEs than the resilient patients, as would be predicted by scar theories of depression vulnerability (Burcusa and Iacono, 2007), but number of MDEs was not associated with interhemispheric SCSR connectivity. Our findings therefore confirm the significance of the subgenual cingulate cortex to the pathophysiology of MDD by demonstrating for the first time that attenuated interhemispheric SCSR connectivity is associated with resilience to recurrent MDEs.

Patients who are currently in the depressed state have repeatedly been shown to demonstrate increased connectivity to the subgenual cingulate cortex that normalizes with treatment (reviewed by (Dichter, Gibbs and Smoski, 2014; Dutta, McKie and Deakin, 2014)). Findings from studies which investigated resting-state connectivity to the subgenual cingulate in populations vulnerable to MDD are less consistent with respect to the direction of abnormal connectivity. For example, Gaffrey and colleagues (2012) described elevated resting-state connectivity between the subgenual and posterior cingulate cortices in patients with a history of preschool onset MDD. In contrast, Herringa and colleagues (2013) found that lower subgenual cingulate-hippocampal connectivity was associated with a history of childhood maltreatment, a known risk factor for MDD, in otherwise healthy adolescents. Our findings suggest abnormally low resting-state functional connectivity of the anterior SCSR may reflect a compensatory mechanism in those patients who remain resilient to MDEs, similar to functional compensation mechanisms found in patients with brain lesions (Zahn, Schwarz and Huber, 2006).

The lower interhemispheric SCSR connectivity we observed in the resilient MDD patients may appear to contradict studies which report normalization of resting-state subgenual cingulate functional connectivity and cerebral glucose metabolism with treatment (Dichter, Gibbs and Smoski, 2014; Dunlop and Mayberg, 2014). These studies typically look at treatment-related changes in recently remitted patients, however, in contrast to the patients studied here who were in stable remission (≥6 months) at the time of scanning. The risk for experiencing a recurrent MDE is elevated during the first 6 months following remission from the depressed state (Solomon et al., 2000). If indeed the abnormally low interhemispheric functional connectivity of the anterior SCSR in resilient MDD patients observed here reflects a compensatory mechanism, this may not emerge until later in the course of recovery. Normal functional connectivity to the anterior SCSR in the recurring episode MDD patients may reflect a
failure to engage, or to continue engaging, this mechanism. Alternatively, connectivity to the SCSR may be linearly associated with depression status, with connectivity to the SCSR ranging from abnormally high in currently depressed patients to abnormally low in patients who remain resilient to recurrent MDEs. Our findings also appear inconsistent with our previous interpretation of subgenual cingulate-amygdala resting-state functional disconnection as a primary vulnerability factor for melancholic MDD (Workman et al., in press). However, the pattern of lower subgenual cingulate-amygdala connectivity we observed in the melancholic MDD patients was independent of vulnerability or resilience to recurring MDEs (see Supplemental Results). We tentatively interpret this as supportive of our original interpretation of lower subgenual cingulate-amygdala connectivity as a signature of primary vulnerability to melancholia (Workman et al., in press), although this merits further investigation.

To our knowledge, this is the first report of abnormalities in interhemispheric SCSR connectivity in MDD. Clues pertaining to the significance of this finding can be found in reports of psychosurgical interventions for MDD and in lesion studies. The subcaudate tractotomy (and the related limbic leucotomy), in which white matter is lesioned at a site below the caudate and posterior to the orbitofrontal cortex, was historically used to treat chronic MDD with moderate success (Schoene-Bake et al., 2010). A tractography study conducted in healthy volunteers with a seed placed in the subcaudate tractotomy lesion site revealed fiber tracts spanning the left and right subgenual cingulate cortices (Schoene-Bake et al., 2010), suggesting disruption of these tracts may be related to clinical improvement in current MDD patients. Relatedly, chronic bilateral DBS applied to the white matter of the subgenual cingulate cortices in a treatment-resistant MDD group resulted in sustained remission in some patients (Mayberg et al., 2005). Although the exact mechanism by which DBS works has yet to be elucidated, the leading explanation is that inhibition occurs at the sites of stimulation (Mayberg et al., 2005). Patients with damage to the ventromedial prefrontal cortex, a large swathe of cortex along the medial wall of the frontal lobe which typically encompasses the subgenual cingulate, reported lower depression severity relative to a sample of control participants with damage to other brain regions (Koenigs and Grafman, 2009). Furthermore, damage to the ventromedial prefrontal cortex has been associated with emotional deficits including diminished guilt (Koenigs and Grafman, 2009), which may be excessive or overgeneralized in current MDD patients (American Psychiatric Association, 2000). Taken together, damage to subgenual cingulate white matter pathways and to the ventromedial prefrontal cortices has previously been shown
to modulate depressed mood as well as guilt, a distinctive symptom of MDD. The lower interhemispheric anterior SCSR connectivity we have reported in the resilient MDD group relative to the recurring episode MDD and HC groups is in keeping with these findings.

3.5.2. Limitations

Several limitations of the current study need to be discussed. First, the decision to use a seed-based approach to analyze our resting-state fMRI data entailed the selection of an *a priori* ROI which consequently constrained our results. This concern is mitigated, however, by the known importance of the subgenual cingulate cortex and adjacent septal region to MDD as has been detailed throughout. Second, the pattern of connectivity we observed in the resilient MDD patients represents a step towards the identification of a biomarker for resilience to recurrent MDEs, but establishing the marker’s validity will require replication of these findings using predictive measures such as a receiver operating characteristic curve analysis.

3.5.3. Conclusions

We demonstrated a distinctive pattern of attenuated interhemispheric resting-state SCSR connectivity in MDD patients resilient to recurrence. To our knowledge, this is the first resting-state fMRI signature of resilience to recurrence in patients who are remitted from the depressed state. The pattern of connectivity observed in the resilient MDD patients represents a potential target for therapeutic interventions aimed at improving resilience to future MDEs. Future longitudinal studies should aim to replicate these findings and to investigate whether this signature can predict who will develop MDEs in populations without a history of MDD that are nonetheless vulnerable.
3.6. Supplemental Information

3.6.1. Supplemental Results

In an earlier cross-sectional resting-state fMRI study which included the participants studied here, our group found subgenual cingulate-amygdala resting-state functional disconnection to be distinctive of remitted depressed patients with a history of melancholic major depressive episodes (MDE) compared to non-melancholic and healthy control groups (Workman et al., in press). We argued that subgenual cingulate-amygdala functional disconnection is a signature of primary vulnerability for melancholic major depressive disorder (MDD). In view of the present findings suggesting lower interhemispheric connectivity between the subgenual cingulate cortices/septal regions (SCSR) promotes resilience to recurring MDEs, we wanted to determine whether the network of lower connectivity we previously observed in the melancholic remitted MDD (rMDD) patients is better understood as promoting resilience. To this end, we first extracted the mean Fisher Z-transformed correlation coefficients from the amygdala cluster as described previously (Workman et al., in press) for each participant in the current study. These data were then entered into a two-way ANOVA in SPSS 20 with between-subjects factors for group (resilient or recurring episode MDD) and for subtype (melancholic or non-melancholic). Results were considered significant at p<0.05 two-tailed.

For subgenual cingulate-amygdala resting-state connectivity, we observed a main effect of subtype ($F(1,43)=7.1, p=0.01$) but no main effect of group ($F(1,43)=0.001, p=0.97$) and no subtype by group interaction ($F(1,43)=0.33, p=0.57$). Subsequent post-hoc pairwise comparisons revealed lower subgenual cingulate-amygdala connectivity in the melancholic rMDD group (M=0.12, SD=0.17) compared to the non-melancholic rMDD group (M=0.25, SD=0.11, $p=0.01$, mean difference=-0.12, 95% CI [-0.21,-0.03]). These results suggest the network of lower functional connectivity we previously reported in the melancholic rMDD patients is independent of vulnerability or resilience to recurring MDEs, which is in keeping with our original interpretation of subgenual cingulate-amygdala functional disconnection as a primary vulnerability factor for melancholia.
<table>
<thead>
<tr>
<th>Raters</th>
<th>Kappa Value</th>
<th>ICC Value</th>
<th>ICC Value</th>
<th>ICC Value</th>
<th>ICC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZ &amp; KL</td>
<td>0.60</td>
<td>0.63</td>
<td>0.45</td>
<td>0.96</td>
<td>0.98</td>
</tr>
<tr>
<td>RZ &amp; JG</td>
<td>–</td>
<td>0.91</td>
<td>0.80</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>KL &amp; JG</td>
<td>1.00</td>
<td>0.86</td>
<td>0.80</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>KL &amp; CW</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.96</td>
<td>0.99</td>
</tr>
<tr>
<td>Mean</td>
<td>0.80</td>
<td>0.80</td>
<td>0.68</td>
<td>0.96</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Table S3.1 Inter-rater reliability for the SCID-I, MADRS, and PSR scales. Reliability for the SCID-I mood disorders module subtype diagnosis is given as a kappa value. Reliability for the MADRS and PSR are given as intra-class correlation (ICC) values (two-way mixed with absolute agreement). RZ, KL, and JG completed the recommended training for the SCID-I for DSM-IV-TR, and RZ, KL, and CW completed the recommended training for the PSR. The SCID-I was modified to allow lifetime diagnoses of MDD subtypes, including melancholic and atypical specifiers. The MADRS was used to assess depression severity at the time of the clinical interview, and was modified to allow for retrospective assessment of the last and most severe MDE. The PSR was used to assess the severity of and impairment caused by depressive symptoms present at each follow up interview and retrospectively throughout the follow up period. The Kappa values for the SCID-I subtype diagnoses reflect moderate to perfect agreement (Landis and Koch, 1977), and ICC values for the MADRS (both current and previous MDE) and PSR reflect moderate to excellent agreement (Fleiss, 1986). ICC, intra-class correlation; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder; MDE, major depressive episode; PSR, Psychiatric Status Rating; SCID-I, Structured Clinical Interview-I.
Chapter 4.

Unhappy to help? Guilt and altruism in remitted major depression

Authorship Contributions
Clifford I. Workman conceived of and designed the study, conducted the data analysis and interpretation, drafted the article, and approved the final version. Rebecca Elliott aided in the conception and design of the study, data interpretation, and provided critical revisions and final approval for the article. Jorge Moll aided in interpretation and provided critical revisions and final approval for the article. Karen E. Lythe, Jennifer A. Gethin, Paula D. Trotter, and Emma J. Thomas aided in data collection and provided critical revisions and final approval for the article. Roland Zahn aided in the conception and design of the study, data analysis and interpretation, and provided critical revisions and final approval for the article.

Submission
This manuscript was prepared for submission to The Journal of Abnormal Psychology (http://www.apa.org/pubs/journals/abn/). The reference style, however, was chosen for consistency with the other chapters presented in this thesis. The journal’s preferred referencing style will be adopted prior to submission.
4.1. ABSTRACT

Guilt is thought to motivate altruistic behaviour. Excessive guilt and self-blaming emotional biases are characteristic of major depressive disorder (MDD) and remain detectable once symptoms have remitted, suggesting their importance as trait vulnerability factors. The role of guilt in altruistic choices, however, is elusive. Moreover, it is unclear how excessive guilt and self-blame affect altruistic choices in individuals with vulnerability to MDD. The current research examined these questions across two separate studies. Fifty-six remitted MDD and 30 healthy control (HC) participants (Study 1), and 30 remitted MDD and 29 HC participants (Study 2), underwent two blocks of emotion priming (Study 1: guilt vs. indignation; Study 2: guilt vs. baseline). Participants provided explicit and implicit choice preferences in response to dilemmas in both blocks (Study 1: selfish options were pitted against reciprocal altruism options [i.e., towards friends/colleagues] in one condition and social options [i.e., without altruism] in the other; Study 2: selfish options were pitted against reciprocal altruism options in one condition and pure altruism options [i.e., towards strangers] in the other). Explicit preference for reciprocal altruism and social options was lower in rMDD patients compared to the HC group. Explicit and implicit preferences for pure but not reciprocal altruism options were increased by guilt priming irrespective of group. These findings suggest a causal role of guilt in pure but not reciprocal altruistic choices and provide the first experimental measure of reduced preference for social interactions in MDD.
4.2. INTRODUCTION

“Morality is not properly the doctrine of how we may make ourselves happy, but how we may make ourselves worthy of happiness.”

Although happiness does not play a central role in Kantian ethics, Kant’s suggestion that heeding one’s moral duties is a requirement for being worthy of happiness draws attention to the complex relationship between moral behaviour and happiness. It is well established that moral emotions are disrupted in major depressive disorder (MDD), which is characterized by symptoms including persistent low mood and/or loss of interest or pleasure in otherwise enjoyable activities (American Psychiatric Association, 2000). Excessive or overgeneralized guilt is a distinctive symptom of depression which is observed cross-culturally (Sartorius et al., 1980). Importantly, guilt is a moral emotion in that it motivates behaviours aimed at improving the welfare of others (Zahn, de Oliveira-Souza and Moll, 2013). Self-blaming moral emotions including guilt may also be elevated in people who are not currently depressed but are vulnerable to experiencing major depressive episodes (MDEs). Green and colleagues (2013b), for example, recently demonstrated a bias towards experiencing self-blaming moral emotions in remitted MDD (rMDD) patients, who are known to be at an elevated lifetime risk for developing future MDEs (American Psychiatric Association, 2000). In healthy volunteers, guilt was positively correlated with self-reported altruistic behaviours (see (O’Connor et al., 2007)). Attempts to investigate such a relationship in current MDD could be made difficult by the presence of other symptoms (O’Connor et al., 2007). Characterizing the relationship between self-blaming moral emotions and altruism in patients remitted from the depressed state, however, could provide a novel measure of social functioning or reveal trait markers of depression vulnerability.

Several lines of evidence suggest altruistic behaviour is dysfunctional in MDD. The Ultimatum Game (UG), in which one player (the receiver) either accepts or rejects proposals from another player (the proposer) to divide sums of money, has proven a popular paradigm for investigating the relationship of depression to altruistic behaviour. In a study of healthy volunteers, participants acting as receivers were more likely to reject unfair offers in the UG after undergoing sadness induction relative to a neutral condition (Harlé and Sanfey, 2007). Furthermore, rejection of unfair offers in the UG by
healthy volunteers was associated with reduced serotonin following acute tryptophan depletion (Crockett et al., 2010b) and with lower platelet serotonin (Emanuiele et al., 2008), a neurochemical system whose dysfunction is implicated in the pathophysiology of MDD. Conversely, acutely increasing serotonin with a selective serotonin reuptake inhibitor increased the likelihood of accepting unfair offers in a cohort of healthy participants (Crockett et al., 2010a). These participants also judged moral harms as less acceptable in response to a moral dilemma task (Crockett et al., 2010a). Comparatively few studies have been conducted in currently depressed patients and have yielded findings which variously support or contradict the literature in healthy volunteers. A handful of studies using the UG have shown current MDD patients to reject unfair offers more often than control participants (Radke et al., 2013; Scheele et al., 2013), as would be predicted from studies in healthy participants. However, Destoop and colleagues (2012) observed no differences between current MDD patients and controls regarding rates of rejection to unfair offers in the UG. Furthermore, Harlé and colleagues (2010) reported that current MDD patients accepted more unfair offers compared to controls, as Crockett and colleagues (2010a) observed after acutely increasing serotonin in healthy participants. Interestingly, one study found that, while in the role of proposer during the UG, current MDD patients made larger offers compared to controls (Destoop et al., 2012). These discrepancies may reflect limitations in comparing studies which investigated participants with or without clinical diagnoses of MDD, or could be a consequence of having studied MDD patients with heterogeneous symptom profiles. Investigating altruistic behaviour in patients who are vulnerable to MDD but are not currently in the depressed state provides a way around the latter issue, although few studies have attempted this. Fujiwara (2009) found that providing charitable financial support was associated with risk for subsequently developing MDD. However, another study found that rMDD patients did not perform differently to controls on the UG or other behavioural-economical paradigms (Pulcu et al., 2015). Additional research is needed to determine whether altruistic behaviour is impaired in people vulnerable to MDD.

Motivated by findings of elevated self-blaming moral emotion in MDD patients, O'Connor and colleagues ((2007), (2012)) formulated a “hyperaltruism hypothesis” which suggests these emotions may give rise to pathological manifestations of altruistic behaviour in MDD patients. In patients with current MDD, who may experience symptoms aside from elevated self-blame such as lack of motivation or social withdrawal, such manifestations could be obscured. They further argue that the
increasing prevalence of MDD (Sartorius, 1986) may serve an evolutionary purpose, with hyperaltruism in MDD promoting evolutionary fitness at the group level (O’Connor et al., 2007; O’Connor et al., 2012). This case is made in reference to theories of group selection (Gintis et al., 2008) and gene-culture coevolution (see (Wilson and Wilson, 2007)). Briefly stated, these theories suggest that individuals within a social group contribute to their group’s evolutionary fitness, and that individual fitness is then determined in part by the fitness of one’s group. These theories are typically employed to provide an evolutionary mechanism in support of the contentious view that humans are capable of engaging self-sacrificing behaviour without any expectation, conscious or otherwise, of reciprocation (Joyce, 2006). In what follows, we will class these types of behaviours as “pure altruism” and will use this term to refer to altruistic acts directed towards strangers. In contrast to pure altruism, the less contentious concept of “reciprocal altruism” refers to self-sacrificing behaviours which benefit another person who is expected, consciously or otherwise, to reciprocate with self-sacrificing behaviour at a later point in time ((Trivers, 1971); e.g., expecting a lift to the airport from a friend to whom you provided a ride last week). In what follows, we will use the term “reciprocal altruism” to refer to helping behaviours directed towards unrelated individuals with whom one could reasonably be expected to interact with again in the future (i.e., friends and colleagues). If the hyperaltruism theory of MDD and its evolutionary implications are correct, one would expect increased pure and/or reciprocal altruism in patients with MDD who are in remission from symptoms, as stable hyperaltruism traits are more likely to be of evolutionary relevance. Using a charitable donations task, however, Pulcu and colleagues (2015) found that rMDD patients and control participants donated comparably to charities and, moreover, current MDD patients made lower donations compared to the control group. Additionally, alternative accounts suggest that the increasing prevalence of depression may be better explained by, for example, polygenic mutation-selection balance (Keller and Miller, 2006). Thus, additional work is required to test the predictions inherent in the hyperaltruism theory of MDD.

We propose an alternative hypothesis building on the hyperaltruism hypothesis that considers these recent empirical findings in MDD. According to this hypothesis, preference for altruism (e.g., reciprocal and pure altruism) as a function of self-blaming moral emotions can be modelled as an inverted U-shaped curve (Figure 4.1). This hypothesis predicts that increasing self-blame increases preference for altruistic behaviours until reaching a threshold, represented as the peak of the inverted U-shaped
curve, after which self-blame is maladaptive and reduces preference for altruistic behaviours. These predictions link research findings showing self-blame promotes altruistic behaviour in healthy volunteers (Tangney, Stuewig and Mashek, 2007; O'Connor et al., 2012) while current MDD patients who experienced elevated altruistic guilt nevertheless made lower charitable donations (Pulcu et al., 2015). Patients with rMDD have also been shown to experience elevated altruistic guilt and biases towards self-blaming emotions despite recovery from the depressed state (Green et al., 2013b), consistent with the view that vulnerability to MDD is associated with proneness towards making internal attributions of blameworthiness (Abramson, Seligman and Teasdale, 1978). Despite this, performance in rMDD patients was indistinguishable from control participants on several behavioural economical paradigms thought to measure aspects of altruism (Pulcu et al., 2015). This hypothesis therefore predicts that patients with rMDD will demonstrate similar preferences for altruistic behaviours to healthy controls although situated on opposite sides of the inverted U-shaped curve. If this hypothesis is correct, one would predict that experimentally inducing guilt in healthy volunteers would increase preference for altruistic behaviours. In contrast, experimentally inducing guilt in MDD patients, in whom guilt priming would be predicted to trigger depressogenic cognitions (Teasdale, 1983), would be expected to decrease preference for altruistic behaviours. Priming feelings of indignation/anger towards others, on the other hand, would be expected to reduce guilt since previous work showed that priming feelings of indignation increased the likelihood of making external causal attributions (Keltner, Ellsworth and Edwards, 1993) and vice versa (Neumann, 2000). Experimentally inducing indignation would therefore be predicted to have the opposite pattern of effects on preferences for altruistic behaviours in each group.

The aim of the current research was to investigate the influence of modulating guilt on preference for altruistic choices (reciprocal or pure altruism) in rMDD patients and healthy control (HC) participants. As is described in detail below, the modulation of guilt was accomplished using an emotion priming paradigm. Within each emotion priming condition, participants responded to dilemmas pitting selfish options against prosocial options, meant here to refer to any option involving a social component including options to behave altruistically. These prosocial options encompassed options to behave socially but with a self-serving component (e.g., asking a friend to socialize), options to engage in reciprocal altruism (e.g., helping a friend), and options to engage in pure altruism (e.g., helping a stranger). Choice preference for each dilemma was measured both explicitly and implicitly. Based on our
hypotheses, we predicted that rMDD patients would experience increased altruistic guilt relative to the HC group but no group differences in explicit or implicit preference for prosocial options would be observed during the baseline emotion priming condition. Guilt priming was predicted to increase preference for altruistic options in the HC group while decreasing it in rMDD patients. Indignation priming was expected to produce an exact opposite pattern of changes, with decreased preference in the HC group and increased preference in the rMDD group for altruistic options. We included dilemmas with an option to behave socially with a self-serving component in order to determine

**Figure 4.1** According to our hypothesis, explicit and implicit preference for altruistic choices as a function of self-blaming moral emotions can be modeled as an inverted U-shaped curve. This hypothesis predicts that rMDD patients will experience more self-blame than HC participants at baseline (black circles) despite demonstrating similar preferences for altruistic behaviours (dashed line). For reference, we have also included a circle representing cMDD patients, who may experience excessive or overgeneralized guilt and would therefore be predicted to sit to the far right on the curve. Guilt priming would be predicted to increase preference for altruistic choices in the HC group (i.e., shifting towards the peak) while indignation priming would be predicted to decrease preference for altruistic choices by priming external causal attributions (i.e., shifting away from the peak). The opposite pattern of changes would be predicted for the rMDD patients, with guilt priming decreasing (i.e., shifting away from the peak) and indignation priming increasing (i.e., shifting towards the peak) preference for altruistic behaviours. cMDD, current major depressive disorder; HC, healthy control; rMDD, remitted major depressive disorder.
whether any effects we might observe could be the consequence of an underlying reduction in preference for social interactions irrespective of selfish or altruistic motivations. Alternatively, according to the original hyperaltruism hypothesis of MDD, rMDD patients would be expected to prefer altruism options more than the HC group at baseline, with preference increasing in both groups during guilt priming and decreasing during indignation priming.

4.3. METHOD

4.3.1. Participants

The current research was conducted across two separate studies (hereon “Study 1” and “Study 2”), each of which received approval from the South Manchester National Health Service Research Ethics Committee (Ref Nos: 07/H1003/194 and 10/H1014/8). Participants from both studies provided written informed consent after all study procedures were explained in full. The current research was carried out as part of two larger independent research projects with comparable inclusion and exclusion criteria and participants from the projects were invited to take part only if they were either currently enrolled or had previously provided consent to be contacted about future research. Participants for these larger projects were recruited with online and print advertisements and were compensated for time and travel expenses. Importantly, the Structured Clinical Interview-I for DSM-IV-TR (SCID-I) was used in both projects to assess current psychiatric status and to diagnose past MDEs (First et al., 2002). We recruited from a pool of 363 participants in total who were eligible to take part in at least one of the two research projects (96 rMDD patients and 48 HC participants from the first project, and 138 rMDD patients and 81 HC participants from the second project). For Study 1, 125 participants were contacted about taking part (71 rMDD patients, 54 HC participants). Of these, 39 participants were unable to take part because they were no longer eligible (N=15) or could not be scheduled (N=24). In total, 56 rMDD patients and 30 HC participants completed Study 1. For Study 2, 157 participants were contacted about taking part (76 rMDD patients, 81 HC participants). Of these, 96 participants were unable to take part because they were no longer eligible (N=55) or could not be scheduled (N=41). In total, 30 rMDD patients and 31 HC participants completed Study 2. After completing Study 2, data were excluded for two HC participants who either failed to follow the instructions provided with the Altruistic Choices Task (described in detail below) or revealed a previously undisclosed first-degree family history of MDD.
All participants across both Studies were subject to the following inclusion criteria: 18 years of age or older, English as a fluent (written and spoken) or first language, and normal or corrected-to-normal vision and hearing. In addition, rMDD patients must have had at least one past MDE lasting at least two months, a diagnosis of MDD according to the DSM-IV-TR (American Psychiatric Association, 2000), and must have been in remission for at least six months prior to enrolment in either Study. The exclusion criteria were: current or relevant past Axis 1 disorders (e.g., substance abuse), recent use of psychotropic medications (four weeks, or eight weeks for fluoxetine), current suicidality or self-harming behaviours, history of medical including neurological disorders know to influence brain functioning, a Montgomery-Åsberg Depression Rating Scale (MADRS) score >10 (depression cut-off; (Montgomery and Åsberg, 1979; Zimmerman, Posternak and Chelminski, 2004)), and a score on the Global Assessment of Functioning (GAF) scale indicative of impaired psychosocial functioning (American Psychiatric Association, 2000). In addition, HC participants could not have any history of Axis 1 disorders or any first-degree family history of relevant psychiatric disorders.

In Study 1, the rMDD patients and HC group were well-matched on age, years of education, and sex (Table 4.1). Furthermore, the rMDD patients and HC group did not differ with respect to scores on the MADRS, used to measure the severity of current depressive symptoms. The rMDD patients did, however, have lower scores than the HC group on the GAF ($t(84) = 2.45, p = .02, r = .26$), a measure of psychosocial functioning. Despite this, average scores for both groups were between 81 and 90 which is indicative of good overall functioning with absent or minimal depressive symptoms (American Psychiatric Association, 2000). In Study 2, the rMDD patients were well-matched to the HC group on age (Table 4.1), but had fewer years of education ($t(57) = 2.37, p = .02, r = .30$) and a smaller proportion of males relative to females (Contingency Coefficient = .32, $p = .01$). In contrast to Study 1, the rMDD patients also had higher MADRS scores compared to the HC group ($t(57) = 3.58, p < .0008, r = .43$). The average MADRS scores for both groups, however, were within the normal range (i.e., <6; (Snaith et al., 1986)). As in Study 1, the rMDD patients also had lower GAF scores compared to the HC group ($t(57) = 3.18, p = .002, r = .39$), but the average GAF scores for both groups were within a range indicative of good overall functioning.

