The role of peer rejection in adolescent depression: genetic, neural and cognitive correlates

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Abstract

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Adolescent depression is a major public health problem, which is associated with educational problems, long-term psychiatric illness and suicide. One major source of stress during adolescence is peer rejection. In this thesis, I investigate the nature of the relationship between peer rejection and adolescent depression. In a review of longitudinal and experimental studies, I describe a bi-directional relationship between peer rejection and depressive symptoms. I then outline how genetic, cognitive and neural vulnerability may modify the effects of peer rejection on adolescent depression. Finally, I introduce five empirical chapters which test these hypotheses using different methodological approaches. The first study is a molecular genetic analysis of a sample of adolescents with and without a diagnosis of mood disorder. I report an interaction between diagnostic group, environmental stress (though not peer rejection specifically) and 5HTTLPR genotype on symptoms of anxiety, which supports the role of genetic factors in modifying the relationship between environmental stress and adolescent mood disorder. The second study is a behavioural study of negative attention biases in a typically developing sample of adolescents. I report a negative attention bias in adolescents with low (versus high) self-esteem. Although the data do not support a causal role for attention biases in adolescent depression, such biased cognitions could also moderate responses to peer rejection, maintaining affective symptoms. A final set of three fMRI datasets investigates how neural circuitry may influence depressed adolescents' responses to peer rejection at three distinct stages: i) expectation of peer feedback, ii) the receipt of peer rejection, iii) emotion regulation of peer rejection. Data show distinct behavioural and neural differences between depressed patients and healthy controls during expectation and reappraisal of peer rejection, although heightened emotional reactivity immediately following the receipt of peer rejection did not differentiate behavioural or neural responses in adolescents with and without depression.

List of publications

A paper based on Chapter 1 has been accepted by *Depression and Anxiety*:

> B. Platt, K. Cohen-Kadosh, J.Y. Lau (in press) The role of peer rejection in adolescent depression. *Depression and Anxiety*, DOI: 10.1002/da.22120

A paper based on Chapter 2 is currently being prepared for submission:

> B. Platt, J.Y. Lau, J. Bemis, E. Gorodetsky, D.S. Pine, D. Goldman, M. Ernst (in prep.) The relationship between serotonin transporter gene polymorphisms (5HTTLPR) and symptoms of depression in adolescents with mood and anxiety disorders.

A paper based on the fMRI studies reported in Chapters 4, 5 and 6 has been published in *Child Psychiatry and Human Development*:

> A.D. Haddad, B. Platt, A.C. James and J.Y. Lau (2012) Anxious and Non-Anxious Adolescents' Experiences of Non-Clinical Magnetic Resonance Imaging Research. *Child Psychiatry and Human Development*, DOI: 10.1007/s10578-012-0350-x

A paper based on Chapter 6 is currently being prepared for submission:

> B. Platt, C.A. Campbell, A.C. James, R. Norbury, S.E. Murphy, M. Cooper, J.Y. Lau (in prep.) Learning to reappraise peer rejection in adolescents with and without depression.

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"For the strength of the Pack is the Wolf, and the strength of the Wolf is the Pack" - Rudyard Kipling.

Contents

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Chapter 1

Review of peer rejection and adolescent depression

Adolescence is a vulnerable period for the development of depression. Adolescent depression is associated with poorer educational and psychosocial outcomes and long-term psychiatric problems. Psychosocial stress is a major predictor of adolescent depression and diathesis-stress models seek to uncover the biological and psychological factors which may explain why some adolescents are more vulnerable to the effects of psychosocial stress than others. In this review of the literature, I focus on one specific source of psychosocial stress during adolescence - peer rejection. I present a framework for understanding the role of peer rejection in adolescent depression. Using this framework, I summarise longitudinal and experimental studies, which suggest a bi-directional relationship between peer rejection and adolescent depression. I then consider the role of genetic factors in adolescent depression (though not specifically in relation to responses to peer rejection). Taking a molecular genetic approach, I review data on the association between the 5HTTLPR polymorphism, environmental stress (more generally),

and symptoms of mood disorder in children and adolescents. I then consider how cognitive biases may contribute to the relationship between peer rejection and adolescent depression. I describe cognitive bias modification studies which have helped illustrate the causal role of cognitive biases in adolescent depression. I also describe an emerging literature which suggests that some cognitive biases (particularly in attention) may serve to heighten depressed adolescents' perception of social threat. Finally, I address depression-linked differences in neural responses to peer rejection. I begin by describing data on the neural correlates of peer rejection in typically developing adolescents. I then outline how neural circuitry may influence depressed adolescents' responses to peer rejection at three distinct stages: i) expectation of peer feedback, ii) the receipt of peer rejection, iii) emotion regulation of peer rejection. This review provides the context for a series of empirical studies, which use diverse approaches to studying the relationship between peer rejection and adolescent depression.

1.1 The vulnerability of adolescence

Major Depressive Disorder (MDD) is a mood disorder characterised by sustained feelings of sadness and lack of positive affect which interfere with social functioning (Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [9] and International Statistical Classification of Diseases and Related Health Problems (ICED-10) [375]). Emotional symptoms are accompanied by a range of physical and behavioural symptoms (e.g. sleep disturbances, fatigue, changes in appetite or weight, psychomotor retardation, and tearfulness) and cognitive disturbances (impaired decision making and concentration, feelings of worthlessness, pessimism about the future, and suicidal ideation) [253]. A clinical diagnosis of depression is based on

symptom severity, persistence, and degree of social and functional impairment.

Depression is the most common mental health problem during adolescence [376] and there appears to be a peak rise in depression during adolescence. Around 8% of adolescents suffer from MDD [31], although one-year point prevalence rates rise dramatically from around 2% in early adolescence (ages 13-15), to 15% in middle adolescence (ages 15-18) [148], suggesting middle-adolescence is a particularly vulnerable time. Although the symptoms of depression are largely similar in adults and adolescents, there may be small differences in the way that depression presents itself during adolescence [52]. For example, depression may manifest as irritability and anger [31] or behavioural problems [254] during adolescence. For many adolescents these symptoms subside, for others they persist and predict a significant number of adult depressive conditions [187, 281].

Since adolescence is defined by biological and social factors (the onset of adolescence is generally regarded as puberty and the offset as the age when social roles become adult-like [76, 342]) which vary between cultures [34], applying chronological ages to this period of development provides merely a marker of adolescence. In this thesis my adolescent samples are aged 12-18.

Depression may account for the fact that suicide is the leading cause of death in female adolescents [31, 131]. Adolescent depression also predicts poorer educational and psychosocial outcomes [31, 104], as well as long-term psychiatric problems such as substance abuse [189] and suicidal behaviour [365]. Given these costs to the individual and to society, identifying effective prevention and treatment is needed to reduce this disease burden. To achieve this, more needs to be understood about the aetiology of depression at this developmentally-sensitive juncture.

Psychosocial stress is a powerful predictor of adolescent depression [350]. As adolescence involves unique upheaval, with young people spending more time with their peers than with their families [43, 74, 199, 240] and exerting a great deal of energy forming peer networks [341], peer rejection may be a particularly potent source of psychosocial stress. For the purposes of this review, I use the term peer rejection to encompass social exclusion (where a person is refused social contact) and ostracism (where a targeted individual is denied social connection). This is consistent with a recent review of emotional reactions to rejection [32]. As social networking sites proliferate, so too does the potential for peer rejection, and understanding how peer interactions come to shape typical and atypical adolescent emotional development is critical.

Of note, there are a number of other forms of rejection that may play a role in adolescent depression, but which are beyond the scope of the current thesis. Firstly, although the impact of romantic relationship breakup on adolescent depression [244] and suicidality [108] has been investigated, these studies are few. Secondly, parental rejection [224, 245] and lack of parental support [237, 307] are associated with adolescent maladjustment and depressive symptoms. However, recent evidence suggests that compared to peer rejection, parental rejection plays a less significant role in the development of depression, particularly during mid to late adolescence [40].

Substantial biological change during adolescence may explain why adolescents in general are more vulnerable to the effects of peer rejection than adults or children [315]. For example, the limbic regions associated with the affective processing of social stimuli are influenced by gonadal hormones, which surge during middle to late adolescence [256, 279]. Protracted maturation of brain regions involved in the cognitive control of emotional respond-

ing may also explain heightened emotional reactivity [58, 201, 256, 334]. While peer rejection may be considered a relatively 'normal' event in adolescence, for many it can yield negative effects on well-being. In Section 1.2 I provide a comprehensive review of studies (including novel experimental paradigms) which investigate the role of peer rejection in adolescent depression.

Another critical question is why some adolescents react more negatively to peer rejection than others. Diathesis-stress models assume that psychosocial stressors such as peer rejection are experienced more negatively by those who possess particular (biological or psychological) vulnerabilities and have been widely implicated in adolescent depression [40, 148]. There is established evidence for a genetic component to adolescent depression, and the adult literature suggests that the effects of psychosocial stress on depressive symptoms may be moderated by genetic factors [59, 331]. That is, the presence of certain genotypes can enhance vulnerability towards stressors. Neural factors may also account for differences in response to peer rejection in adults with low self-esteem [92, 144, 274, 335]. Finally, cognitive factors may also influence the effect of peer rejection on affect [56]. Given that the impact of peer rejection may vary across development [2, 313–315], Section 1.3, Section 1.4 and Section 1.5 discuss emerging adolescent data on the role of these factors in moderating the impact of peer rejection on depression.

Specifically, Section 1.3 discusses the contribution of genetic factors to adolescent depression, which provides the context for a study of the effects of the 5HTTLPR polymorphism on symptoms in adolescents with depression and anxiety, reported in Chapter 2. Section 1.4 describes the role that cognitive vulnerability may play in moderating the effects of peer rejection on adolescent depression, providing the context for an experimental study

of negative attention biases in adolescents and young adults with low self-esteem (Chapter 3). Section 1.5 describes data from emerging functional magnetic resonance imaging (fMRI) studies of peer rejection in adolescence, and how neural factors may influence the effect of peer rejection on adolescent depression. This section provides the context for an fMRI study of depressed adolescents' responses to peer rejection at three distinct stages: i) expectation of peer feedback (Chapter 4), ii) the receipt of peer rejection (Chapter 5), and iii) emotion regulation of peer rejection (Chapter 6).

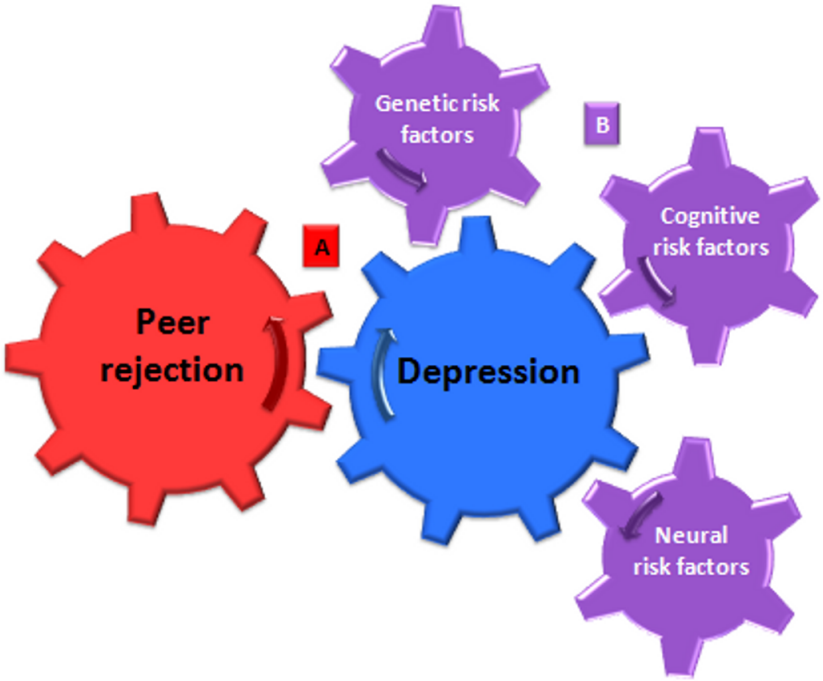

Figure 1.1: Framework for understanding the role of peer rejection in adolescent depression. (A) A bi-directional relationship between peer rejection and depression (B) The potential moderating role of genetic, cognitive and neural risk factors

The framework proposed for understanding how peer rejection influences adolescent depression and the cognitive and neural risk factors that enhance its negative effects is illustrated in Figure 1.1.

1.2 How are peer rejection and adolescent depression related?

Given the social nature of the human species, the ability to detect and respond to social exclusion is thought to be of evolutionary importance [21]. As such, peer rejection causes distress and threatens self-esteem, whereas peer acceptance generally generates positive affect and heightens self-esteem [370]. These automatic responses to peer rejection are thought to be important in shaping prosocial behaviour [370]. Numerous studies of adults have confirmed the potential for peer rejection to elicit distress (see Williams, 2007 for a review [370]). However, peer rejection also elicits maladaptive depressogenic responses [331], which when repeatedly elicited could lead to the development of depression [370]. This may be because emotional resources are depleted [370], or because repeated peer rejection may indicate that current coping strategies are inadequate and social withdrawal may be more adaptive [7]. With growing knowledge of the detrimental effects of peer rejection on adult self-esteem, I now turn to consider the effects of peer rejection during adolescence, when peer relationships may be even more important.

Although studies of unselected adolescents [42, 157, 179, 219] and adolescents with depression [40, 171, 173, 175] demonstrate long-standing and unambiguous support for a correlation between peer rejection and depressive symptoms, the direction of these effects is less clear. Does peer rejection precede depression, or does depression elicit more rejecting experiences? In this section, highlighted in Figure 1.1A, I discuss evidence for a bi-directional relationship between peer rejection and depressive symptoms.

1.2.1 Longitudinal studies of peer rejection

Longitudinal studies have attempted to address the question of the temporal nature of peer rejection and depressive symptoms (see Table 1.1). Naturally occurring peer rejection is measured at time 1, and depressive symptoms are followed up at time 2. Many though not all [246] longitudinal studies suggest that peer rejection temporally predicts adolescent depressive symptoms [67, 105, 110, 163, 261, 289, 317, 324, 361, 372]. Strengths of these studies include the fact that sample sizes are relatively large, improving the power to detect effects. Furthermore, depressive symptoms are generally assessed using standardised self-report measures such as the children's depression inventory (CDI) [192] which are often complemented with parent- or teacher-reports. A (small) majority of studies control for depressive symptoms at time 1 [105, 163, 289, 317, 324, 361, 372], strengthening the extent to which depressive symptoms at follow-up could be explained by the longitudinal effects of peer rejection. Although all studies reported measured depressive symptoms during adolescence, some assessed peer rejection during early-middle childhood [67, 105, 163, 246] or late childhood [261, 317, 372], rather than adolescence [110, 289, 324], limiting interpretations about the effects of adolescent peer rejection on depressive symptoms. Time between assessment of peer rejection and depressive symptoms varies between 6 months [361] and 8 years [246], making comparisons between studies difficult. Although one study relies on just a 2-item self-report measure of rejection [361], the majority of studies use a widely reported peer nomination measure of rejection [66]. Some limitations of this peer nomination measure [66] deserve mentioning.

The Coie et al. (1982) measure of peer rejection requires participants to nominate their most liked and disliked classmates [66]. For each peer, the

number of nominations in each category (liked, disliked) is divided by the total number of classmates, to produce a value between 0 and 1 for peer acceptance (liked) and rejection (disliked), or a continuous value if rejection scores are subtracted from acceptance scores. Although it is the most commonly cited observational measure of peer rejection and demonstrates high reliability and validity [46, 66, 286, 317], there may be limitations to its use in adolescence, compared to early childhood, due to structural differences in the school environment and the increasing influence of opposite-sex peers during adolescence (many childhood studies only measure same-sex nominations) [286]. Whereas children at primary school remain in the same configuration throughout the school day, adolescents socialise in different classroom configurations [286], and with peers from outside school, which may reduce the validity of classroom peer nominations. A final criticism of the peer nomination measure is that rather than measuring peer rejection per se, it reflects peer popularity. Although these variables are likely to correlate with each other, being disliked by peers may not necessarily result in peer rejection.

Study	Sample	Measure of peer rejection	Outcome measure	Follow-up time	Summary of results
Coie et al. (1995) [67]	1147 children (grade 3)	Peer nomination [66]	Psychiatric symptoms	3, 5, and 7 years	Rejection was the best predictor of later internalising problems in females. Boys who experienced rejection and were aggressive also developed internalising problems
Vernberg et al. (1990) [361]	73 young adolescents	2-item self-report	Depressive symptoms	6 months	Rejection predicted later depressive symptoms and depressive symptoms predicted later peer rejection
Nolan et al. (2003) [261]	240 children (age 11)	Self-, mother- and teacher-report (CBCL)	Depressive symptoms	1 and 2 years	Rejection predicted later depressive symptoms but the relationship was not reciprocal
Prinstein et al. (2004) [289]	158 adolescents (age 15-17)	Peer nomination [66]	Depressive symptoms	17 months	Rejection predicted depressive symptoms for girls, only when moderated by attributional style or peer importance
Wivliet et al. (2010) [372]	310 children (age 11)	Clique isolation (social network analysis) and self-report	Depressive symptoms	3 years	Rejection predicted current depressive symptoms and clique isolation predicted later depressive symptoms
Shochet et al. (2011) [324]	504 young adolescents (mean age 13.3)	3 rejection items from PSSM	Depressive symptoms	1 year	Rejection predicted current depressive symptoms and for girls, it predicted later depressive symptoms
Hoza et al. (1995) [163]	236 children (grade 3-5)	Peer nomination [228]	Internalising and externalising symptoms	2 years	Passive rejection predicted internalising problems. Isolated rejection predicted externalizing problems
Fontaine et al. (2009) [105]	585 children (grade 1-3)	Peer nomination [66]	Depressive symptoms	4-7 years	Loneliness mediated the effect of rejection on depressive symptoms
Sentse et al. (2010) [317]	1023 children (age 11)	Peer nomination [66]	Internalising symptoms	2 years	Peer rejection predicted later internalising problems. Parental acceptance did not buffer the effects of peer rejection
French et al. (1995) [110]	501 adolescents (age 13-14)	9-item peer rating scale (2 peers report) and 5-item teacher-report	Depressive symptoms	2 years	Peer rejection predicted later depressive symptoms (but only for anti-social adolescents)
Mrug et al. (2012) [246]	362 ADHD patients (age 7-9)	Peer nomination [66]	Depressive symptoms	6 or 8 years	Rejection predicted other psychiatric symptoms but not depressive symptoms

Table 1.1: Longitudinal studies of adolescent peer rejection and depression

Notes: CBCL-YSR Child Behavior Checklist [3]; PSSM - Psychological Sense of School Membership [125]

More generally, although longitudinal research enables the temporal associations of real-life peer rejection and depressive symptoms to be studied, the experience of naturally-occurring peer rejection is likely to vary as a function of the severity of the rejection (e.g. exclusion versus bullying) and certain contextual factors (e.g. presence versus absence of other sources of social support). These potential moderators may result in an under- or over-estimate of the role of peer rejection in adolescent depression. In contrast, experimental studies enable standardised delivery of peer rejection and an assessment of their negative effects. However, in experimental designs, negative emotional responses must act as a proxy for depressive symptoms, since ethical restrictions prevent the manipulation of peer rejection to the extent that it produces more persistent depressive symptoms. Thus, longitudinal and experimental studies offer complementary means for understanding causal relationships between peer rejection and depression. Longitudinal studies of peer rejection and adolescent depression were the focus of a special issue of Development and Psychopathology [62] and more recent findings are summarised by Nolan and colleagues [261]. The focus of the remainder of this review is on experimental studies of peer rejection.

1.2.2 Experimental studies of peer rejection

A number of tasks have been developed to simulate peer rejection in order to assess its consequences on affective outcomes (see Table 1.2 for a summary of studies using these paradigms in adolescent samples). Despite significant variation across tasks, numerous data show that the receipt of peer rejection elicits negative emotional responses in typically developing adolescents [2, 8, 140, 292–295, 313, 315, 328, 345], sometimes to a greater degree than in adults or children [2, 313, 315, 345].

Paradigm	Study	Sample	Outcome measure(s)	Summary of results
Cyberball	Abrams et al. (2011) [2]	79 adolescents aged 13-14, ∼50% female 41 children aged 8-9, ∼50% female 46 students (mean age=20), 74% female	i) Distress (NTQ) ii) Mood (enjoyment; single item)	Rejection worsened mood and increased distress. Rejection affected adolescents' feelings of belonging more than childrens' or adults'.
	Sebastian et al. (2010) [313]	26 female adolescents aged 11.9-13.9 (mean=12.8, sd=0.59) 25 female adolescents aged 14.0-15.8 (mean=15.0, sd=0.53) 26 female adults aged 22.2-47.1 (mean=27.4, SD=6.2)	i) Distress (NTQ), ii) Mood (NTQ), iii) Anxiety (STAI)	Participants felt more excluded following rejection (compared to inclusion). Rejection worsened mood, anxiety and distress for both groups of adolescents compared to adults.
	Sebastian et al. (2011) [315]	19 female adolescents aged 14-16 (mean=15.44, sd=0.81) 16 female adults (mean age=28.70, sd=3.91)	i) Distress (NTQ), ii) Mood (NTQ)	Peer rejection increased feelings of exclusion, reduced mood and increased distress. The effect of rejection on exclusion was stronger for adolescents than adults.
	White et al. (2012) [367]	10 insecurely attached adolescents aged 11-15, 10% female 13 securely attached adolescents aged 11-15, 54% female	Distress (NTQ)	No difference between groups in the amount of distress reported during rejection.
Chatroom	Davey et al. (2011) [80]	17 youths with depression aged 15-24 (mean=18.7, sd=2.2), 65% female 19 healthy youths aged 15-24 (mean=19.3, sd=2.9), 63% female	Retrospective rating of response to positive feedback (single item)	Patients reported less reward from positive feedback than controls.
	Guyer et al. (2008) [141]	14 children and adolescents with anxiety disorder (mean age=12.30, sd=2.76), 71% female 14 healthy children and adolescents (mean age=12.58, sd=2.54), 71% female	Predicted interest of peers (single item for each of 40 peers)	Patients anticipated more negative feedback from peers than controls. The effects were restricted to salient (versus non-salient) peers.
	Guyer et al. (2009) [142]	34 healthy children and adolescents aged 8.9-17.5 (mean=13.6, sd=2.4), 47% female	Predicted interest of peers (single item for each of 40 peers)	Participants expected salient peers to provide more positive feedback than non-salient peers. There was no effect of gender or age on anticipated peer feedback.

Paradigm	Study	Sample	Outcome measure(s)	Summary of results
Chatroom (continued)	Guyer et al. (2012) [140]	36 healthy children and adolescents aged 8.6-17.5 (mean=13.54, sd=2.5), 44% female	Affective response (single item for each of 40 peers)	Peer rejection reduced affect compared to peer acceptance. There were more negative response to rejection from salient (versus non-salient) peers.
	Lau et al. (2012) [200]	12 children and adolescents with anxiety disorder (mean age=11.88, sd=2.48), 67% female 12 healthy children and adolescents (mean age=12.23, sd=2.44), 67% female	Affective response (single item for each of 40 peers)	No main effect of peer rejection was reported. There were more negative response to rejection from salient (versus non-salient) peers.
	Silk et al. (2012) [328]	60 children and adolescents aged 9-17 (mean=13.2, sd=2.5), 53% female	Retrospective rating of mood and exclusion/inclusion (6 items)	Participants felt angrier, sadder, more excluded, less happy and less included following rejection (versus acceptance).
Social Judgement	Gunther-Moor et al. (2010) [138]	12 children aged 8-10 (mean age=9.7, sd=0.9), 58% female 14 adolescents aged 12-14 (mean age=13.3, sd=0.8), 57% female 15 adolescents aged 16-17 (mean age=17.1, sd=0.6), 47% female 16 young adults aged 19-25 (mean age=21.7, sd=1.9), 50% female	Predicted interest of peers (Yes/No response for each of 120 peers)	Young adults predicted peers would be more interested in them than did children and 12-14 year old adolescents. There was no difference in reaction time to predict rejection versus acceptance and no effect of age on reaction time to predict rejection (versus acceptance).
Survivor	Reijntjes et al. (2006) [294]	186 children and adolescents aged 10-13 (mean=11.5, sd=0.73), 51% female	i) Mood (Self-Assessment Manikin [197]), ii) Observed passivity, distraction and approach behaviour	Peer rejection worsened mood compared to peer acceptance. Rejected participants spent less time on distraction activities and more time looking at folders of previous participants than those accepted. The latter effects were stronger for those with more symptoms of depression.

Paradigm	Study	Sample	Outcome measure(s)	Summary of results
Survivor (continued)	Reijntjes et al. (2006) [295]	186 children and adolescents aged 10-13 (mean=11.5, sd=0.73), 51% female	i) Mood (PANAS), ii) Observed emotion regulation behavioural strategies, iii) Self-reported cognitive reactions (5 items)	Peer rejection worsened mood compared to peer acceptance. Participants who showed a reliable change in mood showed improvements when they engaged in distraction activities and deterioration when they showed passive behaviours. Participants showed worsening mood if they engaged in 'cognitive analysis'.
	Reijntjes et al. (2007) [292]	142 children and adolescents aged 10-13 (mean=11.2, sd=0.66), 49% female	i) Mood (single item), ii) Feedback preference (single item)	Peer rejection worsened mood compared to peer acceptance and was associated with a preference for negative feedback. More symptoms of depression predicted a preference for negative feedback following peer rejection but not acceptance.
	Reijntjes et al. (2009) [293]	142 children and adolescents aged 10-13 (mean=11.2, sd=0.66), 49% female	i) Mood (PANAS), ii) Cognitive appraisal of feedback (7-items)	Peer rejection worsened mood compared to peer acceptance. Participants who showed a reliable change in mood following rejection (versus those who showed no change) did not differ in the number of depressive symptoms but took longer to return to baseline mood. Children with more depressive symptoms had more negative appraisals of rejection than those with fewer symptoms.
Yale Interpersonal Stressor task	Allwood et al. (2011) [8]	56 healthy children and adolescents aged 7-16 (mean=12.0, sd=2.4), 52% female	i) Positive affect (4 items), ii) Negative affect (2 items)	Reduced positive affect and increased negative affect after stressor tasks. No comparison of the two stressor tasks.
	Stroud et al. (2009) [345]	43 healthy adolescents aged 13-17, 47% female 39 healthy children aged 7-12, 56% female	i) Positive affect (4 items), ii) Negative affect (2 items)	Peer rejection reduced positive affect and increased negative affect. Stressors had a greater effect on physiological response in adolescents than children.

Table 1.2: Experimental studies of peer rejection and adolescent depression

Notes: NTQ=Need Threat Questionnaire [371]; PANAS=Positive and Negative Affect Schedule [363]; STAI=State Trait Anxiety Inventory [337]

Cyberball Williams and colleagues developed the earliest experimental manipulation of peer rejection: 'Cyberball' [371]. A virtual ball-throwing game, participants 'play' online with two unknown peers. After several trials, the peers appear to exclude the participant from play. In fact, the participant is playing against a pre-set computer program and fictitious peers. In this task exclusion from a group acts as a proxy for peer rejection [231]. Compared to the inclusion phase, exclusion increases adolescents' negative mood, anxiety, and threatens feelings of belonging central to self-esteem [2, 313, 315].

One limitation of original Cyberball task is that the exclusion phase always follows the inclusion phase, meaning that responses to exclusion may also be the product of fatigue or experience with the task. Critics have also highlighted that Cyberball fails to control for violation of expectancy [333]. That is, responses probed by Cyberball may reflect responses to expectancy violations emerging from sudden and unexpected exclusion from the ball-throwing game rather than peer rejection specifically. Adaptations of Cyberball have attempted to address these problems of order effects and expectancy violation [315, 333].

Chatroom and the Social Judgement task Whereas Cyberball elicits peer rejection in a gaming context, 'Chatroom' [141] and the Social Judgement task [333] have been developed to measure the effects of peer rejection during online social communication. As the vast majority of adolescents and young adults have at least one social networking profile [271] and the Internet is now a common platform for adolescent social communication [287], such experimental paradigms go some way to understanding how peer rejection in social networking sites influences adolescent emotion. In the

Chatroom task, participants are told that they are taking part in a study of online social interaction, and based on a photo, indicate their preference about which unknown peers they would like to chat to. Then these peers make a decision as to whether to reject or accept the participant's request, based on a photo taken of the participant. In the Social Judgement task, participants simply predict, and get feedback about, which peers like them. In both tasks feedback is generated randomly by the computer and the peers are child actors.

One advantage of the Chatroom and Social Judgement tasks is that they enable expectations of social evaluation to be examined, although so far, the impact of these effects on depressive symptoms has not been identified [138, 142]. For adolescents, rejection in the feedback phase of Chatroom elicits greater negative mood than acceptance, and the response is amplified for 'desirable' peers i.e. those who the participant was particularly interested in [140, 200]. An adaptation of the paradigm, 'Chatroom Interact', adds ecological validity to the traditional Chatroom paradigm by including age- and gender-specific peer profiles and by manipulating peer feedback in relation to specific topics of interest to adolescents [328]. Using this paradigm, Silk and colleagues observed increased feelings of anger, sadness, and exclusion as a result of peer rejection versus peer acceptance [328].

Survivor Another computerised manipulation of peer rejection combines both social communication and gaming, and has been used to measure young adolescents' appraisals of peer rejection and consequential coping strategies ('Survivor') [294]. Based on the TV show of the same name, participants are led to believe that they will be competing in a number of challenges against unknown peers, to become the 'Survivor Champion'. Having had

their photo taken and answered personal questions about their interests, academic performance, and romantic relationships, all players vote on who should be eliminated from the next round. In actual fact the peers are confederates and the participant is randomised to receive negative (eliminated at first round) or positive (survived first round) feedback. For young adolescents who took part in Survivor, peer rejection lowered mood [292–295], led to dwelling on negative feedback [292] and elicited maladaptive coping strategies [294]. Their computerised format enables these novel tasks to deliver standardised peer rejection experiences that are not only relatively easy to re-create and more ethically-appropriate, but also simulate a common format of adolescent social communication.

Yale Interpersonal Stressor task A non-computerised task, the child and adolescent version of the Yale Interpersonal Stressor task (YIPS-C), measures the effects of face-to-face peer rejection [346]. Participants chat with same-aged peers on a wide range of salient adolescent topics. In reality the peers are trained confederates, and given specific topics to discuss. Towards the end of the conversation, the confederates use verbal and non-verbal means to exclude the participant. Compared to previous tasks described, YIPS-C has increased ecological validity, while standardising delivery across all participants. Although peer rejection in the YIPS-C task has been shown to negatively impact on mood in adolescents, data are yet to show that this is more so than a non-rejection based performance stressor [8, 345]. As for the computerised tasks described previously, the effects of the YIPS-C task are perhaps also limited by interactions with unknown peers, and a reliance on participants not only believing that these peers are real, but being sensitive to their feedback.

Together, the experimental paradigms described suggest that peer rejection plays a causal role in eliciting negative emotional responses. This complements findings from longitudinal studies of the temporally predictive role of peer rejection in adolescent depression [67, 110, 261, 289, 317, 324, 361, 372]. The negative emotional responses elicited by experimental manipulations of peer rejection are limited in their capacity to infer the temporal dynamics of depression, since their effects are relatively short-term. Nevertheless, these peer rejection paradigms described also lend themselves well to comparing patterns of responding between adolescents with and without depression (Table 1.2).

Experimental studies in clinical (or analogue) populations

In the Social Judgement task, Davey and colleagues found that depressed youths (aged 15-24) showed less enjoyment from peer acceptance than healthy controls, although a peer rejection condition was not included in this study [80]. Although no other studies have investigated responses to peer rejection in clinically depressed adolescents, studies of variation in symptoms of depression in unselected adolescents, offer valuable insights into differential peer rejection responses relevant to adolescent depression. For example, adolescents with more depressive symptoms were more likely to use ruminative coping strategies [294] and negative appraisals [293] in response to rejection, although there was no evidence of worsened mood following rejection compared to adolescents with less depressive symptoms [294].

Because symptoms of depression are themselves relatively rare in the general population, low self-esteem (a cognitive symptom of depression) may better capture depressive symptomology in unselected samples. Young adults (aged 18-24) with low self-esteem estimated the amount of negative

feedback to be greater than did those with high self-esteem in the Social Judgement task [334]. A final study found no evidence that insecurely-attached adolescents (who are at greater risk of developing depression [6]) reported greater distress in response to rejection than securely-attached adolescents [367]. Findings from studies of peer rejection in adolescents with anxiety disorders are also worth noting: although adolescents with Social Anxiety Disorder (SAD) predicted peers to be less interested in chatting to them than did healthy controls [141], no differences emerged in the immediate affective response to peer rejection between anxious and healthy adolescents [200]. These findings tentatively suggest that sensitivity to rejection is largely specific to adolescents with mood symptoms, rather than being linked more to co-morbid social fears and worries.

1.2.3 A bi-directional relationship between peer rejection and adolescent depression

Interpersonal theories propose that in addition to peer rejection eliciting symptoms of depression, depressive symptoms may also predict later peer rejection [361]. One possibility is that depressed adolescents are more attracted to people who confirm their negative self-beliefs, rather than those who provide positive feedback that contradicts self-beliefs [349]. Another possibility is that depressed individuals experience more rejection because of increased reassurance seeking [72]. This effect may be driven by depressed adolescents having negative expectations of social interaction [53].

Negative expectations of future events are a fundamental feature of depression according to cognitive models [25, 193]. A review by Ruble and colleagues (1993) suggests that lower expectations of future outcomes may explain why adolescent females are more susceptible to depression than males

[305], although this was based largely on studies of expectations about future academic (rather than interpersonal) success. A large cross-sectional study demonstrated that negative expectations of peer interaction may modify the effects of early attachments on adolescent depression [217]. In Chapter 4 I report the results of an experimental study to test the hypothesis that depressed adolescents do expect more negative peer feedback than non-depressed adolescents.

Observations of interpersonal interaction have found that depressed adolescents are more often rejected than healthy controls [69, 172]. Compared to healthy adolescents, depressed adolescents' behaviours were associated with a decrease in positive behaviour from a partner [159]. Similarly, adolescents rated depressed peers less positively than non-depressed peers in videos of their interactions [277]. Longitudinal studies suggest adolescent depressive symptoms predict subsequent rejection sensitivity [236] and peer rejection [361]. Correlational studies suggest that interest in negative feedback correlates with depressive symptoms and predicts peer rejection [175], and that depressive symptoms and reassurance seeking interact to predict peer rejection [171]. To date no experimental studies have tested the hypothesis that negative expectations are causally related to subsequent peer rejection.

In summary, peer rejection and adolescent depressive symptoms are closely associated. First, longitudinal and experimental studies suggest that peer rejection predicts depressive symptoms and negative affect respectively. Second, experimental studies also show that faced with the same peer rejection experience, symptomatic or at-risk adolescents show more negative expectations of feedback and appraisals of feedback compared to others. Third, symptomatic adolescents may also be more likely to attract rejection experiences. The interdependence of peer rejection and negative emotional

responses which may maintain the depressive state is illustrated in Figure 1.1A. These findings raise the question of what underlying biological and psychological factors may explain these destructive cycles.

1.3 The role of genetic factors in adolescent depression

Evidence for a genetic component to depression comes from studies which suggest that MDD runs in families [123, 297]. Symptoms of depression are found more often in offspring of parents with an affective disorder compared to offspring of parents with no psychiatric history [147]. Studies of child probands with a diagnosis of MDD suggest that the risk of the disorders in first degree relatives is at least twice that in healthy controls [297]. Evidence from twin studies of paediatric MDD suggests moderate to high heritability [202, 207, 297]. These quantitative genetic studies of paediatric depression pave the way for molecular genetic studies, which aim to identify the specific genes which contribute to psychiatric disorder [204].

Molecular genetic studies of depression

Molecular genetic approaches to adult mood disorders have generally focused on gene variants of the serotonergic system [225, 276], although see [288, 311] for reviews on the role of the glutamatergic system, and molecular genetic studies of a polymorphism of the glutamate receptor 1 gene [60, 241]. Serotonergic approaches draw on evidence of the treatment efficacy of Selective Serotonin Reuptake Inhibitors (SSRIs) [71, 211], which increase the availability of serotonin in the synapse. Furthermore, reducing serotonin levels by tryptophan depletion in patients who have recovered from

MDD is associated with relapse [84]. The delay between administration of SSRIs and reductions in clinical symptoms of depression is thought to reflect the fact that the effects of SSRIs are mediated by reversal of negative cognitive biases and attenuated neural activity in the amygdala [112, 152–155, 263, 323, 354, 362]. Indeed, a recent fMRI study demonstrated that in response to SSRI treatment, reductions in amygdala reactivity to fearful faces temporally preceded improvements in symptoms of depression [118]. Variation in molecules that regulate serotonin, such as the serotonin transporter (5HTT), may therefore influence the development of mood and anxiety disorders [210]. Since 5HTT may be influenced by naturally-occurring variation in the DNA sequencing of the genes from which they are coded, the most studied serotonin gene associated with mood disorders is 5HTT [195, 264].

The flow of serotonin from the pre-synaptic cleft to the post-synaptic receptor is controlled largely by the serotonin transporter (5HTT), a protein located in the pre-synaptic membrane which transports serotonin back into the pre-synaptic cell. 5HTT itself is encoded by a single gene, SLC6A4 [55]. Transcription of the gene is in part controlled by a sequence in the promotor region (5HTTLPR), where a variable number tandem repeat (VNTR) produces two variants of 5HTTLPR: the short 'S' allele and the long 'L' allele. The long allele is associated with significantly greater production of 5HTT mRNA and protein (high expressing) than the short allele (low expressing). Whilst the 5HTTLPR polymorphism has traditionally been considered functionally biallelic [164], recent evidence suggests further allelic variation within the L allele, such that an A-G substitution generates L_A and L_G alleles with differing expression of 5HTT mRNA [164]. Since the L_G allele shows similarly low 5HTT mRNA expression to the S allele,

many paediatric studies pool together the carriers of the S/L_G alleles with those of the SS and L_GL_G homozygotes [63, 109, 259].

The S/L_G alleles may be more common in adult patients with MDD, at least when they are seen in the clinic [68, 276] but not when they are ascertained from the community [59]. Though not without controversy, molecular studies of healthy adults have found an association between the S/L_G allele of 5HTTLPR and symptoms of neuroticism [316], harm avoidance [248] and depression [59, 124]. A similar association between the 5HTTLPR polymorphism has been reported in adult patients with a diagnosis of mood or anxiety disorder. For example, the S/L_G alleles are associated with higher symptom severity than the L_AL_A genotype in adult patients diagnosed with panic disorder [218]. Animal data also show a clear effect of genetic modulation of serotonin activity on the behavioural correlates of depression and anxiety [162, 216]. Accumulating evidence from the adult literature suggests that genetic factors may also enhance the effects of psychosocial stress on depressive and anxious symptoms [59, 308, 312, 316, 331]. That is, the presence of the short allele increases depressive responses to stressors such as recent negative life events and child abuse.

The 5HHTLPR polymorphism and adolescent depression

Figure 1.1B presents a framework for understanding how genetic factors, such as the 5HTTLPR polymorphism, may influence adolescent depression. Fewer studies have investigated the role of this gene on mood outcomes in children and adolescents - and results are inconsistent. Some evidence suggests that the effect of 5HTTLPR on depressive symptoms may vary with development. In a longitudinal study of children from infancy to age 16 [178], there was no association between genotype and anxiety-related behavioural

traits in most age groups, but there was an association in children aged 13-14 and 15-16. Specifically, the high expressing genotype was associated with increased anxiety problems, compared to the low expressing genotype. Not only do these findings suggest developmental differences across childhood and adolescence, but the direction of findings during adolescence (that the high expressing genotype confers greater risk) is inconsistent with adult studies which suggest that the low expressing genotype confers greater risk for psychopathology.

These studies are supported by animal data [373] in which developmental dissociations in genetic effects on behaviour have been found [11]. For example, Wolff and colleagues found that 5-HT1A knockout mice, compared to wild-type mice, displayed memory impairment on a spatial task when tested in young adulthood [373]. However, when tested in old age, 5-HT1A knockout mice seemed to show facilitation on this task. Examination of genotypic modulation of behaviour in human youths could be crucial for models of adolescent psychopathology [134, 209].

A comprehensive review of child and adolescent molecular genetic studies of the 5HTTLPR polymorphism as it relates to mood disorders is provided in Chapter 2. To summarise, just four studies investigated the frequency of the 5HTTLPR genotype in depressed and non-depressed groups, with two finding that carriers of the low expressing alleles were more often depressed than those with the high expressing alleles [100, 258]. Child and adolescent studies of the 5HTTLPR polymorphism and depressive symptoms also have mixed findings, perhaps because they have mainly focused on typically developing samples. Although counterintuitive to the supposition that mood disorders represent the extreme end of a continuous distribution of symptoms, previous studies also suggest that genetic variants may influence

affected and unaffected adolescents differently [205, 206]. Finally, several studies support the hypothesis that genetic vulnerability moderates the effects of psychosocial stress on anxiety- and depressive-traits in paediatric samples, although no large scale molecular genetic studies have looked at genotype variants interacting with peer rejection.

In summary, there is growing evidence for a genetic role in adolescent depression (Figure 1.1B). Preliminary studies suggest that the 5HTTLPR polymorphism may explain why some adolescents react more negatively to environmental stress than others, although investigating its relation to peer rejection specifically is thwarted by the need for relatively large samples in molecular genetic studies. **In Chapter 2 I describe a study which investigates the main effects of 5HTTLPR polymorphisms, and interactions with environmental stress, on depressive symptoms in adolescents with and without mood disorders.**

1.4 Are cognitive biases implicated in maladaptive responses to peer rejection?

In this section I consider how cognitive biases may contribute to negative responses to peer rejection (Figure 1.1B). Biases towards negative information processing are widely acknowledged in prominent theories of adult [25, 234, 369] and adolescent depression [1, 168]. A review of 48 prospective studies demonstrated the important role of cognitive factors such as hopelessness, negative schemas, and rumination, in modifying the effects of life stress on adolescent depression [1]. As illustrated in Figure 1.1B, these cognitive vulnerabilities may also explain heightened emotional responses to peer rejection specifically.

Adolescents with depression exhibit negative attributional styles, meaning that they attribute negative events such as peer rejection to internal ("I am not a likeable person"), stable ("I have never been well-liked") and global ("I am no good at anything") causes, and positive events such as being invited to a party to external ("they are just being nice"), unstable ("this is just a one-off invitation") and specific ("at least one person likes me") causes [196]. Negative attributional style has been shown to moderate the relationship between peer rejection and depressive symptoms in unselected adolescents [289]. Negative cognitive schemas about the self also moderate the effect of peer rejection on depressive symptoms in adolescent psychiatric patients [40].

Analogous to adult findings of depression, the tendency to select negative interpretations of ambiguous social situations may also characterise adolescent depression. Following a negative mood induction female adolescents at risk of developing depression interpreted ambiguous words and sentences more negatively than low-risk female adolescents [83]. These between-group effects were restricted to non-socially-threatening ambiguous words and sentences. There are no studies yet to investigate whether this bias influences responses to peer rejection specifically, although the behavioural data in Chapter 4 which describe depressed and non-depressed adolescents' expectation of peer evaluation aims to address this limitation.

Cognitive bias modification paradigms

Critical questions remain over whether cognitive biases are causally linked to depression. Cognitive bias studies in individuals at risk of depression, or who have recovered from depression, go some way towards supporting the notion that cognitive biases are not simply the by-product of depression.

Prospective studies are able to test temporal links between cognitive biases and depression, while experimental paradigms can test the causal role of cognitive biases. Several experimental paradigms, referred to as Cognitive Bias Modification (CBM) paradigms, have been developed to test the causal effects of interpretive biases (CBM-I) [22, 133, 233]. These paradigms aim to reverse the effect of negative cognitive patterns on depression.

In the most common paradigm, ambiguous situations (AS [233]), participants read or hear a variety of social scenarios that are ambiguous (for example, "Having finished painting the lounge, you invite friends around to dinner. As they walk into the room, you can see that they are surprised. Their reaction is one of..."). Participants are then asked to complete a word fragment that resolves each scenario. The researcher systematically targets the word fragment such that the solution is positive (for example, "pleasure") or benign (for example, "confusion") rather than negative (for example, "horror"). As a result, correct completion of the scenario encourages a benign resolution of ambiguity, which over multiple training trials, becomes reinforced, and presumably, increasingly habitual.

A meta-analysis of CBM-I studies found that benign training increased positive interpretive biases and mood, particularly for participants with an emotional disorder [221]. Studies of depressed adults confirmed that even in a sample of those who have difficulties with motivation and concentration, CBM-I appears to be effective [33, 198]. CBM-I also reduces negative biases in healthy children [249, 250] and adolescents [208, 220, 309, 310], although just one study reports a mood improvement [220]. Disparity could be due to the fact that these adolescents are not symptomatic to begin with, or because multiple sessions are required for training effects on mood to be found. Future CBM-I studies that are tested in clinically depressed adolescents

are important to highlight the potential for CBM-I paradigms to change negative cognitive patterns in general, but around scenarios that are highly salient for adolescents such as peer rejection, in particular.

Attention biases and depression

Recent reviews suggest that negative attention biases may also play a role in adult depression [44, 85]. That is, that depressed adults are quicker to attend to emotionally threatening information in their environment than non-depressed adults. This contrasts with previously held ideas about depression being a disorder associated with strategic, rather than early-stage, cognitive biases [369]. Attentional biases towards negative facial stimuli have been observed in depressed [95, 128, 129, 215, 298, 348] and dysphoric [38, 319] adults, using multiple measures of attention bias. These measures are outlined and discussed in Chapter 3. It should be noted that not all studies find evidence of a negative attention bias in adult depression [128, 180, 222, 243]. Methodological issues associated with measuring attention biases may account for these findings, and are also discussed in Chapter 3. Nevertheless, the emerging literature provides persuasive evidence that negative attentional biases do indeed underlie adult depression. This cognitive vulnerability may render these individuals more vulnerable to the effects of psychosocial stress [234].

To date just two studies have investigated whether attention biases are associated with adolescent depression [177, 257]. The first study of children and adolescents aged 9-17 found no evidence of a negative attention bias in depressed adolescents compared to healthy controls [257]. However, the small sample (19 depressed patients and 26 healthy controls) may account for this, particularly given that relatively small effect sizes are observed

in studies of attention biases [146]. Replication of this study in a larger sample may help elucidate the nature of emotional attentional processing in depressed adolescents.

The second study observed negative attention biases in a sample of children aged 9-14 at risk of depression (children of depressed mothers) [177], although replication in an older adolescent sample could be valuable, given that middle-late adolescence is the time when depression most commonly occurs [148]. This study supports the notion that negative attention biases are not the by-product of depression and replicates adult studies of attention biases in individuals with a family history of depression [358], those who have recovered from depression or are in remission [176, 347], and those with low self-esteem [77]. More studies in adolescent samples would provide a further rationale for investigating whether negative attention biases modify the effects of peer rejection on adolescent depression.

Cognitive bias modification of attention

As previously described, prospective or experimental studies are needed to test the temporal and causal role of cognitive biases in depression. CBM paradigms have been developed to target attention biases specifically (CBM-A), and are described in more detail in Chapter 3. The efficacy of CBM-A paradigms in reducing negative attention biases and improving symptoms informs the causal relationship between these cognitive biases and depression. Studies in clinically depressed adults are yet to be conducted. However, positive effects of CBM-A have been found in students with relatively high depressive symptoms [366] and Baert et al. (2010, Study 1) [17], previously depressed adults [44], and adults with low self-esteem [77–79]. Beneficial effects of CBM-A training have even been found to reduce self-reported

and physiological markers of student exam stress [79] and the impact of a rejection [78] in healthy volunteers. However, not all CBM-A studies demonstrate beneficial effects. A CBM-A study using the Posner cueing task in students with high depressive symptomology found that training actively worsened symptoms (Baert et al., 2010, Study 2 [17]). CBM-A tasks appear to effectively modify symptoms of anxiety when delivered to healthy [97] and anxious children and adolescents [19, 96, 304]. Only one study demonstrates positive effects of a CBM-A task on depressive symptoms, and this in an anxious sample of adolescents [304]. To date no studies have investigated the effects of CBM-A in healthy adolescents or adolescents with depressive symptoms.

In summary, cognitive vulnerabilities such as negative attributional style and negative interpretative biases characterise adolescent depression, and tentatively, may increase adolescents' depressive responses to psychosocial stress such as peer rejection (although data is sparse). CBM-I paradigms indicate that these biases may play a causal role in the development of depressive symptoms. Speculatively, these cognitive biases may therefore serve to generate more negative social experiences among depressed adolescents, as illustrated in Figure 1.1B). Emerging data suggest that negative attention biases may characterise adolescents at risk of depression, although a lack of CBM-A studies in adolescent populations limits interpretations about the causal role of these biases in adolescent depression. The literature reviewed provides the context for an empirical study of negative attention biases in adolescents with low self-esteem. **In Chapter 3, I investigate the association between negative attention biases and symptoms of depression in adolescents, and the extent to which attention biases play a causal role in the development of adolescent depression.**

1.5 What are the neural substrates underlying maladaptive responses to peer rejection?

Cognitive processing biases may be rooted in differences in neural circuitry functioning, therefore in this section I review depression-linked differences in the neural substrates of responses to peer rejection in adolescence. Neuroscientific methods such as fMRI play an important role in improving our understanding of how the brain mediates the differential impact of peer rejection on typical and atypical emotion regulation and behaviour [142]. Moreover, because differences in brain activity may occur in the absence of behavioural differences, these data could implicate more subtle differences in how adolescents respond to rejecting stimuli [230]. A seminal study of social 'pain' in adults was the first to investigate the neural correlates of peer rejection and ostracism [93]. This paper sparked numerous studies of the neural correlates of peer rejection in adults, using the novel experimental paradigms described in Section 1.2 [36, 86, 87, 94, 194, 274, 333, 335]. These paradigms enable substantial control over extraneous variables and have been able to distinguish, for example, neural responses to peer rejection from violations of expectancy [333]. They also provide an ideal means for exploring neural substrates of peer rejection in adolescents.

Social emotion processing in adolescence

Adolescent models commonly posit three overlapping neural systems involved in stages of social-emotional processing; i) detection of social threat via the fusiform gyrus, superior temporal sulcus (STS), anterior temporal cortex, ii) an initial affective response via the amygdala, ventral striatum, hypothalamus, orbitofrontal cortex (OFC), and iii) cognitive regulation of

the emotional response via the ventrolateral pre-frontal cortex (VLPFC) and dorsomedial PFC (DMPFC) [34, 256, 313, 334]. These models posit that imbalances between heightened affectivity and immature regulatory regions underlie adolescent depression [58, 270], although see Davey and colleagues for a model of how development of regions within the pre-frontal cortex (PFC) contributes to adolescent depression, regardless of affectivity [81]. Although these models provide a compelling platform upon which individual differences may be explained and interpreted, they may also be too simplistic in capturing the complex networks of co-activation between these regions and their roles in other cognitive domains [278].

Regions within these overlapping neural systems continues to develop structurally and functionally throughout adolescence [34, 49] and this ongoing neural development has been implicated in the susceptibility of adolescents to peer rejection via heightened affectivity and impaired ability to regulate emotional reactivity [313]. For example, whilst the insula and anterior cingulate cortex (ACC) are thought to be fully developed by adolescence [120, 230], regions within the PFC do not fully develop until early adulthood [230]. This 'mismatch' in maturational patterns may tentatively result in heightened emotional responses left relatively unchecked by regulatory systems. Studies across varying paradigms suggest that peer rejection in typically developing adolescents appears to elicit neural activation in emotion detection, affectivity and regulation regions [256] (Figure 1.2). For a review see Pfeifer and Blakemore [279]. Whereas adults show heightened activity in the right VLPFC during peer rejection compared to a control condition, adolescents showed the opposite effect [314, 315]. Studies across adolescence have demonstrated than during peer rejection, neural activity in the striatum, ventromedial PFC (VMPFC), OFC, and lateral PFC increases linearly

with age [138].