Across all of the rMDD patients and HC participants from both Studies 1 and 2, the groups were well-matched on age, years of education, and sex (Table 4.1). The rMDD patients, however, had higher scores on the MADRS ($t(143) = 3.10, p = .002, r = .25$).
Table 4.1 Demographic variables in the rMDD patients and HC group for Studies 1 and 2 and across both studies. In Study 1, the rMDD patients and HC group did not differ on the demographic variables (Contingency Coefficient = .02, \( p = .82; \) \( t(84) < 1.11, p > .27, r < .12 \)) with the exception of GAF scores. In Study 2, the rMDD patients and HC group were well-matched on age (\( t(57) = .45, p = .65, r = .06 \)) but differed with respect to years of education, sex, and GAF and MADRS scores. Across studies, the rMDD patients and HC group did not differ with respect to age, years of education, or sex (Contingency Coefficient = .14, \( p = .09; t < 1.76, p > .08 \)) although group differences were present for GAF and MADRS scores. GAF, Global Assessment of Functioning; HC, healthy control; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder.

aGAF scores were lower in the rMDD patients relative to the HC group in Study 1 (\( t(84) = 2.45, p = .02, r = .26 \)), Study 2 (\( t(57) = 3.18, p = .002, r = .39 \)), and across both studies (\( t(143) = 3.82, p < .0002, r = .30 \)).

bIn Study 2, the rMDD patients had fewer years of education compared to the HC group (\( t(57) = 2.37, p = .02, r = .30 \)).

cIn Study 2, the proportion of males relative to females was lower in the rMDD group compared to the HC group (Contingency Coefficient = .32, \( p = .01 \)).
dMADRS scores were higher in the rMDD patients relative to the HC group in Study 2 ($t(57) = 3.58, p < .0008, r = .43$) and across both studies ($t(143) = 3.10, p = .002, r = .25$).

and GAF ($t(143) = 3.82, p < .0002, r = .30$). The average MADRS scores for both groups fell within the normal range, and average GAF scores suggested good overall functioning.

4.3.2. Altruistic Choices Task (ACT)

The Altruistic Choices Task (ACT) was designed to probe the influence of modulating guilt on choice preference in response to dilemmas which pit prosocial options against selfish options. In this context, “choice preference” refers either to a participant’s explicit evaluation of preference for a given option (hereon “explicit choice preference”), or an inferred implicit preference based on performance on the Brief Implicit Association Test (BIAT; Hereon “implicit choice preference”; (Sriram and Greenwald, 2009)). Furthermore, “prosocial option” is meant here to refer to any option which involves a social component irrespective of whether this is associated with an altruistic behaviour. To this end, the ACT consists of two counter-balanced blocks in which participants first completed an emotion priming paradigm (Study 1: guilt or indignation; Study 2: guilt or baseline) and then indicated their explicit and implicit choice preference in response to prosocial dilemmas (Study 1: reciprocal altruism dilemmas and selfish dilemmas with a social component; Study 2: reciprocal altruism and pure altruism dilemmas). The ACT lends itself to a 2 x 2 x 2 design for both explicit and implicit choice preference, with group (rMDD or HC) as a between-subjects variable and emotion priming condition and dilemma type as within-subjects variables. The ACT software was coded in MATLAB (Release 2012a, MathWorks) and is available upon request.

4.3.3. ACT: Emotion Priming Paradigm

The novel emotion priming paradigm used in the current research was designed to increase guilt by priming feelings of guilt (Studies 1 and 2), to decrease guilt by priming feelings of indignation/anger directed towards others (Study 1), and to provide an emotional baseline (Study 2). In each emotion priming condition, participants were asked to read a first-person story and to experience emotions they would expect to feel had the story happened to them. The stories were well-matched in length (guilt priming:
390 words, indignation priming: 388 words, baseline priming: 406 words). The story used in the guilt priming condition described a situation in which the participant injured another person in a car accident resulting from their negligence, the story used in the indignation priming condition described the same situation but from the perspective of the injured person, and the baseline story included elements from the other two emotion priming stories not explicitly intended to evoke an emotional response (see Appendix C). Next, participants were presented with 30 statements each three words in length related to the story they just read and were asked to rate each statement on a 7-point Likert scale according to the emotion the story was intended to prime (i.e., 1=not guilty to 7=very guilty, not angry/indignant to very angry/indignant, or not unpleasant to very unpleasant). In order to sustain the effects of the emotion priming throughout each block, the statements were then individually presented prior to each dilemma in a random and non-repeating order. Between blocks, participants were asked to complete the Serial Sevens subtraction task as a distractor to reduce emotional carry-over effects (Folstein, Folstein and McHugh, 1975). Next, participants completed a second block of emotion priming using the story that was not presented during the first block.

4.3.4. ACT: Dilemmata and Explicit Choice Preference

We designed a novel dilemma task by adapting ecologically-valid scenarios drawn from a normative dataset collected in an independent group of healthy volunteers which has been described in detail elsewhere (see supplemental material in (Green et al., 2013a)). Importantly, the dilemmas used in the current research do not pit competing moral concerns against each other and are therefore not moral dilemmas in the widely used sense. Instead, the design of the dilemma task was informed by theories of reciprocal and pure altruism in that we investigated preference for helping behaviours directed towards friends and colleagues or towards strangers.

Each dilemma describes a scenario in which a selfish option with no social component (nonsocial-selfish) is pitted against a prosocial option (i.e., reciprocal altruism, pure altruism, or selfish with a social component [social-selfish]). A set of example dilemmas is provided in Table 4.2. After reading each dilemma, participants were asked “Would you prefer to…” and then selected one of two options corresponding to their preferred choice. Within each block, participants responded to 30 dilemmas in a random and non-repeating order. Both Studies 1 and 2 included 15 reciprocal altruism dilemmas, Study 1 included 15 social-selfish dilemmas, and Study 2 included 15 pure altruism dilemmas for a total of 45 dilemmas across both Studies. Care
was taken to match the content of the dilemmas across different dilemma types such that the 45 dilemmas designed for the current research are separable into 15 groupings each comprising three matched dilemmas (e.g., see Table 4.2 for one such grouping or Appendices D through F for all dilemmas). Furthermore, the dilemmas were comparable in length (social-selfish dilemmas: $M = 48.9$, $SD = 5.2$ words, reciprocal altruism dilemmas: $M = 46.9$, $SD = 3.2$ words, pure altruism dilemmas: $M = 47.6$, $SD = 3.5$ words; $F(2, 42) = .99$, $p = .38$, $\eta^2_p = .05$) and in semantic coherence within each dilemma (relatedness within social-selfish dilemmas: $M = .88$, $SD = .04$, reciprocal altruism dilemmas: $M = .88$, $SD = .05$, pure altruism dilemmas: $M = .84$, $SD = .04$; $F(2, 42) = ., p = .06$, $\eta^2_p = .13$; (Landauer, Foltz and Laham, 1998)).

4.3.5. ACT: Implicit Choice Preference

Explicit evaluations of choice preference, although standard in studies of moral judgment, may be influenced by a conscious desire to provide socially acceptable responses (Moll et al., 2005). To address this issue, we adapted a measure of implicit attitudes, the BIAT, to serve as a proxy for implicit choice preference. Although this represents a novel application of the BIAT, it bears noting that the longer Implicit Association Test was used successfully to investigate implicit moral attitudes (Luo et al., 2006).

The purpose of each BIAT was to determine whether participants were quicker when associating “{PREFERRED}” with prosocial options (i.e., reciprocal altruism, pure altruism, or social-selfish) or with nonsocial-selfish options. Each set of BIAT stimuli was designed to correspond to a single dilemma, and each BIAT was presented shortly after explicit evaluation of the corresponding dilemma. An example set of BIAT stimuli are presented in Table 4.3 and corresponds to the reciprocal altruism dilemma presented in Table 4.2 (see Appendices D through F for all BIAT stimuli). A detailed description of the design and analysis of the BIAT has been provided elsewhere (Sriram and Greenwald, 2009). Briefly, the BIAT stimuli comprised 16 phrases ranging from one to two words in length and were grouped into four categories containing four phrases each. The first two categories of phrases paralleled the response options given for each BIAT’s corresponding dilemma, and the third and fourth categories were always “{PREFERRED}” and “{NON-PREFERRED}” respectively. Each individual BIAT comprised two blocks and in each block participants saw all 16 phrases in an interleaved, random, and non-repeating order. Participants were instructed to press the “k” key whenever a phrase belonging to a “focal” category appeared on the computer.
<table>
<thead>
<tr>
<th>Category</th>
<th>Dilemmata</th>
<th>Response Options</th>
<th>Would you prefer to…</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social-Selfish Dilemma</td>
<td>You are leaving your place of work to go home and relax after a long day. However, you learn that tonight is the last night cinemas are screening a film you want to see, and you think about ringing a friend to go and see the film with you.</td>
<td>…go directly home and relax?</td>
<td>…see the film with your friend before it leaves cinemas?</td>
</tr>
<tr>
<td>Reciprocal Altruism Dilemma</td>
<td>You are leaving your place of work to go home and relax after a long day. However, you see that your co-worker is having serious car trouble, and you consider offering them a lift home although this would add an hour to your commute.</td>
<td>…go directly home and relax?</td>
<td>…give your co-worker a lift home?</td>
</tr>
<tr>
<td>Pure Altruism Dilemma</td>
<td>You are leaving your place of work to go home and relax after a long day. However, you see a vehicle belonging to someone you’ve not met before broken down outside your office, and you consider offering to give them a lift to the garage.</td>
<td>…go directly home and relax?</td>
<td>…give a lift to the person you’ve not met?</td>
</tr>
</tbody>
</table>

Table 4.2 An example set of matched dilemmas and corresponding response options. All dilemmas are provided in the Appendices.

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Focal in Block 1</td>
<td>Non-Focal in Block 2</td>
<td>Focal in Block 2</td>
<td>Non-Focal in Block 1</td>
</tr>
<tr>
<td>Category Name</td>
<td>{go relax}</td>
<td>{give lift}</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Category Member 1</td>
<td>get rest</td>
<td>provide ride</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Category Member 2</td>
<td>go unwind</td>
<td>drive colleague</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Category Member 3</td>
<td>go relax</td>
<td>give transport</td>
<td>DESIRED</td>
<td>UNDESIRABLE</td>
</tr>
<tr>
<td>Category Member 4</td>
<td>lounge around</td>
<td>give lift</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Table 4.3 BIAT stimuli corresponding to the reciprocal altruism dilemma presented in Table 4.2. All BIAT stimuli are provided in the Appendices.
screen or the “d” key if the phrase did not belong to a “focal” category. Using the example BIAT stimuli presented in Table 4.3, the focal categories for the first block are “{go relax}” and “{PREFERRED}” and the non-focal categories are “{give lift}” and “{NON-PREFERRED}.” In the second block, the focal categories are “{give lift}” and “{PREFERRED}” and the non-focal categories are “{go relax}” and “{NON-PREFERRED}.” Each BIAT recorded the latency between the presentation of each phrase and the corresponding correct keystroke. Participants were therefore instructed to respond and to correct mistakes as quickly as possible. Both Studies 1 and 2 included eight reciprocal altruism BIATs, Study 1 included eight social-selfish BIATs, and Study 2 included eight pure altruism BIATs. We did not include BIATs corresponding to every dilemma in order to reduce administration time.

4.3.6. Standard Measures

We used the 67 item version of the Interpersonal Guilt Questionnaire (IGQ; O'Connor et al., 1997) to assess empathy-based guilt. We were specifically interested in omnipotent responsibility guilt, which arises from exaggerated feelings of responsibility for the welfare of others, and survivor guilt, where one feels badly for being better off than others. We also used the Compassionate Altruism Scale (CAS), a 45-item instrument designed to measure the frequency of self-reported altruistic behaviours directed towards family, friends, and strangers (Vaux, Riedel and Stewart, 1987; Berry et al., 2009).

4.3.7. Statistical Analysis

Explicit choice preference was calculated as the ratio of preferred prosocial options of a given type relative to the total number of dilemmas of that type (i.e., 15). To calculate implicit choice preference, following the optimized standard algorithm (Greenwald, Nosek and Banaji, 2003), we first used the latencies recorded by each BIAT to calculate BIAT D scores where the mean latency across one block (M1) was subtracted from the mean latency across the other block (M2) and divided by the standard deviation for latencies across both blocks: (M2-M1)/SD. The mean latency when associating “{PREFERRED}” with prosocial options always corresponded to M1 and nonsocial-selfish options always corresponded to M2. Positive BIAT D scores denote a stronger implicit association between “{PREFERRED}” and prosocial options (quicker mean latency [M1] relative to nonsocial-selfish options [M2]), whereas negative BIAT D scores denote a weaker implicit association (slower mean latency relative to nonsocial-selfish options). We excluded individual BIAT scores where
participants made 10 or more errors or responded in less than 300ms in at least 10% of
trials across the entire task. As a consequence, some participants had fewer than eight
useable BIAT scores corresponding to a given dilemma type. We therefore excluded all
BIATs corresponding to a given dilemma type in participants who had fewer than four
of eight useable BIAT scores. We then calculated mean BIAT D values, used to
represent implicit choice preference, for each dilemma type from the remaining BIAT D
scores.

For Study 1, explicit and implicit choice preference were entered into two
separate 3-way repeated-measures ANOVAs to investigate effects of group (rMDD or
HC), emotion priming condition (guilt or indignation) and dilemma type (reciprocal
altruism or social-selfish) on choice preference. The same procedure was used for Study
2 to investigate effects of group (rMDD or HC), emotion priming condition (guilt or
baseline) and dilemma type (reciprocal or pure altruism) on choice preference. Analyses
were carried out in SPSS20 (SPSS Inc., Chicago, Illinois) and results were considered
significant at $p = .05$, two-tailed.

4.4. RESULTS

4.4.1. Relationship between explicit and implicit choice preference

If BIAT scores are a suitable surrogate for implicit choice preference, one might
expect BIAT scores to correlate at least weakly with explicit choice preference. Indeed,
explicit and implicit choice preference for reciprocal altruism options were moderately
correlated during both the guilt (Study 1: $r(80) = .34, p = .002$; Study 2: $r(80) = .23, p =
.09$) and indignation (Study 1: $r(81) = .23, p = .04$) priming conditions. However,
explicit and implicit choice preference for reciprocal altruism options were not
correlated during the baseline emotion priming condition ($r(59) = -.02, p = .89$), nor
were explicit and implicit choice preference correlated for pure altruism or social-selfish
options irrespective of the emotion priming condition ($-.02 < r < .15, p > .19$).

4.4.2. Study 1

The rMDD patients had higher omnipotent responsibility guilt scores ($M =
47.60, SD = 6.79$) relative to the HC group ($M = 41.24, SD = 7.81$; $t(82) = 3.87, p <
.0003, r = .39$), as well as higher survivor guilt scores ($M = 69.38, SD = 11.06$) relative
to the HC group ($M = 58.34, SD = 9.54$; $t(82) = 4.55, p < .0001, r = .45$) as measured
with the IGQ. The rMDD patients and HC group did not differ on self-reported
frequency of altruistic behaviours directed towards family members (rMDD group: \( M = 127.54, SD = 25.30 \); HC group: \( M = 121.60, SD = 28.23 \); \( t(84) = 1.00, p = .32, r = .11 \)), friends (rMDD group: \( M = 122.23, SD = 25.33 \); HC group: \( M = 114.17, SD = 25.42 \); \( t(84) = 1.41, p = .16, r = .15 \)), or strangers (rMDD group: \( M = 49.73, SD = 31.77 \); HC group: \( M = 38.77, SD = 28.24 \); \( t(84) = 1.58, p = .12, r = .17 \)) as measured using the CAS.

For explicit choice preference measured using the ACT, main effects of group (rMDD versus HC groups; \( F(1, 84) = 5.44, p = .02, \eta_p^2 = .06 \)) and dilemma type (reciprocal altruism versus social-selfish dilemmas; \( F(1, 84) = 54.38, p < .0001, \eta_p^2 = .39 \)) were observed. There were no significant interactions \( (F(1, 84) < 1.40, p > .24, \eta_p^2 < .02 \) and no main effect of emotion (guilt versus indignation priming; \( F(1, 84) = .03, p = .86, \eta_p^2 = .00 \)). Exploratory post-hoc pairwise comparisons revealed an overall lower explicit preference for both reciprocal altruism and social-selfish options relative to nonsocial-selfish options irrespective of emotion priming condition in the rMDD patients relative to the HC group (mean difference = .08, \( p = .02, 95\% CI [.01, .16] \)). Across all participants, explicit preference was lower for reciprocal altruism options relative to social-selfish options irrespective of emotion priming condition (mean difference = .21, \( p < .0001, 95\% CI [.15, .26] \)). For implicit choice preference measured using BIATs, no significant main effects or interactions were observed \( (F(1, 76) < 1.92, p > .17, \eta_p^2 < .03) \), although a dilemma (reciprocal altruism versus social-selfish dilemmas) by group (rMDD versus HC groups) interaction was marginally significant \( (F(1, 76) = 2.96, p = .09, \eta_p^2 = .04) \).

We next sought to determine whether omnipotent responsibility or survivor guilt measured with the IGQ correlated with the magnitude of change in choice preference from the indignation priming condition to the guilt condition. To do this, we first calculated difference scores by subtracting choice preference during the indignation priming condition from choice preference during the guilt condition. Then, we correlated IGQ scores with the resulting difference scores. Neither omnipotent responsibility nor survivor guilt measured with the IGQ correlated with emotion priming-induced changes in explicit or implicit preference for reciprocal altruism or social-selfish options \( (-.17 < r < .09, p > .14) \).

Given that the rMDD patients had lower GAF scores compared to the HC group, we next investigated whether a relationship exists between explicit or implicit choice preference and psychosocial functioning. However, GAF scores did not correlate with preference for reciprocal altruism options during guilt (Explicit: \( r(86) = .08, p = .45 \);
Implicit: \( r(80) = .03, p = .80 \) or indignation (Explicit: \( r(86) = .09, p = .43 \); Implicit: \( r(81) = -.17, p = .14 \)), nor with preference for social-selfish options during guilt (Explicit: \( r(86) = .04, p = .70 \); Implicit: \( r(81) = .02, p = .85 \) or indignation (Explicit: \( r(86) = .00, p = .97 \); Implicit: \( r(80) = -.16, p = .15 \)). This lack of association suggests the main effect of group for explicit choice preference described earlier is not better attributed to an underlying group difference in psychosocial functioning.

4.4.3. Study 2

As in Study 1, the rMDD patients had higher omnipotent responsibility guilt scores (\( M = 44.60, SD = 6.60 \)) relative to the HC group (\( M = 40.76, SD = 5.73 \; t(57) = 2.38, p = .02, r = .30 \)), as well as higher survivor guilt scores (\( M = 69.17, SD = 11.62 \); \( t(57) = 3.74, p < .0005, r = .44 \)). Again, the rMDD patients and HC group did not differ on self-reported frequency of altruistic behaviours directed towards family members (rMDD group: \( M = 113.10, SD = 30.59 \); HC group: \( M = 115.86, SD = 28.42 \); \( t(57) = .36, p = .72, r = .05 \)), friends (rMDD group: \( M = 112.63, SD = 20.20 \); HC group: \( M = 111.45, SD = 27.66 \); \( t(57) = .19, p = .85, r = .02 \)), or strangers (rMDD group: \( M = 32.70, SD = 25.63 \); HC group: \( M = 41.17, SD = 24.63 \); \( t(57) = 1.29, p = .20, r = .17 \)).

For explicit choice preference, an emotion (baseline versus guilt priming) by dilemma (reciprocal versus pure altruism dilemmas) interaction was observed (Figure 4.2a; \( F(1, 57) = 4.67, p = .03, \eta_p^2 = .08 \)), as were main effects of emotion (\( F(1, 57) = 5.59, p = .02, \eta_p^2 = .09 \)) and dilemma type (\( F(1, 57) = 221.30, p < .00001, \eta_p^2 = .80 \)). There were no other significant interactions (\( F(1, 57) < .43, p > .52, \eta_p^2 < .008 \)) and no main effect of group (\( F(1, 57) = 1.51, p = .22, \eta_p^2 = .03 \)). Post-hoc paired samples t-tests (Bonferroni-corrected \( \alpha = .013 \)) revealed no guilt priming induced change in explicit preference for reciprocal altruism options (\( M = .79, SD = .21 \)) relative to the baseline priming condition (\( M = .80, SD = .18 \); \( t(58) = .59, p = .55, r = .02 \)). However, guilt priming increased explicit preferences for pure altruistic choices (\( M = .51, SD = .22 \)) relative to baseline priming (\( M = .46, SD = .21 \); \( t(58) = 2.83, p = .006, r = .12 \)). Furthermore, explicit preference was higher for reciprocal altruism relative to pure altruism options in both the guilt priming (Reciprocal altruism options: \( M = .80, SD = .18 \); Pure altruism options: \( M = .51, SD = .22 \); \( t(58) = 12.63, p < .0001, r = .58 \)) and baseline priming conditions (Reciprocal altruism options: \( M = .79, SD = .21 \); Pure altruism options: \( M = .46, SD = .21 \); \( t(58) = 14.40, p < .0001, r = .62 \)) irrespective of group.
Figure 4.2 Emotion (baseline versus guilt priming) by dilemma (reciprocal versus pure altruism) interactions from Study 2 for A) explicit choice preference, calculated as the average percentage of reciprocal or pure altruism options preferred relative to selfish options without a social component, and B) implicit choice preference, calculated by averaging the BIAT D scores corresponding to the reciprocal or pure altruism dilemmas within each participant. The points in each plot represent averaged choice preference scores and corresponding standard errors.

The results for implicit choice preference were remarkably similar to those for explicit choice preference. An emotion by dilemma interaction was again observed (Figure 4.2b; $F(1, 55) = 6.55, p = .01, \eta_p^2 = .11$), as were main effects of emotion ($F(1, 55) = 5.17, p = .03, \eta_p^2 = .09$) and dilemma ($F(1, 55) = 16.45, p < .0002, \eta_p^2 = .23$). In contrast to the results for explicit choice preference, the main effect of group was marginally significant ($F(1, 55) = 3.93, p = .052, \eta_p^2 = .07$). There were no additional significant interactions ($F(1, 55) < .66, p > .42, \eta_p^2 < .01$). Post-hoc tests (Bonferroni-corrected $\alpha = .013$) revealed no guilt priming induced change in implicit preference for reciprocal altruism options ($M = -.04, SD = .17$) from the baseline priming condition ($M = -.04, SD = .21$; $t(56) = .20, p = .84, r = .02$). In contrast, a guilt priming induced increase in implicit preference for pure altruism options was observed ($M = .02, SD = .18$) relative to baseline priming ($M = .12, SD = .20$; $t(57) = 3.58, p < .0008, r = .25$) irrespective of group. Implicit preference was lower for reciprocal altruism options compared to pure altruism options during both the guilt priming (Reciprocal altruism options: $M = -.04, SD = .17$; Pure altruism options: $M = .12, SD = .21$; $t(56) = 4.87, p < .0001, r = .38$) and baseline priming conditions (Reciprocal altruism options: $M = -.05,$
Similar to Study 1, neither omnipotent responsibility nor survivor guilt correlated with magnitude of guilt priming induced change in explicit or implicit preference for reciprocal altruism options relative to baseline, nor with change in implicit preference for pure altruism options relative to baseline (\(-.13 < r < .16, p < .21\)). However, guilt priming induced changes in explicit preference for pure altruism options relative to baseline correlated positively with both omnipotent responsibility (\(r(59) = .49, p < .0001\)) and survivor (\(r(59) = .36, p = .005\)) guilt.

Lastly, whilst the rMDD and HC groups were well-matched on demographic variables in Study 1, there were differences in Study 2 (Table 4.1). Despite this, there was no main effect of group for either explicit or implicit choice preference in Study 2, thereby ruling out an effect of these variables on the positive effects reported in the study.

4.4.4. Across both studies

We investigated reciprocal altruism choice preference during guilt priming when collapsing across both studies. Explicit choice preference for reciprocal altruism options during the guilt priming condition was lower in the rMDD patients (\(M = .73, SD = .23\)) compared with the HC group (\(M = .83, SD = .15; t(143) = 2.98, p = .003, r = .24\)). However, no group differences were observed for implicit choice preference for reciprocal altruism options during the guilt priming condition (rMDD: \(M = .02, SD = .18\); HC: \(M = .03, SD = .19; t(143) = .31, p = .75, r = .03\)).

4.5. DISCUSSION

As predicted, survivor and omnipotent responsibility guilt were elevated in the rMDD group compared to the HC group in both Studies 1 and 2. Furthermore, the moderate correlations we observed between explicit and implicit choice preference suggests these measures provide related although not identical information. Intriguingly, we did not find group by emotion interactions for explicit or implicit choice preferences in either study, contrary to our predictions. Instead, we observed main effects of group and dilemma for explicit choice preference in Study 1 and emotion by dilemma interactions for both explicit and implicit choice preference in Study 2. The main effects from Study 1 demonstrated that the rMDD patients explicitly preferred prosocial options less than the HC group, and participants explicitly preferred reciprocal altruism options less than social-selfish options irrespective of group. These results demonstrated
that rMDD patients show reduced preference for reciprocal altruism options compared with the HC group, but that this is associated with and possibly driven by a reduced explicit preference for prosocial options overall. These findings were not associated with potential confounds such as psychosocial functioning and residual symptoms. The interactions seen in Study 2 indicated that guilt priming increased both explicit and implicit preference for pure altruism options but not for reciprocal altruism options. Guilt priming, therefore, did not modulate preference for reciprocal altruism options in either study, showing that guilt plays a selective role in motivating pure altruism but not reciprocal altruism. Taken together, these findings do not support our proposed hypothesis for understanding altruistic choice preference in MDD. These findings also failed to support the hyperaltruism hypothesis of MDD (O’Connor et al., 2007; O'Connor et al., 2012). Indeed, considering the present findings and previous work in MDD patients who made similar or lower charitable donations compared to a HC group (Pulcu et al., 2015), there has been a consistent failure to find evidence in support of hyperaltruism in MDD. In spite of this, the present research yielded results which are both clinically relevant and of significance for the field of moral psychology more broadly.