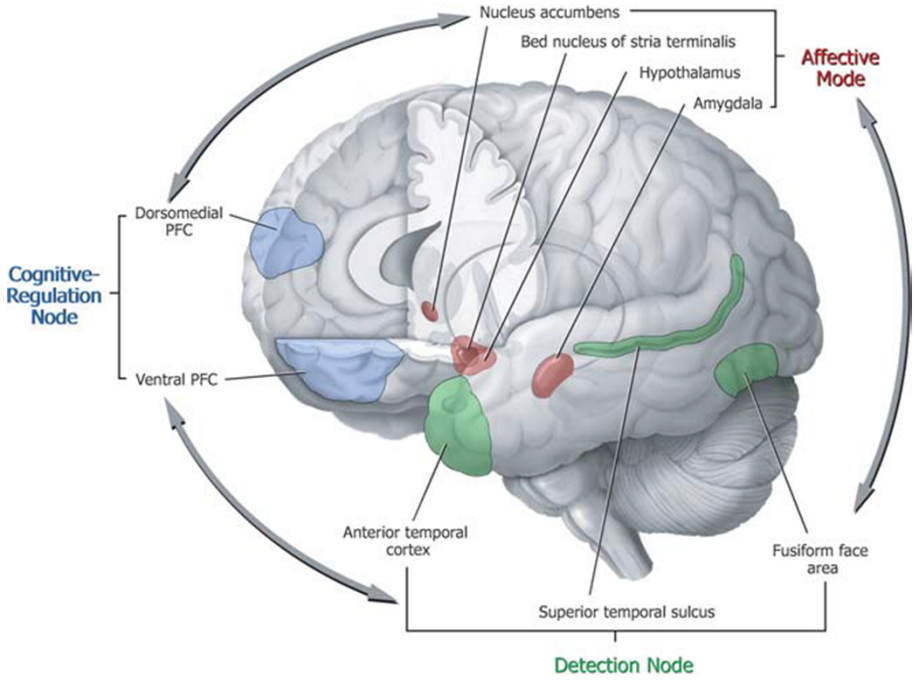

Figure 1.2: The Social Information Processing Network (SIPN) model of emotional processing [256]. *Image included with permission from Eric Nelson*

Based on emerging fMRI data, I suggest that neural sensitivity to peer rejection may play an important role in the development of adolescent depression (see Figure 1.1B). More specifically, I propose that neural sensitivity may influence depressive responses in three distinct stages: i) expectation of peer feedback, ii) the receipt of peer rejection, iii) emotion regulation of peer rejection. The final three Chapters of this thesis address neural sensitivity to peer rejection during each of these phases (Chapters 4 , 5 and 6).

Negative expectations of peer feedback

According to cognitive models, negative expectations of future events are a fundamental feature of adult depression [25, 193]. As described in Section 1.2, negative expectations of future events have also been associated with adolescent depression [217, 305]. Speculatively, expectation of negative social feedback may drive maladaptive social behaviour which elicits future peer rejection, although this has not been empirically tested. Before the precise role of negative expectations in adolescent depression can be investigated, more studies are needed to determine whether depressed adolescents do in fact hold negative expectations of peer feedback. Relatively few studies have investigated the extent to which negative expectations relate to interpersonal interactions specifically [217].

The novel experimental peer rejection paradigms described in Section 1.2 offer a unique tool for investigating adolescents' expectations of a standardised interpersonal event. For example, in the Chatroom paradigm [141] adolescents are asked to predict how interested they anticipate peers to be in chatting to them, although just two experimental studies have taken advantage of this to date [141, 142]. The Chatroom paradigm has been shown to detect individual differences in adolescents' responses to anticipated peer evaluation [141]. Guyer and colleagues demonstrated that socially anxious adolescents anticipated significantly more negative feedback from peers than did healthy controls [141].

The application of experimental peer rejection paradigms to neuroimaging methods enables more subtle effects of peer feedback expectations to be investigated. For example, using the Chatroom paradigm, Guyer and colleagues found no effect of peer desirability on expected peer feedback measured using affective ratings [142]. However, fMRI data identified neu-

ral differences between feedback expectations of desirable and undesirable peers [142]. The study above is the only one to date to investigate neural responses to anticipated peer feedback in typically developing adolescents [142]. Neural activation in the NA, hypothalamus, hippocampus and insula was heightened when typically developing adolescents expected feedback from desirable (versus non-desirable) peers. This study demonstrates the similarity between the brain regions engaged in the receipt of peer rejection, and those engaged during the expectation of peer feedback [142].

The only study to date of the neural correlates of peer feedback expectations in an atypical adolescent sample was another study by Guyer and colleagues [141]. Compared to healthy controls, anxious adolescents showed heightened amygdala activity during the expectation of peer rejection from desirable (versus undesirable) peers [141]. Anxious adolescents show sustained amygdala activation following the receipt of peer rejection, whereas healthy controls show deactivation. To date, no study has investigated depressed adolescents' neural responses to anticipated peer feedback.

In a study reported in Chapter 4, behavioural responses and brain activation correlates of peer feedback expectations are compared in a group of adolescents with and without depression using functional neuroimaging. The Chatroom paradigm has been described previously. Participants predict and then receive feedback about which peers are interested in talking to them in an online Chatroom. I test the hypothesis that depressed adolescents expect more negative feedback from peers, and that whilst doing so, brain regions associated with social emotional processing and peer rejection are heightened, compared to healthy controls.

Peer rejection

Neuroimaging may also be a useful tool for investigating the extent to which depressed adolescents show heightened emotional reactivity to peer rejection itself. Some behavioural studies suggest that depressed adolescents experience significantly longer durations and higher frequencies of negative affect than healthy controls [321, 322], although it is not clear whether these data represent heightened emotional reactivity, or dysfunctional regulation of emotion.

There is little evidence from behavioural data of an association between adolescent depression and heightened emotional reactivity to peer rejection. One experimental study of typically developing adolescents found no evidence that negative affect following rejection was heightened in adolescents with more symptoms of depression, compared to those with few symptoms [294]. Attachment type is another measurement of risk for depression in paediatric samples, however there is no evidence that insecurely attached adolescents report greater distress in response to rejection than securely-attached adolescents [367]. However, to date no study has investigated whether adolescents with a clinical diagnosis of depression report more negative affect in response to rejection than healthy controls. It may be that peer rejection only has observable effects in individuals with many symptoms of depression.

Compared to behavioural data, neuroimaging methods may provide more sensitive measures of individual differences in emotional reactivity. The models of social emotional processing described above posit that negative social stressors such as peer rejection elicit activity in regions involved in emotion detection, affectivity and emotion regulation [34, 256, 313, 334]. Early fMRI studies of emotional reactivity in clinically depressed adolescents focused on amygdala function and demonstrated mixed findings. For

example, as Yang and colleagues (2010) [377] highlight, whilst some studies showed heightened amygdala activation during emotion recognition in depressed youths compared to healthy controls [107, 205, 299, 377], others report decreased amygdala activity in depressed youths [28, 351].

There is a substantial literature on the neural correlates of peer rejection in typically developing adolescents (see Sebastian and colleagues for a review [313]). Peer rejection elicits neural activity in emotion detection regions such as the occipital and fusiform cortex [232, 315], temporal cortex [315], parietal lobe [232] and superior frontal gyrus (SFG; Brodmann Area (BA) 8-9) [232]. Affective regions activated during adolescent peer rejection include the insula [230, 232], ACC [230, 232, 315], amygdala [232], striatum [230, 232], precuneus [232], caudate/putamen [138, 315]. Finally, regulatory regions include the right inferior frontal gyrus (IFG)/VLPFC [138, 230, 232], left IFG/VLPFC [315], dorsolateral PFC (DLPFC) [232] and DMPFC [230, 232]. Interestingly, heightened neural activity in the DMPFC and precuneus appears to remain even when rejection is observed rather than experienced first-hand [231].

Studies of typically developing adolescents suggest that neural responses to rejection may be associated with depressogenic markers. Neural activity in affective and regulatory regions correlates with markers such as low self-worth [138], rejection sensitivity [230], and symptoms of depression [229]. Davey and colleagues conducted the only study to date on depressed youths' (aged 15-24) neural responses to peer feedback [80]. However, they report only responses to positive (but not negative) feedback. Compared to healthy controls, depressed adolescents showed heightened neural activity in the amygdala during receipt of peer acceptance feedback (compared to neutral feedback).

To date no study has investigated the neural correlates of peer rejection in adolescents with a diagnosis of depression. **In Chapter 5 I investigate depressed and non-depressed adolescents' behavioural and neural responses immediately following peer rejection.** In the same sample as in Chapter 4, I use the Chatroom paradigm to elicit behavioural and neural reactions to peer rejection. Based on emerging evidence that reduced positive affect may better characterise depression than heightened negative affectivity [80], I also explore the neural correlates of peer acceptance.

Emotion regulation of peer rejection

The inability to down-regulate negative emotions may explain why depressed adolescents are more susceptible to the effects of peer rejection than non-depressed adolescents. Emotion regulation is the process of modifying an emotional response, whether that be in the magnitude, valence, duration or any other component of the emotion [136]. Adults who have recovered from depression show more spontaneous use of ineffective emotion regulation strategies than never-depressed adults [90]. When instructed to use emotion regulation strategies, depressed adults report finding it more difficult than non-depressed adults [24], although there is no evidence that they are less successful at down-regulating negative emotions than non-depressed adults [24, 90, 102, 170].

Emotion regulation can be difficult to study behaviourally because it relies on participants' introspections about their own (often brief) emotional experiences. Advances in neural imaging enable the study of how the brain mediates the emotion regulation process (for reviews see [15, 270]). Ochsner and colleagues' neural model of emotion regulation proposes the recruitment of three major regions during cognitive reappraisal; 1) the DLPFC,

posterior PFC and inferior parietal lobe (involved in selective attention and working memory), which may be involved in holding in mind the reappraisal or attending to reappraisal-related information, 2) the dorsal ACC (dACC), which is likely to be involved in tracking how well reappraisal is modifying emotion, and 3) the VLPFC, which may play a role in selecting goal-appropriate responses from semantic memory [270]. The recruitment of these regions is likely to regulate emotion by modifying responses in three major affective regions; 1) the amygdala (the most commonly modulated region), 2) the ventral striatum, 3) the insula and VMPFC (less commonly modulated) [270].

FMRI studies suggest that although depressed adults are as able to use cognitive reappraisal to effectively regulate negative emotional experiences as non-depressed adults, they show subtle differences at the neural level [24, 102, 158, 170]. Regions which respond differently in depressed and non-depressed adults during emotion regulation include the middle frontal gyrus (BA10), dACC, amygdala and insula. Given that regions within the PFC are known to continue to develop during adolescence [57, 120, 227, 282, 320, 353], and in particular that regions associated with mentalising continue to develop during adolescence [49], studying cognitive reappraisal in adolescent populations is of theoretical and clinical importance [238, 270].

Few studies have investigated spontaneous emotion regulation in adolescence. One study of a large community sample of 487 adolescents and 630 adults found that adolescents used cognitive coping strategies less often than adults [115]. Furthermore, only three studies have explored the ability of children and adolescents to use emotion regulation strategies under instruction [213, 238, 282]. These data suggest that children and adolescents are able to effectively use emotional regulation to reduce emotional responses

to negative films and photos. As has been found in adult studies, regions of the PFC including the orbital PFC (BA11), medial PFC (BA10), lateral PFC (BA9), VLPFC (BA47; BA45), and VMPFC appear to be involved in reappraisal. Affective regions such as the ACC, insula, and thalamus show reduced activity during reappraisal.

Previous studies suggest that adolescents with more depressive symptoms report using emotion regulation strategies less often than adolescents with fewer depressive symptoms [113]. However, to date no experimental studies have investigated whether explicit emotion regulation is disrupted in adolescents with depression. Despite peer rejection being a salient source of stress during adolescence, to date the only experimental studies of explicit emotion regulation in children and adolescents have measured responses to sad or generally negative stimuli [213, 238, 282], rather than rejection-specific stimuli. **In Chapter 6 I investigate whether depressed and non-depressed adolescents are able to down-regulate emotional responses to peer rejection. Furthermore, I explore whether depressed adolescents show different neural activity during emotion regulation, compared to non-depressed adolescents.**

To summarise data on the neural correlates of peer rejection, evidence from typically developing adolescents supports the notion that brain regions proposed by social emotional models of adolescence are involved in neural processing of peer rejection. Preliminary evidence suggests that the ACC and regions within the PFC are attenuated during peer rejection in young adults with low self-esteem. Figure 1.1B illustrates how neural sensitivity may potentially influence the relationship between peer rejection and adolescent depression, although this hypothesis requires further investigation in clinical adolescent populations. In order to address this limitation, I have

conducted an fMRI study of peer rejection in adolescents with and without a diagnosis of depression. Between group differences in neural activity are measured during three phases of peer feedback: expectation of peer feedback (Chapter 4), the receipt of peer rejection (Chapter 5) and emotion regulation of peer rejection (Chapter 6).

Summary

Adolescent depression is a major public health problem, for which models are relatively underdeveloped. Peer rejection is a salient source of stress during adolescence, and the studies reviewed here suggest a bi-directional relationship between peer rejection and depressive symptoms, which may contribute to the aetiology and maintenance of adolescent depression (Figure 1.1A). A number of genetic, cognitive and neural risk factors may explain individual differences in responses to peer rejection (Figure 1.1B). In this thesis I explore the relationship between peer rejection and adolescent depression using the framework proposed in Figure 1.1. I investigate whether genetic factors moderate the effects of environmental stress on symptoms of mood disorder in adolescents (Chapter 2). I then explore negative attention biases in a sample of adolescents and young adults with low self-esteem (Chapter 3). Across three Chapters I then report findings from an fMRI study of peer rejection, which test the hypothesis that depressed adolescents demonstrate neural sensitivity during expectation of peer rejection (Chapter 4), receipt of peer rejection (Chapter 5), and emotion regulation of peer rejection (Chapter 6).

Chapter 2

The 5HTTLPR polymorphism and adolescent mood disorder

Growing evidence suggests a role for genetic factors in the aetiology of adolescent depression. Studies from the adult literature suggests that the 5HTTLPR polymorphism may modify the effects of environmental stress on depressive symptoms. Molecular genetic studies of the main effects of 5HTTLPR in child and adolescent samples are more mixed. Moreover, few have considered gene x environment interactions or the role of these in explaining symptoms in clinical samples. In this chapter I present data from a study of the 5HTTLPR polymorphism in adolescents with and without a diagnosis of mood disorder. As well as testing for differences in the frequency of the 5HTTLPR alleles between the groups, I measure the interaction between 5HTTLPR genotype and environmental stress on anxious and depressive symptomology. In a sample of 139 adolescent patients with mood disorder and 147 psychiatrically healthy controls, there was no evidence that

genotype varied between patients and controls. Neither was there a main effect of genotype on symptomology in either group. However, there was a significant interaction between group, 5HTTLPR genotype and environmental stress on self-reported anxiety symptoms. For patients with high socioeconomic status, the high expressing genotype conferred greater risk for symptoms of anxiety. There was no effect of genotype on symptoms in patients with low socioeconomic status, or controls with either high or low socioeconomic status. Genetic vulnerability may modify the effects of environmental stress on adolescent psychopathology but the direction of these effects is unclear. Limitations of the methodology and their implications for future studies are discussed.

2.1 Introduction

In Chapter 1 I outlined a genetic component to adult and adolescent depression. I explained that molecular genetic approaches to adult mood disorders have generally focused on gene variants of the serotonergic system [225, 276], and the serotonin transporter gene (5HTT) specifically [195, 264]. Transcription of the gene is in part controlled by a sequence in the promotor region (5HTTLPR), where a VNTR produces two variants of 5HTTLPR: the short 'S' allele and the long 'L' allele. The long allele is associated with significantly greater production of 5HTT mRNA and protein (high expressing) than the short allele (low expressing). As described in Chapter 1, recent evidence suggests that within the L allele, an A-G substitution generates L_A and L_G alleles with differing expression of 5HTT mRNA [164]. Since the L_G allele shows similarly low 5HTT mRNA expression to the S allele, many paediatric studies pool together the carriers of the S/L_G alleles with those of the SS and L_GL_G homozygotes [63, 109, 259].

Adult studies suggest that the S/L_G alleles confer greater risk for mood disorder than the L_A allele. The S/L_G alleles may be more common in adult patients with MDD [68, 276]. Though not without controversy, molecular studies of healthy adults have found an association between the S/L_G allele of 5HTTLPR and symptoms of neuroticism [316], harm avoidance [248] and depression [59, 124]. A similar association between the 5HTTLPR polymorphism has been reported in adult patients with a diagnosis of mood or anxiety disorder [218].

Accumulating evidence from the adult literature suggests that genetic factors may also enhance the effects of psychosocial stress on depressive and anxious symptoms [59, 308, 312, 316, 331]. That is, the presence of the short allele increases depressive responses to stressors such as recent negative life events and child abuse. Preliminary evidence from animal and human studies suggests that the association between 5HTTLPR genotype and mood disorders may vary across development. Tentatively, genetic factors may influence the effect of psychosocial stress, such as peer rejection, on depressive and anxious symptoms in child and adolescent samples (Figure 1.1B).

Before presenting data from my own study, I first provide a comprehensive review of studies which have investigated 5HTTLPR genotype in relation to symptoms of depression and anxiety in paediatric samples. Paediatric MDD shows strong co-morbidity with anxiety, both within and between episodes [16]. About 8% of adolescents suffer from anxiety [242], which also runs in families [122, 265]. Since research also implicates shared genetic influences contributing to the strong associations observed between paediatric anxiety and MDD [99], it is common for genetic studies of children and adolescents to include both anxious and depressed patients.

To my knowledge 20 studies have investigated 5HTTLPR genotype in

relation to symptoms of depression and anxiety in paediatric samples and have reported mixed findings (Table 2.1). As Table 2.1 highlights, these studies vary in methodology in numerous ways. This includes sample selection (clinical versus community samples), sample size (the smallest study included just 44 participants [29] and the largest over 4,000 [12]), and the age of the sample (some participants were as young as 4 months and others up to 20 years old). They also vary in outcome measure (from self-reported symptoms to neural responses to emotion stimuli), inclusion of environmental measures (to assess genetic effects in the presence or absence of these stressors), and in analytic technique (whether they are comparing frequencies of genotypes across diagnostic group or comparing mean levels of symptoms in different genotype groups).

Studies investigating the distribution of genotypes across diagnostic or high-trait groups have been inconclusive. One paediatric study of the frequency of 5HTTLPR genotype reports the S/L_G alleles to be more frequent in MDD patients compared to healthy controls [258], while another found that the S/L_G alleles were more frequent in females with many depressive symptoms compared to those with fewer depressive symptoms and with males [100]. However, at least one other study has failed to find differences in allele frequency of adolescent MDD patients compared to healthy controls [205].

Study	**Sample**	**N**	**Age (sd)**	**Behavioural outcome measure**	**Environmental stress measure**	**Genotype frequency**			**Main effect of genotype (direction of short allele) on outcome measure (*Interaction*)**
						L/L	**L/S**	**S/S**	
Araya et al. (2009) UK [12]	Community	4334	7	Emotional symptoms (SDQ subscale)	Stressful life events & maternal depression	0.34[2]	0.5[2]	0.17	None
Arbelle et al. (2003) Israel [13]	Community	98	7-8	Shyness (items from KSADS; MTAB; ABC)		0.33	0.49	0.18	Decreased shyness ($F_{2,98}$=4, p<0.03, η^2=0.078)
Aslund et al. (2009) Sweden [14]	Community	1482	17-18	Depressive symptoms (DSRS)	Maltreatment	0.32	0.50	0.22	*Fewer symptoms for maltreated females (OR=3.73, CI=1.16-12.07, p<0.03. Adjusted R^2=0.091)*
Battaglia et al. (2005) Italy [20]	Community	45	8.8 (0.72)	Attention to emotional faces (N400 waveform ERP response)		0.29	0.51	0.20	*Reduced attention to angry faces for shy children ($F_{4,72}$=3.57, p<0.01, η^2=0.18)*
Becker et al. (2007) Germany [27]	Disadvantaged children	305	15	Harm avoidance (JTCI/12-18); Internalising problems (CBCL and YSR)	Early life stress	0.31	0.47	0.13	None
Beevers et al. (2010) USA [29]	Community	44	18.9 (0.92)	Attention to positive and negative photos (eye-tracking)		0.20	0.55	0.27	Increased attention to positive stimuli ($F_{6,123}$=2.40, p<0.05, partial η^2=0.11)
Chipman et al. (2007) Australia [61]	Community	674	15-18	Depressive symptoms (SMFQ)	Childhood adversity and stressful life events	0.33	0.51	0.17	*Fewer symptoms of depression for those with high family adversity (β=1.56, SE=0.57, p<0.01)*
Cicchetti (2007) USA [64]	Maltreated children and adolescents (low SES)	337	16.7 (1.3)	Depressive symptoms (DISC & YSR)	Level of maltreatment	0.5	0.39	0.12	*Higher YSR score for those who were sexually abused ($F_{6,310}$=3.47, p<0.01).* No genotype frequency group differences.
Cicchetti et al. (2009) USA [63]	Maltreated children and adolescents (low SES)	850 (478 and 372)	6-13	Suicidal ideation (SI) (1 item from the CDI) Depressive symptoms (CDI score without SI item)	Level of maltreatment	-	-	-	*Higher SI for those who had 1-2 forms of maltreatment ($F_{1,306}$=8.22, p<0.05, d=0.38[1]).* No genotype frequency group differences.

Study	Sample	N	Age (sd)	Behavioural outcome measure	Environmental stress measure	Genotype frequency L/L	L/S	S/S	Main effect of genotype (direction of short allele) on outcome measure (*Interaction*)
Eley et al. (2004) UK [100]	Community	377	10-20	Depressive symptoms distinguished 15% children with the highest scores from 15% with the lowest scores (SMFQ)	Family social adversity	0.17	0.54	0.29	*Increased frequency in females from high depression group (OR=0.56, CI=0.32-0.96, p<0.05). Increased frequency in females with high family adversity (OR=2.82, CI=1.12-7.12, p<0.05)*
Fox et al. (2005) USA [109]	Community	73	7	Behavioural inhibition (POS)	Social support	0.25	0.52	0.23	*Increased risk for behavioural inhibition for those with low social support (t_{61}=-2.167, p<0.05, adjusted R^2=0.036)*
Jorm et al. (2000) Australia [178]	Community	660	4 months to 16 year	Depressive and anxiety symptoms (DSM-III criteria)		0.33	0.50	0.17	*Fewer symptoms for those aged 13-14 and 15-16 (F=5.51, p<0.01) & (F=2.09, p<0.05) respectively*
Kaufman et al. (2004) USA [183]	Maltreated children and adolescents	101 (57 and 44)	10(2.3)	Depressive symptoms (MFQ)	Social support	0.42	0.41	0.17	More depressive symptoms (χ^2=12.3, df=2, p<0.005). *More depressive symptoms for those maltreated (χ^2=10.0, df=2, p<0.01, d=1.38[1]). More depressive symptoms for those maltreated with low social support (χ^2=59.9, df=7, p<0.0001).* No genotype frequency group differences.
Lau et al. (2009) UK [205]	Anxiety/MDD patients	31	13.6	Amygdala responses to emotional faces (fMRI)		0.26[3]	0.48[3]	0.38	*Increased activity to fearful faces in healthy subjects ($F_{1,31}$=5.24, p<0.05, d=0.95).*
	Controls	33	13.7			0.36[4]	0.51[4]	0.25	*Reduced activity to fearful faces in patients ($F_{1,27}$=14.17, p<0.01, d=1.61)*
Nobile et al. (2004) Italy [258]	MDD patients	68	12.1 (2.3)	-	-	0.25	0.43	0.32	Higher frequency of S allele in MDD patients (χ^2=5.91, df=1, p<0.05, OR=1.81, CI=1.12-2.94)
	Controls	68	12.1(2.3)			0.37	0.50	0.13	
Nobile et al. (2007) Italy [259]	Community	589	10-14	Rule breaking and Aggressive behaviour (CBCL)	SES	0.34	0.49	0.18	Less aggressive behaviour ($F_{1,579}$=4.87, p<0.05). *Less aggressive behaviour for those with low SES ($F_{1,579}$=5.06, p<0.05)*

Study	Sample	N	Age (sd)	Behavioural outcome measure	Environmental stress measure	Genotype frequency L/L	L/S	S/S	Main effect of genotype (direction of short allele) on outcome measure (*Interaction*)
Nobile et al. (2009) Italy [260]	Community	548	10-14	Depressive symptoms (CBCL)	Family structure	0.33	0.49	0.18	More depressive symptoms ($F_{1,538}$=4.51, p<0.05, partial η^2=0.008). *More depressive symptoms in children from single parent families ($F_{1,538}$=4.16, p<0.05, partial η^2=0.008)*
Olsson et al. (2005) Australia [273]	Community	752	14-24	Ruminative anxiety (CIS-R subscale). Somatic anxiety (CIS-R subscale). Persistent binge drinking (Retrospective diary)	Attachment type	0.32	0.48	0.20	*Increased ruminative anxiety for insecurely attached (OR=0.77, CI=0.62-0.97, p<0.03). Reduced binge drinking for securely attached (OR=0.74, CI=0.64-0.86, p<0.001)*
Sjoberg et al. (2006) Sweden [330]	Community	200	16-19	Depressive symptoms (DSRS)	Family social adversity	(0.31)	(0.45)	(0.23)	*More depressive symptoms in females. Less depressive symptoms in males (p<0.01). More depressive symptoms in females with increased family adversity*
Stein et al. (2008) USA [338]	Community	150	18.8 (1.5)	Anxiety sensitivity (ASI)	Maltreatment	-	-	-	Higher anxiety sensitivity in those emotionally abused (t_{148}=2.18, p<0.03, d=0.36[1])

Table 2.1: Studies of 5HTTLPR genotype and anxiety- and depression-related traits in paediatric samples

Notes: ABC - Achenbach Behaviour Checklist; ASI Anxiety Sensitivity Index; BI Behavioural Inhibition; CBCL Child Behavior Checklist; CIS-R Clinical Interview Schedule-Revised; DISC Diagnostic Interview Schedule for Children; DSRS Depression Self-Rating Scale; ERP Event Related Potential; JTCI Junior Temperament and Character Inventory; KSADS Kiddie Schizophrenia and Affective Disorders Schedule; MFQ Mood and Feelings Questionnaire; MTAB - Martin Temperament Assessment Battery; POS - Play Observation Scale; SDQ Strengths and Difficulties Questionnaire; SMFQ Short Mood and Feelings Questionnaire; YSR Youth Self Report

[1] I calculated this effect size based on information reported in the paper
[2] L_AL_A (0.26), L_GL_A (0.07), L_GL_G (0.01), SL_A (0.44), SL_G (0.06)
[3] L_AL_A (0.16), L_GL_A (0.10), L_GL_G (0), SL_A (0.45), SL_G (0.03)
[4] L_AL_A (0.27), L_GL_A (0.09), L_GL_G (0), SL_A (0.48), SL_G (0.03)

The majority of studies have explored mean depressive- and anxiety-traits in unselected children and adolescents carrying S/L_G alleles versus L_A alleles [20, 100, 109, 183, 258, 259, 338], finding higher levels in the former group. However, several other studies fail to find any effect of 5HTTLPR genotype on risk for mood or anxiety disorder [12, 27]. Interestingly, a handful of studies report that the L_AL_A genotype confers greater risk for symptoms than the S/L_G carriers [13, 14, 61, 63, 178, 259]. Effect sizes vary greatly among studies, from large (Cohen's d>0.8) [183, 205], to small (partial $\eta^2 = 0.008$) [260].

It should be noted that a wide range of depressive- and anxiety-traits have been studied, including shyness and behavioural inhibition [13, 109], aggressive behaviour [259], harm avoidance [27], anxiety sensitivity [338], suicidal ideation [63] and behavioural and neural measures of attention to threat [20, 29, 205]. This range in measures limits comparisons between studies. Standardised measures of anxious or depressive symptoms provide more valid measures of the influence of genotype on psychopathology, and were used in around half of the studies [12, 14, 27, 61, 63, 64, 100, 178, 183, 260, 330].

Another reason for the inconsistent findings of 5HTTLPR on depression risk is that not all studies have considered the role of environmental stressors in modifying the effects of genes [13, 20, 29, 178, 205, 258]. In line with adult studies, the effects of genetic vulnerability on depressive symptoms are likely to be most pronounced in adolescents at high environmental risk for depression [296]. Thirteen of the twenty molecular genetic studies reviewed tested gene x environment interactions (Table 2.1) [12, 14, 61, 63, 64, 100, 109, 183, 259, 260, 273, 330, 338].

These studies measure environmental risk using child-specific indices

such as stressful life events [12, 61], maltreatment or adversity [14, 27, 61, 63, 64, 338], attachment types [273], as well as family-general risks such as low socioeconomic status (SES), poor parental education or low social support [100, 109, 183, 259, 260, 330]. All but two studies found a significant interaction between environmental stress and 5HTTLPR genotype on anxiety- and depressive-traits [12, 27]. Together, these studies provide promising support for the notion that genetic vulnerability influences the effects of environmental stress on paediatric mood and anxiety symptoms.

Evidence from twin studies also supports this notion across a wide range of environmental stressors [88, 203, 325, 326]. One twin study investigated the effect of genetic vulnerability on modifying responses to adolescent peer rejection specifically [41]. Peer-nominations of acceptance and rejection, and teacher-reported depressive symptoms, were collected in a sample of 336 twin pairs (196 monozygotic pairs and 140 dizygotic pairs) aged 6 years. Data suggested that genetic factors may account for around 30% of the variance in peer acceptance/rejection and around the same amount of variance in depressive symptoms, although the majority of variance in both these two scores was explained by non-shared environmental sources. There was also evidence of a gene-environment interaction, albeit in the opposite direction than diathesis-stress models would suggest. Under conditions of peer rejection, genetic factors explained less of the variance in symptoms of depression than under conditions of peer acceptance. While these data are intriguing, it should be noted that this method for investigating gene-environment interaction, does not identify specific genes, as molecular genetic studies do [41, 50].

As mentioned in Chapter 1, mixed findings about the effects of 5HTTLPR genotype on paediatric symptoms of anxiety and depression may be

due to genetic vulnerability influencing affected and unaffected individuals differently. The only paediatric study to investigate that found that greater amygdala activity to fearful faces characterised healthy adolescents carrying the low expressing alleles (in line with previous studies of healthy and depressed adults), but that mood disorder patients carrying the high expressing allele showed greater amygdala activity that those carrying a low expressing form of the allele [205]. Few adult studies have directly compared the effects of 5HTTLPR on symptoms in psychiatric patients and healthy controls [205].

Based on the aforementioned limitations of the existing adolescent literature, I now present data from a study of the 5HTTLPR polymorphism in adolescents with and without a diagnosis of mood disorder. **My first prediction was that the S/L_G alleles would be more frequent in youths with a diagnosis of MDD and/or anxiety disorder, compared to healthy controls.** I also measured the effects of genotype on symptom severity, testing the hypothesis that genotype would influence symptom severity as a function of diagnostic group (affected versus unaffected individuals). **My second prediction was that genotype would determine symptom severity, and that this may be different for patients and controls.** Although adult studies suggest that the low expressing alleles confer risk, the paediatric literature is more mixed. Therefore, I refrained from making any predictions about the direction of these effects.

Given evidence from the adult and paediatric literature that genetic variance may moderate the effects of environmental stress on symptoms of depression, a final aim was to investigate gene x environment interactions in relation to the 5HTTLPR polymorphism and symptoms of depression and anxiety in patients and healthy controls. Whilst Chapter 1 highlighted peer

rejection as a major source of psychosocial stress during adolescence, these data were not available for analysis in the current data set. The large numbers of participants needed to detect significant effects in a molecular genetic study of this kind often compromise the nature of measures of environmental stress that can be collected. Administering a peer rejection measure (either by self-report or by peer nomination) would require significant resources in a sample size of several hundred. As previously described, environmental stress has been measured in numerous ways in previous paediatric studies. Since the relationship between genetic vulnerability and symptoms of depression in psychiatric samples may be more influenced by family-general risks [98, 100] than child-specific risks, and a prospective epidemiological study suggested low SES temporally precedes depression in adults [252], I chose to use SES as an easily obtainable measure of environmental stress. **My final prediction was that the interaction between 5HTTLPR genotype and group would be greater in participants with a low SES background, compared to those from a high SES background.**

2.1.1 The current study

Data were collected at the National Institute of Mental Health (NIMH) and analysed by myself with the permission of Dr Monique Ernst. I investigated the effect of the genetic polymorphism 5HTTLPR on symptoms of depression in a sample of children and adolescents with and without a diagnosis of anxiety or depression. Given the shared genetic contribution to depression and anxiety [99], and the fact that there was significant comorbidity between depression and anxiety in the current sample, I also explored genetic effects on anxiety symptoms. Finally, I also explored the effects of environmental stress on depressive and anxious symptomology. Previous paediatric studies

suggest that the relationship between 5HTTLPR and depressive symptoms may be stronger for girls than boys [100, 147, 330], therefore gender was included as an additional independent variable in the analysis. I made the following predictions about the study findings:

1. The S/L_G alleles of the 5HTTLPR polymorphism would be more frequent amongst patients compared to controls.

2. Symptom severity would vary as a function of 5HTTLPR genotype, and these effects may be different for affected and unaffected adolescents.

3. Variations in socioeconomic status would moderate the influence of 5HTTLPR on patients' symptoms of depression and anxiety.

2.2 Methods

2.2.1 Participants

Participants were recruited through advertisements and didactic presentations at schools, to ongoing studies at NIMH, each approved by the NIMH Institutional Review Board. Patients were 139 youths aged 9-15 with i) significant impairment (score<60) on the Child Global Assessment Schedule [318], ii) a current diagnosis of Generalised Anxiety Disorder (GAD), Social Phobia (SP), Separation Anxiety Disorder (SAD), Specific Phobia, MDD, or Obsessive Compulsive Disorder (OCD) and iii) who were enrolled in an open treatment study (Table 2.2). A sample of 147 healthy controls with no current or past psychiatric diagnosis matched the patient group in terms of age ($t_{284} = 1.72, p = ns$), gender (χ^2=0.78, p=ns) and IQ ($t_{265} = 0.87, p = ns$; Table 2.2). Youths with an IQ<70 were excluded from the study.

		Patients	Controls	Total
Demographic	Number	139	147	286
	Age	12.1 (1.8)	12.5 (1.9)	12.3 (1.9)
	% Female	56.8%	58.5%	57.7%
	IQ	111.1 (13.6)	112.5 (11.8)	111.9 (12.7)
	SES*	35.9 (15.5)	40.21 (18.4)	38.14 (17.1)
Ethnicity	Hispanic/Latino	9	12	21
	Non-hispanic/Latino	105	115	220
	Other	1	1	2
Race	White	97	98	195
	Black or African American	7	21	28
	Asian	4	5	9
	American Indian or Alaska Native	3	2	5
	Native Hawaiian or Other Pacific Islander	1	0	1
	More than one race	14	10	24
	Other	0	1	1
Symptom severity	SCARED-C (sd)*	29.0 (13.9)	11.2 (7.9)	20.1 (14.4)
	SCARED-P (sd)*	28.8 (13.1)	4.5 (5.8)	16.6 (15.8)
	CDI (sd)*	11.6 (8.8)	3.1 (3.0)	7.3 (7.8)
5HTTLPR genotype	$L_A L_A$	28 (20.1%)	37 (25.2%)	65 (22.7%)
	S or L_G carriers	111 (79.9%)	110 (74.8%)	221 (77.3%)

Table 2.2: Demographics, symptom severity and genetic characteristics of study participants

Notes: * = significantly different at $p<0.05$

Table 2.2 presents demographics, ethnicity, race, symptomology, and 5HTTLPR genotype distribution for the sample. The mean age of the sample was 12.3 years (sd=1.9) and 57.7% were male. The mean IQ score was 111.9 (sd=12.7) and the majority were white (74.1%) and of non-hispanic/latino origin (85.6%). The proportion of Hispanic/Latino vs Non-Hispanic/Latino participants did not differ significantly between the groups (χ^2=0.67, p=ns). In order to compare the racial distribution of patient and control group, all non-white racial groups were combined because the small numbers of participants in each group prevented chi-square test being carried out separately. The proportion on white vs non-white participants did not differ significantly between the groups (χ^2=0.42, p=ns).

Psychiatric diagnoses

The Kiddie Schizophrenia and Affective Disorders Schedule (K-SADS-PL) [181] was administered to all participants and their parents by a trained clinician and confirmed diagnostic status. All clinicians had been trained to achieve acceptable reliability (kappa>0.70). Of the patient group, 80 (57.6%) had a diagnosis of GAD, 55 (39.6%) had SP, 52 (37.4%) had SAD, 44 (31.7%) had specific phobia, 26 (18.7%) had MDD, and 2 (1.4%) had OCD (Table 2.3). Most were diagnosed with more than one psychiatric disorder (89/139; 64%). Participants/parents provided written informed assent/consent for the donation of DNA.

2.2.2 Genotyping

Genetic samples of blood or saliva were collected from all participants. DNA extraction, genotyping, and polymerase chain reaction conditions followed published protocols [164]. Stage 1 distinguished short from long alleles and

Current or ongoing diagnoses	Number of patients
GAD	**80 (57.6%)**
GAD only	20 (14.4%)
GAD plus at least one other diagnosis	60 (43.2%)
SP	**55 (39.6%)**
SP only	14 (10.1%)
SP plus at least one other diagnosis	41 (29.5%)
SAD	**52 (37.4%)**
SAD only	8 (5.8%)
SAD plus at least one other diagnosis	44 (31.7%)
Specific phobia	**44 (31.7%)**
Specific phobia only	1 (0.7%)
Specific phobia plus at least one other diagnosis	43 (30.9%)
MDD	**26 (18.7%)**
MDD only	6 (4.3%)
MDD plus at least one other diagnosis	20 (14.4%)
OCD	**2 (1.4%)**
OCD only	1 (0.7%)
OCD plus at least one other diagnosis	1 (0.7%)

Table 2.3: Patient diagnoses

Stage 2 distinguished L_A from L_G alleles using fluorogenic probes designed specifically for these alleles. Genotypes were generated using ABI PRISM 7700 Sequence Detection system software (Applied Biosystems, Foster City, California). Participants belonged to one of six genotypes: SS (N=57; 19.9%), L_GL_G (N=1; 0.3%), SL_G (N=15; 5.2%), L_GL_A (N=24; 8.4%), SL_A (N=119; 41.6%), and L_AL_A (N=65; 22.7%).

2.2.3 Measures

Symptoms of anxiety and depression

Symptoms of anxiety were assessed using the screen for child anxiety related emotional disorders (SCARED) [30]; two 38-item sections completed by the child (SCARED-C) and their parent (SCARED-P). Each section

is scored on a 0-2 scale. Higher scores indicate greater symptom severity. The SCARED has good internal consistency and test-retest reliability [30]. SCARED-C scores were available for 95.0% of the patients and 90.5% of the controls. SCARED-P scores were available for 87.8% of the patients and 83.0% of the controls and were most often reported by the mother. Scores on the SCARED-C and SCARED-P correlated significantly (Pearson's $R = 0.59, p < 0.001$).

Symptoms of depression was assessed using the CDI [192]. The CDI is a 27-item self-report measure specifically for children and adolescents. CDI items are rated on a 3-point scale. Higher scores represent more severe symptoms. The CDI has been used extensively and has demonstrated high internal consistency [192]. CDI scores were available for 98.6% of the patients and 95.2% of the controls. As parental reports of mood symptoms in children and adolescents are generally poor, a parent-rated version of this measure was not included. CDI scores correlated significantly with SCARED-C (Pearson's $R = 0.67, p < 0.001$) and SCARED-P (Pearson's $R = 0.44, p < 0.001$) scores.

All three symptom variables were non-normally distributed (SCARED-P; K-S statistic=0.15, df=226, p<0.001, SCARED-C; K-S statistic=0.09, df=226, p<0.001, CDI; K-S statistic=0.17, df=226, p<0.001). Transformations were unsuccessful in correcting for this therefore ANOVA tests were performed on raw scores and non-parametric tests conducted where possible. As predicted, compared to controls, patients had more self-reported anxiety symptoms ($t_{207.4} = 12.8, p < 0.001$), parent-reported anxiety symptoms ($t_{166.3} = 18.8, p < 0.001$), and self-reported depression symptoms ($t_{167.0} = 10.7, p < 0.001$; see Table 2.2). Mann-Whitney tests confirmed the same findings (all Ps<0.001).

Socioeconomic status (SES)

SES was measured using the Hollingshead scale [160]. The four-factor scale measures parental education, occupation, sex and marital status. Scores range from 8-66, where higher scores indicate higher SES. The scale is one of the most frequently cited measures of SES [65, 89], and has been used in relation to MDD [75, 116]. Correlations with other measures of SES are relatively high; ranging from 0.73-0.87 [130]. Inter-rater reliability is also relatively high; r=0.81-0.91 [65]. Data were available for 90.6% of patients and 91.8% of controls and were normally distributed across the whole sample. The mean SES score was 38.1 (sd=17.2). There was a significant difference in SES between patients and controls ($t_{259} = 2.03, p < 0.05$), with patients reporting lower SES (mean=35.9, sd=15.5) than controls (mean=40.21, sd=18.4). However, there was no significant difference in the variance of SES scores between the two groups ($F = 3.1, p = ns$).

2.2.4 Statistical analyses

All data analyses were conducted in SPSS Statistics (v20.0, IBM Corporation, NY, USA).

Genotype frequency by group

Chi-square analyses of 5HTTLPR genotype and diagnostic group were computed to determine whether the distribution of genotypes was different between affected and unaffected participants. Participants with the $L_A L_A$ genotype (high expressing) were compared to those carrying at least one S or L_G allele (low expressing).

Group x genotype x SES interaction

A 2 x 2 x 2 x 2 ANOVA test was conducted on all three symptom measures: SCARED-C SCARED-P and CDI. Between-groups factors were diagnostic group (patient, healthy), 5HTTLPR genotype (L_AL_A or S/L_G carrier), SES (low, high) and gender (male, female). This statistical model tested interactions between symptoms, genotype and diagnostic group and the potential effects of gender. As age and IQ correlated with SCARED-C (Pearson's $R = -0.15, p < 0.05$ and Pearson's $R = -0.16, p < 0.05$ respectively) and age correlated with SCARED-P (Pearson's $R = -0.14, p < 0.05$), they were included as covariates in statistical models, although there was no evidence they varied by genotype ($t_{284} = 0.47, p = ns$ and $t_{265} = -0.42, p = ns$ respectively).

2.3 Results

2.3.1 Genotype frequency by group

There was no evidence that 5HTTLPR genotype frequency differed between patients and healthy controls (L_AL_A vs S/L_G carriers; χ^2=1.03, p=ns; Table 2.2).

2.3.2 Group x genotype x SES interaction on self-reported symptoms of anxiety

The 2 x 2 x 2 x 2 ANOVA test found no evidence that symptoms of child-reported anxiety differed between females (mean=21.3, sd=14.8) and males (mean=18.42, sd=14.8; $F_{1,162} = 2.71, p = ns$). However, there was a marginally significant effect of age on child-reported anxiety ($F_{1,162} = 3.77, p = 0.05$). A negative correlation between symptoms of anxiety and

age revealed that older adolescents reported fewer symptoms of anxiety than younger adolescents (Pearson's $R = -0.15, p < 0.05$).

The 2 x 2 x 2 x 2 ANOVA revealed a significant interaction between group, 5HTTLPR genotype and SES on child-reported symptoms of anxiety ($F_{1,162} = 4.42, p < 0.05$). Decomposing this 3-way interaction, the effects of group and genotype and their interaction was explored in participants with high SES and low SES. A 2-way interaction characterised those with high SES only ($F1, 73 = 5.14, p < 0.05$; Figure 2.1).

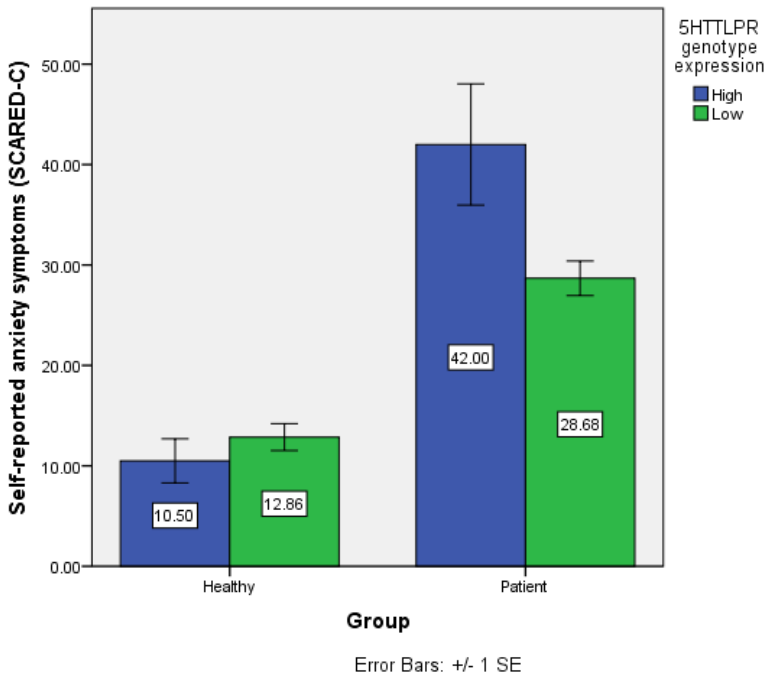

Figure 2.1: Interaction between group and 5HTTLPR genotype on self-reported symptoms of anxiety in participants with high SES

T-tests revealed that for patients with high SES, symptoms of self-reported anxiety were significantly greater in those with the high expressing genotype compared to the low expressing genotype ($t_{49} = 2.96, p < 0.056$,

equal variances not assumed). SCARED-C scores for healthy controls with high SES did not vary as a function of 5HTTLPR genotype (low expression mean=12.86, sd=9.2; high expression mean=10.50, sd=8.2; $t_{59} = 0.9, p = ns$).

Nor did SCARED-C scores for those with low SES differ by genotype in the patient group (low expression mean=28.24, sd=12.6; high expression mean=26.79, sd=13.7; $t_{67} = 0.38, p = ns$) or control group (low expression mean=9.95, sd=7.2; high expression mean=9.70, sd=5.7; $t_{61} = 0.14, p = ns$). This model also demonstrated a significant main effect of SES on self-reported symptoms of anxiety ($F_{1,228} = 5.22, p < 0.05$).

There was no evidence that this 3-way interaction characterised parent-reported anxiety ($F_{1,146} = 1.02, p = ns$) or child-reported depressive symptoms ($F_{1,172} = 2.00, p = ns$). Nor was there evidence of a 2-way interaction between group and genotype for parent-reported anxiety ($F_{1,146} = 1.44, p = ns$) or child-reported depressive symptoms ($F_{1,172} = 1.15, p = ns$). Finally there were no main effects of gender on parent-reported anxiety symptoms ($F_{1,146} = 0.40, p = ns$) or child-reported depressive symptoms ($F_{1,172} = 0.00, p = ns$) and no main effects of age on parent-reported anxiety symptoms ($F_{1,146} = 0.43, p = ns$) or child-reported depressive symptoms ($F_{1,172} = 2.34, p = ns$).

2.4 Discussion

2.4.1 Summary of findings

The first aim of the study was to assess differences in the frequency of 5HTTLPR genotypes between paediatric patients and healthy controls. Results showed no evidence that genotype varied between patients and controls.

The second aim was to test the hypothesis that the 5HTTLPR genotype would determine symptom severity, perhaps to a different degree in patients compared to controls. The data reported found no evidence to support this hypothesis. The final prediction was that amongst patients (but not controls), SES would modify the effects of 5HTTLPR genotype on symptom severity, with low SES being associated with increased symptomology (in those with the low expressing allele). The data in the current study support an interaction between genotype and SES on self-reported anxiety symptoms in patients only, but surprisingly in the opposite direction to that expected. Patients with the high expressing allele (rather than the low expressing) with high SES had highest anxiety scores. Patients with the low expressing allele with high SES had lower anxiety scores. Interpretations and implications of these findings are discussed below.

2.4.2 Interpretations

The failure to find a main effect of 5HTTLPR genotype on symptom severity in youths suffering from anxiety and/or depression contrasts with adult studies which suggest that the low expressing forms of the 5HTTLPR polymorphism are associated with increased depressive symptoms [59, 124], neuroticism [316] and harm avoidance [248], than the high expressing genotype. The findings also contrast with paediatric studies which have found 5HTTLPR genotype to influence symptoms of anxiety and depression [13, 14, 20, 61, 63, 100, 109, 178, 183, 258–260, 338] , although not all paediatric studies report an effect of 5HTTLPR genotype [12, 27].

An alternative explanation for null-effects is that environmental factors may moderate the effects of genotype on paediatric clinical samples. Mixed findings in the adult literature have been attributed to a moderating role of

environmental stress [308, 312, 316]. Preliminary evidence of an interaction between 5HTTLPR genotype and stressful events on paediatric depressive- and anxiety-traits has also been found [59, 63, 70, 147, 338]. Based on these findings, together with the growing diathesis-stress literature, I predicted that the effect of genotype on symptom severity in paediatric patients would be moderated by environmental stressors. The results of this study suggest that SES did moderate the effect of 5HTTLPR genotype on patients' self-reported symptoms of anxiety. However, unlike previous studies of gene x environment interactions, symptom severity was greater in patients with the high expressing 5HTTLPR genotype (L_AL_A) with high SES.

Although in contrast to most adult studies, the association of L_AL_A genotype with increased anxiety symptoms in patients does echo reports of risk associated with the L_AL_A genotype in depressed adolescents [205] and healthy paediatric samples [13, 14, 61, 161, 178, 259]. However, it is unclear from the current study whether the association between the L_AL_A genotype and increased symptoms of anxiety is due to the developmental nature of the study, the clinical nature, or the effect of SES.

The association between high SES and increased anxious symptom severity is more difficult to explain. Indeed, previous studies demonstrate interactions between low SES and 5HTTLPR genotype on symptoms of depression [100] and externalizing behaviours [259]. However, the findings do support an a differential susceptibility approach gene x environment interactions (see Ijzendoorn et al., 2012 for a review [357]). According to findings by Belsky and colleagues, as well as being more susceptible to the effects of negative environments, carriers of the S/L_G alleles benefit more from positive environments, than those with the high expressing, L_AL_A allele [284, 357]. This may explain why patients in the current sample experienced fewer symp-

toms of anxiety if they carried the S/L_G allele and were from a high SES background.

There are some limitations of the Hollingshead measure of SES that are worth mentioning. One limitation is that it incorporates both education and occupation, but weights these factors differently [247]. Furthermore, because it requires the researcher to code a participants' job title based on those listed in the USA census of 1970, the validity of the measure is called into question [65]. Since SES is a 'family-general' [100] measure of environmental stress, future studies of gene-environment interactions using child-specific measures would be useful.

A final finding was that differences in symptom severity, as a function of genotype and SES, occurred in the absence of genotype distribution differences between affected and unaffected individuals. These data suggest subtle ways in which genetic risk and emotional symptoms may interact: notably, while the risk alleles are fairly evenly distributed across affected and unaffected individuals, they might play a greater role in precipitating symptoms in some individuals (patients with high SES) compared to others (healthy youths and patients with low SES). These findings extend those of previous studies [205, 206] and tentatively, suggest that patients may carry a unique constellation of risk factors necessary for the expression of genotype on indices of risk.

The fact that effects were limited to child-reported anxiety (but not parent-reported anxiety) indicate the inconsistency of this effect, but may also be due to invalidity of parents' reports of internalising symptoms in adolescence [4, 165]. A meta-analysis revealed that agreement between parents and children was relatively low (r=0.25 [4]), perhaps because parents' own symptoms influence the reports of their child's symptoms [165].

The heterogeneity of the patient group may explain why effects were seen in relation to self-reported anxious, but not depressive, symptomology. Pooling patient groups has the potential to strengthen statistical power, but it may also carry limitations if genotype is more strongly associated with the aetiology of some disorders than others. An inevitable limitation of molecular genetic studies of psychiatric symptomatology is the difficulty of recruiting a sufficiently large sample. In a child clinical population such as the one described here, the challenge is even greater. In the present study the homogeneity of the sample was compromised by the need for a relatively large sample. I only included patients who had some form of anxiety or depression (largely GAD, SP, SAD or MDD). Although these disorders are characterised by some shared genetic factors, there is some suggestion that anxious and depressive symptoms are influenced by different genetic mechanisms [359]. Studies of more heterogeneous samples, or those which compare patients with different psychiatric diagnoses may help address this limitation.

2.4.3 Future research

The present findings spur suggestions for future research. Where possible, large-scale studies of select psychiatric disorders and samples with minimal comorbidity are needed to investigate relative associations of 5HTTLPR genotype with specific disorders. Replication of the present methodology in other clinical samples may enable meta-analyses to be conducted, thus partly overcoming the limitation of small sample sizes in molecular genetic studies. Greater consistency in the measures of behavioural phenotypes used in studies would also help statistical comparisons. Longitudinal studies investigating the genotype-behaviour relationship across development could

be crucial for developing more sophisticated models of the impact of genotype on behaviour across the lifespan [134, 209], as well as for identifying at-risk children and adolescents.

Ultimately, identifying the biological phenotypes that mediate gene-behaviour relationships would also generate larger effect sizes and be more powerful at detecting associations between genotype and markers of deviant behaviour. The small effect sizes of molecular genetic studies, and the associated need for large, homogenous samples, mean that other methods of study might be more powerful in detecting vulnerability for adolescent depression.