4.5.1. Study 1: Impaired Prosociality and Vulnerability to MDD

The results of Study 1 revealed a lower explicit preference for social interaction in patients with rMDD compared with HC participants. This was not specific to reciprocal altruism but also included a lower explicit preference for self-serving options with a social component, such as calling a friend to invite them to spend the afternoon together. These findings contrast with studies by Fujiwara (2009) and by Pulcu and colleagues (2015) in which depression vulnerability was associated with increased altruism or no difference to altruism in comparison to healthy volunteers, respectively. However, Fujiwara (2009) reported data derived from a survey study, which could have been confounded by, for example, inaccuracies or the desire to provide socially acceptable responses (Pulcu, Zahn and Elliott, 2013). Furthermore, Fujiwara (2009) and Pulcu and colleagues (2015) measured willingness to donate money, to split money, or to cooperate for water as proxies for altruism. The current study, however, measured preference for prosociality including altruism relative to selfishness while alone, rather than a willingness to cooperate for or to distribute resources. Each of these measures may tap into different aspects of altruism, and we suggest that the ACT captures a prosocial component of altruism. Preference for reciprocal altruism options was
unaffected by guilt priming in either study, suggesting either that the emotion priming paradigm was ineffective or that preference for engaging in reciprocal altruism behaviours is not influenced by guilt priming. The latter explanation is corroborated by the finding that the emotion priming paradigm effectively modulated preference for pure altruism options in Study 2.

Interestingly, lower explicit preference for social interactions was observed in the rMDD patients from Study 1 despite disclosing similar levels of self-reported altruistic behaviour compared to the HC group. These findings may indicate that rMDD patients do prefer social interactions less than HC participants but are able to overcome their preferences in day-to-day life. Alternatively, the presence of a group difference for explicit but not implicit choice preference could suggest that rMDD patients are as implicitly motivated to engage in social interactions as HC even if such behaviours are not explicitly preferred. Although additional work is needed to appropriately interpret this relationship, it would seem that a preference for social withdrawal-like behaviour may be present in MDD patients despite symptom remission. The importance of social withdrawal as a symptom of MDD was recently underscored by Zahn and colleagues (2015b), who showed that this symptom often co-occurs with core symptoms of MDD including feelings of inadequacy, hopelessness, and depressed mood. Indeed, Allen and Badcock (2003) have argued that symptoms such as social withdrawal are of central importance to MDD, suggesting that the depressed state evolved in order to provide a mechanism to avoid social risks in people who view themselves as more burdensome than valuable within a social context. Impaired social functioning has been implicated as a risk factor for recurrent MDEs (Burcusa and Iacono, 2007). Given that explicit choice preference for reciprocal altruism options was not associated with GAF scores in Study 1, the ACT may tap into aspects of psychosocial functioning not captured by the GAF. Since the present research was conducted in patients with rMDD, who are at risk for developing future MDEs, the ACT may therefore provide a novel measure of depression vulnerability. Future research should investigate this longitudinally in people with a positive family history of MDD and no history of MDEs. At the minimum, future studies should exercise caution in interpreting results suggestive of impaired altruistic behaviours in MDD if not also accounting for overall preferences for engaging in social situations.
4.5.2. **Study 2: Self-Blame Modulates Pure Altruism Choice Preference**

In Study 2, guilt priming was associated with increases to both explicit and implicit preference for pure altruism options but not for reciprocal altruism options. The magnitude of this increase correlated positively with both survivor and omnipotent responsibility guilt scores. These findings are consistent with previous studies in healthy volunteers which explored the relationship of guilt to altruistic behaviour. Nelissen and colleagues (2011) found a positive correlation between anticipated guilt and the size of monetary offers made during an UG. Relatedly, Ketelaar and Au (2003) used autobiographical memories to induce feelings of guilt which increased cooperation on a Prisoner’s Dilemma game. Using the same guilt induction paradigm, de Hooge and colleagues (2007) showed that guilt increased cooperation on a ten-coin give-some dilemma game. Studies have also investigated the influence of priming other emotions aside from guilt on moral judgments with mixed results. Anger induction, for example, was shown to increase judgments of moral permissibility in one study (Ugazio, Lamm and Singer, 2012) but also increased the severity of judgments about crimes against others in another study (Seidel and Prinz, 2013). Disgust is perhaps the emotion whose relationship to moral decision-making has been studied most extensively. Schnall and colleagues (2008) showed that priming feelings of incidental disgust amplified the severity of moral judgments, whereas reducing feelings of disgust had the opposite effect (Schnall, Benton and Harvey, 2008). Similar findings were reported across several other studies (Wheatley and Haidt, 2005; Ugazio, Lamm and Singer, 2012; Seidel and Prinz, 2013). A recent meta-analysis, however, has argued that the evidence supporting the influence of incidental disgust on moral judgment is weaker than originally believed (Landy and Goodwin, 2015). The authors of the meta-analysis suggest manipulations of disgust may trigger unwanted other-blaming emotions directed at the experimenters, such as anger, which could confound attempts to measure the influence of disgust on moral judgment. Based on the current research, we add that the effectiveness of priming incidental emotions in influencing moral judgment may depend in part upon participants’ familiarity with those who might hypothetically benefit from their decisions.

4.5.3. **Future Directions and Limitations**

Translational cognitive neuroscience is a discipline in which cognitive-anatomical components of mental processes described in basic cognitive neuroscience studies are used to inform and advance clinical neuropsychiatry (Zahn, 2009). For
example, the Event-Feature-Emotion Complex (EFEC) model hypothesizes that moral cognitions and emotions arise from the integration of context-dependent representations stored in the prefrontal cortex, perceptual and social features represented in the temporal lobe, and emotional and motivational states in subcortical regions (Moll et al., 2005). More specifically, this model predicts that the right superior anterior temporal lobe (RSATL) represents context-independent social concepts (Zahn et al., 2007) and the subgenual cingulate and adjacent septal region (SCSR) are involved in the experience of guilt (Zahn et al., 2009c). Based on the EFEC model, Green and colleagues (2012) expected and found self-blame related functional decoupling of the RSATL and SCSR in rMDD patients relative to healthy volunteers which the authors suggest may reflect a mechanism underpinning self-blaming emotional biases in MDD. This finding provided an enriched understanding of the functioning of the SCSR which is of known pathophysiological importance to MDD, even acting as the target site for deep brain stimulation in treatment resistant MDD patients (Mayberg et al., 2005). The EFEC model also informed a recent study which probed altruistic behaviour using a charitable donations task in rMDD patients, who exhibited greater SCSR activation while making donations compared to a HC group despite a lack of behavioural group differences (Pulcu et al., 2014b). Although the current research found group differences in preference for social interactions but not for altruism alone, it is possible that patterns of neural activation associated with preference for altruistic choices might provide a more sensitive marker of group differences.

Several limitations of the current research need to be addressed. First, as has been mentioned, dilemmas may engage hypothetical reasoning about altruism in lieu of online moral cognition. This is particularly concerning when studies use dilemmas that describe extreme situations with which participants are unlikely to be familiar (e.g., whether or not to push a large man in front of a runaway trolley to stop it from hitting five others: (Greene et al., 2001)). However, we took care to create dilemmas based on normative data which describe real world scenarios that participants might reasonably be expected to encounter in day-to-day life (e.g., Table 4.2). Second, by indicating their preferred options, participants were not making moral judgments per se. For example, a participant might prefer the option to go home after a long day of work over giving a lift to a stranded colleague even if they believed going home would not be morally permissible. By measuring choice preference, the current research aimed to explore motivation to engage in prosocial behaviours over selfish behaviours. Further to this point, the emotion priming paradigm was designed to modulate feelings of guilt, which
is known to play a role in motivating altruistic behaviours. Therefore, while judgments of choice preference and of moral permissibility may not necessarily converge, asking participants to judge the former provided a unique measure of psychosocial functioning relevant to the aims of the current Studies. Lastly, as was discussed earlier, explicit measures of choice preference may be confounded by social desirability. Although true, this is also the first study of altruistic or moral decision-making that, to our knowledge, has included both explicit and implicit measures of choice preference.

4.5.4. Conclusions

In summary, Study 1 revealed a lower explicit preference for social interactions in rMDD patients compared to a HC group, while no group differences in preference for pure altruism options were found in Study 2. In other words, rMDD patients preferred social interactions equally to HC participants when driven by pure altruism, but less than the HC participants when driven by expectations of reciprocity or selfish gain. These results were inconsistent both with the predictions made using our hypothesis for understanding altruistic choice preference in MDD (i.e., Figure 4.1) and with the hyperaltruism theory of MDD (O’Connor et al., 2007; O’Connor et al., 2012). We suggest that the ACT may provide a novel measure of preference for social withdrawal, a symptom that often co-occurs with core symptoms of MDD, which may be sensitive to depression vulnerability. The current research also showed that preference for pure altruism but not reciprocal altruism options can be increased by priming incidental feelings of guilt. This finding builds upon work in healthy volunteers which showed that guilt priming increased altruistic behaviour to suggest that the effectiveness of emotion priming paradigms in modulating preference for altruistic behaviours may depend in part upon familiarity with the hypothetical beneficiaries of altruistic options. One potential avenue for future research is to relate performance on the ACT to neuroimaging measures of brain functioning, such as task-based or resting-state fMRI, which may be more sensitive than behavioural measures to underlying group differences (e.g., (Pulcu et al., 2014b)).
Chapter 5.

Resting-State Connectivity within a Social Agency Network is Associated with Implicit Prosociality

Authorship Contributions

Clifford I. Workman conceived of and designed the study, conducted the data analysis and interpretation, drafted the article, and approved the final version. Jorge Moll aided in interpretation and provided critical revisions and final approval for the article. Karen E. Lythe, Jennifer A. Gethin, Emma J. Thomas, and Paula D. Trotter aided in data collection and provided critical revisions and final approval for the article. Shane McKie aided in the design of the statistical analyses and provided critical revisions and final approval for the article. John F. W. Deakin aided in the study’s conception and design and provided critical revisions and final approval for the article. Rebecca Elliott aided in the conception and design of the study, data interpretation, and provided critical revisions and final approval for the article. Roland Zahn conceived of and designed the study, data analysis and interpretation, and provided critical revisions and final approval for the article.

Submission

This manuscript will be formatted for submission to The Journal of Neuroscience (http://www.jneurosci.org/). The reference style was chosen for consistency with the other chapters presented in this thesis. The journal’s preferred referencing style will be adopted prior to submission.
Resting-State Connectivity within a Social Agency Network is Associated with Implicit Prosociality

Clifford I. Workman, B.S.\textsuperscript{1,2}, Jorge Moll, M.D., Ph.D.\textsuperscript{3}, Karen E. Lythe, Ph.D.\textsuperscript{2}, Jennifer A. Gethin, M.Res\textsuperscript{2}, Emma J. Thomas\textsuperscript{1}, Paula D. Trotter\textsuperscript{1}, Shane McKie, Ph.D.\textsuperscript{4}, John F. W. Deakin, F.R.C.Psych., Ph.D.\textsuperscript{1}, Rebecca Elliott, Ph.D.\textsuperscript{1}, Roland Zahn, M.D.\textsuperscript{4,5}

\textsuperscript{1}University of Manchester, Institute of Brain, Behaviour and Mental Health, Neuroscience & Psychiatry Unit, UK
\textsuperscript{2}University of Manchester, School of Psychological Sciences, Neuroscience and Aphasia Research Unit, UK
\textsuperscript{3}Cognitive and Behavioral Neuroscience Unit, D’Or Institute for Research and Education, Brazil
\textsuperscript{4}Institute of Psychiatry, Psychology, and Neuroscience, Department of Psychological Medicine, Centre for Affective Disorders, King’s College London, UK

5.1. ABSTRACT

The capacity for attributing causality to oneself and to others, or “sense of agency”, is essential for engaging in moral cognition and altruistic behaviour. The subgenual cingulate and adjacent septal region (SCSR) has been associated with sense of agency and with experiencing guilt. Activation of the SCSR during altruistic decision making distinguished healthy volunteers from patients with remitted major depressive disorder (rMDD). The relationship between agency, guilt, and altruism, however, is poorly understood. Since moral cognition is thought to emerge from the binding of representations stored across spatially distributed brain regions, the present study investigated whether resting-state SCSR networks were associated with altruistic choice preference during the experience of guilt in rMDD patients and HC participants. Forty-five medication-free rMDD patients and 30 healthy controls underwent resting-state fMRI scanning. Seed-based connectivity to the SCSR was related to performance on a novel task designed to investigate the influence of guilt on explicit and implicit preferences for altruistic and social choices relative to selfish choices. This relationship was investigated across the whole brain and with \textit{a priori} regions of interest implicated in studies of affiliative emotions and social reward processing. Across all participants, connectivity between the SCSR and the right temporoparietal junction was negatively associated with implicit preference for prosocial options (i.e., altruism and social options). This relationship was weaker in the rMDD patients, particularly for altruistic choice preference. We observed a negative relationship between implicit prosociality and connectivity within a social agency network. The nature of this relationship distinguished the rMDD patients from the HC participants and could reflect a vulnerability factor for MDD. Future studies should adapt the task used here to investigate the relationship of task-based connectivity within this network to implicit prosociality.
5.2. INTRODUCTION

The capacity for attributing causality to oneself and to others, also referred to as “sense of agency” (Moll et al., 2007), is a critical component of moral behaviour. Guilt, for example, is thought to motivate reparative behaviours in individuals who have committed moral transgressions (Tangney, Stuewig and Mashek, 2007). Guilt is therefore considered a moral emotion, or an emotion which motivates behaviours aimed at improving the welfare of others (Zahn, de Oliveira-Souza and Moll, 2015). A prerequisite for experiencing guilt is feeling responsible for having committed a moral transgression which may have negatively impacted other agent(s). In other words, without the ability to assign causality to agents, one cannot hold oneself or others responsible for actions. Interestingly, guilt has been shown to positively correlate with self-reported altruistic behaviour in healthy participants (Yi et al., 2005), which is consistent with the view that sense of agency is essential to altruistic behaviour (Tankersley, Stowe and Huettel, 2007). However, the relationship between sense of agency, self-blaming moral emotions including guilt, and altruism is poorly understood.

Patients with major depressive disorder (MDD) may experience guilt to an excessive degree or overgeneralize their feelings of guilt to inappropriate situations (American Psychiatric Association, 2000). Indeed, this represents a distinctive symptom of MDD which is observed cross-culturally (Sartorius et al., 1980). Even patients who have remitted from the depressed state may nevertheless experience a bias towards feeling self-blaming moral emotions including guilt compared to other-blaming emotions such as anger towards others (Green et al., 2013b; Zahn et al., 2015a; Zahn et al., 2015b). In view of data suggesting guilt is positively associated with altruistic behaviour (Yi et al., 2005), it has been proposed that self-blaming biases in MDD may give rise to pathological increases to altruistic behaviour (O'Connor et al., 2011). This could be difficult to observe in current MDD patients, in whom the presence of other symptoms such as social withdrawal may mask changes to preferences for behaving altruistically. Since MDD is a lifetime disorder in the sense that each subsequent major depressive episode (MDE) increases one’s risk for future episodes (Kupfer, 1991), and remitted MDD (rMDD) patients have been shown to experience elevated altruistic guilt (Green et al., 2013b), one might expect rMDD patients to demonstrate increased altruistic behaviour. Preliminary findings, however, do not clearly support this view. Using data from a larger survey study, Fujiwara (2009) found that financial support provided to nonfamily members was associated with risk for developing one’s first
MDE. Pulcu and colleagues (2015), however, observed no differences in altruistic behaviour between rMDD patients and healthy control (HC) participants on several neuroeconomical paradigms.

We recently investigated whether modulating feelings of guilt in rMDD patients and HC participants influenced judgments in response to dilemmas which pitted reciprocal altruism or social options against selfish options (Workman et al., in preparation-a). Reciprocal altruism options described opportunities to help friends or colleagues who might reasonably be expected to reciprocate at a later time (Trivers, 1971), social options included a social component but without any helping behaviours, and selfish options included no social component and no helping behaviours. Rather than demonstrating an increased preference for altruistic behaviours when primed to feel guilt, rMDD patients had explicitly lower preferences relative to HC participants for all “prosocial” options, or options which included a social component (Workman et al., in preparation-a). Although current evidence suggests rMDD patients may not experience pathological changes to altruistic behaviour, this could be a consequence of compensatory mechanisms acting at the neuronal level. Indeed, a recent study reported an enhanced neural response in the subgenual cingulate cortex during charitable donations in rMDD patients compared to HC participants despite a lack of behavioural differences (Pulcu et al., 2014b).

A spatially distributed network of brain regions is thought to underlie the capacity for engaging in moral cognition and experiencing moral emotions (Moll et al., 2005; Zahn, de Oliveira-Souza and Moll, 2015). According to the Event-Feature-Emotion Complex (EFEC) model, sequential representations of events are localized to the frontal cortex, temporal regions represent perceptual features and social concepts, and subcortical and limbic regions represent undirected emotional and motivational states (Moll et al., 2005; Zahn, de Oliveira-Souza and Moll, 2015). These regions do not act in isolation but rather as part of larger networks through which information is bound together to give rise to moral cognitions. For example, social attachment/affiliation and social reward processing are thought to recruit frontal regions such as the medial frontopolar and orbitofrontal cortices, as well as subcortical and limbic structures such as the ventral striatum, ventral tegmentum, septal nuclei, and hypothalamus (Moll et al., 2006; Krueger et al., 2007; Zahn et al., 2009c; Moll et al., 2012; Zahn, de Oliveira-Souza and Moll, 2015). One such limbic region, the subgenual cingulate and adjacent septal region (SCSR), is currently considered the best candidate for selective involvement in moral cognition (Zahn, de Oliveira-Souza and Moll, 2015). Activation
of the SCSR has been associated with making charitable donations (Moll et al., 2006), with both self- and other-blaming emotions (Lythe, personal communication), and with individual differences in guilt proneness (Zahn et al., 2009c), in empathic concern (Zahn et al., 2009a), and in perceptions of one’s family as a distinct group (Rüsch et al., 2014). Lesions to the ventromedial prefrontal cortex (VMPFC), which typically encompasses the SCSR, have been associated with reduced feelings of guilt (Koenigs and Grafman, 2009) and increased stereotypical attitudes (Gozzi et al., 2009). Taken together, the SCSR is currently believed to play roles in both the experience of blame and in representing agency.

Interestingly, aside from its involvement in blame and agency, the subgenual cingulate is also considered a key region in the pathophysiology of MDD (Mayberg, 2003). Our group demonstrated abnormal connectivity between the SCSR and superior anterior temporal lobe during the experience of guilt in rMDD patients (Green et al., 2012; Lythe et al., 2015). According to the EFEC model, atypical integration of the social conceptual information and self-blame represented by these regions could give rise to pathological guilt in MDD. Indeed, we recently showed that increased connectivity between the SCSR and anterior temporal lobe during the experience of guilt was predictive of subsequent recurrence in a group of MDD patients (Lythe et al., 2015). Resting-state functional MRI (fMRI), which is frequently used to identify synchronous patterns of low frequency signal fluctuations across spatially distributed brain regions (Fox and Raichle, 2007), has also revealed abnormal connectivity to the SCSR in MDD. Current MDD patients, for example, demonstrated increased connectivity to the SCSR (reviewed by (Dutta, McKie and Deakin, 2014)), and resilience to recurrent MDEs was associated with reduced interhemispheric SCSR connectivity (Workman et al., in preparation-b). The SCSR is part of the ventral default mode network (DMN), a collection of medial prefrontal brain regions that are active at rest and are thought to be associated with self-referential thought (Buckner, Andrews-Hanna and Schacter, 2008) and implicit goal-related thinking (Bado et al., 2014). Although the relationship of altruism to task-based activation of the SCSR has been investigated in rMDD patients (Pulcu et al., 2014b), the relationship of altruistic behaviour to resting-state SCSR networks in rMDD has not been established.

The present study investigated whether preference for reciprocal altruism and/or social choices, measured both explicitly and implicitly, was associated with patterns of resting-state connectivity to the SCSR in rMDD patients and HC participants. We predicted that choice preference would be associated with connectivity to the SCSR,
specifically connectivity to regions implicated in social attachment and reward (Moll et al., 2006; Krueger et al., 2007; Zahn et al., 2009c; Moll et al., 2012; Zahn, de Oliveira-Souza and Moll, 2015), and that these associations would differ between the rMDD patients and HC participants. Since the SCSR is associated with functions critical to moral cognition and altruism, as has been discussed, if group differences in the relationship between SCSR connectivity and choice preference were observed, this could indicate that rMDD patients experience dysfunction within an implicit moral sensitivity network (Moll et al., 2007).

5.3. METHODS

5.3.1. Participants

The present study obtained ethical approval from the South Manchester National Health Service Research Ethics Committee (Ref No: 07/H1003/194). After the study procedures were explained in full, participants provided informed consent (verbal for telephone screening and written consent at the start of each study visit). Both print and online advertisements were used to recruit participants as part of the larger UK Medical Research Council-funded “Development of Cognitive and Imaging Biomarkers Predicting Risk of Self-Blaming Bias and Recurrence in Major Depression” project (Lythe et al., 2015; Zahn et al., 2015b). Telephone screening was used to preliminarily assess eligibility in 707 participants (a copy of the telephone screening document can be obtained at: http://www.translational-cognitive-neuroscience.org/start/test-materials). Participants who were eligible after the telephone screening were invited to complete a clinical interview supervised by a senior psychiatrist (RZ) which probed psychiatric, clinical, and family histories. Past major depressive episodes (MDE) were diagnosed with the Structured Clinical Interview-I for DSM-IV-TR (SCID-I; (First et al., 2002); inter-rater reliability ranged from moderate to perfect agreement, Table S2.1). Inclusion criteria were: right handed, native English speaker, at least 18 years of age, and normal or corrected-to-normal hearing and vision. Additionally, rMDD patients must have had a moderate to severe DSM-IV-TR diagnosed MDE (World Health Organization, 1992; American Psychiatric Association, 2000) and must have been in remission for at least 6 months. The exclusion criteria were: current or relevant past Axis I disorders (e.g., substance abuse), a Montgomery-Åsberg Depression Rating Scale (MADRS) score >10 (Montgomery and Åsberg, 1979; Zimmerman, Posternak and Chelmins, 2004), recent exposure to psychotropic medications (8 weeks for fluoxetine, 4 weeks otherwise), history of major medical or
### Reasons for Telephone Pre-Screening Exclusions

<table>
<thead>
<tr>
<th>Reason</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI contraindications</td>
<td>77</td>
</tr>
<tr>
<td>Psychiatric disorders other than MDD</td>
<td>54</td>
</tr>
<tr>
<td>Current antidepressants or other centrally active medications</td>
<td>52</td>
</tr>
<tr>
<td>Withdrawal after telephone pre-screening</td>
<td>33</td>
</tr>
<tr>
<td>Not meeting full screening criteria for MDD</td>
<td>30</td>
</tr>
<tr>
<td>Family history of MDD/bipolar/schizophrenia (HC group)</td>
<td>26</td>
</tr>
<tr>
<td>Substance or alcohol abuse</td>
<td>23</td>
</tr>
<tr>
<td>Current antihypertensive or statin medications</td>
<td>20</td>
</tr>
<tr>
<td>Left-handed</td>
<td>20</td>
</tr>
<tr>
<td>Non-native English speaker</td>
<td>19</td>
</tr>
<tr>
<td>Thyroid function problems</td>
<td>19</td>
</tr>
<tr>
<td>Fulfilling criteria for current MDD</td>
<td>13</td>
</tr>
<tr>
<td>History of cancer</td>
<td>7</td>
</tr>
<tr>
<td>Not remitted for long enough (&gt;6 months)</td>
<td>7</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>5</td>
</tr>
<tr>
<td>No reason recorded</td>
<td>5</td>
</tr>
<tr>
<td>Other general medical conditions</td>
<td>5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4</td>
</tr>
<tr>
<td>Out of age range (18 – 65 years)</td>
<td>4</td>
</tr>
<tr>
<td>Excluded because of age-matching (HC group)</td>
<td>3</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>3</td>
</tr>
<tr>
<td>History of stroke</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>1</td>
</tr>
</tbody>
</table>

Total excluded after the telephone pre-screening: 431 / 707

### Reasons for Clinical Interview Exclusions (remitted MDD patients)

<table>
<thead>
<tr>
<th>Reason</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unable to schedule for additional visits</td>
<td>10</td>
</tr>
<tr>
<td>Fulfilling criteria for a bipolar disorder</td>
<td>6</td>
</tr>
<tr>
<td>Fulfilling criteria for current social anxiety disorder</td>
<td>6</td>
</tr>
<tr>
<td>Not meeting full criteria for MDD</td>
<td>5</td>
</tr>
<tr>
<td>Fulfilling criteria for past substance abuse</td>
<td>4</td>
</tr>
<tr>
<td>Not remitted for long enough (&gt;6 months)</td>
<td>3</td>
</tr>
<tr>
<td>Residual symptoms of post-traumatic stress disorder</td>
<td>3</td>
</tr>
<tr>
<td>Probable personality disorders</td>
<td>2</td>
</tr>
<tr>
<td>Fulfilling criteria for current generalized anxiety disorder</td>
<td>1</td>
</tr>
<tr>
<td>MRI contraindications</td>
<td>1</td>
</tr>
<tr>
<td>Withdrawal after the clinical interview</td>
<td>1</td>
</tr>
</tbody>
</table>

Total number of remitted MDD patients excluded after the clinical interview: 42 / 138

### Reasons for Clinical Interview Exclusions (HC group)

<table>
<thead>
<tr>
<th>Reason</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unable to schedule for additional visits</td>
<td>6</td>
</tr>
<tr>
<td>Probable or definite positive first degree family history of MDD</td>
<td>4</td>
</tr>
<tr>
<td>Fulfilling criteria for a past MDE lasting less than two months</td>
<td>1</td>
</tr>
<tr>
<td>Fulfilling criteria for current adjustment disorder</td>
<td>1</td>
</tr>
<tr>
<td>Fulfilling criteria for current MDD</td>
<td>1</td>
</tr>
<tr>
<td>Fulfilling criteria for current social anxiety disorder</td>
<td>1</td>
</tr>
<tr>
<td>Non-native English speaker</td>
<td>1</td>
</tr>
<tr>
<td>Past depressive episode not fulfilling criteria for a past MDE</td>
<td>1</td>
</tr>
</tbody>
</table>

Total number of HC participants excluded after the clinical interview: 16 / 64

---

Table 5.1 Reasons for exclusion of volunteers from the current study. Of the 707
volunteers who completed the telephone pre-screening, 276 were eligible (184 remitted MDD patients, 92 HC participants). Of these, 202 participants agreed to complete the clinical interview after having reviewed the study’s participant information sheet (138 remitted MDD patients, 64 HC participants). Following the clinical interview, 144 participants were eligible to complete the remaining study visits (96 remitted MDD patients, 48 HC participants). Of these, 102 participants underwent resting-state fMRI scanning (63 remitted MDD patients, 39 HC participants). fMRI, functional magnetic resonance imaging; HC, healthy control; MDD, major depressive disorder; MDE, major depressive episode.

neurological disorders, or contraindications for MRI scanning. HC participants also must not have had any history of Axis I disorders and no first-degree family history of relevant psychiatric disorders (e.g., mood disorders or schizophrenia). A thorough overview of reasons for exclusions is provided in Table 5.1. 102 participants underwent resting-state fMRI scanning, of which 75 also completed the Altruistic Choices Task and were included into this study (45 remitted MDD patients, 30 HC participants).