2.4.4 Summary

In conclusion, the present findings support an interaction between 5HTTLPR genotype, SES and paediatric mood disorder on affective symptom severity. Although they provide preliminary evidence to suggest that biological vulnerability may influence adolescent psychopathology, questions about the influence of peer rejection on adolescent depression specifically are difficult to answer using molecular genetic approaches. In the following chapters, I investigate group differences in cognitive and neural correlates of peer rejection.

Chapter 3

Attention biases and self-esteem in adolescents and young adults

Adolescent depression has been associated with negative cognitive biases. Recent evidence suggests that negative interpretive biases may be causally related to adolescent depression, and that attributional biases may modify the effects of peer rejection on adolescent depression. However, cognitive models of adolescent depression have not yet addressed the role of negative attention biases. In this chapter I briefly review paradigms for measuring attention biases in depression, and summarise findings from the emerging adolescent literature. I then describe adaptations of these paradigms which enable causal associations to be studied, and their findings in young adult samples. I report data from a study of 149 adolescents and young adults who completed dot-probe task measures of attention bias before and after a cognitive bias modification training session. Data suggest that negative attention biases are associated with low self-esteem and symptoms of de-

pression, although there was no evidence of a causal role of these biases, and no evidence that attention biases were modified by age. Methodological issues associated with the measurement and modification of attention biases are discussed and directions for future research identified.

3.1 Introduction

In Chapter 1 (Section 1.4), I described how negative cognitive biases may modify the effects of peer rejection on adolescent depressive symptoms (see Figure 1.1B). Indeed, preliminary evidence supports the notion that attributional biases and self-schemas modify the effect of peer rejection on depressive symptoms [40, 289]. Furthermore, data from novel experimental paradigms supports the causal role of interpretive biases in adolescent depression [208, 220, 309, 310]. However, whether a negative attention bias characterises depression, and the extent to which negative attention biases moderate the effect of peer rejection on adolescent depression, is unknown.

Attentional biases towards negative facial stimuli have been observed in depressed [95, 128, 129, 215, 298, 348] and dysphoric [38, 319] adults, though other studies have failed to find evidence of negative attention biases in adult depression [128, 180, 222, 243]. These effects may be due to task differences in how attention biases are measured. Therefore before describing adolescent studies of attention bias, I first outline the tasks most commonly used to measure attentional biases. I then tentatively explore the temporal-causal link between attention biases and depressive symptoms by describing data from emerging cognitive bias modification of attention paradigms (CBM-A).

This brief review provides the context for a study investigating the nature and causal role of negative attention biases in adolescents with low self-esteem. Understanding more about the role of attentional biases in

adolescent depression is important in laying the foundations for future studies of whether negative attention biases modify the effects of peer rejection on adolescent depression.

3.1.1 Measuring attention biases

Attention biases have been measured using several tasks (see DeRaedt and Koster, 2010 for a review of these in relation to depression [85]). The most common are the emotional Stroop task [344], the visual search task [151, 355], the dot-probe task [222] and Posner's exogenous cueing paradigm [285]. Across all four tasks, participants' reaction times to emotionally negative and emotionally neutral stimuli are compared.

The emotional Stroop task The emotional Stroop task is a modification of the original Stroop task [344], which has been used widely in cognitive psychology [368]. The emotional Stroop involves asking participants to name the colour of each of a series of words. These words vary not only in colour but in emotional valence. Speeded reaction times are expected to emotionally neutral words, since the meaning of the word does not detract attention away from the colour. Delayed reaction times to emotional words are expected because the meaning of the word is thought to distract attention momentarily from the colour of the word. However, there are numerous difficulties with interpreting data from the emotional Stroop task [85]. For example, Stroop effects may represent non-attentional interference effects [5] or cognitive avoidance [82, 85].

Across the following three paradigms, facial (rather than word) stimuli are typically employed, because of their known salience and their ecological

validity in conveying real-life emotional content [37, 39, 215, 272].

The visual search task The visual search task requires participants to search for a target emotional stimulus amongst an array of other stimuli [151, 355]. Negative attention biases are characterised by speeded reaction times to identify a negative stimulus in an array of neutral stimuli, compared to a neutral stimulus in an array of negative stimuli. Trials typically involve exposure of stimuli for around 10 seconds. Benefits of the visual search task include the fact that it involves relatively few trials (typically 112), and may be more engaging than other tasks.

The dot-probe task In the dot-probe task, a negative attention bias is characterised by speeded reaction times to identify a probe which appears in the same location as a negative stimulus, compared to a neutral stimulus. In a typical trial, participants are simultaneously exposed to a negative and a neutral stimulus. A probe subsequently appears on the screen in the location of either the negative stimulus (congruent trial) or neutral stimulus (incongruent trial) and participants are required to identify a characteristic of the probe. Commonly, this characteristic relates to the orientation of two dots (':' or '..'), although other stimulus characteristics have been used. Reaction times to the probe in congruent and incongruent trials are calculated. Negative attention biases are characterised by fast reaction times to the probe when it is in the same location as the negative image (i.e. congruent trials), and slower reaction times to the probe when it appears in the location of the neutral image (i.e. incongruent trials).

Posner's cueing task Posner's cueing task addresses the limitation of the dot-probe and visual search tasks in not being able to distinguish between

speeded engagement and slowed disengagement from a negative stimulus [85]. In contrast to the dot-probe task, on each trial participants are required to detect a single (emotionally neutral) target, which appears either on the left or right of a fixation cross. A single cue (emotionally negative or neutral) precedes the target either on the same side (valid trial) or opposite side (invalid trial) as the target. Negative engagement bias is measured by speeded reaction times to the valid trials when the cue is negative (compared to neutral). Negative disengagement is measured by slowed reaction times to the invalid trials when the cue is negative (compared to neutral).

Methodological issues surrounding the measurement of attention biases may have implications for the interpretation of findings from attention bias studies. For example, some studies have failed to observe negative attention biases in adults with depression [128, 180, 222, 243]. Whilst this could be seen as evidence against the existence of negative attention biases in adult depression, another possibility is that methodological flaws explain these null-effects. Two of these papers used the word-based Stroop task [128, 243], which has been criticised in a recent review of measures of attention biases in depression [85]. Another study used schematic faces [180]. Although schematic faces control for confounding variables associated with real-life facial stimuli (such as luminance), the null-effects in this study could be due to a failure of the schematic faces to depict social threat.

3.1.2 Attention biases in adolescents

Developmental studies have the potential to improve our understanding of when the negative attention biases consistently observed in adults emerge. Uncovering attentional processes during critical periods of vulnerability for

depression could in turn inform interventions to prevent cognitive vulnerabilities from triggering depressive episodes. As described in Chapter 1, just two studies have measured attention biases in adolescents. One study failed to find a negative attention bias in adolescents with depression [257], although this study was perhaps underpowered to detect a significant difference. A second study observed an negative attention bias in children aged 9-14 at risk of depression [177]. Studies of older adolescents are important given that this is when depression most commonly arises.

3.1.3 Cognitive bias modification of attention (CBM-A)

As previously described, CBM-A tasks have been designed in order to better understand the causal mechanisms between attentional processing and depression [146]. Repeated training aims to create benign or negative biases, which if they produce changes in mood or depressive symptomology, support a temporally causal link between negative attention biases and depression.

The original CBM-A paradigm used a modified version of the dot-probe task, to train attention focus away from, or towards of threat cues [223]. In contrast to the original dot-probe task, where congruent and incongruent trials are presented with equal frequency, CBM-A training involves systematically increasing the frequency of incongruent trials. By this means, attention is trained away from threat by pairing the neutral stimulus and the probe. Repeated pairing of the probe with the neutral stimulus shifts attention towards neutral information and away from negative. The number of trials and sessions administered in dot-probe CBM-A varies from a single session of 160 trials [10] to 10 sessions of 750 trials [10, 18].

The dot-probe CBM-A task appears to effectively modify symptoms of anxiety when delivered to children [19, 96, 97] and adolescents [96, 304].

The task also appears to reduce symptoms in students with relatively high depressive symptoms in some studies [17, 366], but not all (e.g. Baert and colleagues, 2010, Study 2 [17]). Although the task may reduce depressive symptoms in adolescents with anxiety disorder [304], to date no studies have tested its efficacy in clinically depressed adolescent populations.

The Posner cueing task version of CBM-A trains attention away from threat by increasing the frequency of invalid negative trials (trials where the probe appears in the opposite location of a negative stimulus) from 50% to 90%. Training towards positive information involves increasing the frequency of valid positive trials (trials where the probe appears in the same location as the positive stimulus) from 50% to 90%. Although this CBM paradigm enables training both away from negative stimuli and towards positive stimuli [17], it also requires a relatively large number of trials (for example, Bar-Haim and colleagues found effects after two training sessions of 384 trials each [19]).

Recently, a CBM-A paradigm based on the visual search task [151] has been developed to more directly target rejection biases, in young adults with low self-esteem [77]. In this task participants are presented with a grid of 15 frowning (rejecting) faces and a single smiling (accepting) face. Participants learn to disengage from rejection and selectively attend to acceptance by identifying the smiling face as fast as they can. An advantage of this task is that training effects have been reported in a single session of 112 trials (Dandeneau and colleagues, 2007, Study 2b [79]). Across a series of studies, Baldwin and colleagues show that, compared to a control training task, the paradigm is effective in reducing the vigilance towards rejection which is characteristic of adults with low self-esteem [77–79], reducing self-reported and physiological markers of stress [79], reducing the impact of a rejection

manipulation [78], and increasing self-esteem [78, 79]. To date no studies have investigated the efficacy of the visual search CBM-A task at reducing vigilance to rejection and depressive symptoms in adolescent populations.

3.1.4 The current study

In a single-session (and replicating Dandeneau and colleagues, 2007, Study 2b [79]), the present study sought to address two gaps in the adolescent literature. The first aim was to measure attention biases in typically developing adolescents, investigating whether negative attention biases are associated with low self-esteem and symptoms of depression. Self-esteem is a person's positive or negative attitude towards themselves, with low self-esteem reflecting low self-worth and dissatisfaction with the self [301]. Self-esteem negatively correlates with depressive symptoms in adolescence [166], and predicts current [166] as well as future adolescent depression [275]. Furthermore, as described in Chapter 1, because symptoms of depression are relatively rare in the general population, low self-esteem (a cognitive symptom of depression) may better capture depressive symptomology in unselected samples. **The first hypothesis was that negative attention biases (as measured by the dot-probe task) would be associated with low self-esteem and increased symptoms of depression.**

Based on assumptions about the causal role of attention biases, the second aim was to determine whether a developmentally appropriate CBM-A task could effectively modify negative attention biases and improve mood in typically developing adolescents. I selected the visual search CBM-A task because of studies supporting its efficacy in modifying biases in adults with low self-esteem [77–79], and because the task is likely to be more engaging for an adolescent population. In order to replicate the findings of Dande-

neau and colleagues (2007, Study 2b [79]) I chose to use the same control training task as they do. This visual search control task uses the same sized grid as the experimental condition, however the stimuli are flowers rather than faces and the search is for a physical rather than emotional characteristic. This task therefore controls for the visual search element of the experimental condition. I chose to use the dot-probe paradigm as a measure of attention bias because of its established use in depressed adults [85] and adolescents [177, 257]. The aim of using different tasks for measuring and training attention biases was to see whether training effects generalise across tasks. **The second hypothesis was that a CBM-A training session (using the visual search task) would significantly reduce negative attention bias and improve mood. I hypothesised that these effects may be more pronounced in those with lower self-esteem or more symptoms of depression, who were more likely to show an initial negative attention bias.**

Given that adolescence has been identified as a developmentally risky period for depression [148], a final aim was to investigate whether the association between attention biases and low self-esteem/symptoms of depression varied across development. Although there is debate as to when elaborative biases emerge during development [149], little research has investigated the effects of age on the relationship between attentional biases and low self-esteem or symptoms of depression. To address this I included both adolescents (aged 13-17) and young adults (aged 19-24) in my sample. **I hypothesised that attention biases may vary across development, although no predictions were made about the direction of these effects.** Neither were predictions made about the influence of development on the effects of CBM-A training.

In summary, my hypotheses for the current study were as follows:

1. Negative attention bias would be associated with low self-esteem and increased symptoms of depression.

2. A CBM-A training session would reduce negative attention bias and improve mood, possibly to a greater extent in participants with low self-esteem or increased symptoms of depression.

3. The association between negative attention biases and low self-esteem/symptoms of depression may vary across development.

3.2 Methods

3.2.1 Participants

A community sample of 113 adolescents (aged 13-17) and 52 young adults (aged 19-24) were recruited through local secondary schools and public and online advertisements in Oxfordshire, UK (N=165). Sample size was based on Dandeneau and colleagues (2007, Study 2b; N=147 [79]). Participants aged 16 and above provided written informed consent and participants aged 13-15 provided written assent (written informed consent was provided by their parents). Ethical approval for the study was provided by the University of Oxford, Central University Research Ethics Committee (CUREC).

3.2.2 Procedure

The study session lasted around 45 minutes. On arrival, participants completed computerised self-esteem and depressive symptoms questionnaires followed by a dot-probe measure of attention bias and a measure of state mood.

Participants were then randomly allocated to the experimental or control training condition, although I oversampled participants in the experimental training condition (N=100) compared to the control condition (N=49) (for an explanation see Section 3.4). Attention bias and state mood were measured again after training.

3.2.3 Measures

Self-esteem The Rosenberg Self-Esteem Scale (RSES) [301] is one of the most widely used measures of self-esteem and was developed for use in adolescent samples. The questionnaire contains ten items, scored on a four-point likert-scale (scored 0-3). A mixture of positive (e.g. 'on the whole, I am satisfied with myself') and negative (e.g. 'at times, I think I am no good at all') statements are included. RSES scores range from 0-30, with lower scores indicating low self-esteem. In adolescent samples RSES has high internal consistency (Cronbach's $\alpha = 0.77 - 0.88$) [35, 302] and concurrent validity with the single-item measure of self-esteem 'I have a high self-esteem' in undergraduate students ($r = 0.75$) [300]. The RSES also showed high internal consistency in the current sample (Cronbach's $\alpha = 0.88$).

Self-esteem scores were available for 144 participants (97%) of the current sample. Self-esteem scores ranged from 5 to 30 and were normally distributed (Table 3.1). Following Dandeneau and colleagues (2007, Study 2a [79]), a median-split was used to distinguish participants with 'high' and 'low' self-esteem scores. The ten participants whose score was the same as the median (median=19) were assigned to the high self-esteem group.

Depressive symptoms Symptoms of depression were assessed in adolescents using the CDI, which has been described previously (see Chapter 2,

Section 2.2.3). In the current study, item 9 which assesses suicidal ideation was not administered because of concerns about instigating thoughts of suicide. This is consistent with other studies of child and adolescent samples [150, 262, 332]. The CDI demonstrated high internal consistency in the current sample (Cronbach's $\alpha = 0.85$).

In adults, symptoms of depression were assessed using the Beck depression inventory (BDI)-II [26], a 21-item self-report measure of depressive symptoms. BDI items are rated on a 4-point scale. Higher scores indicate more severe symptoms. The BDI has previously demonstrated high internal consistency (Cronbach's $\alpha = 0.90$) [343]. In the current sample the BDI also demonstrated high internal consistency (Cronbach's $\alpha = 0.86$).

Standardised CDI and BDI scores, available for 142 participants (95%) were generated to compare depressive symptoms across the whole sample (Table 3.1). These scores were not normally distributed (K-S test statistic $= 0.13, df = 142, p < 0.05$). A median-split (median=-0.29) was used to distinguish participants with 'high' and 'low' symptoms of depression.

State mood The Positive and Negative Affectivity Scale (PANAS) [363] was used to assess changes in mood. The 20-item scale provides both a positive affect (pleasurable experiences) and negative affect (distress) score between zero and ten. The scale has high reliability in adult samples (Cronbach's $\alpha = 0.85 - 0.89$) [73], as well as adolescent samples (Cronbach's $\alpha = 0.83$) [114]. In the present sample, pre-training PANAS scores were available for 148 participants (99%). Pre-training positive affect ranged from 1.3 to 10.0 and was normally distributed (Table 3.1). Pre-training negative affect ranged from 0 to 6.9 but was not normally distributed (K-S test statistic $= 0.16, df = 148, p < 0.05$; Table 3.1).

Attention bias Attention bias was assessed using a modified version of the dot-probe task developed by Bradley and colleagues [37] (Figure 3.1). Stimuli were five male and five female adult grey-scale faces (participants were shown stimuli of their own gender) taken from the NimStim dataset (www.macbrain.org/resources). An angry and neutral expression of each face was available, and stimuli were presented in side-by-side angry-neutral or neutral-neutral pairs. Angry-neutral pairs were shown with the angry face on both the left hand side and right, therefore in total there were ten angry-neutral face pairs and five neutral-neutral face pairs of each gender (15 face pairs in total). The face pairs were of resolution 505 dpi, measured 200 by 257 pixels, and were displayed on a black background.

Angry-neutral face pairs had two trial types: congruent and incongruent. Trials were congruent when the angry face appeared on the same side as the probe and incongruent when they appeared on the side of the neutral face (Figure 3.1). Since there were also two probe orientation types (':' or '..'), and two probe positions (right or left), there were 120 (15 x 2 x 2 x 2) trials in total. Trials were presented in two blocks of 60 trials, preceded by 12 practice trials which gave participants 'correct' or 'incorrect' feedback.

In each trial participants were shown a fixation cross ('+') in the centre of the screen for 1000ms, followed by a face-pair for 500ms, followed by a probe for 100ms in one of the two face-pair positions (Figure 3.1). Face-pairs and probes were presented in a random order.

Participants were asked to identify the orientation of the probe as quickly and accurately as possible by using the 'z' key for vertical orientation (':') and the 'm' key for horizontal orientation ('..'). Participants were given 1000ms to make their decision. The inter-trial interval varied randomly between 500ms and 1250ms. The task was programmed in E-Prime 2 (Psy-

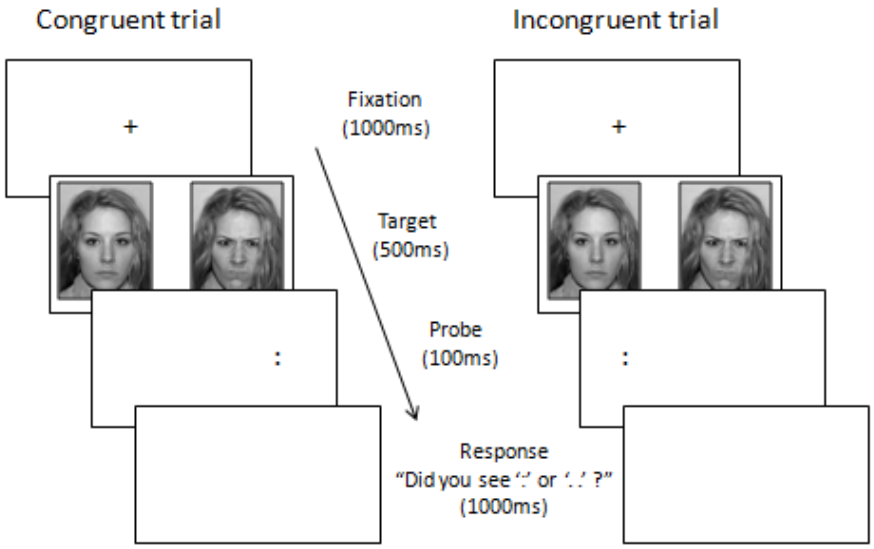

Figure 3.1: Dot-probe task parameters

chology Software Tools, Inc., Pittsburgh, PA).

Attention bias was calculated from angry-neutral trials only, and involved subtracting mean reaction times to congruent trials, from those to incongruent trials, such that positive scores represented a negative attention bias [79, 222]. Attention bias scores were available for the whole sample and ranged from -58.9 to 59.7 (Table 3.1). Attention bias scores were normally distributed.

3.2.4 CBM-A training

The CBM-A training tasks were replications of Dandeneau and colleagues (2007, Study 2b [79]; Figure 3.2). Both the experimental and control tasks involved identifying a distinguishing feature in a 4 x 4 grid, over six practice trials and a single block of 112 experimental trials. In both conditions,

trials began with a fixation cross ('+') which appeared in the centre of the screen for 1000ms. This was followed by the 4 x 4 grid, which appeared for 10,000ms. Participants were asked to identify the target stimuli using the left button of the mouse. Practice trials provided participants with feedback as to whether they were 'correct' or 'incorrect' in identifying the target image.

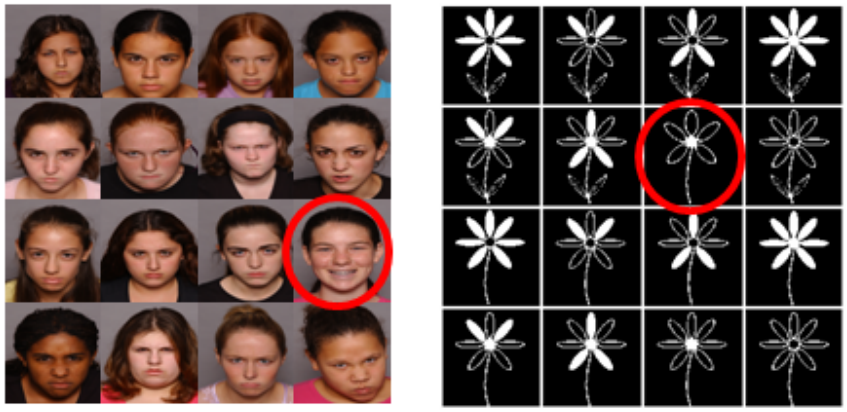

Figure 3.2: Experimental and control CBM-A training tasks

Stimuli in the experimental training condition were modified from the original task to include 16 adolescent (instead of adult) faces (congruent with the gender of the participant; Figure 3.2) selected from Nelson [255]. Each face was available with a smiling (accepting) expression, as well as an angry (rejecting) expression. Angry faces had closed mouths.

The faces were of resolution 300 dpi, measured 8.5 x 8.5mm on the screen, and presented in colour on a gray background. Individual trials involved presenting one smiling face in a 4 x 4 grid of 15 angry faces (100 x 100 pixels). Each of the smiling faces was presented seven times in each of

the 16 positions (112 trials). Participants were asked to identify the smiling face as quickly and accurately as possible.

Stimuli in the control condition were the black and white drawings of 5-petalled and seven-petalled flowers (Figure 3.2). They were of the same resolution and size as the adolescent face stimuli reported above and presented for the same duration. Participants were asked to identify the 5-petalled flower in a grid of 15 seven-petalled flowers. The training tasks were programmed in E-Prime 2 (Psychology Software Tools, Inc., Pittsburgh, PA).

3.2.5 Data preparation and statistical analysis

Data preparation

Dot-probe data from seven participants was incomplete and therefore removed. Following dot-probe preparation reported elsewhere [79], trials were excluded from analysis if the response was inaccurate or if reaction time was less than 200ms and greater than two standard deviations from each participant's mean reaction time [291, 303]. Across both dot-probe sessions, participant accuracy ranged from 57.92% to 98.75% (mean=84.72, sd=8.68). Results remained the same regardless of whether participants who scored less than 75% accuracy were included or not.

Three participants (all in the control condition) were excluded because they obtained <20% accuracy on the training task. Data from six male participants were then removed in order to make sure the experimental and control groups were matched for gender. Of note, all the results reported below remained unchanged when these six male participants were included.

Statistical analysis

Group differences in baseline characteristics were assessed using chi-square and t-tests. Performance accuracy and reaction times were used to compare the two training conditions in ANOVA tests.

A 2 x 2 ANOVA test was used to investigate whether self-esteem (high, low) and age (adolescent, adult) predicted attention bias. The same 2 x 2 ANOVA was repeated for the effect of depressive symptoms (high, low) instead of self-esteem.

A 2 x 2 x 2 ANOVA was used to test the effects of CBM-A condition (experimental, control) and interactions with self-esteem (low, high) and age (adolescent, adult) on change in attention bias (pre- post-training). Change scores were computed by subtracting pre-training from post-training bias, such that positive scores indicated an increase in negative attention bias and negative scores a decrease in negative attention bias. A similar 2 x 2 x 2 ANOVA was used to test interactions with depressive symptoms (low, high) rather than self-esteem. Finally, both ANOVA tests were repeated on change in negative affect and change in positive affect (pre- post-attention training).

3.3 Results

3.3.1 Group characteristics

The final sample was 149 adolescents and young adults. Table 3.1 describes baseline characteristics of participants in the two conditions. The mean age of the sample was 17.61 (sd=2.2) with no significant difference between the experimental and control conditions ($t_{143} = 0.94, p = ns$). Of the total sample, 78.5% were female and there were no significant differences in the

proportion of females between the two groups ($\chi^2 = 1.8, p = ns$). Neither were there differences between the conditions in self-esteem ($t_{142} = 0.26, p = ns$), standardised depressive symptoms ($t_{77.7} = 1.78, p = ns$), pre-training attention bias ($t_{142} = 0.31, p = ns$), pre-training positive affect ($t_{146} = 0.60, p = ns$), or pre-training negative affect ($t_{146} = 0.39, p = ns$). Because standardised depressive symptom scores and negative affect scores were non-normally distributed, Mann-Whitney tests were also conducted to determine whether their distributions differed between the experimental and control CBM-A conditions. These tests confirmed that there were no differences between participants assigned to the two conditions in standardised depressive symptoms (p=ns) or negative affect (p=ns).

Mean (sd)	**Total (N=149)**	**Experimental (100)**		**Control (49)**	
		Adolescents (75)	Young adults (25)	Adolescents (29)	Young adults (20)
Age	17.61 (2.2)	16.43 (0.8)	21.14 (1.5)	16.32 (0.8)	20.00 (1.3)
Female	78.5%	90.5%	87.5%	86.2%	73.3%
Self-esteem	19.3 (5.4)	18.34 (4.8)	21.76 (5.0)	17.69 (5.2)	22.10 (6.6)
Depressive symptoms	0	-0.12 (0.9)	-0.10 (0.9)	0.29 (1.2)	0.12 (1.2)
Baseline attention bias	-1.02 (25.1)	-1.24 (26.4)	1.43 (21.2)	5.06 (27.5)	-12.07 (18.2)
Baseline positive affect	5.78 (1.7)	5.60 (1.7)	6.08 (1.8)	5.57 (1.6)	6.37 (1.6)
Baseline negative affect	0.97 (0.8)	1.77 (1.4)	2.13 (2.1)	2.36 (1.8)	1.40 (1.5)
CBM-A trial accuracy[1]	96.3% (4.9)	97% (4.2)	98% (1.5)	94% (4.5)	95% (8.1)
CBM-A trial RT[1]	2783.4ms (661.9)	2721.4ms (590.2)	2303.1ms (499.9)	3177.0ms (580.6)	3045.6ms (784.6)

Table 3.1: Participant charactersitics

[1]Significant difference between those in the Experimental (N=100) and Control (N=49) groups, at $p < 0.001$

3.3.2 Equivalence of CBM-A conditions

T-tests revealed that compared to the experimental condition (mean accuracy=97%, sd=3.7; mean RT=2616.8ms, sd=595.0), participants in the control condition were less accurate (mean accuracy=94%, sd=6.2; $t_{65.4} = 3.57, p < 0.001$) and took longer to correctly identify the CBM-A target (mean RT=3123.4ms, sd=666.8; $t_{147} = 4.69, p < 0.001$).

3.3.3 Pre-training attention bias

Self-esteem A 2 x 2 ANOVA revealed a main effect of self-esteem (high, low) on pre-training attention bias ($F_{1,140} = 3.84, p = 0.05$), such that participants with low self-esteem had significantly higher negative attention bias (mean=4.58, sd=23.9) than participants with high self-esteem (mean=-5.95, sd=23.9; Figure 3.3). There was no evidence of a main effect of age (adolescent, adult; $F_{1,140} = 0.71, p = ns$), or an interaction between age and self-esteem ($F_{1,140} = 0.71, p = ns$) on pre-training attention bias.

Depressive symptoms A 2 x 2 ANOVA also revealed a main effect of depressive symptoms (high, low) on pre-training attention bias ($F_{1,138} = 3.88, p = 0.05$), such that participants with more symptoms of depression had significantly higher negative attention bias (mean=3.35, sd=24.1) than those with fewer symptoms (mean=-5.65, sd=23.9). There was no evidence of an interaction between age and depressive symptoms ($F_{1,138} = 0.06, p = ns$) on pre-training attention bias.

3.3.4 CBM-A training effects

Change in attention bias Change in attention bias was normally distributed. There was no main effect of training condition on change in at-

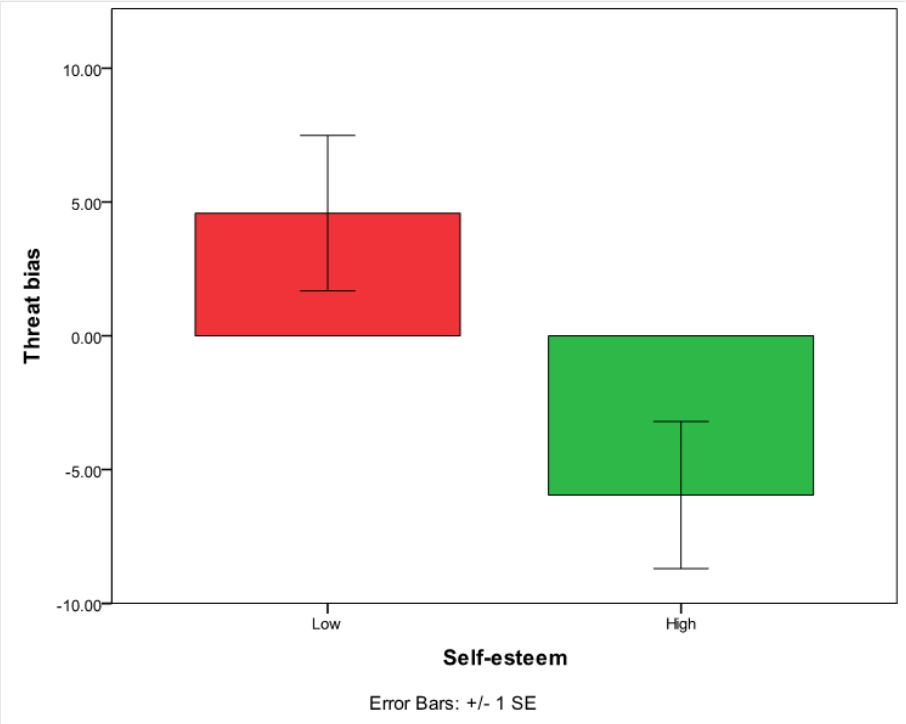

Figure 3.3: Effect of self-esteem on pre-training negative attention bias

tention bias ($F_{1,136} = 2.3, p = ns$) and no interaction between condition and self-esteem on change in attention bias ($F_{1,136} = 0.35, p = ns$). However, there was an interaction between condition and age ($F_{1,136} = 4.35, p < 0.05$; Figure 3.4). T-tests revealed that for adolescents, there was no difference in change in attention bias between those in the experimental condition (mean=2.4, sd=33.3) and those in the control condition (mean=-1.7, sd=38.7; $t_{102} = 0.54, p = ns$). However, adults in the control condition showed a significant increase in attention bias (mean=14.7, sd=33.7), whereas those in the experimental condition did not (mean=-4.35, sd=27.7; $t_{43} = 2.08, p < 0.05$). There was no interaction between condition, age and self-esteem on change in attention bias ($F_{1,136} = 0.74, p = ns$).

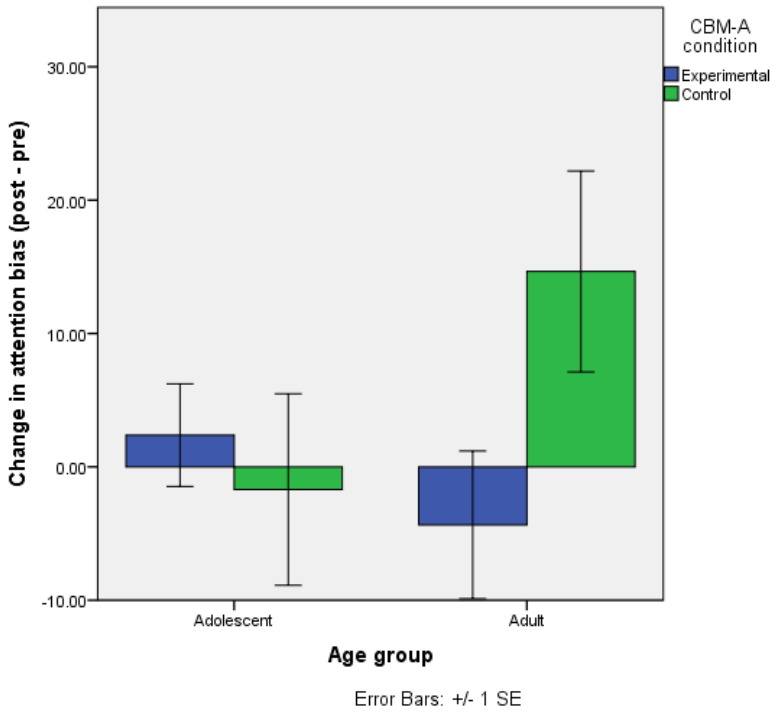

Figure 3.4: Interaction between age and condition on change in attention bias

A similar 2 x 2 x 2 ANOVA revealed no interaction between condition and depressive symptoms on change in attention bias ($F_{1,134} = 0.05, p = ns$) and no interaction between condition, depressive symptoms and age ($F_{1,134} = 0.53, p = ns$).

Change in negative affect Change in negative affect was non-normally distributed (K-S test statistic $= 0.14, df = 147, p < 0.05$). A 2 x 2 x 2 ANOVA revealed a main effect of condition ($F_{1,134} = 5.0, p < 0.05$). T-tests confirmed that the reduction in negative affect was greater in the experimental CBM-A group (mean=-0.31, sd=0.8) compared to the control group (mean=-0.04, sd=0.8; Figure 3.5). However, transforming change in negative affect using the function (log(Change in negative affect + 1)), reduced

the main effect of condition to ($F_{1,134} = 1.8, p = ns$). There was no significant interaction between condition and self-esteem ($F_{1,134} = 1.16, p = ns$), condition and age ($F_{1,134} = 0.7, p = ns$) or condition, age and self-esteem ($F_{1,134} = 1.69, p = ns$) on change in negative affect.

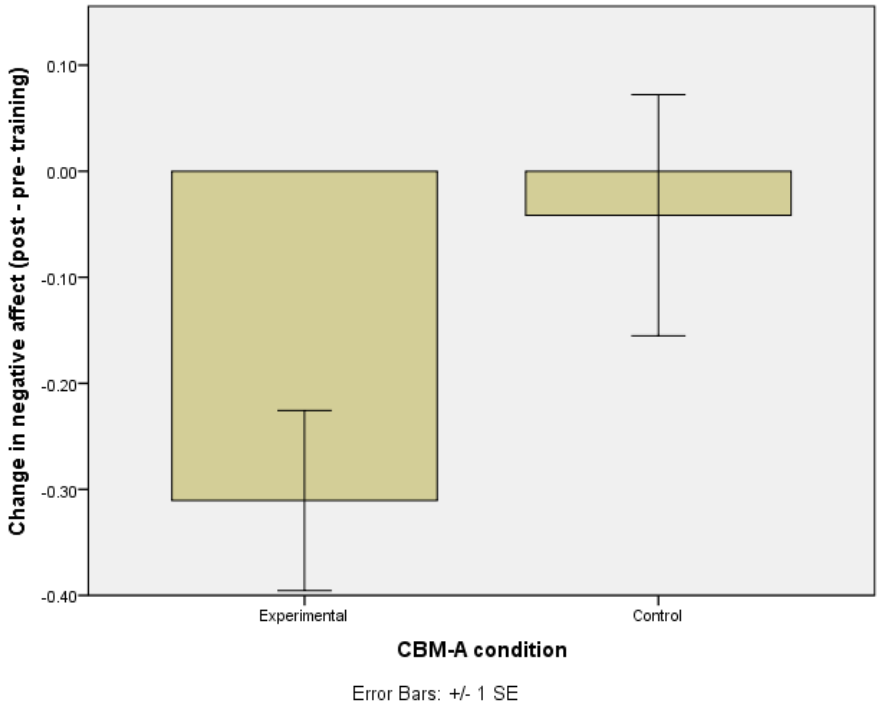

Figure 3.5: Effect of CBM-A on change in negative affect

A 2 x 2 x 2 ANOVA also revealed no interaction between condition and depressive symptoms on change in negative affect ($F_{1,132} = 1.77, p = ns$) and no interaction between condition, depressive symptoms and age ($F_{1,132} = 1.30, p = ns$).

Change in positive affect Change in positive affect was normally distributed. A 2 x 2 x 2 ANOVA revealed no main effect of condition on change in positive affect ($F_{1,134} = 2.2, p = ns$), although t-tests revealed a

trend for increased positive affect in the experimental condition (mean=0.13, sd=1.0) compared to the control condition (mean=-0.08, sd=0.8; $t_{145} = 1.3, p = ns$). There was no interaction between condition and self-esteem ($F_{1,138} = 1.92, p = ns$), condition and age ($F_{1,134} = 0.5, p = ns$), or condition, age and self-esteem ($F_{1,134} = 0.30, p = ns$) on change in positive affect.

A similar 2 x 2 x 2 ANOVA also revealed no interaction between condition and depressive symptoms on change in positive affect ($F_{1,132} = 0.29, p = ns$) and no interaction between condition, depressive symptoms and age ($F_{1,132} = 0.14, p = ns$).

3.4 Discussion

3.4.1 Summary of findings

The present study supports the primary hypothesis, that negative attention biases characterise adolescents and young adults with low self-esteem and symptoms of depression. Contrary to predictions, there was no evidence to support the second hypothesis, that CBM-A training (based on the visual-search paradigm) would reduce negative attention bias. Non-statistically significant trends suggested that the experimental CBM-A training reduced negative affect and increased positive affect. In relation to the third hypothesis, there was no effect of age on pre-training attention bias, although the control condition was associated with increased negative attention bias in young adults (compared to adolescents). Finally, there was evidence that the control training condition elicited slower response times and more inaccurate responses than did the training condition.

3.4.2 Interpretations

By demonstrating a negative attention bias in adolescents with low self-esteem and symptoms of depression, the present study replicates findings from the adult literature [77–79]. Furthermore, the data replicate findings from a study of negative attention biases in adolescents at risk of depression [177]. Since depression has traditionally been associated with later attention biases and difficulty disengaging from negative stimuli [38, 129], 1000ms exposure times are often necessary to detect attention biases in depressed samples [85, 127, 129, 215]. However, the present study demonstrates that it is also possible to observe them at shorter exposures (i.e. 500ms). More generally, the study makes an important contribution to developmental models of depression by demonstrating that negative attention biases are associated with adolescent depression. The findings provide further rationale for investigating the presence of negative attention biases in adolescents with a current diagnosis of depression. To date only one study has addressed this issue, in a relatively small sample [257]. The findings also provide further rationale for investigating the extent to which negative attention biases may moderate the effects of peer rejection on adolescent depression (Figure 1.1B).

The CBM-A paradigm provided the opportunity for testing whether negative attention biases may play a causal role in adolescent depression. The present study failed to replicate the beneficial effect of CBM-A training on negative attention bias for those with low self-esteem previously found [79], although there was tentative evidence to suggest it had modest effects on positive and negative affect. One explanation for the null effects is that negative attention biases do not temporally precede negative affect. Another possibility is that negative attention biases modify responses to peer rejec-

tion. However, complexities with the measurement of attention biases, the methods for training them, and incongruence between the two, also mean that the null effects may reflect methodological limitations.

One issue is that whilst the dot-probe task provides a measure of attentional engagement with threat, it has been argued that the visual search task trains engagement with positive stimuli and disengagement from threat (rejection). Therefore, the null effects may be explained by the fact that training engagement of positive stimuli does not affect disengagement from threat i.e. that they are two distinct processes. This would explain why mood effects were seen in the absence of attention bias change. Measures of disengagement, such as the Posner cueing task, may provide more accurate measures for assessing the effects of the training task. An alternative would have been to use the Posner cueing task instead of the dot-probe task, or to use a dot-probe CBM-A task instead of the visual search task. However, the dot-probe CBM-A task generally involves more trials than the visual search and is perhaps less engaging to adolescents. In addition, I was keen to replicate the methods of Dandeneau and colleagues (2007, Study 2b [79]) as closely as possible and to see whether training effects in one task generalised to another.

Another issue is that the stimuli in the training task were of adolescent faces, whereas adult faces were used in the dot-probe task. Therefore it is possible that the null results can be explained by the fact that attentional training of adolescent faces did not generalise to adult stimuli. Adult faces were chosen for the dot-probe because they were standardised stimuli selected from a validated data-set. Adolescent faces were selected for the CBM-A task in order to increase the salience and ecological validity of the task, given previous reports of the importance of using self-relevant stimuli

[45, 85, 146]. A similar issue it that it is possible that the wide age-range of the sample meant the stimuli were not relevant to all participants. For example, the adolescent training stimuli may not have been sufficiently salient to younger adolescents and young adults in order to instigate an attentional change in bias.

Given these methodological limitations, I am cautious in the interpretations of these null effects, and instead use them to highlight the fragility of CBM-A data. As demonstrated by a meta-analysis, the effects of CBM-A training tasks are also relatively small [146]. The current sample size was based on a study by Dandeneau and colleagues (2007, Study 2b; N=147 [79]). However, the effect size in Dandeneau and colleagues study was very small (Cohen's $f^2 = 0.03$; Stéphane Dandeneau, personal communication). Based on this effect size, and power of 0.8, one would need a sample of size of 27,480 participants to detect as significant difference between experimental and training condition. Nevertheless, as the research literature grows, meta-analyses may help to provide more high-powered analyses and knowledge of optimal dot-probe parameters could ensure greater reliability (see Bar-Haim, 2010 for a review of the optimal parameters of CBM-A training paradigms [18]).

Contrary to predictions, the present analysis found no evidence that age modified the link between negative attention biases and self-esteem/depressive symptoms. This may be because the sample was too small to detect significant developmental effects, because the difference in mean age between the adolescents and adults was relatively small (4 years), or because attention biases emerge during early adolescence, a developmental period that was not represented in the current sample. The dichotomising of participants as 'adolescents' or 'adults' is somewhat arbitrary and may therefore have con-

founded the results. Replications of the current study in more restricted age groups from early adolescence to adulthood could inform the debate about when cognitive biases emerge.

The final set of findings relate to problems with the control condition. Indeed, my initial concerns about the validity of the control task were the reason that I oversampled for participants in the experimental condition. Although there were no effects of age on the ability of the CBM-A experimental condition to reduce negative attention biases, the findings suggest that negative attention bias was increased in adults in the control condition. This finding fits with other data reported here which suggest that the control task was significantly harder to perform than the experimental task. Specifically, reaction times and performance accuracy were significantly lower in the control condition compared to the experimental condition. Although this may not affect the null effects of the current study, since negative attention biases in the control condition would have *favoured* a positive effect of the experimental condition, it may cause a problem for the interpretation of the findings of Dandeneau and colleagues [77–79]. For example, Dandeneau and colleagues' (2007, Study 2b [79]) finding of a beneficial effect of training on attention bias may have been driven by increased attention bias in participants trained using the control condition. Therefore although Dandeneau and colleagues do not report performance data for their samples, I recommend that these results be interpreted with caution.

Indeed, there are some other limitations of the control task that are worth noting. Firstly, whilst the task does control for the visual search of a stimulus, its non-facial aspect means that positive effects in the experimental condition could be the result of a facial search, rather than an emotional search. Faces have preferential processing [156] and therefore an ideal control

condition would be a non-emotional facial search, or a reversed emotion facial search, such as looking for an angry face in a grid of happy faces. Others have noted the influence that luminance and other low-level features have in visual processing [226], therefore an alternative control condition should also control for these.

3.4.3 Future research

Given the infancy of the attention bias literature in relation to adolescent depression, there are numerous avenues for future research. As previously described, studies of restricted age groups and younger children and adolescents could help identify the emergence of negative attention biases and developmental periods of vulnerability to attention biases. Studies of depressed adolescents are needed to confirm that the negative attention biases seen in at-risk samples characterise depressive episodes as well. Additionally, exploration of the optimal parameters for CBM-A tasks for children and adolescents should be explored before the task is developed further. More suitable control training conditions for the visual search CBM-A task should also be developed and validated.

Importantly, it is recommended that future studies of CBM-A tasks also report evidence of negative attentional biases in the samples which they test. Several recent CBM-A studies in adults report positive effects of training, but fail to report whether they observed an initial negative attention bias (e.g. [44, 366]). This leads to difficulties in interpreting the extent to which attentional biases contribute to depression in these samples.

Together, further examination of the association between negative attention biases and adolescent depression, and thorough testing of the causal nature of these biases, paves the way for future studies of the role of negative

attention biases in modifying the effects of peer rejection on adolescent depression. As a result, these studies will help inform whether the modifying role of cognitive biases proposed in Figure 1.1B, relates to attentional biases specifically.

More generally, little research has investigated the biological correlates of negative attentional biases in depression. Given that the biological correlates of attentional processes are relatively well understood, investigating genetic and neurological correlates of attention biases may help understand how biological and cognitive vulnerabilities interact to trigger a depressive episode [85].

3.4.4 Summary

In summary, the present study sought to investigate whether negative attention biases observed in adults at risk of depression could be replicated in an adolescent sample. Data collected from 149 adolescents and young adults show negative attention biases in participants with low self-esteem and symptoms of depression. These data suggest that negative attention biases may play a role in adolescent depression. However, whereas adult data have implicated a causal role for negative attention biases, there was no evidence that the current CBM-A task was causally associated with negative attention biases. Numerous complexities associated with the tasks used to measure and modify attention biases mean that further examination of these effects is needed before firm conclusions about the theoretical and clinical implications of CBM-A can be drawn. The present study also provides novel data which highlights some of the limitations of the control task designed by Dandeneau and colleagues.

Work based on Chapters 4, 5 and 6 has been published in the following article: Haddad, A.D., Platt, B. James, A.C. and Lau, J.Y. (2012) Anxious and non-anxious adolescents' experiences of non-clinical magnetic resonance imaging research. *Child Psychiatry and Human Development*. The final publication is available at http://link.springer.com/article/10.1007%2Fs10578-012-0350-x

Chapter 4

Neural correlates of the anticipation of peer feedback

Negative expectations of peer feedback may explain why depressed adolescents are more likely to be exposed to peer rejection than non-depressed adolescents. Although it is widely acknowledged that depressed adolescents have negative expectations of future success, the extent to which this characterises interpersonal interactions specifically is unknown. Novel experimental peer rejection paradigms offer a unique opportunity to study the behavioural and neural correlates of the expectation of peer feedback in a controlled (i.e. standardised across participants) context. In the present study, 15 clinically-depressed and 15 psychiatrically healthy adolescents were asked, during MRI acquisition, to predict how interested or disinterested unknown peers would be in talking to them. These peers had previously been rated by the participants in terms of desirability. Depressed adolescents expected significantly more negative feedback compared to non-depressed adolescents. Furthermore, depressed adolescents showed heightened activity in the right caudate whilst they predicted peer feedback (independent of peer desirabil-

ity), compared to healthy adolescents. These data tentatively elucidate the neural correlates of anticipated peer feedback in adolescent depression. Furthermore, peer desirability influenced participants' behavioural and neural responses during prediction of peer feedback. Further studies are needed to investigate the causal role of negative expectations of peer feedback in adolescent depression.

4.1 Introduction

In Chapter 1 (Section 1.2), I described evidence from longitudinal and experimental studies that adolescent peer rejection elicits negative emotional responses and may predict future depression. I also described a possible bidirectional relationship between peer rejection and depression, such that depressive symptoms may predict subsequent peer rejection experiences (Figure 1.1A). The mechanisms of the latter pathway remain relatively underinvestigated. Speculatively, by holding negative beliefs about future interpersonal interactions, depressed adolescents may adopt maladaptive social behaviour such as reassurance seeking, or withdrawal, which increase their chances of future peer rejection [53] (Figure 1.1B). In this chapter, I empirically test whether depressed adolescents do indeed expect more negative feedback from peers than healthy controls, and the biological bases of these differences.

According to cognitive models, negative expectations of future events are a fundamental feature of depression [25, 193]. Cross-sectional studies have shown an association between negative expectations and adolescent depressive symptoms [217]. However, many studies of negative expectations involve measuring global expectations of the self, the world and the future using self-reported questionnaires, which may be confounded by mood

symptoms. Other experimental studies have measured expectation of performance in non-social tasks (e.g. a maths problem; see Ruble and colleagues (1993) [305] for a review), finding that girls have lower expectations of their performance than boys, which may explain why girls report more depressive symptoms than boys [305].

Relatively few studies have investigated the extent to which negative expectations relate to interpersonal interaction in adolescents, but drawing on studies of pre-adolescent children, this is a significant characteristic of those with higher depressive symptoms [132, 306, 379]. For example, Rudolph and colleagues provided children with vignettes about possible peer interactions and asked them to select the most likely outcome [1] [306]. Children with elevated symptoms of depression had more negative views of themselves within peer relationships. Given the salience of peers during adolescence [43, 74, 199, 240, 341], expectations of peer feedback may be even more strongly linked to depressive symptoms. Ongoing structural and functional neural development throughout adolescence [34, 49] has also been implicated in the susceptibility of adolescents to peer rejection via heightened affectivity and impaired ability to regulate emotional reactivity [313].

One difficulty with measuring expectations of real-life social interactions is that individual differences in actual peer relationships and history of rejection could confound findings. The novel experimental peer rejection paradigms described in Chapter 1 (Section 1.2) offer a unique tool for investigating adolescents' expectations of peer feedback, in a controlled experimental context. In the Chatroom paradigm [141] participants believe they

[1] "You're on the playground at lunchtime and one of the older kids comes up and starts to pick on you. What do you think the kids in your class might do? (a) They might stick up for me and tell the older kid to leave me alone [supportive], (b) They might just walk away so that they don't get picked on also [indifferent], or (c) They might join in with the older kid and start teasing me [hostile]."-taken from Rudolph et al., 1997 [306]

are taking part in a study of online social interaction. They make ratings of their interest in chatting with a number of fictitious peers. Subsequently, participants are lead to believe that these peers will make a decision about whether they want to chat to the participant or not. Prior to receiving feedback from peers, participants are asked to predict how interested each peer will be in chatting with them. The Chatroom paradigm has been shown to elicit behavioural differences in the expectation of negative feedback between socially anxious adolescents and healthy controls [141]. As predicted, anxious adolescents expected more negative feedback from peers than did healthy controls. However, the extent to which negative expectations of peer rejection characterise adolescent depression has not been investigated in such a context. **In the current study, I tested the hypothesis that depressed adolescents would expect more negative feedback from peers compared to healthy controls.**

Another advantage of experimental peer rejection paradigms is that they can easily be applied to the scanner environment. Neuroimaging studies may offer more subtle information on the mechanisms by which negative expectations of social interaction operate (Chapter 1, Section 1.5). For example, Guyer and colleagues found no behavioural effects during prediction of peer feedback. However, fMRI data suggested that age and gender predicted neural responses while participants rated their expectations of peer feedback [142]. Ultimately, neuroimaging methods may help identify the extent to which neural sensitivity influences the relationship between peer rejection and adolescent depression (Figure 1.1B).

The brain regions involved in processing expectations of peer feedback were investigated for the first time in typically developing adolescents by Guyer and colleagues [142]. Neural activity in the nucleus accumbens (NA),

hypothalamus, hippocampus and insula was heightened when typically developing adolescents predicted feedback from desirable (versus non-desirable) peers in the Chatroom paradigm. The NA has been associated with reward processing, the hypothalamus with regulating stress responses, the hippocampus with memory and context appraisal, and the insula with affective processing of visceral stimuli [142]. The fact that these brain regions showed heightened activity in older adolescents, and particularly in females, fits with the model of social information processing proposed by Nelson and colleagues (Figure 1.2) [256]. Furthermore, the data support the suggestion that the networks involved in processing actual peer rejection [279, 315] may also be activated in the expectation of peer rejection, although interestingly in this study there was no evidence that regions of the PFC associated with emotion regulation were involved. Finally, these regions are congruent with those implicated in the anticipation of reward more generally [103, 190].

A study of anticipated reward and loss in female adolescents found that compared to girls with no family history of depression, girls whose mothers were depressed showed less activation in the insula and putamen during anticipation of reward and increased activity in the insula [126]. The only study to date of anticipated peer feedback in an atypical adolescent sample is another study by Guyer and colleagues [141]. They found that compared to healthy controls, socially anxious adolescents showed heightened activity in the amygdala and left VLPFC when they predicted feedback from peers in the Chatroom, supporting the engagement of both affective and regulatory brain regions during social information processing in adolescence [256]. The authors tentatively argue that expectations of negative feedback may contribute to heightened sensitivity during the receipt of rejection in anxious adolescents. Here I also suggest that these negative expectations may

contribute to heightened exposure to rejection more generally.