The rMDD patients and HC group were well-matched on demographic variables including age, years of education, sex, and MADRS scores (Table 5.2). There was a trend towards lower scores on the GAF scale in the rMDD patients (M=86.6, SD=5.0) compared to the HC group (M=88.6, SD=3.0; t(73)=1.95, p=0.06). GAF scores in both groups were between 81 and 90, however, thus denoting good overall functioning with few or no symptoms (American Psychiatric Association, 2000). Consistent with our findings from Chapter 4, compared to the HC group, the rMDD patients had higher omnipotent responsibility guilt scores (i.e., guilt associated with feelings of responsibility for the wellbeing of others; t(72)=3.56, p=0.001) and higher survivor guilt scores (i.e., guilt associated with perceiving oneself as better off than others; t(72)=4.05, p<0.0002) as measured with the Interpersonal Guilt Questionnaire (O'Connor et al., 1997).

5.3.2. Altruistic Choices Task (ACT)

The Altruistic Choices Task (ACT) has been described in detail elsewhere (Workman et al., in preparation-a). Briefly, participants who were tested with the ACT underwent emotion priming to modulate feelings of guilt before responding to dilemmas which pitted options to either engage socially or to behave altruistically against options.
<table>
<thead>
<tr>
<th>Demographic Variable</th>
<th>Remitted MDD (N=45) Mean (SD)</th>
<th>HC (N=30) Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37.7 (12.1)</td>
<td>39.7 (13.7)</td>
</tr>
<tr>
<td>Years of Education</td>
<td>17.2 (2.5)</td>
<td>16.8 (2.2)</td>
</tr>
<tr>
<td>Sex (Male / Female)</td>
<td>17 / 28</td>
<td>13 / 17</td>
</tr>
<tr>
<td>MADRS Score</td>
<td>0.9 (1.5)</td>
<td>0.6 (1.1)</td>
</tr>
<tr>
<td>GAF Score</td>
<td>86.6 (5.0)</td>
<td>88.6 (3.0)</td>
</tr>
<tr>
<td>Omnipotent Responsibility Guilt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.0 (6.4)</td>
<td>41.1 (7.8)</td>
</tr>
<tr>
<td>Survivor Guilt&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.5 (11.4)</td>
<td>58.2 (9.4)</td>
</tr>
<tr>
<td>Framewise Displacement (mm)</td>
<td>0.24 (0.13)</td>
<td>0.27 (0.15)</td>
</tr>
</tbody>
</table>

**Table 5.2** Demographic variables in the remitted MDD patients and HC group. The remitted MDD patients and HC group did not significantly differ on the demographic variables (Contingency Coefficient<0.06, \( p > 0.63 \); \( t < 0.83, \ p > 0.41 \)), although there was a trend towards lower GAF scores in the remitted MDD patients relative to the HC group (\( t(73) = 1.95, \ p = 0.06 \)). GAF, Global Assessment of Functioning; HC, healthy control; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder.

<sup>a</sup>Significantly different between the remitted MDD and HC groups (\( t(72) = 3.56, \ p = 0.001 \)).

<sup>b</sup>Significantly different between the remitted MDD and HC groups (\( t(72) = 4.05, \ p < 0.0002 \)).

to behave selfishly. Participants responded to dilemmas by indicating their choice preference which was measured explicitly as preference for given options (explicit choice preference) or implicitly using the Brief Implicit Association Test (BIAT; implicit choice preference; (Sriram and Greenwald, 2009)). The ACT was administered over two counterbalanced blocks with guilt primed in one block and a different emotion primed in the other block (indignation or an emotional baseline), although the present study focused only on choice preference measurements acquired during the guilt priming condition because of the aforementioned association between guilt and the SCSR.

5.3.3. *ACT: Emotion Priming Paradigm*

The ACT uses a novel emotion priming paradigm to modulate feelings of guilt, with the guilt priming condition expected to trigger depressogenic cognitions in the rMDD patients (Teasdale, 1983). At the start of the guilt priming condition, participants were asked to read a story told from the first-person perspective in which the participant had grievously injured another person by driving recklessly. Before reading the story,
participants were instructed to experience any emotions they might expect to feel had the story actually happened. Participants were then asked to read 30 brief statements (3 words long) related to the guilt priming story and to rate each statement along a 7-point Likert scale (1=not guilty to 7=very guilty). Individual statements were then presented in a random and non-repeating order immediately before each dilemma to sustain the effects of the guilt priming. Participants who completed the guilt priming condition during the second block of the ACT completed the Serial Sevens subtraction task immediately beforehand as a distractor to reduce emotional carryover across blocks (Folstein, Folstein and McHugh, 1975).

5.3.4. ACT: Dilemmata and Explicit Choice Preference

After reading the guilt priming story and rating corresponding statements, participants were presented with a series of dilemmas which pitted a selfish option without any social component (nonsocial-selfish) against a prosocial option (reciprocal altruism or selfish with a social component [social]). As has been discussed elsewhere, care was taken to ensure the dilemmas were ecologically valid and matched on length and semantic coherence (Workman et al., in preparation-b). Participants responded to 15 reciprocal altruism dilemmas and 15 social dilemmas which were presented in a random and non-repeating order. Each reciprocal altruism dilemma corresponded to a social dilemma that was matched for content. An example set of matched reciprocal altruism and social dilemmas is provided in Table 4.2. In response to each dilemma, participants were asked “Would you prefer to …” and then selected the option they most preferred (i.e., the reciprocal altruism or non-social selfish option, the social or nonsocial-selfish option). Explicit reciprocal altruism choice preference was calculated as the ratio of preferred reciprocal altruism options to 15 (i.e., the total number of reciprocal altruism dilemmas), and explicit social choice preference was calculated as the ratio of preferred social options to 15 (i.e., the total number of social dilemmas).

5.3.5. ACT: Implicit Choice Preference

The BIAT, designed to measure implicit associations between objects or concepts (Sriram and Greenwald, 2009), was adapted in the current study to provide a surrogate measure of implicit choice preference. The purpose of each BIAT was to determine whether participants were quicker to associate the concept “preferred” with a nonsocial-selfish option or with a prosocial option (i.e., reciprocal altruism or social) thus reflecting a stronger implicit preference for that option. Each BIAT corresponds to a single reciprocal altruism or social dilemma (see Table 4.3 for an example set of BIAT
stimuli) and was presented after participants indicated their explicit choice preference in response to the corresponding dilemma. Each BIAT is comprised of 16 phrases (1 to 2 words long) separable into four categories. The first two categories contain four phrases each which represent the response options provided in the corresponding dilemma. The third and fourth categories also contained four phrases each and represented the concepts of “preferred” and “non-preferred”, respectively. Each BIAT consists of two blocks and participants were presented with all 16 phrases in an interleaved, random, and non-repeating order over each block. Participants pressed the “k” key any time words from a “focal” category appeared and the “d” key any time words from a “non-focal” category appeared. With reference to the example stimuli in Table 4.3, in the first block participants were instructed to press “k” when phrases from the “go relax” and “preferred” categories appeared and “d” for everything else. In the second block, participants were instructed to press “k” for words from the “give lift” and “preferred” categories and “d” for everything else. The latency between the presentation of each stimulus item and the appropriate keystroke was recorded for each BIAT, and participants were asked to correct any mistakes as quickly as possible. Participants responded to eight BIATs corresponding to reciprocal altruism dilemmas and eight BIATs corresponding to social dilemmas. Due to restrictions in administration time, it was not possible to include BIATs for each dilemma.

To calculate implicit choice preference, it was first necessary to calculate BIAT D scores for each BIAT using a previously published algorithm (Greenwald, Nosek and Banaji, 2003). BIAT D scores were calculated by taking the mean latency from one block (M1) and subtracting this from the mean latency from the other block (M2) and then dividing this by the standard deviation for all latencies across both blocks (i.e., M2-M1/SD). The mean latency M2 always corresponded to the block in which participants associated “preferred” with nonsocial-selfish phrases, and M1 always corresponded to the block in which participants associated “preferred” with prosocial phrases, such that a positive BIAT D score indicates a stronger implicit association between “preferred” and prosocial phrases (i.e., if participants are slower to associate “preferred” with nonsocial selfish options, or conversely quicker to associate “preferred” with prosocial options, M2 will be larger than M1 thusly yielding a positive BIAT D value). BIAT D scores for individual BIATs were excluded when participants made 10 or more errors, or if they responded in under 300ms to at least 10% of trials. Implicit choice preference was then calculated as the mean BIAT D value for all useable BIATs corresponding to each dilemma type (i.e., reciprocal altruism, social). However, BIATs corresponding to
a given type of dilemma (e.g., reciprocal altruism) were excluded for a participant if fewer than four of eight BIAT D scores were usable.

5.3.6. **MRI Image Acquisition**

MRI scanning was carried out on a 3T Philips Achieva scanner (Philips Medical Systems, Netherlands) with an 8-channel coil. Resting-state echo-planar images (EPI) optimized for detecting ventral frontal signal were acquired for each participant (240 volumes; 40 axial slices; 3mm slice thickness; ascending sequential acquisition; repetition time: 2000ms; echo time: 22ms; field of view: 240x240x120mm; acquisition matrix: 80x80 voxels; reconstructed voxel size: 3mm3; flip angle: 90°). Participants were told to remain awake and still with eyes open throughout the resting-state EPI scan. Afterwards, participants were debriefed to ensure adherence to these instructions. Three-dimensional T1-weighted magnetization-prepared rapid-acquisition gradient-echo (MPRAGE) structural images were also acquired for each participant (160 axial slices; 0.9mm slice thickness; repetition time: 8.4ms; echo time: 3.9ms; field of view: 240x191x144mm; acquisition matrix: 256x163 voxels; reconstructed voxel size: 0.94x0.94x0.9mm; flip angle: 8°).

5.3.7. **Resting-State fMRI Analysis**

The methods used to pre-process the resting-state fMRI data have been provided elsewhere (Workman et al., in preparation-b, in press). EPIs were pre-processed with the SPM8 (http://www.fil.ion.ucl.ac.uk/spm/), DPARSFA (Chao-Gan and Yu-Feng, 2010), and Artifact Detection Tools (ART; http://web.mit.edu/swg/software.htm) MATLAB (MathWorks) toolboxes. Briefly, each EPI underwent the following pre-processing steps: 1. Discarding of the first 10 volumes, 2. Slice timing and head motion correction, 3. Creation of regressors for high-motion volumes with ART, 4. Co-registration of the corresponding MPRAGE image to the EPI, 5. Linear detrending and nuisance covariates regression (24 motion parameters (Friston et al., 1996), white matter and cerebrospinal fluid signal, ART regressors), 6. Normalization with parameters created during segmentation of the co-registered MPRAGE, 7. Smoothing with a 6mm kernel, 8. Band-pass filtering to retain frequencies between 0.01Hz-0.08Hz, 9. Removal of high motion volumes identified by ART, 10. Removal of segments of data with fewer than five contiguous volumes. Each resulting EPI contained at least 150 volumes (i.e., 5 minutes of data).

The pre-processed resting-state EPIs were subjected to a seed-based connectivity analysis. The left SCSR was chosen as the seed region (Montreal Neurological Institute
[MNI] coordinates: -4, 23, -5; 6mm sphere; (Green et al., 2012; Lythe et al., 2015;
Workman et al., in preparation-b, in press)). This region has demonstrated abnormal
guilt-related functional connectivity in rMDD patients (Green et al., 2012; Lythe et al.,
2015) and is in close proximity to an SCSR region associated with individual
differences to guilt proneness and empathic concern in healthy volunteers (Zahn et al.,
2009a; Zahn et al., 2009c). The time course of the left SCSR seed region was extracted
from each EPI and correlated with the time course of all other voxels in the brain,
producing seed-based connectivity maps that were then Fisher Z-transformed to
improve normality.

Connectivity maps for all participants were entered into two separate one-sample
t-tests in SPM8. The first one-sample t-test included explicit reciprocal altruism and
social choice preference as covariates. The second one-sample t-test included implicit
reciprocal altruism and social choice preference as covariates. Contrasts determined
whether connectivity to the SCSR was positively or negatively associated with
reciprocal choice preference ([0 1 0], [0 -1 0]), social choice preference ([0 0 1], [0 0 -
1]), or prosocial choice preference ([0 1 1], [0 -1 -1]). Five a priori regions of interest
(ROIs) were also used: medial frontopolar cortex (mFPC)/left anterior orbitofrontal
cortex (aOFC), bilateral ventral striatum, bilateral ventral tegmental area, bilateral septal
region, and bilateral hypothalamus. These ROIs were chosen because they have been
implicated in studies of social attachment, affiliative emotions, and social reward
processing (Moll et al., 2006; Krueger et al., 2007; Zahn et al., 2009c; Moll et al., 2012;
Zahn, de Oliveira-Souza and Moll, 2015). An overview of how these ROIs were created
has been provided elsewhere (Zahn et al., 2009c), with the exception of the ventral
striatum and mFPC/left aOFC ROIs. The ventral striatum ROI was created by placing
two 7mm spheres at the following MNI coordinates: ±10, 10, -2. The mFPC/left aOFC
ROI was created by placing two 6mm spheres at the following MNI coordinates from
(Moll et al., 2006): 0, 55, 1 (mFPC) and -5, 40, -1 (left aOFC). Both ROIs were then
smoothed with a 6mm kernel. Results were only considered significant if they survived
a voxel-level familywise error (FWE)-corrected threshold of \( p < 0.05 \) across the whole
brain and a priori ROIs. The mean Fisher Z-transformed correlation coefficients were
extracted from any significant cluster and entered into an analysis of covariance
(ANCOVA) which included connectivity to the SCSR seed region as the dependent
variable, group (rMDD or HC) as a between-subjects factor, and covariates for
preference for both reciprocal altruism and social choice preference. Results were
considered significant at \( p < 0.05 \) two-tailed.
5.4. RESULTS

5.4.1. Explicit Choice Preference

No regions demonstrated patterns of connectivity to the SCSR that were positively or negatively correlated with explicit choice preference for reciprocal altruism options, social options, or prosocial options (i.e., both reciprocal altruism and social options).

5.4.2. Implicit Choice Preference

Implicit preference for prosocial options (i.e., both reciprocal altruism and social options) correlated negatively with connectivity between the left SCSR and right temporoparietal junction (rTPJ; Table 5.3, Figure 5.1). No other regions demonstrated patterns of connectivity to the SCSR that were positively or negatively correlated with implicit choice preference. The extracted cluster means from the rTPJ were entered into an ANCOVA as described above. A three-way interaction between group, implicit reciprocal altruism choice preference, and implicit social choice preference was observed ($F(1,58)=4.22$, $p=0.04$, $\eta^2_p=0.07$). A two-way interaction between implicit reciprocal altruism choice preference and implicit social choice preference was also observed ($F(1,58)=6.95$, $p=0.01$, $\eta^2_p=0.11$), as was a main effect for implicit reciprocal altruism ($F(1,58)=10.20$, $p=0.002$, $\eta^2_p=0.15$). No other interactions or main effects were observed ($F(1,58)<2.46$, $p>0.12$, $\eta^2<p<0.04$). Regarding the three-way interaction, parameter estimates revealed that the interaction between implicit reciprocal altruism choice preference and implicit social choice preference was more negative for the HC group than the rMDD patients ($B=-1.99$, $SEM=0.98$, $t=-2.05$, $p=.04$, $\eta^2_p=0.07$). We further investigated the nature of this three-way interaction by plotting SCSR-rTPJ connectivity against implicit choice preference (reciprocal altruism and social; Figure 5.2). For the HC group, the model fit for implicit reciprocal altruism choice preference was $R^2=0.27$, and for implicit social choice preference it was $R^2=0.23$. For the rMDD group, the model fit for implicit reciprocal altruism choice preference was $R^2=0.08$, and for implicit social choice preference it was $R^2=0.13$.

5.4.3. Investigation of Potentially Confounding Variables

Although the rMDD patients and HC group did not significantly differ on any demographic variables, there was a trend towards lower GAF scores in the rMDD patients compared to the HC group. We therefore investigated the relationship of GAF scores to the current findings to ensure they were not driven by heterogeneity in
psychosocial functioning. Connectivity between the SCSR and rTPJ, however, was not correlated with GAF scores ($r=-0.03$, $p=0.79$). Adding GAF scores as a covariate of no interest into the ANCOVA described previously also revealed no main effect of GAF scores ($F(1,57)=0.03$, $p=0.87$, $\eta^2_p=0.0004$). Furthermore, despite including GAF scores into the model, the three-way interaction described previously remained significant ($F(1,58)=4.16$, $p=0.046$, $\eta^2_p=0.07$) as did the two-way interaction ($F(1,58)=6.71$, $p=0.01$, $\eta^2_p=0.11$) and main effect for implicit reciprocal altruism ($F(1,58)=10.02$, $p=0.002$, $\eta^2_p=0.15$). As before, no other interactions or main effects were observed ($F(1,58)<2.43$, $p>0.12$, $\eta^2_p<0.04$). Importantly, the groups were well-matched on framewise displacement (Table 5.1), a measure of relative head displacement between

**Figure 5.1** Connectivity between the left SCSR and right TPJ was negatively correlated with implicit prosocial (i.e., reciprocal altruism and social) choice preference across all participants. The right TPJ cluster is displayed at an uncorrected voxel-level threshold of $p<0.001$ on a whole-brain image which has been cropped to reveal the left SCSR seed region. L, left; R, right; TPJ, right temporoparietal junction; SCSR, subgenual cingulate/septal region.
**Figure 5.2** a) Scatterplot of mean Fisher z-transformed SCSR-rTPJ correlation coefficients extracted from the rTPJ cluster relative to implicit choice preference (reciprocal altruism in red, social in blue) in the remitted MDD patients. For the relationship between SCSR-TPJ connectivity and implicit reciprocal altruism choice preference, the model fit was $R^2=0.08$. For implicit social choice preference, the model fit was $R^2=0.13$. 

b) Scatterplot of mean Fisher z-transformed SCSR-rTPJ correlation coefficients extracted from the rTPJ cluster relative to implicit choice preference (reciprocal altruism in red, social in blue) in the healthy control participants. For the relationship between SCSR-TPJ connectivity and implicit reciprocal altruism choice preference, the model fit was $R^2=0.27$. For implicit social choice preference, the model fit was $R^2=0.23$. 

MDD, major depressive disorder; rTPJ, right temporoparietal junction; SCSR, subgenual cingulate/septal region.
Table 5.3 Regions whose connectivity to the subgenual cingulate/septal region was negatively correlated with implicit prosocial choice preference across all participants. FWE, familywise error; MNI, Montreal Neurological Institute; R, right.

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Regions</th>
<th>Peak MNI Coordinates</th>
<th>Peak z Score</th>
<th>Cluster Size</th>
<th>FWE-Corrected p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>Temporoparietal junction</td>
<td>X: 57 Y: -72 Z: 24</td>
<td>4.78</td>
<td>23</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Table 5.3 Regions whose connectivity to the subgenual cingulate/septal region was negatively correlated with implicit prosocial choice preference across all participants. FWE, familywise error; MNI, Montreal Neurological Institute; R, right.

FWE-corrected at the voxel-level over the whole brain.

contiguous volumes (Power et al., 2012), suggesting the three-way interaction between group and implicit choice preferences is not a consequence of one group having moved more than the other whilst in the scanner.

5.5. DISCUSSION

Consistent with our hypotheses, implicit choice preference for prosocial options (i.e., both reciprocal altruism and social options) was associated with connectivity to the SCSR, and the relationship of connectivity to implicit choice preference for both reciprocal altruism and social options distinguished the rMDD patients from the HC participants. Intriguingly, implicit prosocial choice preference was negatively correlated with SCSR connectivity to the rTPJ but not to the regions involved in social attachment and reward that were predicted. In HC participants, similar relationships were observed between SCSR-rTPJ connectivity and implicit choice preference for reciprocal altruism and for social options. The rMDD patients, on the other hand, demonstrated comparatively weaker relationships between SCSR-rTPJ connectivity and implicit choice preferences, particularly for reciprocal altruism options.

Connectivity between the SCSR and rTPJ, regions which have been implicated in sense of agency (Decety and Lamm, 2007; Gozzi et al., 2009; Rüsch et al., 2014), was associated with implicit but not explicit choice preference. This is in keeping with recent work by Yoder and colleagues (2015) in which implicit but not explicit moral cognition produced greater signal change in the subgenual cingulate associated with psychopathy in incarcerated individuals. Our results therefore suggest that resting-state connectivity within a social agency network is associated with implicit preference for prosociality. This interpretation is generally consistent with the view that the capacity to perceive agency is a prerequisite for altruism (Tankersley, Stowe and Huettel, 2007), although this may be more domain general (i.e., prosociality rather than altruism). We
argued previously that lower explicit preference for prosocial options in rMDD patients relative to HC participants reflects a preference for social withdrawal-like behaviour (Workman et al., in preparation-a), a symptom which often co-occurs with the core symptoms of MDD (Zahn et al., 2015b) and impaired social functioning is a known risk factor for MDD (Burcusa and Iacono, 2007). We speculate that the distinctive relationship between SCSR-rTPJ connectivity and implicit prosocial choice preference we observed in rMDD patients may represent a risk factor for such social withdrawal-like symptoms, which is consistent with work showing differential activation of the rTPJ in individuals experiencing different degrees of social isolation (Cacioppo et al., 2009).

We noted earlier that the SCSR is part of the ventral DMN. In addition to this ventral compartment, the DMN is comprised of dorsal regions including the temporoparietal junction/inferior parietal lobule (Buckner, Andrews-Hanna and Schacter, 2008). A recent meta-analytically informed connectivity study of task-based and resting-state studies reported an association between ventral DMN regions including the SCSR and motivational and emotional states, as was suggested previously, and highlighted the importance of dorsal DMN structures including the rTPJ to social cognition (Amft et al., 2014). Furthermore, several resting-state and task-based fMRI studies have employed seed-based analysis of the rTPJ to demonstrate functional connectivity to medial prefrontal regions including the SCSR (Mars et al., 2012; Bzdok et al., 2013). The present study builds upon these findings by demonstrating that SCSR-rTPJ connectivity is associated with individual differences in implicit prosociality.

The subgenual cingulate has been recruited in previous studies during the experience of blame (Koenigs and Grafman, 2009; Zahn et al., 2009c), and in evaluating agency (Gozzi et al., 2009; Rüsch et al., 2014) as was discussed previously. The rTPJ has also been ascribed a variety of high-level social cognitive functions, such as representing sense of agency, theory of mind, and feelings of empathy (Decety and Lamm, 2007). The clusters reported in these activation-based fMRI studies, however, overlap substantially with rTPJ clusters reported in studies of attention, pointing towards a domain-general function (Decety and Lamm, 2007). For example, the rTPJ was activated more strongly when participants received cues to attend to stimuli but at incorrect locations (Astafiev, Shulman and Corbetta, 2006). We cannot make strong claims about the functioning of the rTPJ based on the present results, although it bears noting that posterior rTPJ clusters such as the one described have been associated more strongly with sense of agency in activation-based fMRI studies (Decety and Lamm,
The rTPJ has also been implicated in studies of moral cognition and altruism. Young and colleagues (2007), for example, observed that activation of both the rTPJ and VMPFC was increased when judging attempted moral harms, and disruption to rTPJ networks altered judgments of moral permissibility (Young et al., 2010). Relatedly, activation of the rTPJ and subgenual cingulate was associated with the interplay between the difficulty or ease of scenarios containing either moral or non-moral content (FeldmanHall, Mobbs and Dalgleish, 2014). Germane to the current study, self-reported altruism was correlated with activation of the rTPJ during an unrelated reaction-time task (Tankersley, Stowe and Huettel, 2007). Furthermore, Morishima and colleagues (2012) reported a relationship between individual differences in preference for behaving altruistically relative to selfishly and gray matter volumes of the rTPJ. In summary, both the SCSR and rTPJ have been implicated in sense of agency among other functions and have been shown to play key roles in moral cognition and in altruism.

The subgenual cingulate has long been considered to play a central role in the pathophysiological processes underpinning MDD (Mayberg, 2003). The SCSR has demonstrated increased metabolic activity and resting-state connectivity in current MDD patients which normalized with treatment (Mayberg, 2003; Dutta, McKie and Deakin, 2014) and is the target site for deep brain stimulation in treatment resistant MDD (Mayberg et al., 2005). We recently reported lower interhemispheric SCSR connectivity in rMDD patients who remained resilient to recurrence compared to patients who experienced a recurrence and to a HC group (Workman et al., in preparation-b). Fewer studies report abnormal functioning of the TPJ in MDD. Two studies, however, report hypofunctioning of the rTPJ in current MDD associated with emotional arousal (Deldin et al., 2000; Moratti et al., 2008). Furthermore, patients with rMDD have demonstrated impairments on tasks designed to probe the functioning of the TPJ (Inoue et al., 2004), and these impairments have been associated with risk for subsequent recurrence (Inoue, Yamada and Kanba, 2006). These studies implicate both the SCSR and, although less directly, the temporoparietal junction in risk for experiencing recurrent MDEs. The present findings additionally suggest the relationship of connectivity between these regions to implicit choice preference may represent a vulnerability factor for MDD, since the rMDD patients studied here were at an elevated risk for experiencing future MDEs (Kupfer, 1991). Additionally, implicit choice preference for reciprocal altruism and social options did not differ between groups (Workman et al., in preparation-a), which is in agreement with previous work in which
rMDD patients activated the SCSR more than HC participants while making altruistic decisions despite a lack of behavioural group differences (Pulcu et al., 2014b).