No study has investigated the neural correlates of depressed adolescents' expectations of peer feedback. **Therefore a second goal of the current study was to investigate whether neural activity during the prediction of peer feedback differed between depressed and non-depressed adolescents.** I hypothesised that depressed adolescents would show heightened activation of the affective neural networks involved in social emotional processing [256], anticipation of reward [126], and peer rejection specifically [315], including the amygdala, NA, hypothalamus, hippocampus and insula.

Guyer and colleagues' findings suggest that peer desirability may play an important role in moderating behavioural and neural responses to during predictions of peer feedback. Firstly, participants expected more negative feedback from undesirable peers than desirable peers [142]. Secondly, the researchers identified numerous brain regions which showed heightened activation when feedback expectations related to desirable (versus undesirable) peers [142]. **A final aim was therefore to investigate the effects of peer desirability on behavioural and neural correlates of peer feedback expectations in depressed and non-depressed adolescents.** I predicted that adolescents would expect more positive feedback from desirable peers and more negative feedback from undesirable peers, and that this effect might be heightened in depressed versus non-depressed adolescents.

To explore the neural correlates of peer desirability during predicted peer evaluation I employed a different analysis strategy from Guyer and colleagues [141, 142]. I compared activity during the expectation of feedback which was independent of peer desirability, with activity during expectation of peer feedback that correlated with peer desirability scores (from pre-scan

ratings). In contrast, Guyer and colleagues compared neural responses during prediction of feedback between high interest and low interest peers, using a median-split variable. Guyer and colleagues justify the use of a median-split variable by reporting that 'about half the time a given photo was a high interest and half the time it was a low-interest stimulus'. However, I hypothesised that peer desirability might be better included as a continuous variable (since this is how it is measured). Covariate fMRI analysis may also convey greater power than median-split designs, in this case because peers rated as moderately desirable may be categorised as being of 'high' or 'low' desirability just because their score is one point either side of the median.

The relative strengths and weaknesses of the various peer rejection paradigms are reviewed in Chapter 1 (Section 1.2). Briefly, whereas the YIPS re-creates real-life social interactions to deliver highly ecologically valid peer rejection experiences, computerised paradigms such as Cyberball [371], Survivor [294], and Chatroom [141] rely on participants believing that the online interaction is real, but enable more standardised delivery of peer rejection experiences that can be used to investigate more subtle behavioural and neural responses to peer rejection. I chose to use the Chatroom paradigm (see Figure 4.1) because of the potential to investigate the effect of peer desirability on expectations of peer feedback, as well as the receipt of peer rejection.

4.1.1 The current study

In this functional neuroimaging study, behavioural responses and brain activation patterns were compared between adolescents with and without depression, while they predicted how interested other peers would be in talking to them (Chatroom study; phase 2; Figure 4.1).

In the first set of analyses, I compared behavioural ratings of expected

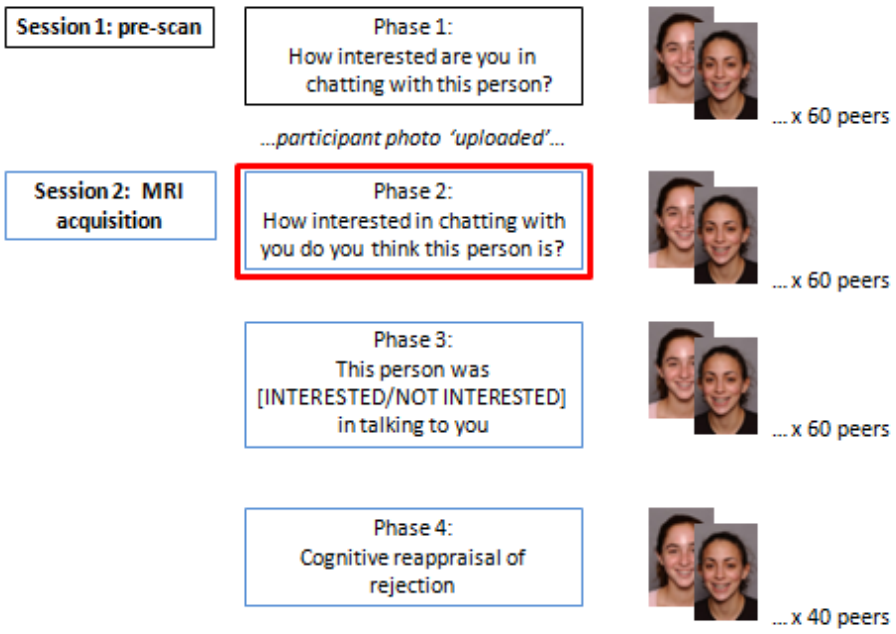

Figure 4.1: The Chatroom paradigm (phase 2).

peer feedback in adolescents with and without depression and investigated whether any group differences were further amplified by peer desirability (PD). I predicted an interaction between patient group and peer desirability such that depressed patients would expect more negative feedback from desirable (versus undesirable) peers, than healthy adolescents. Because of concerns that anxiety may confound how interested participants expected peers to be in talking to them, I also tested for an association between symptoms of anxiety and negative expectations of feedback.

In the second set of analyses, I focused on brain activation patterns during the prediction of peer feedback. Specifically, I sought to investigate which neural regions were responsive to the desirability of the peer, and which were unresponsive to peer salience. To do this, two events were created: i) prediction with all peers weighted equally ('prediction'), and ii)

prediction with behavioural ratings of peer desirability added as a covariate ('prediction + PD'). The first contrast ($prediction + PD > prediction$) explored regions where the action of predicting feedback correlated with peer desirability, over and above regions that simply respond to the prediction of peer feedback. The contrast enabled me to identify regions that were strongly correlated with peer desirability, regardless of whether these correlations were positive or negative. Based on previous studies of typically developing adolescents, I expected this contrast to be associated with activity in the amygdala, hypothalamus, insula, hippocampus and NA. These regions were then used to compare depressed and non-depressed adolescents. Guided by prior data on dysfunctional brain correlates associated with adolescent depression, between-group differences were expected in these regions.

The reverse contrast ($prediction > prediction + PD$) explored activity during the prediction of peer feedback which was independent of (uncorrelated with) peer desirability. Since previous studies have not investigated the neural correlates of predicted peer evaluation alone, I made tentative predictions that regions associated with the receipt of peer rejection would be engaged. These include emotion detection regions (e.g. occipital and parietal lobes), affective regions (e.g. cingulate cortex and insula), and emotion regulation regions (e.g. IFG and frontal pole). I explored group differences in neural activity during predicted peer evaluation, although based on the limited literature, I made no predictions about the location or direction of these effects.

In summary, I made the following predictions about the findings of the current study:

1. Depressed adolescents would expect other peers to be less interested in interacting with them than healthy controls. Across the whole group,

expectations of feedback from desirable peers would be higher than expectations of peer feedback from undesirable peers.

2. Predicting peer feedback would be associated with peer desirability in regions such as the amygdala, hypothalamus, insula, hippocampus and NA. Depressed adolescents would show heightened activity in these regions compared to healthy controls. Predicting feedback alone (regardless of peer desirability) would activate areas such as the occipital and parietal lobes, the cingulate cortex and insula, and the IFG and frontal poles. The association between depression and response in these regions was explored, although no predictions were made about the direction of effects.

4.2 Methods

4.2.1 Participants

Participants were 15 medication-free adolescents aged 15-17 with a diagnosis of MDD according to the DSM-IV [9] and 15 adolescents with no current or previous psychiatric history (Table 4.1).

Recruitment Patients were recruited from Child and Adolescent Mental Health Services (CAMHS) in Oxfordshire, Buckinghamshire and Berkshire. Patients were included if they presented with depression, were aged 12-17, and were medication-free. They were excluded if they had another psychiatric diagnosis (other than an anxiety disorder). Patients who reported current suicidal intent or a suicide attempt in the past 3 months were also excluded, because of concerns that the peer rejection manipulation may exacerbate their suicidal behaviour. Controls were recruited from local Ox-

fordshire state schools. Participants with IQ<70 or MRI incompatibility (e.g. metal dental work, pregnancy or claustrophobia) were excluded. 29 of the 30 participants were right-handed.

Four participants recruited from local schools disclosed depressive symptomology at the screening session and subsequently met criteria for MDD according to DSM-IV. These participants were allocated to the patient group, advised to discuss their feelings with their parents or other adult, and given an information sheet about seeking help. Within the patient group, there was no significant difference in depressive symptoms (as measured by the CDI; see below) between participants identified through CAMHS services (mean=25.73, sd=24.5) and those identified through schools (mean=24.50, sd=5.8; $t_{13} = 0.4, p = ns$). Non-parametric tests confirmed the same findings for median depressive symptom scores of those recruited through CAMHS services (median=27) and those recruited through schools (median=25; p=ns). These statistical tests suggest that there was no significance in depression severity between the two forms of patient recruitment. Some limitations of combining these two groups are discussed in Chapter 6.

Participants aged 16-17 provided written informed consent, as did their parents. Participants under 16 provided written informed assent and written informed consent was provided by their parents. The study received ethical approval from Oxfordshire NHS Research Ethics Committee B (10/H0605/12).

Characteristics Participant demographics are described in Table 4.1. There were no significant differences between the groups in gender, age, IQ, ethnicity or parental education (all Ps>0.05). As expected, patients reported significantly more symptoms of depression (mean=25.4, sd=4.6) than controls (mean=4.5, sd=3.7; $t_{28} = 13.7, p < 0.001$). CDI cut-off scores

		Patients (N=15)	**Controls** (N=15)
Gender	No. female (%)	13 (86.7)	13 (86.7)
Age	Mean (sd)	15.2 (0.9)	15.6 (1.2)
	Range	14-17	14-17
IQ	Mean (sd)	105.0 (15.4)	111.9 (7.7)
Ethnicity	White-British	15	14
	Chinese	0	1
Parental education	Mother	10	12
(No. in higher edu.)	Father	8	7
Depressive symptoms[2]	Mean	25.4 (4.6)	4.5 (3.7)
	Range	14-31	1-13
Anxiety symptoms[2]	Mean	48.8 (4.6)	28.3 (4.5)
	Range	38-55	21-38

Table 4.1: Participant characteristics

are typically 13 in clinical samples and 19 in community samples [352]. The distribution of scores in the patient group corroborates the findings of the psychiatric interview which identified these participants as being depressed. Furthermore, the score is similar to that in a sample of 80 clinically depressed adolescent inpatients [191]. The distribution of scores in the control group is similar to other studies of unselected sample [294] and is indicative of a non-clinical sample. However, since I used a 26-item version of the CDI, comparison with cut-off scores should be interpreted tentatively. The use of these cut-off scores has also been criticised more generally [235]. Finally, patients also reported significantly more symptoms of anxiety (mean=48.8, sd=4.6) than controls (mean=28.3, sd=4.5; $t_{28} = 12.3, p < 0.001$).

Psychiatric comorbidity and treatment Seven of the 15 patients had no comorbid psychiatric diagnosis, six patients had one comorbid anxiety diagnosis (GAD, N=3; OCD, N=1; SP, N=1; Panic Disorder, N=1) and two patients had two additional diagnoses (GAD and SAD, N=1; Panic disor-

[2]Significantly different at $p < 0.001$

der and Phobia (of dogs), N=1). Eleven patients were receiving treatment from primary CAMHS (pCAMHS) services. PCAMHS is an early intervention service provided by mental health workers to address problems such as bullying, low self-esteem and family breakdown from developing into psychiatric disorders which require inpatient treatment. One patient was receiving treatment from CAMHS and three (recruited from local schools) were not receiving any treatment.

4.2.2 Measures

Psychiatric diagnoses The KSADS-PL [182] was used to confirm patient diagnoses and absence of psychiatric disorder in controls. The KSADS was administered by myself or Catherine Campbell, who both had previous experience of psychiatric interviewing and received further training from a child and adolescent psychiatrist (Dr Tony James, Consultant and Adolescent Psychiatrist, Warneford Hospital, Oxford).

Intelligence All four performance measures from the Wechsler Intelligence Scale for Children (WASI-III)[364] were used to assess general cognitive ability in patients and controls. The WASI is suitable for use in children aged 6-16 and provides normative scores based on a representative sample of 2,200 youth in the United States of America. The scale demonstrates good internal consistency [364].

Demographics The parent that accompanied the child to the study screening session provided data on maternal and paternal education (a rough indication of SES), and child ethnicity.

Depressive and anxious symptoms Symptoms of depression were assessed using the CDI, which has been described previously (see Chapter 2, Section 2.2.3 and Chapter 3). In the current study, item 9 which assesses suicidal ideation was not administered because of concerns about instigating thoughts of suicide. This is consistent with other studies of child and adolescent samples [150, 262, 332]. Depressive symptoms were measured at session 1 and session 2.

Symptoms of anxiety were assessed using the trait version of the Spielberger State Trait Anxiety Inventory for children (STAI-C) [336]. The STAI-C contains 20 items measuring the frequency of anxiety symptoms using a 3-point scale. The STAI-C has shown high internal consistency in previous studies of adolescents (Cronbach's $\alpha = 0.91$)[251].

4.2.3 Procedure

Participants were screened for MRI suitability by telephone and invited for an initial screening if they were suitable. Participants attended a screening session where data were collected on diagnostic status, IQ, and self-reported symptoms of depression and anxiety. Parents attended to provide demographic data and so that the researcher could explain the full nature of the study procedure (including the deception regarding peer feedback). Parents were then given an explicit opportunity to withdraw their child from the study if they thought they might be affected by the peer rejection or deception (no parent chose to do this).

The MRI scan session took place approximately two to three weeks later (range=1-64 days; median=17.5 days; mode=7 days; mean=21.4 days; sd=17.6). The mean number of days between the sessions did not differ between patients (mean=21.7, sd=18.4) and controls (mean=21.0, sd=17.3;

$t_{28} = 0.1, p = ns$). Prior to the scan itself, depression and anxiety symptoms were assessed again. A repeated measures ANOVA confirmed that depressive symptoms did not differ between session 1 (mean=14.97, sd=11.4) and session 2 (mean=14.31, sd=12.5, $F_{1,28} = 0.1, p = ns$). Neither was there an effect of group (patient, control) on change in depressive symptoms (patient mean change=0.29, sd=5.5; control mean change=-0.73, sd=2.3; $F_{1,27} = 0.44, p = ns$). A scan safety screen was also completed prior to scanning, as was reappraisal training (see Appendix B). Reappraisal training lasted 15 minutes and taught participants how to reinterpret negative feedback more positively, a topic explored in Chapter 6. Participants were instructed only to use this training during the final phase of the scan session (reported in Chapter 6), when instructed to do so. Full details of the training are described in Chapter 6.

The MRI scan lasted approximately 45 minutes. During this time participants completed phase 2 (reported in this Chapter), phase 3 (reported in Chapter 5) and phase 4 (reported in Chapter 6) of the Chatroom task (Figure 4.1). Structural MRI data were also collected for fMRI registration purposes. After scanning a manipulation check was performed before participants were fully debriefed about the study (Appendix A).

Peer rejection manipulation ('Chatroom')

Chatroom is an ecologically valid paradigm developed by Guyer and colleagues to measure neural correlates of expected peer feedback and receipt of peer rejection and acceptance [141]. For the purposes of this thesis, the Chatroom paradigm was amended to include a cognitive reappraisal of rejection phase (phase 4; see Chapter 6).

The Chatroom paradigm runs across two sessions (see Figure 4.1). At

the screening session participants are told they are taking part in an investigation of Internet-based communication using novel software. They are informed that during the scan session, they will be given the opportunity to chat online with peers from across the UK who are also taking part in the study. Phase 1 requires participants to rate of a scale between 0 and 10 how interested they are in talking to each of 60 peers. Participants are then photographed (or asked to send the researcher a photo before the next session). Participants are told that their photo will be shown to the peers, who will complete the same rating process so that they can be paired with peers of mutual interest.

The remaining phases are completed at the second session during MRI acquisition. Phase 2 concerns expectations of peer feedback, and asks participants to rate on a scale from 0 to 10 how interested they think each of the peers will be in chatting with them. Collecting these behavioural measures enables corroboration of the psychological process involved (expectations of peer feedback). A description of phase 3 (receipt of peer rejection) and phase 4 (reappraisal of peer rejection) are provided in Chapter 5 and Chapter 6 respectively.

Previous studies suggest no adverse effects of the paradigm in typically developing adolescents [140, 142, 328] and socially anxious adolescents [141, 200]. No adverse reactions were observed during either of the study sessions and although participants were informed they should contact the researchers afterwards if they felt upset, none did.

The paradigm involved several aspects of deception. The 'peers' were photos of child actors and the participants' photos were immediately destroyed. Peer rejection and acceptance was allocated at random and participants did not have to chat to peers. Assessment of successful deception was

based on a manipulation check completed immediately after the MRI session, when participants were expecting to enter the Chatroom. Four items prompted participants to retrospectively describe their thoughts about the study at various points. Participants were asked, "[After session 1] what did you think the study was about?", "[After session 1] how did you feel about going into the Chatroom?", "[After the MRI scan] what do you think the study is about now?", "[After the MRI scan] how do you feel about going into the Chatroom now?". Although these standardised questions were asked to all participants, assessment of successful deception was ultimately subjectively measured by the researcher.

The manipulation check suggested that all 30 participants believed the feedback from peers was real. When asked how they felt about going into the Chatroom after session 1, typical responses included "I'm looking forward to it", "I'm scared. [I] don't know what to say" and "Weird, but I won't see them [the peers]". When asked how they felt about going into the Chatroom after session 2, responses included "Ok, a bit apprehensive, now I know some people aren't interested in me" and "Excited". None of the participants expressed (explicitly or implicitly) that they did not believe it was real.

The Chatroom paradigm was administered in Presentation software (v0.70, www.neurobs.com). Stimuli were 60 colour smiling head shots selected from a database owned by Eric Nelson [255]. An initial set of 40 stimuli depicted adolescents (20 female) aged 12-17 to match participant inclusion criteria, and are reported elsewhere [140–142, 200]. Twenty more stimuli were added to increase the number of trials per condition and resulting statistical power. Given that many of the existing stimuli were relatively young in appearance, and the sample recruited were in middle-adolescence,

head shots of smiling young adults (age 18-21 years; 10 female) were chosen from the same database to boost the appropriateness of stimuli for the older adolescents. Both adolescent and young adult stimuli varied in race and ethnicity. Although there are known neural differences in the way that individuals process same-sex and opposite-sex faces [290], and preferential facial processing of same-race versus opposite-race stimuli [378], due to the limited number of stimuli available for use, I was unable to control for this in the current study. Stimuli were presented on a personal computer.

During each phase of the Chatroom, the 60 faces of peers appeared in a random order in the centre of the computer screen. In phase 1 the peer face was presented simultaneously with the text 'How interested are you in chatting to this person? 0=uninterested, 10=very interested' and a sliding bar for participants to make their ratings. Phase 2 was identical except that participants were asked 'How interested do you think this person will be in chatting with you? 0=uninterested, 10=very interested'. In phase 1 and phase 2, each trial lasted 5 seconds. Inter-trial intervals varied randomly between 2, 3, and 4 seconds.

4.2.4 Data acquisition and analysis

Behavioural analysis

To address the first hypothesis, that depressed adolescents would predict other peers to be less interested in interacting with them than healthy controls, the mean score from phase 2 behavioural ratings was averaged (across the 60 peers). This variable is hereafter labelled 'expected interest'.

To determine whether peer desirability influenced expectations of peer feedback, participants' phase 1 ratings of their interest in each peer were used as 'desirability' scores. For behavioural analysis, a dichotomous variable was

created based on these scores. For each participant, the 60 peers were ranked in terms of how interested the participant was in chatting to them. The 30 peers with the highest ratings were labelled 'desirable peers'. The 30 peers with the lowest ratings were labelled 'undesirable peers'.

To investigate the effects of peer desirability on expectations of peer feedback, and whether this differed between patients and controls, a mixed-measures ANOVA test with peer desirability (desirable, undesirable) as a repeated-measures variable and group (patient, control) as a between-group variable was conducted.

Phase 1 ratings were also averaged (across the 60 peers) to create the variable 'interest in peers'. T-tests were conducted to compare interest in peers between patients and controls (Table 4.2).

fMRI data acquisition, pre-processing and analysis

MRI data were acquired on a 3T TIM Trio scanner at the Oxford Centre for Clinical and Magnetic Resonance (OCMR; Oxfordshire, UK). Stimuli were projected onto a screen that was viewed by participants using angled mirrors. Participant responses were recorded using an MRI compatible button box. The three phases of the MRI scan were performed as separate runs and the researcher spoke to the participant before each run to remind them of the upcoming task requirements.

The functional MRI data were acquired on a transverse plane with a voxel resolution of 3 x 3 x 3mm, repetition time (TR)=3000ms, echo time (TE)=30ms, Flip angle=87°. Field maps were acquired for registration using dual echo 2D-gradient echo sequences with echos at 5.19ms and 7.65ms, and TR=488ms (64 x 64 x 64mm grid; voxel resolution of 3mm isotropic). T1-weighted FLASH structural images were acquired with the following pa-

rameters: voxel resolution of 1 x 1 x 1mm , TR=2040ms, TE=4.7ms, flip angle=8°, inversion time (TI)=900ms.

Data were analysed using FSL version 5.0 [169]. The following pre-statistical processing was applied to echo-planar imaging (EPI) data: slice time correction, motion correction (MCFLIRT), non brain removal (BET), spatial smoothing (FWHM=5mm), mean based intensity normalisation of all volumes of the same factor, high pass temporal filtering (90ms). Time series statistical analysis was carried out using FILM with local autocorrelation correction [374]. Registration to high resolution and standard space images was carried out using FLIRT. Motion outliers were partialled out in one subject's data (absolute motion=7.67mm, relative motion=1.35mm). None of the remaining subjects demonstrated significant movement in the scanner (absolute movement>3mm).

The first 25 volumes were removed to allow for T1 equilibrium effects. First-level analysis of phase 2 involved creating the following two explanatory variables (EVs): 'prediction' (peer photo displayed and participant rates expected feedback), 'prediction + PD' (same as 'prediction' but with standardised de-meaned peer desirability scores from phase 1 included as a covariate). Inter-trial intervals (ITI) provided baseline blood-oxygen-level-dependent (BOLD) activity and the onset of each EV was convolved with the hemodynamic response function using a variate of the gamma-function. Neural activity where predicted peer feedback correlated with peer desirability (over and above regions which responded to the process of predicting peer feedback) was investigated with the contrast ($prediction + PD > prediction$). Activity during the prediction of peer feedback which was independent of (uncorrelated with) peer desirability was investigated with the contrast ($prediction > prediction + PD$).

At the higher-level analysis, a mixed-effects model was used to compare neural activity during predicted peer feedback between patients and controls. Whilst the two groups were modelled as separate EVs, a single-group variance was applied. Automatic outlier-deweighting was also applied. Key contrasts were: (mean activation; whole-group), (patients>controls), and (patients<controls).

To begin with, each higher-level analysis was run at a (corrected) cluster-based threshold of Z=2.3, p<0.05 [111]. Given relatively subtle effects were expected, the same higher level analysis was run at an uncorrected threshold of Z=2.3, p<0.005. After extracting percent BOLD signal change within significant clusters of interest, SPSS (v20.0, IBM Corporation, NY, USA) was used to perform appropriate between-group ANOVAs, t-tests and correlations. Corresponding Brodmann Areas (BA) were identified by transforming MNI coordinates into Talairach space.

4.3 Results

Since all participants appeared to believe the Chatroom task was real, no subject's data were excluded from the analysis.

4.3.1 Behavioural data

Table 4.2 describes the behavioural data from phases 1 and 2, which were analysed using SPSS (v20.0, IBM Corporation, NY, USA).

Group differences in expectations of peer feedback Participants' predictions of peer interest ranged from 0.4 to 7.4 and followed a normal distribution (D=0.14, p=ns). A repeated-measures ANOVA revealed a main effect of group on peer feedback expectations ($F_{1,28} = 5.99, p < 0.05$; Table

Chatroom phase	Behavioural measure	Patient mean (sd)	Control mean (sd)
Phase 1	Interest in peers	4.10 (2.0)	5.24 (1.2)
Phase 2	Expected interest	4.29 (1.6)	5.04 (0.6)
(scan)	-Desirable peers	4.17 (1.8)	5.40 (1.2)
	-Undesirable peers	3.67 (1.8)	4.63 (1.2)

Table 4.2: Chatroom behavioural data from phase 1 and phase 2

4.2). There was no evidence that symptoms of anxiety (measured at session 2) correlated with feedback expectations in either the patient group (Pearson's $R = 0.01, p = ns$) or control group (Pearson's $R = -0.08, p = ns$).

Peer desirability and expectations of peer feedback The repeated-measures ANOVA also revealed a main effect of peer desirability on predicted peer interest ($F_{1,28} = 4.09, p = 0.05$), such that participants anticipated desirable peers to be more interested in talking to them than undesirable peers (Table 4.2). There was no interaction between participant group (patients, controls) and peer desirability ($F_{1,28} = 0.18, p = ns$).

Interest in chatting with peers Interest in peers ranged from 0.2 to 7.9 and was normally distributed ($D = 0.13, p = ns$). T-tests revealed there was a non-statistically significant trend for patients to be less interested in chatting to peers compared to controls although this did not reach statistical significance ($t_{28} = -1.91, p = 0.07$). Participants' mean interest in chatting with peers did however correlate negatively with symptoms of depression (Pearson's $R = -0.36, p < 0.05$).

4.3.2 Neural analysis of peer feedback expectations

Peer desirability The hypothesis that whilst predicting feedback from peers, regions such as the amygdala, hypothalamus, insula, hippocampus

and NA would be associated with peer desirability, was tested by analysing mean activation across the sample to the contrast ($prediction + PD > prediction$). At the corrected threshold of Z=2.3, p<0.05, six significant clusters of activation were identified. These covered 1) the precuneus and posterior cingulate, 2) the lateral occipital cortex, 3) right middle temporal/parietal lobe, 4) left middle temporal/parietal lobe, 5) right frontal lobe and ACC, and 6) left IFG (see Table 4.3).