Several limitations pertaining to the current study need to be discussed. First, we employed an emotion priming paradigm to investigate the influence of guilt on choice preference. Future studies should investigate whether the relationship between connectivity and implicit prosociality that we observed here persists without priming participants to feel guilt. Second, SCSR-rTPJ connectivity was associated with implicit choice preference, which was inferred based on performance on the BIAT. One could argue that our measure of implicit choice preference may be independent of explicit choice preference and consequently difficult to interpret. However, we previously observed significant moderate correlations between explicit and implicit choice preference during the guilt priming condition (Workman et al., in preparation-a). This suggests these measures are indeed related, although they do not provide identical information. Lastly, our decision to use a seed-based approach to analyze our resting-state fMRI data consequently constrained our results to networks which include the SCSR. Given the known importance of the SCSR to MDD (Mayberg, 2003; Mayberg et al., 2005; Koenigs and Grafman, 2009) and its relationship to sense of agency (Gozzi et al., 2009; Rüsch et al., 2014), to the experience of guilt (Zahn et al., 2009c; Green et al., 2012; Lythe et al., 2015), and to empathy and altruism (Zahn et al., 2009a; Pulcu et al., 2014b), the decision to use a seed-based analysis of SCSR connectivity allowed us to contextualize our findings within these clinical and non-clinical literatures.

5.5.1. Conclusions

In summary, the present study revealed a negative relationship across all participants between implicit prosocial choice preference and connectivity of the SCSR to the rTPJ. In other words, since both of these regions have been linked to sense of agency which plays a pivotal role in altruism and in social cognition more generally, we suggest that implicit prosociality is related to connectivity with a social agency network. The nature of the relationship between connectivity and implicit choice preference differed between the rMDD patients and HC participants. Specifically, whereas HC participants demonstrated comparable relationships between connectivity and implicit preference for both reciprocal altruism and social options, this relationship was weaker in rMDD patients for implicit social choice preference and weakest for implicit reciprocal altruism choice preference. We have suggested that the nature of this relationship in rMDD patients may reflect a vulnerability factor for MDD, although
additional research is needed to test this assertion. Future studies could adapt the ACT for use in task-based fMRI studies to investigate connectivity within this social agency network outside of the resting-state. Techniques such as transcranial magnetic stimulation could be used in this context to investigate whether disrupting the functioning of the rTPJ is associated with dysfunction within this social agency network or with changes to implicit prosociality.
Chapter 6.

Discussion

6.1. Purpose

The central aim of this thesis was to investigate the relationship of moral cognition and emotions to the pathophysiology of major depressive disorder (MDD). I used two complementary approaches across four studies in order to address this aim: First, in Chapters 2 and 3, I investigated resting-state functional connectivity to the subgenual cingulate and adjacent septal region (SCSR) in patients with remitted MDD (rMDD) and in healthy control (HC) participants. The SCSR is considered central to the functional neurocircuitry of MDD (Mayberg, 2003; Mayberg et al., 2005; Ressler and Mayberg, 2007). The SCSR has also been implicated in studies of guilt, agency, and altruism (Gozzi et al., 2009; Zahn et al., 2009c; Green et al., 2012; Pulcu et al., 2014b; Rüscher et al., 2014; Lythe et al., 2015). Since MDD is thought to arise from dysfunction within networks rather than individual brain regions (Mayberg, 2003; Seminowicz et al., 2004), and moral cognition and emotions are thought to emerge from binding across networks (Moll et al., 2005), investigating resting-state SCSR connectivity allowed me to identify networks of potential relevance both to MDD and to moral cognition and emotions. Second, in Chapter 3, I investigated the relationship between moral cognition and emotions using the novel Altruistic Choices Task (ACT) paradigm. Specifically, I investigated the influence of experimentally increasing and decreasing feelings of guilt on explicit and implicit preferences for altruism (either directed towards friends and colleagues or towards strangers) or for social options not motivated by altruism relative to selfish options. Patients with current and remitted MDD may experience excessive guilt and self-blaming biases which has been suggested to increase altruistic behaviour (American Psychiatric Association, 2000; Yi et al., 2005; O'Connor et al., 2012; Green et al., 2013b; Zahn, de Oliveira-Souza and Moll, 2013). This relationship has not been conclusively demonstrated, however (Fujiwara, 2009; Pulcu et al., 2015). The ACT was therefore designed to characterise the influence of manipulating a moral emotion, namely guilt, on moral cognition, specifically preferences for engaging in altruistic acts. In Chapter 4, I investigated the relationship of moral cognition and emotions to the pathophysiology of MDD by relating altruistic choice preference during guilt priming as measured with the ACT to resting-state connectivity within SCSR networks.
This discussion opens with a brief summary of the findings presented in Chapters 2-4. Next, the theoretical and clinical implications of these findings are considered. Specifically, I discuss the methodological advantages offered by the ACT before addressing what the ACT results presented in Chapter 3 mean for the hyperaltruism hypothesis of MDD and for the Event-Feature-Emotion Complex (EFEC) model of moral cognition and emotions. I then discuss the importance of considering depressive subtypes to future neuroimaging investigations of MDD before focussing on vulnerability and resilience to MDD. After addressing these implications, I present a number of limitations to the current thesis and attempt to address these where possible. Next, I lay out a plan for future research motivated by the findings presented in Chapters 2-4 before offering concluding remarks.

6.2. Summary of the Findings

In Chapter 2, I aimed to investigate whether patients with a history of melancholic major depressive episodes (MDE) could be distinguished from non-melancholic MDD and HC groups on the basis of resting-state functional connectivity to the SCSR. A seed-based analysis was carried out using a SCSR region of interest (ROI) previously implicated in cross-sectional and longitudinal studies of depression vulnerability (Green et al., 2012; Herringa et al., 2013; Lythe et al., 2015). This approach allowed me to investigate networks which specifically include the SCSR, a region which has been implicated in studies of guilt proneness (Zahn et al., 2009c) and in the pathophysiology of severe and familial forms of MDD (Mayberg, 2003; Ressler and Mayberg, 2007). I reported lower connectivity within a network which included the SCSR, amygdala, and parahippocampal gyrus in rMDD patients with a history of melancholic MDEs compared to the non-melancholic and HC groups. Interestingly, Mayberg’s (2003) limbic-cortical model of MDD considers these regions part of a limbic compartment associated with the vegetative and somatic symptoms which are commonly present in melancholic MDD. The pattern of SCSR-amygdala functional disconnection in the melancholic rMDD patients was not better accounted for by group differences in demographic variables and was not associated with number of previous MDEs, suggesting this may be a primary vulnerability factor for melancholia rather than a consequence of “scarring” from previous MDEs. To my knowledge, this represents the first resting-state functional MRI (fMRI) signature of primary vulnerability to the melancholic subtype of MDD.
In Chapter 3, I aimed to investigate whether patients who remained resilient to recurring MDEs over a 14 month follow up period were distinguishable from recurring episode MDD patients and a healthy control group on the basis of resting-state functional connectivity to the SCSR. A longitudinal task-based fMRI connectivity study recently reported abnormally low connectivity between a right anterior temporal lobe seed region and the SCSR in patients who remained resilient to recurring MDEs (Green et al., 2012; Lythe et al., 2015). The approach used by Lythe and colleagues (2015) to characterise task-based connectivity precluded further investigation of connectivity to the SCSR, but this constraint does not apply to investigations of resting-state connectivity to the SCSR. The seed-based resting-state functional connectivity maps calculated for Chapter 2 were again used. Lower interhemispheric SCSR connectivity distinguished the resilient MDD patients from the recurring episode MDD and HC groups. This pattern of connectivity was not associated with potentially confounding variables such as residual depressive symptoms. Consistent with the present findings, a seed-based structural connectivity study in HC participants used an SCSR ROI selected because it was the target site for psychosurgical interventions historically used to treat MDD to reveal tracts spanning the left and right subgenual cingulate cortices (Schoene-Bake et al., 2010). Furthermore, deficits in interhemispheric coordination have been repeatedly associated with MDD. Electroencephalography was used to demonstrate asymmetrically reduced left frontal activation in both current and remitted depressed patients relative to healthy controls (Henriques and Davidson, 1990, 1991). Interhemispheric connectivity has also been investigated with resting-state fMRI in current MDD using the recently developed technique “voxel-mirrored homotopic connectivity.” These studies have shown lower interhemispheric connectivity between a number of regions including the medial frontal cortices, but not the SCSR, in patients with current MDD compared to healthy volunteers (Guo et al., 2013; Wang et al., 2013; Lai and Wu, 2014). Taken together, although abnormally low interhemispheric connectivity has been observed in current MDD, it would appear that lower interhemispheric SCSR connectivity is a compensatory mechanism which may be a neural signature of resilience to recurring MDEs.

In Chapter 4, I aimed to characterise explicit and implicit preferences for options to socialise or to behave altruistically relative to selfish options in rMDD patients and HC participants while undergoing emotion priming to experimentally modulate feelings of guilt. Guilt is a moral emotion thought to motivate altruism (Zahn, de Oliveira-Souza and Moll, 2013) and excessive guilt and self-blaming biases have
been reported in current and remitted MDD (American Psychiatric Association, 2000; Green et al., 2013b). It has been suggested that elevated guilt in MDD could give rise to pathological manifestations of altruism (O’Connor et al., 2012) although experimental evidence is inconclusive (Fujiwara, 2009; Pulcu et al., 2015). Using the ACT, I found that rMDD patients explicitly preferred prosocial options (i.e., social options and altruism directed towards friends or colleagues) less than healthy volunteers. Irrespective of group, guilt priming increased explicit and implicit preferences for altruism options directed at strangers. Although elevated guilt was detected in the rMDD patients and the guilt priming paradigm effectively modulated preference for behaving altruistically towards strangers, these results are not consistent with the view that elevated guilt in MDD gives rise to increased altruism. Instead, I suggested that in MDD patients the ACT may provide a novel measure of preference for social withdrawal, a symptom which often co-occurs with the core symptoms of MDD (2015b). Furthermore, I suggested that guilt plays a causal role in motivating altruism directed towards strangers but not towards friends and colleagues.

In Chapter 5, I aimed to investigate whether explicit and/or implicit preferences for prosocial options (i.e., social options and altruism directed towards friends or colleagues) during the experience of guilt were associated with resting-state connectivity to SCSR networks across the rMDD and HC participants. I further investigated whether the nature of the relationship between choice preference and SCSR connectivity could distinguish the rMDD patients and HC participants. In addition to being a central region to the pathophysiology of MDD, the SCSR has been associated with the experience of self-blaming moral emotions such as guilt (Zahn et al., 2009c; Green et al., 2012; Lythe et al., 2015), with sense of agency (Gozzi et al., 2009; Rüsch et al., 2014), and with altruism (Pulcu et al., 2014b). Since moral cognition and emotions are thought to arise from coordinated activity across spatially distributed brain regions (Moll et al., 2005; Zahn, de Oliveira-Souza and Moll, 2015), I investigated whether prosocial choice preference correlated with connectivity within resting-state SCSR networks using the seed-based connectivity maps calculated for Chapter 2. Across all participants, implicit preference for prosocial options was negatively associated with connectivity between the SCSR and right temporoparietal junction (TPJ), another region linked to sense of agency (Decety and Lamm, 2007). Furthermore, the relationship of SCSR-TPJ connectivity to implicit preferences for social options and for altruism towards friends and colleagues was weaker in the rMDD patients compared to the HC participants, particularly for implicit preference for
altruism. Taken together, implicit prosociality was negatively associated with connectivity between regions which have been implicated in sense of agency. Furthermore, the relationship of connectivity to implicit choice preferences distinguished the rMDD patients from the HC participants and may reflect a vulnerability factor for MDD.

6.3. Theoretical and Clinical Implications

6.3.1. Theoretical Implications

In order to answer the questions posed in Chapters 4 and 5, I designed a novel Altruistic Choices Task to measure the influence of modulating feelings of guilt on explicit and implicit preferences for altruistic and social options. In designing the ACT, I was careful to consider methodological issues which are commonly present in emotion priming and moral judgment studies (Kahane and Shackel, 2010). As was discussed in Chapter 4, participants who completed the novel emotion priming paradigm read stories told from the first person perspective in order to experimentally increase or decrease feelings of guilt. Short priming sentences were then presented throughout the task to sustain the effects of the emotion priming. The approach typically employed to experimentally induce feelings of guilt is to ask participants to recall guilt-evoking autobiographical memories (Ketelaar and Au, 2003; de Hooge, Zeelenberg and Breugelmans, 2007; Nelissen et al., 2011). In my view, the novel emotion priming paradigm included with the ACT provides a more standardised approach to experimentally induce feelings of guilt. The autobiographical memories used to elicit feelings of guilt in previous studies can vary dramatically between participants. For example, de Hooge and colleagues (2007) note that guilt-evoking autobiographical memories ranged from having been unfaithful to a significant other to having accidentally broken another person’s possession. Another benefit to the ACT is that its dilemmas are highly ecologically valid since they are based on descriptions of actual scenarios encountered by participants from another study (see supplemental material in (Green et al., 2013a)). This is in contrast to previous studies in which participants were asked to respond to moral dilemmas which described scenarios with which participants were unlikely to be familiar (Greene et al., 2001; Greene et al., 2004; Koenigs et al., 2007; Greene et al., 2008; Greene et al., 2009). For example, participants were asked whether it is morally acceptable to sacrifice one life to save several others. Although interesting, scenarios such as this may trigger hypothetical reasoning in lieu of real-world moral cognition (Casebeer, 2003; Moll et al., 2005). Lastly, only a handful of studies have
investigated explicit and implicit moral cognition simultaneously (Harenski and Hamann, 2006; Harenski et al., 2010; Yoder et al., 2015). An additional benefit to the ACT, then, is that it provides a straightforward means of measuring both explicit and implicit aspects of moral cognition.

The ACT was designed in order to test the hyperaltruism hypothesis of MDD against my hypothesis about the relationship of guilt to altruism. The hyperaltruism hypothesis states that elevated guilt and self-blaming biases in MDD (Green et al., 2013b) could increase altruistic behaviour in some patients (O'Connor et al., 2012). Since rMDD patients did not demonstrate increased altruistic behaviour on several neuroeconomical paradigms (Pulcu et al., 2015), as would have been predicted by the hyperaltruism hypothesis, I proposed an alternative hypothesis which suggests that the relationship between self-blaming moral emotions and altruistic choice preference can be modelled as an inverted U-shaped curve (Figure 4.1). According to this hypothesis, rMDD patients and HC participants demonstrate similar preferences for altruism but are situated on opposite sides of the inverted U-shaped curve. This prediction is consistent with reports of increased altruistic guilt in rMDD patients despite normal preferences for altruistic behaviour (Green et al., 2013b; Pulcu et al., 2015). I further predicted that experimentally increasing and decreasing feelings of guilt would increase and decrease altruistic choice preference in the HC group while resulting in an opposite pattern of changes to choice preference in the rMDD patients. In contrast to the predictions inherent in my hypothesis, however, modulating guilt had no effect on preference for altruistic choices directed towards friends and colleagues in either the rMDD patients or the HC participants. Furthermore, while experimentally increasing guilt did increase explicit and implicit preferences for altruism directed towards strangers, this occurred irrespective of group. Instead, rMDD patients demonstrated a lower explicit preference for all options which involved a social component compared to the HC group.

Importantly, these data also fail to provide support for the hyperaltruism hypothesis of MDD. In my view, the present findings, when considered together with previous work suggesting rMDD patients are neither more nor less altruistic than HC participants (Pulcu et al., 2015), represent a consistent failure to provide empirical data in support of the hyperaltruism hypothesis (O'Connor et al., 2012).

The results presented in Chapters 4 and 5 are also relevant to the EFEC model which was discussed at length in section 1.3.4 of the Introduction (Moll et al., 2005; Zahn, de Oliveira-Souza and Moll, 2015). According to the EFEC model, if moral emotions such as guilt play a role in motivating altruistic decisions, this should be
evident when probing the influence of these moral emotions on preferences for altruistic relative to selfish options (Zahn, de Oliveira-Souza and Moll, 2015). Indeed, as was reported in *Chapter 4*, I found that experimentally increasing guilt also increased explicit and implicit preferences for altruism when directed towards strangers but not towards friends and colleagues. These data suggest that the effectiveness of guilt in motivating altruistic behaviours will depend on who stands to benefit from these behaviours. In *Chapter 5*, I reported that resting-state connectivity between the left SCSR and right TPJ was negatively correlated with implicit prosociality across all participants. It would appear, then, that connectivity to the TPJ is not selectively associated with altruistic choice preference *per se*, but rather is associated with overall preference for engaging in social situations. This interpretation is consistent with evidence suggesting the TPJ may serve a domain-general function which supports social cognition (Decety and Lamm, 2007) and with the EFEC model’s interpretation of the functioning of the TPJ as playing a nonspecific role in moral cognition. Indeed, moral judgment was not impaired in patients with Alzheimer’s Disease (Mendez, Anderson and Shapira, 2005) in whom atrophy may affect posterior cortical regions including the TPJ (Zahn, de Oliveira-Souza and Moll, 2015). Importantly, however, rMDD patients showed a weaker relationship than HC participants between SCSR-TPJ connectivity and implicit preference for social options, and weaker still between SCSR-TPJ connectivity and implicit preference for options to behave altruistically towards friends and colleagues. SCSR-TPJ connectivity may therefore demonstrate different relationships to implicit choice preferences depending on depression status and whether choices include an altruistic component. The EFEC model should consequently reconsider incorporating the TPJ into the fronto-temporo-subcortical network which it suggests gives rise to moral cognition and emotions. As is discussed in section 6.4.2, an interesting avenue for future studies would be to administer the ACT to patients with Alzheimer’s disease in whom the TPJ has atrophied. The ACT may be more sensitive to dysfunction in the TPJ than the moral dilemma tasks that have been administered to Alzheimer’s patients previously (Mendez, Anderson and Shapira, 2005).

### 6.3.2. Clinical Implications

Neuroimaging studies of MDD do not typically distinguish between depressive subtypes (e.g., (Greicius *et al.*, 2007; Sheline *et al.*, 2010)). The syndromic heterogeneity of MDD, however, may hamper attempts at identifying its underlying neuropathology. Subtyping MDD patients according to the presence of melancholic
features, which include persistent low mood and psychomotor and vegetative symptoms (American Psychiatric Association, 2000), is a particularly promising way forward since melancholia is associated with consistent clustering of symptoms and with biological homogeneity (Parker et al., 2010). Indeed, in Chapter 2, I reported that lower subgenual cingulate-amygdala connectivity distinguished the melancholic rMDD patients from the non-melancholic and HC groups. As was mentioned in section 6.2, this network overlaps with the limbic compartment of Mayberg’s (2003) limbic-cortical model of MDD, and the limbic compartment is associated with the psychomotor and vegetative symptoms which commonly occur in melancholia (American Psychiatric Association, 2000). This finding is consistent with a previous study that reported functional abnormalities in the subgenual cingulate which distinguished melancholic patients with current MDD from non-melancholic MDD patients and HC participants (Pizzagalli et al., 2004). These findings support the view that there is a neurobiological basis for the melancholic subtype of MDD and underscore the importance of considering depressive subtypes in future neuroimaging studies of MDD. The latter point is particularly relevant for studies investigating the functioning of the subgenual cingulate in MDD, which may demonstrate subtype-specific abnormalities.

To my knowledge, the lower interhemispheric SCSR connectivity I reported as distinguishing the resilient MDD patients from the recurring episode and HC groups is the first resting-state fMRI signature of resilience to recurrence in MDD. It is not, however, the first fMRI signature of resilience. Farb and colleagues found that patients with rMDD who underwent fMRI scanning during a sad mood induction paradigm demonstrated higher activity in the medial prefrontal cortex which predicted recurrence over an 18 month follow up period (Farb et al., 2011). The same study found that stable remission was associated with increased activity in the visual cortex. It is intriguing that so few studies have identified neural signatures of resilience to MDD. This suggests either that comparatively few studies are carried out in populations vulnerable to MDD or that findings from these studies are often negative or weak. For example, whereas abnormal amygdala functioning has been consistently associated with biased emotional processing in current MDD patients, the evidence for such impairments in rMDD is weaker (Elliott et al., 2011). There has historically been a tendency in the literature to investigate the functioning of specific regions rather than characterising dysfunction at the network-level. It has been argued, however, that functional interactions within limbic-cortical networks may underpin heterogeneity in MDD and may provide insight into depression vulnerability (Mayberg, 2003; Seminowicz et al., 2004). I would
therefore encourage future neuroimaging studies of depression vulnerability to consider employing resting-state or task-based connectivity approaches when analysing fMRI data. I would add that cross-sectional neuroimaging studies of vulnerability to MDD must exercise caution when interpreting group differences as indicative of vulnerability. Previous studies have reported both increased and decreased connectivity to the SCSR in participants vulnerable to experiencing future MDEs (Gaffrey et al., 2012; Herringa et al., 2013). It would be a mistake to assume that because connectivity is abnormally increased or decreased that it must reflect an underlying vulnerability factor. Consider the results presented in Chapter 3, for example, in which abnormally low SCSR connectivity was associated with resilience to recurring MDEs. In section 6.4.2, I propose a series of studies aimed at clarifying the relationship between depression status and SCSR connectivity.

In addition to the tendency in the literature to investigate the functioning of brain regions rather than networks in MDD, there is also a tendency to administer neuropsychological tasks which measure dysfunction within cognitive domains that are not selectively impaired in MDD. For example, in section 1.2.2 of the Introduction, I reviewed evidence indicating that a number of cognitive domains are disrupted in current MDD including executive functioning, autobiographical recall, and attentional and memory biases (Clark, Chamberlain and Sahakian, 2009; Elliott et al., 2011; Roiser, Elliott and Sahakian, 2012). After recovery from the depressed state, however, many of these neuropsychological dysfunctions are no longer detectable (Clark, Chamberlain and Sahakian, 2009). I argued that characterising dysfunctions at the interface between cognition and emotion in individuals at risk of developing MDD could yield novel markers of depression vulnerability. I further argued that measures of moral cognition and emotions could prove particularly useful towards this end. The findings presented in Chapter 4, in which I investigated the relationship between moral cognition and emotions in rMDD patients and HC participants, generally support this view. Patients with rMDD, who are at an elevated lifetime risk of developing MDEs (Kupfer, 1991), demonstrated a lower explicit preference for all options which included a social component. I interpreted this as reflecting a preference for social withdrawal-like behaviour in the rMDD patients and suggested this may reflect a vulnerability factor for future MDEs. In section 6.4.2, I outline several potential experiments using the ACT aimed at further characterising the interface between emotion and cognition in other psychiatric disorders.
6.4. Limitations and Future Directions

6.4.1. Limitations

Each of the experimental chapters presented in this thesis relied on retrospective assessment to establish whether or not participants previously experienced MDEs and whether or not past MDEs could be classified according to the melancholic specifier (American Psychiatric Association, 2000). Namely, the Structured Clinical Interview-I for DSM-IV-TR (SCID-I) was used to diagnose past MDEs and the Montgomery-Åsberg Depression Rating Scale (MADRS) was used to assess the severity of the last and most severe MDE. One potential limitation following from this approach is that retrospective assessments may be subject to memory biases. For example, patients may not recall all symptoms that were present during previous MDEs or to what degree they were experienced with full accuracy. Furthermore, patients may recall earlier MDEs with poorer accuracy relative to more recent MDEs. Regarding Chapter 2, in which we investigated patients with and without a history of melancholic MDEs, it should be noted that the retrospective reporting of symptoms was unlikely to be biased towards particular subsets of symptoms such as those associated with the melancholic subtype of MDD (Zahn et al., 2015b). Regarding Chapter 3, in which we investigated patients with a history of MDEs who did and did not experience a recurrence over a 14 month follow up period, this concern is somewhat mitigated by the fact that follow up psychiatric assessments were carried out in the recurring episode MDD patients whilst patients were still in the depressed state whenever possible. Although it is not possible to rule out with certainty that the patients I studied misreported their psychiatric histories, this was an acceptable risk in view of the strengths conferred by assessing patients outside of the depressed state. Studying rMDD patients allowed us to investigate the underlying pathological features of MDD without the potentially confounding presence of current depressive symptomology. Relatedly, co-morbid personality disorders were excluded with greater confidence in the rMDD patients than is possible in patients with current MDD due to the obscuring presence of depressive symptoms. Lastly, since MDD is a highly recurrent disorder (Kupfer, 1991), studying patients remitted from the depressed state allowed us to investigate vulnerability factors for MDD.

Relatedly, it cannot be stated with certainty that the remitted status of the MDD patients studied is not simply a proxy for other personality or psychological factors that are more relevant in characterising the patient group. Personality traits are stable characteristics present in a variety of contexts that shape the way in which one perceives
and interacts with the environment (American Psychiatric Association, 2000). Theories of personality typically incorporate at least two dimensions, including neuroticism and extraversion (Eysenck, 1991). Patients with a history of recurrent depression had higher neuroticism personality scores, which encompasses feelings including anxiety, guilt, and low mood, compared to patients with only one past MDE (Duggan et al., 1995). Trait extraversion (i.e., sociability), which was lower in rMDD patients compared to healthy controls, predicted symptom course over a 1 year follow up period (Spinthon et al., 2011). Remitted patients with recurrent MDD also reported receiving poor social support (Wilhelm et al., 1999) which has been shown to predict recurrent MDEs (Lewinsohn, Hoberman and Rosenbaum, 1988). Relatedly, attachment theory has been applied to the study of depression vulnerability. Attachment theory, which suggests that security in one’s interpersonal relationships influences emotional well-being and ability to trust, states that individuals may develop secure or insecure (e.g., anxious/avoidant and anxious/ambivalent) attachment styles (Hazan and Shaver, 1994). Insecure attachment styles were associated with the development of depressive symptoms in adolescents exposed to stressful life events (Kobak, Sudler and Gamble, 1991; Hammen et al., 1995) and with the development of MDEs in at-risk women over a 1 year follow up period (Bifulco et al., 2002). Psychological well-being has also been shown to be associated with vulnerability to depression. Psychological or eudaimonic well-being is thought to include six components: autonomy, environmental mastery, personal growth, positive relations with others, purpose in life, and self-acceptance (Ryff and Keyes, 1995). Individuals with low psychological well-being measured across these components were twice as likely to develop a depressive episode across a 10 year longitudinal follow up period (Wood and Joseph, 2010). Psychological well-being was also impaired in patients in remission from mood and anxiety disorders compared to healthy volunteers (Rafanelli et al., 2000). Lastly, and particularly relevant for the resting-state findings presented in this thesis, rumination has been linked to depression vulnerability. Rumination, or compulsively focusing on one’s distress and its causes (Nolen-Hoeksema, 1991), has been shown to predict the onset of new MDEs (Nolen-Hoeksema, 2000) and was correlated with resting-state functional connectivity within the default mode network in rMDD patients (Jacobs et al., 2014). Unfortunately, I was unable to collect data on personality traits, psychological well-being, attachment, or rumination as part of the current research due to the increases this would have entailed to participant burden. The specificity of the findings reported in this thesis can therefore only be confirmed in future studies. Such studies could include scales such as the
Revised NEO Personality Inventory (Costa and McCrae, 1992) to measure trait neuroticism and extraversion, The Ryff Scales of Psychological Well-Being (Ryff and Keyes, 1995) to measure the different components of psychological well-being, the Attachment History Questionnaire (Pottharst, 1990) to measure childhood attachment, and the Ruminative Response Scale (Nolen-Hoeksema, 1991) to measure rumination. It is important to note, however, that the frameworks described above are not without limitations, particularly as they relate to the current research. Regarding personality, as was argued in Chapter 2, melancholic patients may be characterised by a melancholic personality type which is detectable even outside of the depressed state (Hecht et al., 1998) and such personality features could underlie the pattern of distinctive resting-state functional disconnection we observed in the melancholic rMDD patients. Importantly, self-report measures of personality may be influenced by current mood state (Chmielewski and Watson, 2009) and the construct of neuroticism in particular incorporates a number of depressive symptoms (Ormel, Rosmalen and Farmer, 2004), and the current research controlled for the presence of residual depressive symptoms. Relatedly, measures of psychological well-being are known to correlate negatively with depressive symptoms (Ryff and Keyes, 1995), which were controlled for in the current research. Regarding rumination, the study that reported a relationship between rumination and connectivity within the default mode network reported no such relationship with connectivity to a left SCSR seed region (Jacobs et al., 2014) in close proximity to the seed region used in the current research. Furthermore, rumination is closely linked to the experience of self-blaming moral emotions (Joireman, 2004; Orth, Berking and Burkhardt, 2006), and guilt was measured in the current research using the Interpersonal Guilt Questionnaire (O'Connor et al., 1997) and is reported in each chapter. Furthermore, it is unlikely that an alternative framework is better suited to explain the results of the longitudinal resting-state fMRI study presented in Chapter 3 since the resilient MDD patients were compared to patients who had been diagnosed with a MDE over the follow up period (American Psychiatric Association, 2000).

Additional limitations to the current study are broadly separable according to whether they are related to the resting-state fMRI data or to the Altruistic Choices Task. With respect to the resting-state fMRI data, one potential limitation that has been discussed at length in previous chapters is the use of a seed-based approach to analyse the data. By using a seed-based approach, I consequently constrained the possible results of my resting-state fMRI analyses to networks which include the SCSR. There are alternative approaches which allow for data driven analysis, such as independent
components analysis, Granger causality analysis, graph theoretical analysis (e.g., small worldness, centrality), or measures of local connectivity (i.e., regional homogeneity and amplitude of low-frequency fluctuations) (Margulies et al., 2010). However, as I argued previously, constraining the results of the resting-state fMRI analyses to networks which include the SCSR allowed me to relate the findings to an extensive literature in current and remitted MDD patients in which connectivity to the SCSR is reportedly disrupted (Gaffrey et al., 2012; Herringa et al., 2013; Dutta, McKie and Deakin, 2014) and to a literature in which the SCSR is implicated in social and moral cognition (Moll et al., 2006; Gozzi et al., 2009; Zahn et al., 2009a; Pulcu et al., 2014b; Rüsch et al., 2014; Zahn, de Oliveira-Souza and Moll, 2015). Additionally, since the resting-state fMRI data were acquired in patients who had already experienced at least one MDE, and vulnerability to MDD increases with each subsequent episode (Kupfer, 1991), I was unable to conclude with certainty whether the abnormal patterns of resting-state SCSR connectivity I observed in the melancholic rMDD patients and in the resilient MDD patients represent primary or secondary vulnerability factors. If indeed the abnormal patterns of resting-state SCSR connectivity I described are a consequence of scarring from previous MDEs (Burcusa and Iacono, 2007), then connectivity within these networks should be correlated with the number of previous MDEs. Since this relationship was not observed, however, it is more likely that these patterns of connectivity represent primary vulnerability factors. Two additional limitations pertain specifically to the resting-state fMRI analyses carried out in Chapter 5. First, I only related SCSR connectivity to choice preferences during the guilt priming condition. Although the effects of experimentally increasing guilt on the relationship of choice preference to connectivity are not fully understood, it bears noting that implicit preference for prosocial options (i.e., social options and altruism towards friends and colleagues) did not differ across emotion priming conditions (Workman et al., in preparation-a). It is therefore unlikely that guilt priming influenced the relationship between connectivity and implicit prosociality. Second, since I investigated resting-state rather than task-based connectivity, I was unable to make strong claims about the functional significance of regions captured within the SCSR networks I described. As is argued in detail below, future studies should adapt the ACT for use in activation-based fMRI studies, which would allow for stronger claims to be made about the significance of regions which are functionally connected to, or disconnected from, the SCSR.

With respect to the Altruistic Choices Task, one potential limitation is that choice preference was measured in response to hypothetical dilemmas. Although moral
dilemmas have proven a popular paradigm for investigation moral cognition, these dilemmas often describe scenarios which are far removed from the types of situations people are likely to encounter in day-to-day life, such as whether it is appropriate to push someone in front of a train to stop it from hitting five others (Greene et al., 2001; Casebeer, 2003; Kahane and Shackel, 2010). As I argued, however, one of the advantages of the ACT is that it includes dilemmas that were designed based on normative data in order to obtain a high level of ecological validity. Another potential limitation to the ACT is that its dilemmas did not pit competing moral concerns against each other (e.g., whether it is morally permissible to kill one person to save five others). Rather, the dilemmas pitted options to behave selfishly (e.g., going straight home after work) against options to engage socially with or without altruistic motivations (e.g., calling a friend to spend time together after work, offering a friend a ride home after work, or offering a stranger a ride home after work). I was therefore able to investigate whether rMDD patients demonstrated a preference for avoiding social situations overall, the relevance of which is underscored by recent work suggesting the importance of social withdrawal and dysfunction to MDD (Burcusa and Iacono, 2007; Zahn et al., 2015b). Another potential limitation to using dilemmas which require participants to provide explicit responses is that they may be confounded by a desire to provide socially acceptable responses. This concern is mitigated by the fact that an implicit measure of choice preference was used. That said, implicit choice preference by its very nature cannot be measured explicitly, so it is possible that the implicit measure may not be related to overt choice preference. As I reported in Chapter 3, however, implicit choice preference for options to behave altruistically towards friends and colleagues was moderately correlated with explicit choice preference during the guilt and indignation priming conditions. This suggests that the explicit and implicit measures are related although not identical. Due to restrictions in administration time, I could not directly measure feelings of guilt or indignation throughout each emotion priming block. I was therefore unable to confirm that the effects of the emotion priming were sustained throughout each block. Guilt priming did, however, effectively increase explicit and implicit preferences for options to behave altruistically towards strangers but not towards friends and colleagues. As I have argued, this suggests that the guilt priming paradigm is effective but only under certain circumstances. Relatedly, I could not include all three emotion priming conditions and all three types of dilemmas into a single study as the increase in administration time could not be justified. I was therefore unable to determine how participants would respond to social-selfish dilemmas during
an emotional baseline, or whether indignation priming modulates explicit and implicit preferences for pure altruism. The ACT software was designed with flexibility in mind, such that swapping between emotion priming conditions and dilemma types is easily done, so it would not be difficult to investigate this in a future study.

6.4.2. Future Directions

In Chapter 2, I suggested that resting-state subgenual cingulate-amygdala functional disconnection represents a primary vulnerability factor for melancholia. In Chapter 3, I reported that lower interhemispheric SCSR connectivity was a distinguishing feature of patients who remained resilient to recurring MDEs. It is curious that lower connectivity to the SCSR could be associated with vulnerability in one instance and with resilience to recurrence in another. Since lower subgenual cingulate-amygdala connectivity was not associated with risk for future MDEs in the melancholic rMDD patients, nor with number of previous MDEs, I maintain that the original interpretation was appropriate. Future studies should, however, attempt to validate these neural signatures. I suggest, for example, acquiring resting-state fMRI data in never-depressed individuals who are at risk for developing MDD themselves, and then following these participants longitudinally to see whether lower subgenual cingulate-amygdala connectivity is predictive of the subsequent development of melancholic MDEs. Several never-depressed populations at elevated primary risk for MDD could be recruited to this end. For example, first degree relatives of patients with early onset recurrent MDD are at greater risk of experiencing MDEs (17.4 - 24.2%; (Weissman et al., 1984; Kupfer et al., 1989)) than individuals without a family history (4.8%; (Weissman et al., 1984)). Alternatively, one could recruit samples with and without personality traits thought to increase vulnerability to MDD, such as neuroticism. Neuroticism was predictive of developing MDEs and was related to genetic risk for MDD in a longitudinal twin study (Kendler et al., 2006). Stressful life events, such as physical abuse or the loss of loved ones, have also been associated with vulnerability to MDD (Shrout et al., 1989). Kendler and colleagues (2010) found a genetic basis for sensitivity to stressful life events in triggering the onset of MDEs in a study of female twins. Early traumatic events in particular have been linked to an increased incidence of depression. Bifulco and colleagues (1991) found that 64% of women who reported experiencing childhood sexual abuse went on to experience a depressive episode. The same group found that childhood abuse and neglect were associated with the development of early onset MDD (Bifulco et al., 1998). Although I
argued the resting-state functional disconnection we observed in the melancholic patients in Chapter 2 is a primary vulnerability factor for MDD, an interesting direction for future research would be to investigate connectivity in several of the populations described above (e.g., first degree relatives and early life trauma) in order to clarify whether environment plays a role in giving rise to the subgenual-amygdala disconnection we observed.

Relatedly, I argued in Chapter 2 that lower interhemispheric SCSR connectivity may represent a compensatory mechanism which emerges later in the course of recovery and increases resilience to recurring MDEs. Alternatively, connectivity to the SCSR may exhibit a linear relationship to depression status such that connectivity to the SCSR is higher in current MDD patients and lower in resilient MDD patients. To test this, one could carry out a treatment study in currently depressed patients in which resting-state fMRI data are acquired and clinical interviews conducted before treatment, immediately after treatment, and again at 3 and 6 months post-treatment (Solomon et al., 2000). One could then characterise the relationship between interhemispheric SCSR connectivity and change to depressive symptomology. If depressive symptoms consistently decreased throughout the follow up period, but interhemispheric SCSR connectivity remained stable from post-treatment to 3 months before decreasing at the 6 month time point, this would suggest the emergence of a compensatory mechanism. If connectivity decreases linearly across all four time points, and this is associated with change to depressive symptoms, this would support the alternative interpretation. Our group recently developed a software package which supports real-time fMRI-based neurofeedback which can be used to train participants to regulate connectivity between brain regions (Basilio et al., 2015). Using this toolbox, our group reported enhanced activation of brain regions implicated in the experience of affiliative emotions in participants who underwent neurofeedback (Moll et al., 2014). A recent study found that neurofeedback can produce lasting changes to connectivity within resting-state networks (Yuan et al., 2014). An interesting challenge for a future study would be to design neurofeedback paradigms to modulate subgenual cingulate-amygdala connectivity and to modulate interhemispheric SCSR connectivity. One could then acquire resting-state fMRI data before and after neurofeedback to investigate whether this produces lasting changes to connectivity within these networks. For example, one could investigate whether using neurofeedback to increase subgenual cingulate-amygdala connectivity strengthens resting-state connectivity between these regions in never-depressed individuals vulnerable to experiencing melancholic MDEs. One could
then prospectively monitor these patients to determine whether increased subgenual cingulate-amygdala connectivity improves resilience to MDD relative to participants who completed a control condition. Relatedly, one could investigate whether using neurofeedback to reduce resting-state interhemispheric SCSR connectivity could be used as a novel intervention in currently depressed patients, or as prophylactic intervention in patients who are remitted from the depressed state but are nonetheless vulnerable to future MDEs.

In Chapters 4 and 5, I found that explicit prosociality was lower in rMDD patients compared to HC participants and implicit prosociality was negatively correlated with connectivity between the SCSR and TPJ across all participants. Furthermore, the nature of the relationship between SCSR-TPJ connectivity and implicit choice preferences distinguished the rMDD patients from the HC group. Patients with rMDD were recruited for this research because they have been shown to experience elevated altruistic guilt (Green et al., 2013b), and I aimed to characterise the influence of modulating guilt on choice preference. As was discussed earlier, however, neither my hypothesis nor the hyperaltruism hypothesis accurately predicted the influence of modulating guilt on altruistic choice preference. The relationship between pathological experiences of guilt and altruistic choice preference is therefore not fully understood. One complementary approach for future research would be to repeat the studies described in Chapters 4 and 5 in individuals in whom guilt may be impaired. Individuals with psychopathy could be studied, for example, since psychopathy is characterised by deficits in remorse and shame (Cleckley, 1988). Alternatively, one could recruit a cohort of individuals with chronic anger disorders, in whom guilt would be predicted to be impaired (Keltner, Ellsworth and Edwards, 1993; Neumann, 2000). Another potential avenue for future research is to investigate whether patients with lesions in or atrophy to the SCSR or TPJ demonstrate abnormal implicit prosociality. For example, one could administer the ACT to patients with Alzheimer’s disease, in whom atrophy commonly affects posterior cortical regions including the TPJ (Zahn, de Oliveira-Souza and Moll, 2015). Alternatively, one could administer the ACT to patients with frontotemporal dementia in whom neurodegeneration has been shown to affect the functioning of the SCSR (Moll et al., 2011). As was mentioned previously, one limitation to relating choice preference to resting-state functional connectivity is that strong claims cannot be made about the functional significance of any networks that were identified. I would therefore suggest adapting the ACT for use in activation-based fMRI studies. One could then apply transcranial magnetic stimulation or cathodal...
transcranial direct current stimulation to the TPJ throughout scanning in order to determine how disrupting the functioning of the TPJ impacts implicit prosociality.

6.5. Conclusions

The primary objective of this thesis was to investigate the relationship of moral cognition and emotions to the pathophysiology of major depressive disorder (MDD). In Chapters 2 and 3, I identified resting-state networks including the SCSR, a region implicated both in MDD and in moral cognition and emotions, which were associated with vulnerability and resilience to MDD. Specifically, I argued that lower subgenual cingulate-amygdala connectivity represents a primary vulnerability factor for melancholia, and lower interhemispheric SCSR connectivity represents a compensatory mechanism which enhances resilience to recurring MDEs. In Chapter 4, I investigated the relationship between moral cognition and emotions and found that rMDD patients had lower explicit preferences for prosocial options compared to HC participants irrespective of emotion priming condition and, across all participants, guilt priming increased preferences for altruism towards strangers but not towards friends and colleagues. In Chapter 5, I investigated the relationship of moral cognition and emotions to the pathophysiology of MDD by relating choice preference measured using the ACT to SCSR connectivity and found a negative relationship between implicit prosociality and SCSR-TPJ connectivity. Taken together, abnormal SCSR connectivity and choice preference were observed in the rMDD patients, and the relationship between SCSR connectivity and choice preference distinguished the rMDD patients from the HC group. I presented a number of potential theoretical and clinical implications stemming from the current findings before addressing the limitations to the current research. As is discussed in section 6.4.2, I believe these findings set the stage for a number of interesting future research projects that will enhance our understanding of how the brain supports moral cognition and emotions.
References


Bechara, A., Tranel, D., et al. (1996). Failure to respond autonomically to anticipated future outcomes following damage to prefrontal cortex. *Cerebral Cortex, 6*(2), 215-225.


Rumination explains why shame but not guilt is maladaptive. *Personality and 
dysfunction in autism. *Mental Retardation and Developmental Disabilities 
NY: Free Press.
Rejections of Ultimatum Offers. *Organizational Behavior and Human Decision 
Processes, 68*(3), 208-224.
prefrontal cortex abnormalities in melancholia. *Molecular Psychiatry, 9*(4), 393-
405.
semantic impairment induced by transcranial magnetic stimulation. *Current 
Biology, 20*(10), 964-968.
*Research explorations in adult attachment* (pp. 9–38). New York, NY: Peter 
Lang.
Power, J. D., Barnes, K. A., et al. (2012). Spurious but systematic correlations in 
Power, J. D., Mitra, A., et al. (2014). Methods to detect, characterize, and remove 
motion artifact in resting state fMRI. *Neuroimage, 84*, 320-341.
Power, J. D., Schlagger, B. L., et al. (2015). Recent progress and outstanding issues in 
motion correction in resting state fMRI. *Neuroimage, 105*, 536-551.
*Neuropsychopharmacology, 35*(1), 192-216.
Pujol, J., Batalla, I., et al. (2012). Breakdown in the brain network subserving moral 
judgment in criminal psychopathy. *Social Cognitive and Affective Neuroscience, 7*(8), 917-923.


Sato, T., Sakado, K., et al. (1996). Importance of the melancholic type of personality for research into the premorbid personality of depression. In C. Mundt, M. J. Goldstein, K. Hahlweg and P. Fiedler (Eds.), *Interpersonal Factors in the Origin and Course of Affective Disorders* (pp. 48-64). London: Gaskell.


Appendices

A. Blame Bias Study Protocol

Experimental Protocol

Development of Cognitive and Imaging Biomarkers
Predicting Risk of Self-Blaming Bias
and Recurrence in Major Depression

Funded by MRC Clinician Scientist Fellowship to RZ (reference: G0902304)

NHS REC: Study 2ab: The architecture of social knowledge: fMRI, EEG &
Neuropsychology in Participants with or without a History of Depression

The University of Manchester, UK

School of Psychological Sciences, Division of Psychology

Dr. Roland Zahn  Chief investigator (all studies)
Dr. Karen Lythe  Postdoctoral Research Associate (all studies)
Jennifer Gethin  PhD student (EEG and fMRI studies)
Dr. Wael El-Deredy  Co-principle investigator (EEG study)
Prof. Matt Lambon-Ralph  Co-investigator (all studies, cognitive neuroscience
expertise)

School of Medicine, Division of Psychiatry

Professor Bill Deakin  Co-investigator (all studies, clinical neuroscience
expertise)
Dr. Rebecca Elliott  Collaborator (all studies, affective neuroscience expertise)
Professor Alistair Burns  Fellowship Sponsor and Mentor

Biomedical Imaging

Professor Steve Williams  Collaborator (GABA Spectroscopy study)
Dr. Rishma Vidyasagar  Collaborator (GABA Spectroscopy study)
Dr. Laura Parkes  Collaborator (GABA Spectroscopy study)

Biostatistics
Professor Graham Dunn Collaborator (statistical advice on survival analysis of longitudinal data)

**International collaborators**

*Neuroscience Institute, LABS D’OR, Rio de Janeiro, Brazil*

Dr. Jorge Moll International collaborator (all studies: moral cognitive neuroscience expertise)

*University of Zurich, Switzerland*

Dr. Nicolas Rüsch International collaborator (BIAT and self-stigma studies)

*Northwestern University, USA*

Professor Galen Bodenhausen International collaborator (BIAT study)

Alumni with significant contributions to development of study instruments:

Dr. Sophie Green Honorary Postdoctoral Research Associate

Project Web Page: http://www.medicine.manchester.ac.uk/blamebiases

Project email address: blamebiases@manchester.ac.uk

South Manchester REC ref no: 07/H1003/194

WTCRF Study Number 08/0150
1 Study Outline

Phone screening interview → **Exclusion**

**Inclusion**

Day 1
Face-to-face interview → **Exclusion**

**Inclusion**

Day 2
MRI scan and post-MRI moral sentiment task

Day 3
Action tendencies task (via email)

Day 4
Conceptual Social Knowledge Differentiation task (via email)

**EEG study**

**Healthy Control**
End of study participation

**Remitted MDD**

Day 5
3 month follow-up (Phone call)

Day 6
6 month follow-up interview (Face-to-face if possible)

Day 7
14 month follow-up interview (Face-to-face if possible)

End of study participation
2 Inclusion/Exclusion Criteria

2.1 Inclusion criteria

All:
- Age range: 18+
- Right-handedness
- Native speakers of English

Vulnerable to MDD group:
- Life time MDD (DSM IV) at least 2 months and requiring treatment, > 6 months remission, Montgomery-Asberg Depression Scale (MADRS) \( \leq 10 \) (cutoff of 10 on the MADRS is equivalent to the HRSD cutoff of smaller or equal to 7 used to define remission in clinical studies (Zimmermann et al., J Psychiatr Res. 2004 Nov-Dec;38(6):577-82).

Normal controls:
- Age-matched and education matched to respective study population.

2.2 Exclusion criteria:

All:
- Acute suicidality
- Impairments of vision or hearing which cannot be corrected during the experiment
- History of learning disabilities or developmental disorders or of manic or hypomanic episodes, of schizophreniform symptoms or schizophrenia, of substance abuse, repeated self injuries, severe OCD, PTSD, manifest eating disorder, primary anxiety disorder with only secondary depression
- History of neurological disorders
- History of Major medical disorders (significant heart insufficiency, severe COPD, uncontrolled hypertension or diabetes, endangiitis obliterans, severe vascular encephalopathy, hypo- or hyperthyroidism, severe liver or kidney disorders, rheumatoid disorders and all other medical conditions affecting brain function, blood flow or metabolism).
- no exposure to centrally active or psychotropic medications within 4 weeks of testing (8 weeks for fluoxetine)
• MRI contraindications

**Normal controls:**

• History of MDD, Bipolar Disorder or Schizophrenia in first degree relative
• No history of psychiatric disorders (Determined by the SCID-NP Research Version - module A, and the MINI screen, plus MINI module L), or neurological disorders, or use of psychopharmacological treatment.
  • From 6th February 2012 the MINI screen and MINI module L were replaced by SCID screen for Modules F, E and H (anxiety disorders, substance use disorders and eating disorders), plus B/C Psychotic Screening Module (for SCID).

3 **Screening**
Participants will respond to adverts and be asked to provide a contact number where they can be reached for a phone screening interview.

3.1 **Phone Screening**
The first point of contact is a phone screen session, for which oral consent is required. A statement shall be read to the participant at the beginning of the phone call providing details of what the phone screen entails and asking them to orally consent to this screen.

Phone screening for major medical illnesses, substance abuse, major axis I disorders, history of psychiatric symptoms and treatments. If participants fulfil inclusion criteria and provide informed consent then first appointment can be made.

Information sheets and consent forms to be sent out via post, e-mail or fax. Information sheets must be received and signed at least 24 hours before the first study day.

*See document(s):*
• ASKP_Phone Screen_study 2ab_v5_12Mar2012.doc
• 4_PIS_fMRI_neuropsych_H Part w hist_noMDD_study2_v4_unbld_conupdt_2ndamwchgsacc_4may11_jennyadded
• Consent formMRI_neuropsych_hist_noMDD_study2_v5_2ndamendmentwchangesaccepted.doc
4 Day 1: Face-to-Face interview

4.1 Psychiatric and Neurological Examination

If potential participants pass the phone screen, the next stage is to undergo psychiatric and neurological examination by Roland.

- Consent
- Urine drug screen
- Psychiatric and Neurological Examination (see clinical history & exam checklist for study 1&2) which includes phenomenological psychopathology semi-structured interview (translated AMDP-System, 3rd edition; Faehndrich & Stieglitz, 2007) for symptoms: Feeling of Loss of Feeling, Blunted Affect, Felt Loss of Vitality, Hopelessness, Feelings of Inadequacy, Feelings of Guilt, Anxiety, Depressed Mood, Lack of Drive, Inhibition of Drive, Motor Restlessness.
- For rMDD - Age at onset, Episode duration, Total illness duration, medical history including all medical records
- Mood Disorders Part (A) of SCID-I Research Version Patient = P
- On 7th feb we changed from using MINI for psychotic symptoms and anxiety disorders, etc, to using the SCID for both. A new SCID screening document was created to cover anxiety, substance use and eating disorders:
  - Scid.Screen_modules_FEH_3Feb2012.doc
  - Scid_4a_bc_module_psychotic_screen Jan 2007 final_3Feb2012.doc
- For age>50, Addenbrooke’s Cognitive Examination Final Revised Version A (2005)
- Global Assessment of Functioning (SCID-I Research Version)
- MADRS
- Weissman Family History Screen (shortened version) to screen for MDD and general psychiatric disorders.
- Beck Depression Inventory
- Life events questionnaire
- Life base

See document(s):
- Checklist_study2ab_updated_7Feb2012.doc
4.2 Standard Psychological Scales and Neuropsychological Assessment
If potential participants pass though the psychiatric and neurological assessment phase, then they will be eligible to take part in the rest of the study including standard psychological scales and tests of executive function.

See document(s):
- Checklist_study2ab_updated_7Feb2012.doc

5 Day 2: MRI and experimental tasks

See document(s):
- Followup_Checklist_study2ab_17August2011.doc
- MRC_project_mri_protocol_v3_12August2011.doc
- mri_patient_declaration.pdf

Consent is taken again and an MRI patient declaration is completed

5.1 Positive and Negative Affect Schedule.
This is for the last few days.

See document(s):
- PANAS_Scale_pastfewdays_premri_S2ab_Pxxx.xls

5.2 Details of best friend information
The following statement will be presented:

Please type in the first name of your best friend (what you call her/him). She/He should be the same gender as you. Your friend should not be related to you either genetically or by marriage. You shouldn’t have a sexual relationship with him/her.

How many months/years have you known this person?
Relative to what you know about other peoples’ relationships and your relationships with other people, please rate how close you feel
to your best friend? (1= not close at all; 7 = extremely close)
(Adapted from Berscheid, Sneider, Omoto, 1989)
Please circle the diagram which best describes your relationship
with your best friend- (use of Venn-diagram of Aron, Aron,
Smollan, 1992).
Please rate how intense your friendship was within the past year;
(1=not intense at all; 7=extremely intense)

See document(s):
• pre-ratings_study2_final_S2ab_Pxxx.xls

5.3 Practice fMRI paradigm
Before the fMRI paradigm, the participants in study 2 are first required to complete a
practice session outside of the scanner so that they can become accustomed to the
assignment of responses to two different keys. The practice session uses 12 social
concepts, (negative or negated positive). Response keys are randomised across
participants.

See document(s):
• Task Instructions_practice_index finger is very.es
• Task Instructions_index finger is mildly.es

5.3.1 Task Instructions
The following instructions shall be given to participants on paper before starting the
practice task.

The functional anatomy of social knowledge
Instructions for fMRI Experiment

Thanks for participating in this study and helping us to explore the
neuroanatomical basis of social knowledge.

In some trials, you will see short statements and visual patterns on
a screen.
Patterns look like this:
You don’t have to make a decision on the patterns, or press any buttons.

In other trials, you will see short statements describing your social behaviour towards your best friend, such as:

[your name] does act tactlessly towards [your best friend’s name]

In other trials you will see statements describing social behaviour of your best friend towards you, such as:

[your best friend’s name] does act tactlessly towards [your name]

Take care to read the sentences properly as in some, “does not act” (e.g. “does not act generously”) is used, and in others, “does act” (e.g. “does act stingily”) is used. The important thing to note is that the social behaviours are always negative in nature. That is, in all cases, either you or your best friend is acting in a fashion that is counter to social values.

Please make a decision about how you would feel about the described behaviour. You will have two choices (mildly unpleasant / very unpleasant) assigned to either the left or right button. (The experimenter will tell you the correct assignment).

Please make your judgments always about your own feelings and from your own perspective. Please make your decision as quickly and as accurately as possible. The statements will be shown for 5 seconds and then disappear. You have to make your decision within this time. Between the statements you will see an asterisk in the centre of the screen. Please try to keep your eyes focused on the
centre of the screen. Short eye movements to other locations to give your eyes some rest are acceptable.

There will be a practice run of the task outside of the scanner. Please ask any questions at this time. In the scanner, anatomical pictures of your brain will be taken first, then 3 functional runs (around 14 minutes each) will be performed. Before each run, you’ll see a countdown (5 to 1). You will do the same task 3 times on different statements.

When moving you into the scanner it is very important for us to make sure that your head is centred in the head coil. Also please help us by keeping your head still during the testing. If you move your head too much, the results cannot be used. It is also important not to talk (unless it is an emergency) once the scanning begins to minimize head movement.

See document(s):
- paper instructions_fMRI_study2_3_Jul_08.doc

5.4 fMRI paradigm

90 items are negative concepts such as ‘stingy’ and ‘tactless’ and the other 90 are negated positive concepts. For example, “Sam does not act generously towards Tom”. Thus all stimuli present behaviours which are counter to social values. The negative and negated positive statements for each condition (self-agency, other-agency) are distributed equally across the three runs (90 negative social concepts, e.g ‘stingy, and 90 not positive e.g ‘not generous’), along with 90 null events. Altogether, the same social concept appears twice, but only once within one run.

Stimuli are statements written on three lines presented visually in vertical order in the centre of the screen for 5000ms. After a response has been given, the statement disappears and an asterisc appears in its place.

The task requires the participant to make a decision based on how unpleasant they find the social behaviour to be, with the options of ‘mildly unpleasant’ and ‘very unpleasant’. The response options of ‘mildly unpleasant’ and ‘very unpleasant’ will be assigned to the left finger (index finger) and right finger (middle finger) glove-box keys in a counterbalanced fashion (half of the subjects with reversed response-finger assignment).
See document(s):

- run1_cumulative_naru_box_2august11.es
- run2_cumulative_naru_box_2august11.es
- run3_cumulative_naru_box_2august11.es

5.4.1 fMRI paradigm design

The fMRI paradigm will consist of three runs of 820 seconds = 13 minutes 40 seconds. This includes a 10 second count down before each run, 5 TRs (2 secs) as 5 dummy scans during the count-down, and 90 stimuli x (4000msec mean interstimulus interval + 5000 msec stimulus duration).

The MR-scanner repetition time (TR) will be 2000 ms. The mean interstimulus interval of 4000 ms will be jittered in 500 ms steps from 2000 ms to 6000 ms, resulting in 9 ISIs; 10 stimuli per ISI.

Each Run will consist of 410 Repetitions (including 5 dummy scans during count down) of 2000 TR totalling 820 seconds (13 mins, 40 secs). The Philips Achieva Scanner drops the dummy scans automatically and does not save this as part of the Par_Rec file.

### Run 1

<table>
<thead>
<tr>
<th>ISI</th>
<th>self - agent</th>
<th>best friend – agent</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2500</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>3000</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3500</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>4000</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4500</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5000</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5500</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6000</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>sum=30</td>
<td>sum=30</td>
<td>sum=30</td>
</tr>
</tbody>
</table>

### Run 2
<table>
<thead>
<tr>
<th>ISI final</th>
<th>self - agent</th>
<th>best friend – agent</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2500</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>3000</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>3500</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>4000</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>4500</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5000</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>5500</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6000</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>sum=30</td>
<td>sum=30</td>
<td>sum=30</td>
<td></td>
</tr>
</tbody>
</table>

Run 3

<table>
<thead>
<tr>
<th>ISI final</th>
<th>self - agent</th>
<th>Best friend – agent</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>2500</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3000</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3500</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4000</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4500</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>5000</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5500</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>6000</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>sum=30</td>
<td>Sum=30</td>
<td>sum=30</td>
<td></td>
</tr>
</tbody>
</table>

5.4.2 MRI protocol

See document(s):

- MRC_project_mri_protocol_v3_12August2011.doc
- Spectroscopy SSC_voxel placement.docx

5.5 Post-MRI task: Moral Sentiment Task (only negative action version)
Following the fMRI paradigm, participants will be required to complete a task through use of Excel Macros. The Moral Sentiment Task is sorted by column H before starting.

With respect to the stimuli that are of the other-agency condition, participants will be asked:

> How would you feel about (proper name of best friend) acting i.e. ‘tactlessly’ towards you? (N=90 negative other-agency)

With respect to stimuli that are of the self-agency condition, participants will be asked:

> How would you feel about yourself acting i.e. ‘tactlessly’ towards (proper name of best friend)? (N=90 negative self-agency)

The response choices are as follows:

> Please select the feeling that you would experience most strongly
  Shame
  Guilt
  Anger/Indignation towards oneself
  Anger/Indignation towards the other
  Contempt/Disgust towards oneself
  Contempt/Disgust towards the other
  No Feeling
  Other Feeling

There are three scales that need to be completed for each statement:

> How unpleasant did you find the feelings that you experienced? (1 (not at all) to 7 (extremely unpleasant) point Likert scale)

> Please estimate how many different outcomes of this behaviour there are? (7 step visual analogue scale, Very Few=1, Very Many=7) [5]

> In how much detail does this statement describe a characteristic set of social behaviours? (7 step visual analogue scale, little detail=1, great detail=7 [6])

**NB:** From 23-8-12 the task will no longer ask for how much detail the statement describes a set of social behaviours.
From 14th June 2012 this task has been altered to include a counter for the statements.

6  Day 3: Post-scanning ratings – Action Tendencies Task
An excel sheet for the Action Tendencies Task is sent to participants by email. Using the same stimuli as in the fMRI paradigm and the post-MRI Moral Sentiment Task, participants are asked:

“to choose the action that you would most strongly feel like doing in response to that behaviour. Please make sure you only check one box otherwise we are not able to use your data. You also need to rate how responsible you would feel and how much control you would have.”

The task is sorted by column H before sending out.

See document(s):

- 1st_action_tendencies_study2_v1_2Aug11_S2ab.xls

7  Day 4: Optional task – Conceptual Social Knowledge Differentiation Task
An excel sheet for the Conceptual Social Knowledge Differentiation Task is sent to participants by email. There are two versions, only one of which is sent to each participant following randomisation.

“Please imagine one specific example of behaving in the described way and then choose the concept/s which you would use to best describe this example of behaviour. Please check at least one concept.

If there are several concepts describing the example of behaviour equally well, please check all of the ones which equally apply. If you think that none of the listed concepts describes this example of
behaviour well, please try to find at least one concept which describes one possible aspect of the overall behaviour.”

Before sending the task, negative items and positive items are sorted by column I.

See document(s):
- 2nd_conceptsocknow_and_bestfriendinfo_negfirst_final.xls
- 2nd_conceptsocknow_and_bestfriendinfo_postfirst_final.xls

8 Follow-up interviews
On the MRI day participants are given a date for their 3 month follow-up phone call, and told that they will receive an email reminding them of this appointment a week before it is due.

See document(s):
- Followup_Checklist_study2ab_17August2011.doc

8.1 Day 5: 3 month follow-up email
See document(s):
- Followup_Checklist_study2ab_17August2011.doc
- LIFE_DSM_IV_current_1201_modified_Karen_5Jan2012.doc

8.2 Day 6: 6 month follow-up interview
See document(s):
- Followup_Checklist_study2ab_17August2011.doc
- LIFE_DSM_IV_current_1201_modified_Karen_5Jan2012.doc

8.3 Day 7: 14 month follow-up interview
See document(s):
- Followup_Checklist_study2ab_17August2011.doc
- LIFE_DSM_IV_current_1201_modified_Karen_5Jan2012.doc
B. NORD Study Protocol

*Neurobiology of Resilience to Depression: A Neuropsychological and Neuroimaging Investigation*
*Research Protocol (version 06, 14 May 2012)*

**Background**
Depression is the mental health disorder associated with the greatest amount of disability worldwide. At least one in six people in the UK will experience depression at some point in their lives, with a steady increase in reported rates. Although many episodes of depression respond to treatment, for most patients vulnerability to depression is a lifelong trait. Both acute and chronic uncontrollable life stress have been reliably associated with the onset and recurrence of depression. However, it is clear that some resilient individuals do not become depressed despite exposure to major adverse life events. There has been little systematic study of the factors determining resilience to depression in human participants. Identifying neurobiological markers associated with resilience in the face of stressful life events has important implications for understanding the mechanisms of depression. Pharmacological and behavioural treatments for depression typically target decreasing vulnerability; promoting resilience offers a distinct but complementary approach, with potential for depression prevention in at-risk groups.

**Stressful life events and depression**
Neurobiological studies of resilience in humans have typically focused on responses to acute life-threatening stressors, specifically identifying factors which protect against post-traumatic stress disorder (PTSD). Putative neurochemical and neuroanatomical signatures have been associated with resilience to acute trauma as have distinct cognitive profiles. However, there have been very few studies of resilience in the face of more widely-experienced stressful life events and difficulties associated with depression. Discrete stressful life events (eg. relationship breakdown) precede the majority of initial depressive episodes (up to 80%). Chronic life stress (eg. ongoing marital difficulty) is also a major predictor of depression. Recent research has provided significant insights into the neurobiological basis of depression, but the neurobiological and neurochemical underpinnings of resilience to stress are far less well understood. Resilience can be defined as an individual’s successful adaptation and functioning in the
face of stress or trauma. There is now clear evidence for biological determinants of resilience (neuroendocrine markers, genetic factors and neuroimaging indices of brain structure and function), that interact with environmental influences. Research suggests that in the presence of enough stress triggers, the majority of individuals develop at least some degree of depression. In a recent Manchester community sample, only 30% of those with 4 or more stressful life events had no history of depression. Thus, extremely resilient individuals represent a significant minority of the population who may be characterised by a distinct neurobiological profile.

**Resilience and stress hormones**
The hypothalamo-pituitary-adrenal (HPA) axis consists of a feedback system regulating circulating cortisol and reactions to stress in humans. Resilience to PTSD has been associated with altered concentrations of stress hormones including reduced DHEA and DHEA/cortisol ratios. Depression is also associated with HPA axis dysregulation including elevated plasma and salivary cortisol levels, increased urinary free cortisol excretion, decreased corticosteroid receptor function as well as adrenal and pituitary enlargement. However there remain conflicting results in the role of HPA axis activity in mediating resilience or vulnerability to depression. One factor in resolving these inconsistencies may be in the balance between cortisol and DHEA. We will measure cortisol and DHEA in resilient and vulnerable participants, including the waking cortisol response.

**Cognitive features of resilience to depression**
Studies of PTSD have suggested that superior cognitive functioning, particularly cognitive flexibility and active problem solving, may be important in resilience. Distinct cognitive styles have also been proposed in resilience to other psychiatric disorders. Based on our previous work, we propose two particular aspects of cognitive function that may underpin resilience:

1. **Emotional processing bias.** Healthy controls show biases to process positive information. In many studies, depressed patients have demonstrated a bias towards remembering and processing sad information, including autobiographical material. We propose that positive affective biases in memory and attention may be particularly marked in resilient participants.

2. **Constructive response to failure.** Studies of responses to negative feedback during task performance suggest that healthy volunteers tend to use accurate negative feedback
constructively to improve performance, while depressed patients do not. Constructive response to failure is a component of effective problem-solving. Strategic, problem-solving approaches to life stress may act as a coping mechanism. We therefore propose that constructive response to negative feedback may be a distinct cognitive mechanism associated with resilience to depression.

Recent studies have shown that psychopathology of certain moral emotions is associated with major depressive disorder. The expression of these moral emotions varies; some are exaggerated and experienced out of context (e.g. guilt), whereas shame, for example, leads to attempts to deny, hide or escape from the shame-eliciting situation leading to interpersonal separation and social withdrawal. We will investigate these moral emotions, which could provide insights into the resilience to depression, by utilising experimental social economic decision-making games.

**Neuroimaging of resilience**

Functional magnetic resonance imaging (fMRI) has been used to explore neurobiological features associated with stress. fMRI studies of responses to aversive anxiogenic stimuli suggest critical roles for ventromedial prefrontal cortex (vmPFC), including ventral anterior cingulate (vACC) and medial orbitofrontal cortex (mOFC), and amygdala in stress responses. Kalisch et al (2005) have shown that using strategies to reduce anxiety (resilient behaviour) is associated with increased vmPFC function. We propose that increased mPFC responses and reduced amygdala responses to negative emotional stimuli and performance feedback may represent neuroimaging markers for resilience.

**Research location**

The research will be based at the Neuroscience and Psychiatry Unit (NPU), University of Manchester. The NPU was recently the leading centre in the NewMood Study ([www.newmood.ac.uk](http://www.newmood.ac.uk)) which has carried out complementary research in remitted and currently depressed patients compared with controls. We have questionnaires (n=2004) and analysed DNA samples (n=1493) from a large community sample. We will identify suitable participants for the proposed study from the existing database and anticipate being able to recruit all 260 participants from this cohort. We also plan to recruit through the public website, and through advertisements, Facebook etc., if necessary. The fMRI study will be sited in the Magnetic Resonance Imaging Facility at Hope Hospital, where we have conducted previous studies.
Research plan

Aims and objectives:
We will study groups of patients with high and low numbers of stressful life events and with and without a history of depression. We aim to obtain detailed neuropsychological profiles of these groups and associated neuroimaging data. This will allow us to identify distinct cognitive and neurobiological signatures associated with resilience to depression. Further, we aim to explore the neurochemical basis of these effects using biochemical assays.

Hypotheses are that, compared to other groups:
1) Resilient participants will show a specific pattern of cognitive performance, characterised by positive emotional bias in memory and attention, constructive response to negative feedback, active problem solving and cognitive flexibility.
2) Resilient participants will demonstrate enhanced vmPFC and ACC responses to negative emotional stimuli and task failure and decreased limbic responses. Task-related connectivity from mPFC to limbic regions will be enhanced.
3) Resilient participants will show elevated DHEA levels and DHEA/cortisol ratios with a more rapidly responsive, and shorter lasting, waking cortisol response.
4) Resilience, measured by cognitive performance and functional imaging responses, will be influenced by functional polymorphisms in the serotonergic system (e.g. by the 5-HTTLPR) and by the CB1 gene.

Participants:
We will recruit participants from our existing NewMood database of 2004 participants. The database will be searched for (a) participants in the relevant age-group (30 to 50), and (b) who have expressed a willingness to be contacted again for future research studies. Potential participants will be contacted initially by their e-mail, telephone or postal address given to the NewMood study. They will be given a link to a secure, online screening questionnaire survey, which is hosted by the University of Manchester (http://www.mhs.manchester.ac.uk/surveys/TakeSurvey.aspx?SurveyID=7lKK8834). The first page contains information about the study followed by a brief on-line consent form (relating only to the on-line screening survey); participants will be unable to complete the screening questionnaire unless they have actively given consent. On-line screening through a secure website, rather than using a postal questionnaire, has two
main advantages: (1) it prevents any personal data being lost in the post, and (2) it eliminates any human error during data-input, thus ensuring that any subsequent analysis uses accurate data.

Based on responses to the on-line screening survey, the participants will be provisionally assigned to a number of different groups: those who appear to be unsuitable to be included in the full study, and those who can be provisionally allocated to one of the six groups listed below; these people will be invited for an interview to confirm group allocation. Invitations will be sent by e-mail and then followed-up, if necessary, with another e-mail and/or a telephone call, using the contact details that respondents give on the screening questionnaire. We will use only the contact details provided and not seek information from other sources (e.g. telephone directory) thus, if a participant does not leave any contact details, no effort will be made to contact them. Prior to interview, participants will be sent a Participant Information Sheet for the full study. At the interview, participants will be given the opportunity to ask questions about the study, and sign a paper copy of the Consent Form. However, everyone who completes the on-line screening questionnaire, even if they are unsuitable for the full study (i.e. do not fall into one of the 6 groups below), will be invited to contribute to part of the study, by answering further on-line questionnaires and/or neurocognitive testing at a separate interview; these data will be analysed anonymously and will provide additional information about thinking styles/personality traits that may cause individuals to be more resilient or vulnerable to depression, thus enriching the data-set.

Four groups will be identified for a factorial analysis, initially based on scores on a shortened life events and difficulties scale (LEDS) and self-reported depression history:
1. Low scores on LEDS, no history of depression (baseline group, N=40)
2. Low scores on LEDS, history of recurrent depression but currently in remission (extremely vulnerable group, N=40)
3. High scores on LEDS, history of recurrent depression but currently in remission (vulnerable group, N=40)
4. High scores on LEDS, no history of depression (resilient group, N=40)

After detailed interviewing confirming the above groupings, we will also identify fifth and sixth groups with
5. High scores on LEDS, history of mild, transient depression in response to specific life events but currently symptom free (N=40)
6. Low scores on LEDs, history of mild, transient depression in response to specific life events but currently symptom free (N=40)
These will serve as additional control groups.

40 participants from each group will undergo a detailed assessment using personality measures and neuropsychological tests chosen to assess functions that may be relevant to resilience. A subset of 20 participants from groups 1-4 will also undergo an fMRI scan using cognitive challenges designed to probe medial prefrontal and limbic function.

As a result of the interview some participants will not fall clearly into one of the above 6 groups, due to experiencing an intermediate number of stressful life events, but they will have fully participated in the study so far having completed computerised neuropsychological tests and on-line questionnaires. A subset of these participants will also be offered an fMRI scan with cognitive challenges, similar to those employed with the other groups, but specifically exploring decision-making – another mental process that is thought to be affected during episodes of depression. This group of individuals could provide additional information on the resilience to depression.

Screening and preliminary interviews:
We have questionnaire measures (n=2004) and extracted and genotyped DNA (n=1493) from a large community sample, including information about life events and depression history. The sample was enriched by targeted recruiting of people with a history of depression and 1044 had a self-reported depression history. Participants included in the database have indicated their willingness to be contacted for future research. In initial on-line screening, we will identify participants who fulfil the criteria for groups 1-4 described above. For the groups without significant stress (1 and 2), we will recruit those with no more than 2 stressful life events in their lifetime. For the groups with significant life stress (3 and 4), we will recruit those with at least 5 (originally 4) events. Preliminary analysis of our database suggests that we should be able to recruit sufficient numbers from our existing database, even assuming a proportion have been lost to further study. Although we will use self report with crude scores to identify individuals likely to fall into each group, classification will be finalised only after a comprehensive face-to-face interview by a researcher qualified to administer the full LEDS and Childhood Trauma Questionnaire. Detailed interview will also allow us to identify
participants falling into groups 5 and 6 above who represent an intermediate response to life stress. These participants will have experienced some minor depression defined as meeting the following criteria:

a) No depressive episode not in response to a life event
b) No depressive episode lasting more than 4 weeks
c) No contact with specialist mental health services
d) No significant functional impairment associated with depressive episodes

Following these preliminary interviews we hope to have identified 6 groups of 40 participants each.

**General inclusion/exclusion criteria:**

Additionally, all participants must be aged 29-51 years, have colour vision and acuity within normal (or corrected-to-normal) limits, speak English as their preferred language, and have a current IQ of >85. All participants with a history of significant head injury, neurological disease, electro-convulsive therapy, impaired thyroid function, and steroid use will be excluded, although participants with a thyroid condition which is stable and well controlled and participants using steroidal asthma inhalers will not be excluded. For the 20 participants from each group used in the scanning study, those with any contra-indications for MRI scanning will be excluded. Female and male participants will be included. Female participants who are, or may be, pregnant will be excluded. For the resilient and baseline (i.e. never depressed) groups, we will exclude anyone with self reported history of any psychiatric illness or drug/alcohol dependence. For the vulnerable groups, participants with a history of diagnosed recurrent unipolar depression will be included but we will exclude participants with primary history of any other Axis I psychiatric diagnosis, significant medical disorders or history of drug/alcohol abuse. Participants in the vulnerable groups will not fulfil criteria for current depression; it will be at least 2 months since the end of their last depressive episode and they will be currently unmedicated. The six groups will be matched for age and IQ, using the Wechsler Test of Adult Reading.

**Neuropsychological testing:**
The 260 participants will attend the NPU for a 3 hour session comprising personality measures and neuropsychological testing. All questionnaires and tests will be computerised and participants will perform the tests in a designated quiet testing room,
with comfort breaks as required. In our NewMood study, 3 hour test sessions were well-tolerated.

Questionnaire measures, which can be completed on-line through a secure survey website hosted by the University of Manchester, will include rumination, resilience and personality scales. Neuropsychological tests will include tests designed to address our specific cognitive hypotheses. We will include established tests of emotional bias and negative feedback processing for our core hypotheses. We will also include tests assessing problem solving skills and cognitive flexibility, as these functions have been associated with resilience in the face of acute trauma.

**Biochemical assays and genetic profiling:**

All participants will be asked to provide six salivary samples for measurement of cortisol and DHEA levels, one just before going to sleep at night, one immediately on waking the following morning followed by 4 samples at 15 minute intervals together with precise timings of the samples. We have already analysed DNA from approximately 75% of participants in the NewMood database with genotyping for the relevant SNPs. Any subject without analysed DNA will be asked to provide a further saliva sample for genotyping.

**Functional imaging:**

20 participants from groups 1-4 will additionally participate in the fMRI study. We will also acquire a high resolution structural scan for all participants. A structural scan is necessary to exclude significant brain abnormality and we will employ a structural sequence that will permit supplementary structural analysis, for example to determine whether hippocampal volumes are larger in resilient participants (N=80). Methods for meaningful power calculations have not been developed for imaging studies but it has been argued that at least 12 participants are required for random effects analysis. The trend is for larger studies and we are therefore aiming for a minimum of 18 participants per comparison group (assuming 10% of the data may prove unusable). Participants will be scanned for a total of one hour, during which time they will perform 3 cognitive challenge tasks: tests of attention to emotional words, emotional memory and response to performance feedback.

Participants who are judged to have had in intermediate number (3 or 4) of stressful life events, may also be asked to participate in an fMRI scan. As with the other groups, these participants, (n = 30; 15 control and 15 remitted depressed) will have a structural
scan and, instead of the 3 cognitive challenges mentioned above, they will undertake a shorter challenge designed specifically to explore social economic decision-making.

**Analysis:**

Neuropsychological task data will be analysed using 2 models: a 2x2 factorial approach with depressive vulnerability (present or absent) and life stress (present or absent) as the factors (groups 1-4 included, n=40 for each group) and a continuum model comparing groups with increasing levels of resilience (groups 2-6 included, n=40 for each group). Planned comparisons between the resilient (group 4, n=40) and baseline/intermediate groups (1, 5 and 6 n=40 each) will allow us to explore the cognitive signature associated with resilience.

A similar approach will be applied to the neuroimaging data for the three challenge tasks to explore the effects of resilience, vulnerability and life stress on neuronal responses to different cognitive challenges (n=20 in each group). Imaging data will be analysed using SPM8.

All the data can be analysed using multiple regression techniques to explore the relationships with cortisol levels, DHEA levels, DHEA/cortisol ratios and composite scores on personality measures (total sample n=260 gives considerable power). We will explore possible influences of key functional polymorphisms (5-HTT, CB1) on the main outcome measures by using multiple regression analysis in the total sample of 260.
### C. Altruistic Choices Task: Emotion Priming Stimuli

#### Guilt priming story and corresponding priming statements

"You are driving home late one night during a heavy rainstorm. It’s been a long and hard day and you would like to get home quickly, so you drive slightly over the speed limit. As far as you can tell, there is no one else on the road. The rain is heavy enough that it is hard to see clearly through your windscreen, despite having your windscreen wipers turned up to the maximum speed.

Because the rain is making it hard to see clearly, you do not notice that you are going to drive across a puddle of rain water which has formed on the road. When you hit the puddle, you briefly lose control of your vehicle. Your vehicle begins sliding to the left, and you hit another car that appears to have been parked in the emergency lane. After hitting the parked car, you manage to regain control of your vehicle. Despite hitting the other car, it seems your vehicle can still be driven. You stop your vehicle and look into your rear view mirror to see the car you hit. It does not appear that anyone was in the other car. You consider leaving a note with your contact information, but you realise that the note will likely get washed away in the rainstorm. Instead, you write down the car’s license plate number. You are quite shaken up by the incident, and resolve to file a police report the following morning.

You wake up the next morning and you turn on the television to watch the local news. The top story is about a hit-and-run accident which occurred last night. According to the news report, a person was changing a tyre on the side of their car hidden from the road during last night’s rainstorm when their vehicle was struck. Prior to being struck, the person’s car had been lifted with a jack. When struck, the car fell from the jack and pinned the person’s right arm under the car. The person could not free their arm, but was able to use their mobile to call for emergency help. Unfortunately, their arm was badly injured and had to be removed. You realise that you are the driver that hit the person’s car, and that you are responsible for their arm having been removed."

<table>
<thead>
<tr>
<th>Accident my fault</th>
<th>I disfigured them</th>
<th>Should've driven carefully</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blameworthy for accident</td>
<td>I hurt them</td>
<td>Should've slowed down</td>
</tr>
<tr>
<td>Caused the accident</td>
<td>I mutilated them</td>
<td>Speeding was inexcusable</td>
</tr>
<tr>
<td>Crashed because incompetent</td>
<td>Injury my fault</td>
<td>Dishonourable for leaving</td>
</tr>
<tr>
<td>Liable for wreck</td>
<td>Responsible for amputation</td>
<td>I regret leaving</td>
</tr>
<tr>
<td>Responsible for accident</td>
<td>Responsible for suffering</td>
<td>Should have stopped</td>
</tr>
<tr>
<td>Should've seen them</td>
<td>Drove so hazardously</td>
<td>Shouldn't have left</td>
</tr>
<tr>
<td>Amputation my fault</td>
<td>Drove too fast</td>
<td>Should've called police</td>
</tr>
</tbody>
</table>
"You are driving back to your home during a heavy rainstorm late one evening. The heavy rainfall makes you anxious, so you drive slightly below the speed limit. There do not seem to be any other cars on the road, although it is difficult to see clearly due to the rainfall. You turn your windscreen wipers up to the maximum speed so that you can see the road more easily.

While driving along the road, you hear a loud “pop” sound, and then you feel your car beginning to vibrate abnormally. You think you might have a flat tyre, so you pull off to the left into the emergency lane. After parking your car, you turn the engine off, remove your keys, and get out of the car to see what has happened. You notice that one of the tyres on the passenger side of your car is flat. You go into the boot of your car to get a jack and spare tyre. You use the jack to raise the car from the pavement, and you begin removing the flat tyre. While doing this, you see a car coming down the road. It is driving rather quickly. It hits a puddle of rainwater and the driver appears to lose control of their vehicle. Their vehicle begins to slide, and it collides with your car. The force of the impact pushes your car off of the jack, and the car lands on your arm. Your arm becomes pinned between the car and the pavement, and you are in intense pain.

The driver that struck your car regains control of their vehicle, and slows down to a stop. You shout as loudly as you can for the driver to come and help you. After a few moments, the vehicle drives away. You try to get your arm out from under your car, but you can’t. You remember that your mobile phone is in your pocket, so you take it out and call for emergency help. After about a half an hour, emergency help arrives and is able to free your arm. You are rushed to the hospital, where you undergo surgery on your arm. When you awake the next morning, you learn that the damage to your arm was too great, and that your arm had to be removed."
Driver caused suffering  Enraged by recklessness  Should've helped me
Driver inflicted agony  Furious they sped  They should've stayed

Baseline emotion priming story and corresponding priming statements

"You are driving back to your home during a heavy rainstorm late one evening. Because the rainfall is rather heavy, you drive slightly below the speed limit. There do not seem to be any other cars on the road, although it is difficult to see clearly due to the rainfall. You turn your windscreen wipers up to the maximum speed so that you can see the road more easily.

The rain is making it difficult to see clearly, but you notice a puddle of rainwater on the road ahead of you. The puddle of rainwater has collected in a rather deep pothole, but you cannot see this because the puddle has made the pothole very difficult to detect. You slow down as you cross over the puddle, although you hear a loud “bang” as your tyre hits the pothole. The loud “bang” sound is followed by a “pop,” and then you feel your car beginning to vibrate abnormally. You think you might have a flat tyre, so you pull off to the left into the emergency lane. After parking your car, you turn the engine off, remove your keys, and get out of the car to see what has happened. You notice that one of the tyres on the passenger side of your car is flat. You go into the boot of your car to get a jack and spare tyre. You use the jack to raise the car from the pavement, and you replace the flat tyre with your spare tyre. After lowering the jack, you place the flat tyre and jack into the boot of your car, and you return to the driver’s seat.

After starting your car’s engine, you look into your rear view mirror before pulling back onto the road from the emergency lane. You see that another vehicle is coming down the road. You wait until the other vehicle has passed before pulling back onto the road, and then you carry on driving home. After arriving at home, you decide to get some rest. The next morning, you decide to turn on the television to watch the local news. The top story is about the rainstorm from the previous night. Despite a large accumulation of rainfall, there have been no reports of serious property damage or an increase in the number of car accidents. After watching the news, you make an appointment at a nearby garage to have your tyre changed."

It poured rain  Inspected flat tyre  Watched speed limit
It rained hard  Lowered the jack  Watched vehicle pass
It was raining  Put jack back  Wipers were on
Rain hit windscreen  Raised my car  Carried on driving
Rained while driving  Removed flat tyre  Drove to mine
Rainfall was heavy  Stored flat tyre  I drove home
Stormy that evening  Checked my speedometer  Left emergency lane

203
<table>
<thead>
<tr>
<th>Changed my tyre</th>
<th>Checked rear view</th>
<th>Merged onto road</th>
</tr>
</thead>
<tbody>
<tr>
<td>Got out jack</td>
<td>Drove under limit</td>
<td>Returned to mine</td>
</tr>
<tr>
<td>Got spare tyre</td>
<td>Waited to merge</td>
<td>Went back home</td>
</tr>
</tbody>
</table>
D. Altruistic Choices Task: Reciprocal Altruism Dilemmas and BIATs

Reciprocal Altruism Dilemma 01
- You are leaving your place of work to go home and relax after a long day.
- However, you see that your co-worker is having serious car trouble, and you consider offering them a lift home although this would add an hour to your commute.
- Would you prefer to…
  - … go directly home and relax?
  - … give your co-worker a lift home?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category Name</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mem. 1</td>
<td>go relax</td>
<td>give lift</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>get rest</td>
<td>provide ride</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>go unwind</td>
<td>drive colleague</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>go relax</td>
<td>give transport</td>
<td>DESIRED</td>
<td>UNDESIGNED</td>
</tr>
<tr>
<td></td>
<td>lounge around</td>
<td>give lift</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Reciprocal Altruism Dilemma 02
- You are at home watching your favourite film on Saturday afternoon.
- However, you receive a panicked phone call from your co-worker, and you are asked if you would come in right away to help complete a project before a deadline that evening.
- Would you prefer to…
  - …continue watching your favourite film?
  - …leave right away to help complete the project?

Reciprocal Altruism Dilemma 03
- You will soon leave to attend a performance by your favourite musician, for which you have a single ticket.
- However, you remember previously agreeing to pick up your friend from the airport this evening, and you must choose whether to miss the musical performance to pick up your friend.
- Would you prefer to…
… attend the performance by your favourite musician?
… pick up your friend from the airport?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>attend performance</td>
<td>get friend</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>see musician</td>
<td>transport pal</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>attend gig</td>
<td>drive mate</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>see concert</td>
<td>get friend</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>attend performance</td>
<td>collect friend</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Reciprocal Altruism Dilemma 04

- You have saved some money you plan to use for travelling.
- However, you learn your friend has recently lost their job and is concerned about being able to pay their mortgage, and you consider using the money you saved to pay your friend’s mortgage.
- Would you prefer to…
  - … help to pay your friend’s mortgage?
  - … keep your money saved for travelling?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>pay mortgage</td>
<td>keep money</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>cover debt</td>
<td>retain income</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>lend money</td>
<td>keep money</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>pay mortgage</td>
<td>keep earnings</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>give loan</td>
<td>retain cash</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Reciprocal Altruism Dilemma 05

- You are sitting down at your place of work to eat a lunch you prepared yesterday.
- However, you notice that a co-worker who is currently having money problems is going without lunch, and you think about offering to share your lunch though there is just enough for one person.
- Would you prefer to…
  - … share your lunch with your co-worker?
  - … keep the lunch you prepared just for yourself?
Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>share lunch</td>
<td>keep lunch</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>give food</td>
<td>retain food</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>split lunch</td>
<td>keep meal</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>share meal</td>
<td>withhold lunch</td>
<td>DESIRED</td>
<td>UNDESIRIED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>share lunch</td>
<td>keep lunch</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Reciprocal Altruism Dilemma 06

- You plan to spend your evening resting after an extremely stressful day.
- However, you receive a phone call from a friend saying their babysitter has cancelled, and you are asked if you could babysit that evening while they visit a family member in the hospital.
- Would you prefer to…
  - … rest after your extremely stressful day?
  - … babysit for your friend?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>get rest</td>
<td>babysit child</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>go relax</td>
<td>watch toddler</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>go unwind</td>
<td>mind child</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>get rest</td>
<td>babysit infant</td>
<td>DESIRED</td>
<td>UNDESIRIED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>lounge around</td>
<td>babysit child</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Reciprocal Altruism Dilemma 07

- You wake up to learn your job has given employees an optional day off due to bad weather in the area, and you look forward to staying indoors.
- However, you learn the route you take to work hasn’t been affected by the weather, and you know your help is needed to finish a big project.
- Would you prefer to…
  - … stay in and take the day off from work?
  - … go in to work to help finish the big project?

Reciprocal Altruism Dilemma 08
• You are preparing to go home and watch television after working a double shift to complete an office-wide project.

• However, you learn that your colleague’s computer has erased their share of the project, and you know they could use your help redoing the work they completed.

• Would you prefer to…
  o … go home and watch television?
  o … help your colleague redo their work that was erased?

Reciprocal Altruism Dilemma 09

• You recently moved into a new flat just large enough to comfortably fit in your belongings.

• However, you have a friend who recently became unemployed after being made redundant and they cannot afford their rent, and you realise you could offer to let them stay with you temporarily.

• Would you prefer to…
  o … let your friend stay with you temporarily?
  o … keep your new flat just for yourself?

Reciprocal Altruism Dilemma 10

• You have a voucher for a free item from an expensive bakery and you are picking out your favourite cake.

• However, you remember that your friend’s birthday is tomorrow, and you consider using the voucher for a birthday cake though you dislike their favourite flavour.

• Would you prefer to…
  o … use the voucher for your friend’s birthday cake?
  o … get your favourite cake?

Reciprocal Altruism Dilemma 11

• You are about to take your car out for a daytrip on Saturday to visit one of your favourite places.

• However, your friend calls saying their car has died and they must drive their elderly parents to several doctors’ appointments that day, and you think about lending them your car.

• Would you prefer to…
  o … allow your friend to borrow your car to help their parents?
... take your car for a daytrip to one of your favourite places?

Reciprocal Altruism Dilemma 12
- You purchased return train tickets several weeks ago for a holiday in London next weekend.
- However, you have learned your friend is having surgery that same weekend and wants you there for moral support, and you are considering paying a large fee to rebook your tickets.
- Would you prefer to...
  - ... rebook your tickets to stay and give moral support to your friend?
  - ... take a holiday in London next weekend?

Reciprocal Altruism Dilemma 13
- Your boss appreciates your hard work on a recent project and has offered to let you leave early, so you plan to go home and catch up on sleep.
- However, you have a colleague who is having trouble understanding an assignment, and you consider spending this time helping them.
- Would you prefer to...
  - ... stay to help your colleague understand the assignment?
  - ... leave work early to go home and catch up on sleep?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category Name</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mem. 1</td>
<td>help colleague</td>
<td>go sleep</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td></td>
<td>assist co-worker</td>
<td>get rest</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>help colleague</td>
<td>take nap</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>support colleague</td>
<td>get sleep</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>help co-worker</td>
<td>go sleep</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Reciprocal Altruism Dilemma 14
- You plan to spend your evening eating take-away and reading a new book by your favourite author.
• However, your co-worker lives alone and missed the last few days of work due to a chronic medical condition, and you think about offering to prepare a meal for them.

• Would you prefer to…
  o … spend your evening eating take-away and reading a new book?
  o … prepare a meal for your co-worker who is sick?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>stay home</td>
<td>take meal</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>remain inside</td>
<td>deliver meal</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>keep indoors</td>
<td>provide supper</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>stay in</td>
<td>take dinner</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>stay home</td>
<td>take meal</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Reciprocal Altruism Dilemma 15

• You are deciding what to order at your favourite take-away restaurant on Thursday evening.

• However, you receive a call from a friend whose cooking you dislike, but who is going through a difficult time, and you are asked over for a meal they’ve prepared.

• Would you prefer to…
  o … eat a meal prepared by your friend going through a difficult time?
  o … order take-away from your favourite restaurant?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>support friend</td>
<td>order take-away</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>support friend</td>
<td>order take-away</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>comfort pal</td>
<td>pick-up dinner</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>console mate</td>
<td>order dinner</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>comfort friend</td>
<td>visit drive-through</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>
E. Altruistic Choices Task: Pure Altruism Dilemmas and BIATs

Pure Altruism Dilemma 01
- You are leaving your place of work to go home and relax after a long day.
- However, you see a vehicle belonging to someone you’ve not met before broken down outside your office, and you consider offering to give them a lift to the garage.
- Would you prefer to…
  - … go directly home and relax?
  - … give a lift to the person you’ve not met?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>go relax</td>
<td>transport stranger</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>get rest</td>
<td>take unknown-person</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>go unwind</td>
<td>drive stranger</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>go relax</td>
<td>transport stranger</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>lounge around</td>
<td>drive unknown-person</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Pure Altruism Dilemma 02
- You are at home watching your favourite film on Saturday afternoon.
- However, you hear your doorbell and open your door to find an elderly person you’ve not met before, and they ask for your help finding their pet dog that has just gotten loose.
- Would you prefer to…
  - … leave right away to help find the woman’s dog?
  - … continue watching your favourite film?

Pure Altruism Dilemma 03
- You have just left to attend a performance by your favourite musician, for which you have a single ticket.
• However, you see someone waving for help by a broken-down car while driving down a deserted road, and you must decide whether to miss the performance to pull over and help.

• Would you prefer to…
  o … attend the performance by your favourite musician?
  o … pull over for the person waving for help?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>attend performance</td>
<td>help unknown-person</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>see musician</td>
<td>aid stranger</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>attend gig</td>
<td>help unknown-person</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>see concert</td>
<td>aid unknown-person</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>attend performance</td>
<td>assist stranger</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

**Pure Altruism Dilemma 04**

• You have saved some money you plan to use for travelling.

• However, you learn that a local domestic violence shelter is struggling to pay its bills and may have to shut down, and you consider donating what you've saved to help the shelter stay open.

• Would you prefer to…
  o … help to pay the shelter’s overdue bills?
  o … keep your money saved for travelling?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>support shelter</td>
<td>keep money</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>assist safehouse</td>
<td>retain income</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>support shelter</td>
<td>keep money</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>aid refuge</td>
<td>keep earnings</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>fund safehaven</td>
<td>retain cash</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>
Pure Altruism Dilemma 05

- You are sitting down at your place of work to eat a lunch you prepared yesterday.
- However, you saw a person outside your building earlier in the day who is homeless and looked extremely hungry, and you consider going to see whether they would like to have your lunch.
- Would you prefer to…
  - … give your lunch to the person who is homeless?
  - … keep the lunch you prepared just for yourself?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>feed homeless</td>
<td>keep lunch</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>feed beggar</td>
<td>retain food</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>feed vagrant</td>
<td>keep meal</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>feed homeless</td>
<td>withhold lunch</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>feed tramp</td>
<td>keep lunch</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Pure Altruism Dilemma 06

- You plan to spend your evening resting after an extremely stressful day.
- However, your doorbell is rung by a resident of a nearby community whom you've never met asking if you’ll join others tonight picking up rubbish at a local park, and you consider going.
- Would you prefer to…
  - … help pick up rubbish at the park?
  - … rest after your extremely stressful day?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>pick-up rubbish</td>
<td>get rest</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>remove litter</td>
<td>go relax</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>collect garbage</td>
<td>go unwind</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>pick-up rubbish</td>
<td>get rest</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>pick-up trash</td>
<td>lounge around</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>
Pure Altruism Dilemma 07
• You wake up to learn your job has given employees the day off due to bad weather in the area, and you look forward to staying indoors.
• However, you learn that a nearby nursing home urgently needs snow shoveled from the pavement, and you consider volunteering to help.
• Would you prefer to…
  o … stay in on your day off from work?
  o … volunteer to shovel snow at the nursing home?

Pure Altruism Dilemma 08
• You are leaving to go home and watch television after working a double shift to complete an office-wide project.
• However, you meet a visually impaired person during your commute who is lost, and you are asked if you could help them to get back home which is rather far away.
• Would you prefer to…
  o … help the visually impaired person to get back home?
  o … go home and watch television?

Pure Altruism Dilemma 09
• You recently moved into a new flat just large enough to comfortably fit in your belongings.
• However, you learn that a neighbour you’ve never met is staying in a hotel that doesn’t allow pets after losing their home to a fire, and you consider offering to keep their pet temporarily.
• Would you prefer to…
  o … keep your new flat just for yourself?
  o … keep your neighbour’s pet temporarily?

Pure Altruism Dilemma 10
• You have a voucher for a free item from an expensive bakery and you are picking out your favourite cake.
• However, you saw a person outside the shop who is homeless and looked very hungry, and you consider using the voucher to get them some food instead.
• Would you prefer to…
  o … get food for the person who is homeless?
Pure Altruism Dilemma 11
- You are leaving in your car to take a daytrip on Saturday to visit one of your favourite places.
- However, you are waved down by an elderly man who says he and his wife must go to several doctors’ appointments today but his child is too ill to drive, and you are asked to drive them.
- Would you prefer to…
  - … take your car for a daytrip to one of your favourite places?
  - … take the elderly couple to several doctors’ appointments?

Pure Altruism Dilemma 12
- You purchased return train tickets several weeks ago for a holiday in London.
- However, you meet a parent at the train station who needs to return to London now because their child has fallen ill, and you consider offering them your ticket because the train is fully booked.
- Would you prefer to…
  - … use your ticket for a holiday in London?
  - … give your ticket to the parent whose child has just fallen ill?

Pure Altruism Dilemma 13
- Your boss appreciates your hard work on a recent project and has offered to let you leave early, so you head home to catch up on sleep.
- However, you see a neighbour you’ve never met moving heavy items onto a removal van without help, and you consider stopping to help.
- Would you prefer to…
  - … help the neighbour you’ve never met move house?
  - … go directly home and catch up on sleep?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>help move</td>
<td>go sleep</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>assist lifting</td>
<td>get rest</td>
<td>FAVOURLED</td>
<td>NOT FAVOURLED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>carry boxes</td>
<td>take nap</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>help move</td>
<td>get sleep</td>
<td>DESIRED</td>
<td>UNDESIRER</td>
</tr>
</tbody>
</table>
Pure Altruism Dilemma 14
- You plan to spend your evening eating take-away and reading a new book by your favourite author.
- However, you know of a person who is homeless and spends nights in a nearby shopping centre, and you consider cooking them dinner and taking them warm clothes.
- Would you prefer to…
  - … spend your evening eating take-away and reading a new book?
  - … take food and clothes to the person who is homeless?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>stay home</td>
<td>help homeless</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>remain inside</td>
<td>aid vagrant</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>keep indoors</td>
<td>help tramp</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>stay in</td>
<td>assist beggar</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>stay home</td>
<td>help homeless</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Pure Altruism Dilemma 15
- You are deciding whether to order food from your favourite take-away restaurant on Thursday evening.
- However, you are invited to attend a charity fundraiser dinner being held that evening at a local restaurant you dislike, and you consider attending to show support.
- Would you prefer to…
  - … order take-away from your favourite restaurant?
  - … attend a charity fundraiser dinner at a restaurant you dislike?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>order take-away</td>
<td>attend fundraiser</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>order take-away</td>
<td>attend fundraiser</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>pick-up</td>
<td>support</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>dinner</td>
<td>charity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>---------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>order dinner</td>
<td>support benefit</td>
<td>DESIRED</td>
<td>UNDESired</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>visit drive-through</td>
<td>attend benefit</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>
F. Altruistic Choices Task: Social-Selfish Dilemmas and BIATs

Social-Selfish Dilemma 01
- You are leaving your place of work to go home and relax after a long day.
- However, you learn that tonight is the last night cinemas are screening a film you want to see, and you think about ringing a friend to go and see the film with you.
- Would you prefer to…
  - … go directly home and relax?
  - … see the film with your friend before it leaves cinemas?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category Name</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mem. 1</td>
<td>go relax</td>
<td>see film</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>get rest</td>
<td>visit cinema</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>go unwind</td>
<td>see movie</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>lounge around</td>
<td>see film</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
</tbody>
</table>

Social-Selfish Dilemma 02
- You are at home watching your favourite film on Saturday afternoon.
- However, you notice the weather has become very nice, and you wonder if your friend would meet you to spend the rest of the afternoon outdoors before the weather changes.
- Would you prefer to…
  - … meet your friend to spend the rest of the afternoon outdoors?
  - … continue watching your favourite film?

Social-Selfish Dilemma 03
- You will soon leave to attend a performance by your favourite musician, for which you have a single ticket.
- However, someone just offered you two free tickets to a sports event this evening that they can’t use, and you consider asking a friend to this rather than seeing the musical performance.
- Would you prefer to…
  - … attend the performance by your favourite musician?
  - … go to the sports event with a friend?
Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>attend performance</td>
<td>watch sports</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>see musician</td>
<td>attend match</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>attend gig</td>
<td>catch game</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>see concert</td>
<td>watch contest</td>
<td>DESIRED</td>
<td>UNDESIRIED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>attend performance</td>
<td>watch sports</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Social-Selfish Dilemma 04
- You have saved some money you plan to use for travelling.
- However, you get a call from a friend at a nearby store telling you an expensive item you want is on sale, and you consider meeting your friend and using the money you saved to buy the sale item.
- Would you prefer to…
  - … meet your friend and buy the sale item you want?
  - … keep your money saved for travelling?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>attend sale</td>
<td>keep money</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>purchase cheaply</td>
<td>retain income</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>get bargain</td>
<td>keep money</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>use discount</td>
<td>keep earnings</td>
<td>DESIRED</td>
<td>UNDESIRIED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>attend sale</td>
<td>retain cash</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Social-Selfish Dilemma 05
- You are sitting down at your place of work to eat a lunch you prepared yesterday.
- However, you realise that you have a voucher for a free lunch from a nearby restaurant which expires today, and you consider contacting a friend to see if they would join you at the restaurant for lunch.
- Would you prefer to…
  - … join your friend at a nearby restaurant for a free lunch?
  - … eat the lunch you prepared for yourself?

Corresponding BIAT stimuli:
Social-Selfish Dilemma 06

- You plan to spend your evening resting after an extremely stressful day.
- However, you wonder if you might find it more relaxing to spend the evening amongst friends, and you give thought to inviting a few people over for a quiet evening at your home.
- Would you prefer to…
  - … invite a few people over for a quiet evening at your home?
  - … rest after your extremely stressful day?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>invite friends</td>
<td>get rest</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>invite mates</td>
<td>go relax</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>call buddies</td>
<td>go unwind</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>gather friends</td>
<td>get rest</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>invite friends</td>
<td>lounge around</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Social-Selfish Dilemma 07

- You wake up to learn your job has given employees the day off due to bad weather in the area, and you look forward to staying indoors.
- However, you are contacted by a friend inviting over those willing to go out in the bad weather, and you have to decide whether or not to join your friends.
- Would you prefer to…
  - … stay in on your day off from work?
  - … go to your friend’s social gathering?

Social-Selfish Dilemma 08

- You are preparing to go home and watch television after working a double shift to complete an office-wide project.
• However, you realise you could spend the evening doing a craft project you recently began with a friend, and you consider ringing to see if they’re free to join you.

• Would you prefer to…
  o … do the craft project you recently began with your friend?
  o … go home and watch television?

Social-Selfish Dilemma 09
• You recently moved into a new flat just large enough to comfortably fit in your belongings.
• However, your friend is considering getting a roommate to take a vacant room in their much bigger and cheaper flat, and you think about asking your friend if you could become their roommate.
• Would you prefer to…
  o … stay at your new flat by yourself?
  o … move into your friend’s bigger and cheaper flat?

Social-Selfish Dilemma 10
• You have a voucher for a free item from an expensive bakery and you are about to pick out your favourite cake.
• However, your friends are in the area grabbing drinks, and you consider joining them although you can’t bring a cake, the shop is shutting, and your voucher expires today.
• Would you prefer to…
  o … grab drinks with your friends who are in the area?
  o … get your favourite cake?

Social-Selfish Dilemma 11
• You are about to take your car out for a daytrip on Saturday to visit one of your favourite places.
• However, you have just found out that several of your friends have decided to stay in the area and to spend the day together, and you think about asking to join them.
• Would you prefer to…
  o … take your car for a daytrip to one of your favourite places?
  o … join your friends’ in spending the day together in the area?
Social-Selfish Dilemma 12

- You purchased return train tickets several weeks ago for a holiday in London next weekend.
- However, you have a friend visiting Edinburgh that same weekend who suggested you could join them if you’d like, and you are considering paying a large fee to rebook your tickets.
- Would you prefer to…
  - … take a holiday in London next weekend?
  - … rebook your tickets to join your friend on a trip to Edinburgh?

Social-Selfish Dilemma 13

- Your boss appreciates your hard work on a recent project and has offered to let you leave early, so you plan to go home and catch up on sleep.
- However, you bump into a friend you’ve not seen in a while during your commute, and you consider asking if they would like to go to the pub.
- Would you prefer to…
  - … go to the pub with a friend you’ve not seen in a while?
  - … go directly home and catch up on sleep?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>get drinks</td>
<td>go sleep</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>grab cocktails</td>
<td>get rest</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>get drinks</td>
<td>take nap</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>have pints</td>
<td>get sleep</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>grab beers</td>
<td>go sleep</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Social-Selfish Dilemma 14

- You plan to spend your evening eating take-away and reading a new book by your favourite author.
- However, you wonder if you might have a more enjoyable time getting dinner and drinks with a friend, and you consider ringing your friends to see who is available.
- Would you prefer to…
  - … spend your evening eating take-away and reading a new book?
  - … ring your friends to see who is available for dinner and drinks?
Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>stay home</td>
<td>ring friends</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>remain inside</td>
<td>ring friends</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>keep indoors</td>
<td>call buddies</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>stay in</td>
<td>gather friends</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>stay home</td>
<td>gather mates</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>

Social-Selfish Dilemma 15

- You are deciding what to order at your favourite take-away restaurant on Thursday evening.
- However, you recall some friends talked about getting dinner together that Thursday evening, and you contemplate whether you’d rather meet them for dinner instead.
- Would you prefer to…
  - … order take-away from your favourite restaurant?
  - … eat with your friends who talked about getting dinner together?

Corresponding BIAT stimuli:

<table>
<thead>
<tr>
<th>Category</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>order take-away</td>
<td>meet friends</td>
<td>{PREFERRED}</td>
<td>{NON-PREFERRED}</td>
</tr>
<tr>
<td>Mem. 1</td>
<td>order take-away</td>
<td>meet friends</td>
<td>FAVOURED</td>
<td>NOT FAVOURED</td>
</tr>
<tr>
<td>Mem. 2</td>
<td>pick-up dinner</td>
<td>see buddies</td>
<td>WANTED</td>
<td>UNWANTED</td>
</tr>
<tr>
<td>Mem. 3</td>
<td>order dinner</td>
<td>join pals</td>
<td>DESIRED</td>
<td>UNDESIRED</td>
</tr>
<tr>
<td>Mem. 4</td>
<td>visit drive-through</td>
<td>visit mates</td>
<td>PREFERRED</td>
<td>NON-PREFERRED</td>
</tr>
</tbody>
</table>