The effect of depression on neural activity which correlated with peer desirability was explored in a between-groups comparison. Corrected analysis failed to identify any clusters of activity where activity varied between patients and controls. Uncorrected analysis revealed a small cluster in the right IFG (20 voxels; Z value=3.1; x=42, y=16, z=4) where patients showed greater activity than controls. Uncorrected analysis also revealed relatively small clusters in the right caudate (69 voxels; Z value=3.08; x=6, y=4, z=-4) and the right ACC (Z value=3.33, x=-10, y=28, z=-12) where patients showed less activity than controls.

Region	**BA**	**Cluster size (voxels)**	**Z value**	**x**	**y**	**z**
Precuneus and posterior cingulate	-	7454	6.81	-10	-56	16
Lateral occipital cortex	-	7012	6.93	-40	-78	28
Middle temporal/parietal lobe (R)	-	5603	5.04	50	-52	24
Middle temporal/parietal lobe (L)	-	2841	4.48	36	40	-6
Frontal lobe and ACC (R)	-	815	4.41	-20	18	44
IFG (L)	45	627	5.39	-34	38	8

Table 4.3: Neural regions engaged during the prediction of feedback, which correlated with peer desirability

Region	**BA**	**Cluster size (voxels)**	**Z value**	**x**	**y**	**z**
Occipital, parietal & frontal lobes (R & L)	6, 7, 24, 32	34166	8.05	42	-74	-18
Insula & IFG (R)	44, 45	1484	5.33	40	16	2
Insula & IFG (L)	44	948	5.72	-40	16	0
VLPFC (R)	9	718	4.63	46	38	30
Frontal pole (L)	9	606	4.80	-28	52	20

Table 4.4: Neural regions engaged during the prediction of feedback, which are independent of peer desirability

Notes: BA - Brodmann Area; Coordinates represent the location (in MNI space) of the peak voxel in each cluster.

Predicting peer feedback (independent of peer desirability) The second hypothesis was that predicting peer feedback (regardless of peer desirability) would engage similar regions to those associated with the receipt of peer rejection, including emotion detection regions (e.g. occipital and parietal lobes), affective regions (e.g. cingulate cortex and insula), and emotion regulation regions (e.g. IFG and frontal pole). This hypothesis was tested by analysing mean activation during predicted feedback (versus predicted feedback which correlated with PD).

At the corrected threshold, five clusters of significant activation were identified (see Table 4.4 and Figure 4.2). These regions covered 1) bilateral occipital and parietal lobes, the cerebellum, bilateral pre-motor cortex, prefrontal gyrus, medial frontal gyrus, and SFG, and left cingulate gyrus and bilateral ACC 2) the right IFG and insula, 3) the left IFG and insula 4) right middle frontal gyrus and 5) left frontal pole (BA9).

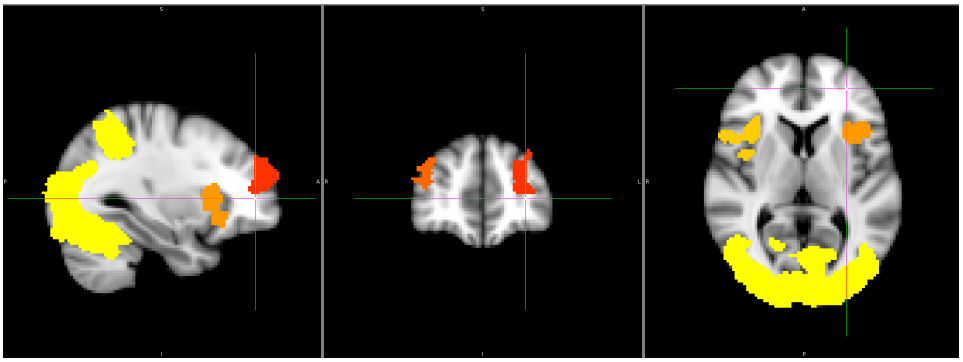

Figure 4.2: Neural regions engaged during prediction of feedback, which correlated with peer desirability. Images thresholded at Z>2.3, p<0.05, corrected.

In order to explore whether neural responses during predicted peer feedback differed between depressed and non-depressed adolescents a between-groups analysis of the same contrast was conducted. Uncorrected analysis revealed one such cluster in the right caudate (116 voxels; Z value=3.32;

x=6, y=4, z=-4), engaged more so by patients than healthy controls, although this cluster was not significant when analysis was corrected for multiple comparisons. The extracted percentage of signal change within this region for predicted peer feedback (relative to fixation baseline) was then performed in post-hoc analysis. T-tests revealed that whilst predicting peer feedback (compared to baseline), patients showed heightened activation (mean=0.20, sd=0.15) whereas controls showed deactivation (mean=-0.22, sd=0.08; $t_{28} = 9.57, p < 0.0001$; Figure 4.3).

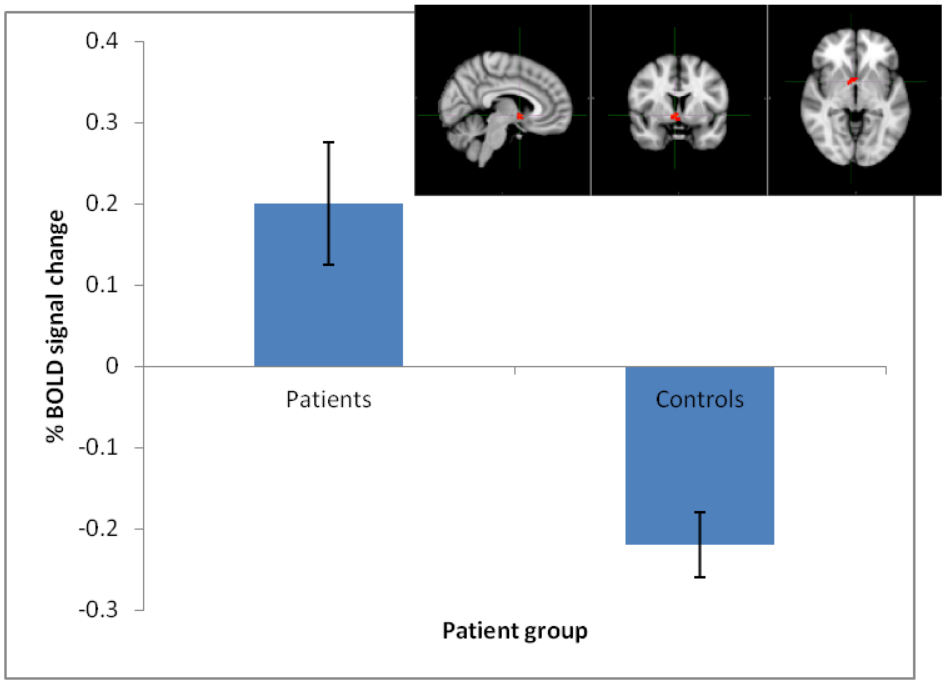

Figure 4.3: Neural activity in the right caudate engaged during prediction of peer feedback. Images thresholded at Z>2.3, p<0.05, uncorrected.

4.4 Discussion

4.4.1 Summary of findings

The present study provided support for the primary hypothesis, that depressed adolescents would expect more negative feedback than healthy controls. There was a significant difference in the expectation of peer feedback between patients and controls such that depressed adolescents anticipated more negative feedback from peers than non-depressed adolescents. There was an effect of peer desirability on expectations of peer feedback, such that participants anticipated more positive feedback from desirable (compared with undesirable) peers, although this effect was the same for patients and healthy controls. Interestingly, there was a negative correlation between participants' mean interest in chatting with peers and symptoms of depression.

There was partial support for the second hypothesis, that during the prediction of peer feedback, activity in regions such as the amygdala, hypothalamus, insula, hippocampus and NA would be associated with the desirability of the peers. Although these specific regions were not directly engaged in the current study, other regions associated with emotional affectivity were. These included the ACC and left IFG. Furthermore, contrary to previous studies in typically developing adolescents[142], the right frontal lobe was also associated with predicted feedback as it related to peer desirability. There were only modest effects of patient group on activity in these regions.

Analysis of predicted peer feedback (independent of peer desirability), revealed that brain regions associated with emotion generation and regulation were engaged. These included the medial and SFG, the left cingulate

gyrus and bilateral ACC, bilateral IFG and insula, the right middle frontal gyrus and the left frontal pole (BA9). Between-group differences suggested that right caudate activity was heightened in patients compared to controls.

4.4.2 Interpretations

Negative expectations may explain how adolescent depressive symptoms predict subsequent peer rejection [53]. Although some studies have associated negative expectations with adolescent depression [305], these have largely been related to non-social tasks [217]. Drawing on questionnaire studies of pre-adolescent children [132, 306, 379], negative expectations of peer interactions are a characteristic of individuals with higher depressive symptoms. Given the salience of peers during adolescence [43, 74, 199, 240, 341] and the ongoing neural development of the social brain in adolescence [34, 49], expectations of peer feedback may be even more strongly linked to depressive symptoms in this period.

This study is the first to demonstrate that depressed adolescents expect more negative feedback from peers, compared to non-depressed adolescents. The only other study to investigate the association between adolescent peer feedback expectations and psychopathology, found that socially anxious adolescents anticipated peers to be less interested in talking to them, compared to healthy controls [141]. I did not exclude depressed patients who had comorbid anxiety disorder from the current study. There was no correlation between anxiety symptoms and expected peer feedback. A final issue which may influence the interpretation of these behavioural findings is that depressed adolescents reported more negative responses to both expected peer feedback and interest in talking to peers. Whilst it is possible then that negative expectations of peer feedback reflect more general blunted responses

in depression, see Chapter 5 for data which suggest depressed adolescents may experience more pleasure from positive peer experiences.

The study also identified the neural correlates of anticipated peer feedback. Across the group, neural activity in a network of regions responded to prediction of peer feedback (independent of peer desirability). This included the medial and SFG, the left cingulate gyrus and bilateral ACC, bilateral IFG and insula, the right middle frontal gyrus and the left frontal pole (BA9). These regions are similar to those involved in social emotion processing more generally [34, 256], and the receipt of peer rejection specifically [313]. Importantly, between-group analysis suggested that during the prediction of feedback (regardless of peer desirability), right caudate activity was heightened in patients compared to controls. This region has previously been implicated in the anticipation of risk and reward in non-social tasks [103]. This is the first study to investigate neural differences to during expectations of peer feedback in adolescent depression. Although this effect was weak, it tentatively suggests subtle neural differences between depressed and non-depressed adolescents during the anticipation of peer rejection. Speculatively, this may influence the way in which depressed adolescents respond during the receipt of peer rejection, although the next Chapter addresses this question more directly.

A third finding was that peer desirability influenced behavioural and neural responses during peer feedback expectations. Across the whole sample, participants anticipated more positive feedback from peers they had identified as more desirable. Reciprocity is an important feature of positive social interactions and although children as young as 6 years old demonstrate reciprocity in their social interaction, the ability to detect and respond to the actions of peers continues to develop throughout adolescence [139], perhaps

because of neural development in 'mentalising' networks during this time [49]. Contrary to predictions, this effect did not differ between patients and controls and was not associated with symptoms of depression. These findings replicate those of Guyer and colleagues, who found that peer desirability positively correlated with expectations of peer feedback in a sample of typically developing adolescents [142].

As a result of these behavioural data, neural data probed brain regions which responded to peer desirability during prediction of peer feedback. Whereas previous studies had included peer desirability as a dichotomous variable [141, 142], I measured peer desirability on a continuous scale. I sought to investigate regions that were more generally involved in the expectation of peer feedback, and within these, regions which tracked peer desirability. Whilst participants predicted how interested peers would be in talking to them, numerous emotional processing regions showed a correlation with peer desirability. These included the ACC and left IFG. Guyer and colleagues found similar (but not identical) neural regions in their analysis of predicted feedback from desirable versus undesirable peers [142]. Since neural activity during anticipated peer evaluation varies with peer desirability, I recommend that future studies of peer feedback include peer desirability as a covariate.

One limitation of this approach to exploring the effects of peer desirability is that it assumes that during the scan session, participants are able to accurately remember the ratings which they gave peers in session 1. Without asking participants to recall their previous ratings of peer desirability, I cannot be sure that peer desirability as measured at session 1 accurately reflects desirability at session 2. The fact that peer desirability contributed significantly to peer feedback expectations at both the behavioural and neu-

ral level suggests that the ratings collected in phase 1 were at least salient markers of peers, although it is also possible that the peer desirability measure to some extent reflect fear of the peer, rather than undesirability per se. Peer desirability ratings were collected at time 1 to improve the believability of the Chatroom paradigm. Future studies may consider asking participants to recall their ratings in order to assess their memory of peer ratings, or repeating the peer desirability data collection again at phase 2.

One unexpected finding of the current study was the failure of predicting peer feedback to activate the amygdala. The amygdala has been associated with affective processing in typically developing adolescents [256, 313] and depressed adolescents [377]. It has been implicated in the neural processing of anticipated [141] and actual peer rejection [200] in anxious adolescents. A study of the neural correlates of peer acceptance in depressed adolescents also reported amygdala activation [80]. One possibility is that the Chatroom was not provocative enough in the current sample. Although the Chatroom task has previously been found to elicit amygdala activity in anxious adolescents [141, 200], the same findings did not characterise typically developing adolescent samples [140, 142]. Studies of adolescents using other peer rejection paradigms, such as Cyberball [229–232, 315] and the Social Judgement Task [138] have failed to find evidence that the amygdala is involved in the neural processing of rejection.

4.4.3 Strengths and limitations

The fact that all participants appeared to believe the Chatroom task was real, increases the validity of their responses during anticipation of peer feedback. The experimental design of the current study minimised the extent to which confounding variables, such as the way peer feedback is defined,

could account for the results. Furthermore, depressed and non-depressed adolescents were matched for key factors believed to influence emotional processing, including gender and age.

Issues surrounding the sample selection are also worth mentioning. Due to concerns that the Chatroom paradigm could exacerbate depressive symptoms in patients with more severe depression, I chose to select participants who did not report current suicidal ideation or recent self-harm (see Chapter 5 for a discussion of ethical issues associated with the Chatroom paradigm). Due to the concern that anti-depressant medication may confound MRI data, medicated patients were also excluded. These restrictions led to a sample of depressed patients with mild to moderate, rather than severe, MDD. This may explain the subtlety of neural differences between patients and controls. It also means that interpretations about the role of negative expectations of peer feedback in adolescent depression are restricted to non-severe cases.

Whilst fMRI has enabled further exploration of the effect of peer rejection on emotion regulation and behaviour, interpretations of fMRI data should be treated with caution. Since fMRI measures blood-oxygen-level-dependent (BOLD) changes (rather than direct neural activity), its accuracy and temporal precision in detecting brain mechanisms are somewhat limited. Furthermore, the direction of group differences is not always consistent between studies, raising questions over whether greater or weaker activity is problematic.

A limitation of this phase of the Chatroom task is that it probes elaborative processes involved in the prediction of peer feedback, rather than more automatic processes. Since participants complete these trials in a separate block from the actual peer feedback trials, and are told explicitly that they

are required to think about how they expect peers to evaluate them, their behavioural and neural responses may reflect more reflexive processes associated with peer feedback expectations and the results may therefore not be generalisable to unexpected or automatic anticipation of peer feedback. A strength of administering peer feedback expectations and actual peer feedback in separate runs, is that it allows the two processes to be separated. In contrast, other neural studies have administered peer feedback expectations and actual peer feedback one after another for one peer at a time [333, 335].

4.4.4 Future research

More established studies in typically developing adolescents are important in order to determine how neural correlates differ in psychiatric samples. Just one study has investigated the neural correlates of peer feedback expectations in typically developing adolescents [142]. Since this study was an MRI study, and therefore relatively small in sample size, there was limited statistical power to explore interactions between age, gender, and peer desirability. Larger studies investigating behavioural effects may help to understand the complex processes involved in atypical prediction of peer feedback.

As previously described, future studies of the effects of peer desirability on expectations of peer feedback are important, given the preliminary evidence in this study that peer desirability modifies adolescents' responses to predicted peer feedback. Particularly in experimental paradigms, where participants anticipate feedback from unknown peers, the extent to which participants wish to receive positive and negative feedback is an important factor to consider. Although the neural correlates of predicted peer feedback and peer desirability are similar to those of another study [142], differences

between the studies highlight the importance of the statistical models used. Without good reason to believe that participants make dichotomous decisions about their desirability to interact with peers, I recommend that future studies consider including peer desirability as a continuously modelled variable.

The current study makes an important contribution to the literature by demonstrating an association between adolescent depression and negative expectations of peer feedback, and the neural correlates of this relationship (Figure 1.1B). However, further studies are needed to test the speculative hypothesis that negative expectations of peer feedback modify the effect of depression on future peer rejection. Studies which experimentally manipulate peer expectations may help to elucidate the causal role of negative expectations of peer feedback.

4.4.5 Summary

In summary, the present study demonstrates that depressed adolescents hold more negative expectations of peer feedback than healthy adolescents. Furthermore, it identifies the neural correlates of peer feedback expectations, and tentatively suggests subtle neural differences in how depressed and non-depressed adolescents predict peer feedback. Behavioural and neural correlates of predicted peer feedback were influenced by peer desirability, although there was no evidence that this differed between depressed adolescents and healthy controls. The study is the first to examine the cognitive and neural correlates of peer feedback expectations in depressed adolescents. Future studies which explore the mechanisms by which negative expectations of peer feedback contribute to adolescent depression could help to further develop diathesis-stress models of adolescent depression.

Work based on Chapters 4, 5 and 6 has been published in the following article: Haddad, A.D., Platt, B. James, A.C. and Lau, J.Y. (2012) Anxious and non-anxious adolescents' experiences of non-clinical magnetic resonance imaging research. *Child Psychiatry and Human Development*. The final publication is available at http://link.springer.com/article/10.1007%2Fs10578-012-0350-x

Chapter 5

Neural correlates of peer rejection

Emotional reactivity describes short-lived behavioural and physiological responses to salient stimuli. Depression is associated with increased emotional reactivity to negative emotional experiences and reduced reactivity to positive experiences. However, relatively little is known about the extent to which emotional reactivity characterises *adolescent* depression. Given that peer rejection may be a salient source of stress during adolescence, the current study sought to test whether depressed adolescents showed heightened emotional reactivity to peer rejection, compared to healthy controls. Participants (15 clinically depressed adolescents and 15 healthy controls) had previously rated how interested they were in talking to peers and had predicted how interested the peers would be in talking to them (Chapter 4). In the current study, participants received feedback about how interested the peers were in talking to them in an online Chatroom, whilst they underwent neuroimaging. FMRI data supported previous studies which suggest that regions associated with emotion processing such as the insula and ventrolateral

pre-frontal cortex were engaged during peer rejection and peer acceptance in all adolescents. However, in contrast to expectations, depressed adolescents showed no behavioural or neural evidence of disrupted emotional reactivity in response to peer rejection, compared to non-depressed adolescents. Furthermore, depressed adolescents reported feeling more positive in response to peer acceptance than healthy controls. Alternative explanations for these findings and implications of the data for models of adolescent depression are discussed.

5.1 Introduction

In Chapter 1 I described how adolescent depression is strongly predicted by psychosocial stress [350], and that one major source of psychosocial stress during adolescence is peer rejection [315] (Figure 1.1A). Longitudinal studies suggest that peer rejection in unselected samples predicts later depressive symptoms, and experimental studies complement these by showing that peer rejection elicits negative emotional responses in typically developing adolescents. However, depressed adolescents' responses to peer rejection have not yet been investigated. I proposed that biological and psychological factors may determine the effects of peer rejection on adolescent depression (Figure 1.1B). In the previous chapters, I considered how genetic factors may moderate the effects of environmental stress (though not peer rejection specifically) on adolescent mood disorder (Chapter 2). I also described how negative cognitive biases may underlie adolescent depression, and potentially moderate the effects of peer rejection on adolescent depression (Chapter 3). In this chapter I explore whether depressed adolescents are more sensitive to the effects of peer rejection because of heightened emotional reactivity.

5.1.1 Emotional reactivity

Emotions are adaptive, fleeting reactions to salient stimuli associated with physiological as well as behavioural changes [51]. Emotion regulation is the process of modifying an emotional response, whether that be in the magnitude, valence, duration or any other component of the emotion [135, 136]. The term emotion regulation refers to both explicit (effortful) forms of emotion regulation, as well as implicit (automatic) forms [143]. Explicit emotion regulation, for example using cognitive reappraisal, is a topic discussed in the next chapter (Chapter 6). Disrupted implicit emotion regulation is a characteristic of adolescent depression [137, 282, 322], resulting in heightened emotional reactivity to meaningful stimuli. Specifically, depression is associated with both increased reactivity to negative stimuli, as well as reduced reactivity to positive stimuli [135].

Pervasive negative mood states are thought to heighten depressed adolescents' emotional reactivity to negative stimuli [51]. Self-report and observational data suggest that adolescents with depression experience disturbances in negative emotion [174, 321, 322, 327]. These relate to both longer durations and higher frequencies of negative affect than healthy controls [321, 322], although the extent to which these responses reflect poor emotion regulation is unknown.

An alternative, but not mutually exclusive view, is that depressed adolescents experience reduced emotional reactivity to positive stimuli [135]. Adolescent depression is also characterised by loss of interest in previously enjoyed activities, reduced libido, energy, self-confidence and appetite [80]. Reduced positive affect may even better characterise depression than heightened negative affectivity [80]. One experimental study suggested that depressed adolescents experienced shorter duration and reduced frequency of

positive affect compared to healthy controls [322].

Together, these studies suggest disrupted emotional reactivity in adolescent depression. Given the salience of peers during adolescence, I now turn to consider evidence that adolescent depression is associated with emotional reactivity to interpersonal interactions specifically.

Peer feedback

Studies of typically developing adolescents have measured emotional reactivity during peer rejection using self-report measures of affect. One experimental study of unselected adolescents compared negative affect following rejection in adolescents with many and few symptoms of depression [294]. However, there was no evidence that depressive symptoms influenced emotional reactivity to peer rejection. Attachment type is another measurement of risk for depression in paediatric samples, however there is no evidence that insecurely attached adolescents report greater distress in response to rejection than securely-attached adolescents [367]. However, to date no study has investigated whether adolescents with a clinical diagnosis of depression show heightened emotional reactivity to rejection than healthy controls. It may be that peer rejection only has observable effects in individuals with more severe symptoms of depression.

Reviews of emotional development in adolescence suggest that lack of reward from peer acceptance may also to contribute to adolescent depression [339, 340]. Numerous studies report the positive effects of peer acceptance on mental health [317]. Interestingly, reduction in the perception of peer acceptance (as opposed to actual peer acceptance) during late childhood may predict dysphoria during adolescence [188]. The only experimental study of emotional reactivity to peer acceptance in adolescent depression

found that depressed adolescents reported significantly less enjoyment from positive feedback than did healthy controls [80].

The first aim of the current study was to test the hypothesis that depressed adolescents (compared to healthy controls) would show more negative responses to experimentally-induced peer rejection and peer acceptance. Measuring affective responses to naturally occurring events is limited by the fact that variables associated with the nature of emotion eliciting stimuli (for example the severity, duration, or type) may confound affective responses. Studies which measure emotional reactivity using standardised stressors minimise the contribution of confounding variables [293]. The experimental paradigms described in Chapter 1 provide the opportunity for studying emotion reactivity in response to (standardised) peer feedback specifically. Using the Chatroom paradigm (see Figure 5.1), behavioural responses to positive and negative feedback from peers were measured in the same group of depressed and non-depressed adolescents who predicted peer feedback in Chapter 4.

5.1.2 Neural correlates of emotional reactivity

As previously described, neuroscientific methods such as fMRI play an important role in improving our understanding of how the brain mediates the differential impact of peer rejection on typical and atypical emotion regulation and behaviour [142]. Particularly since emotional reactivity is characterised by physiological changes as well as behavioural changes [51], neuroimaging methods provide an opportunity to corroborate behavioural data on emotional reactivity. Biological measures may also reflect distinct aspects of emotional reactivity compared to behavioural measures.

Functional neuroimaging studies have substantially contributed to our

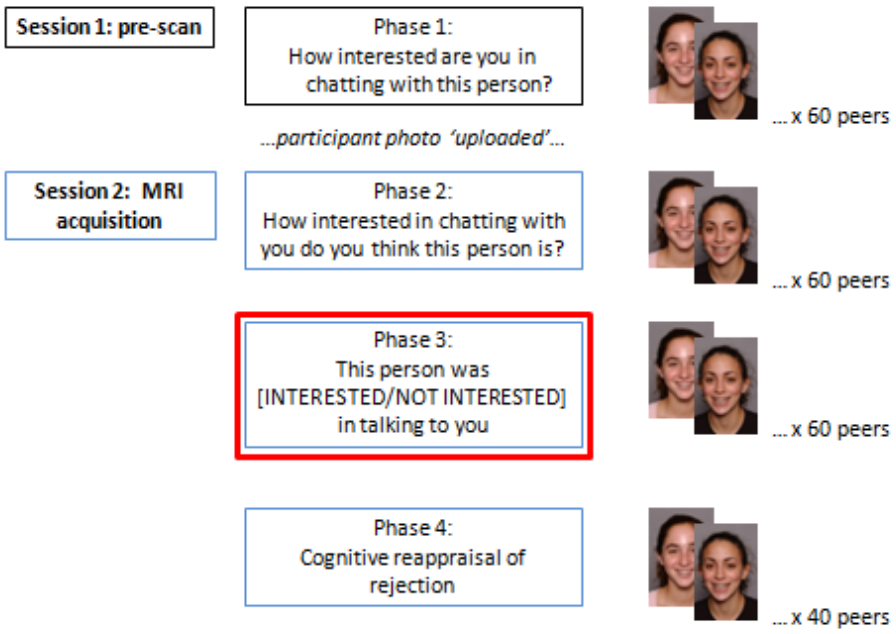

Figure 5.1: The Chatroom paradigm (phase 3).

knowledge of the brain mechanisms underlying adult emotion processing, including emotional reactivity and regulation (see Ochsner et al., 2012 for a review [270]). Key brain structures involved in emotion processing are the amygdala (involved in the perception of threatening stimuli), the ventral striatum (involved in predicting reward), the VMPFC (which tracks how the stimulus relates to current goals), and the insula (implicated in negative affective states with a visceral component e.g. disgust) [270]. However, the neural correlates of emotion reactivity and regulation are relatively unexplored in adolescence, despite the fact that adolescence may be a critical time for the development of regulatory abilities needed to adapt to the changing social environment [270].

Early fMRI studies of emotional reactivity in clinically depressed adolescents were based on adult findings and focused on elucidating differences in

amygdala function. However, mixed findings have been demonstrated. For example, as Yang and colleagues (2010) [377] highlight, whilst some studies showed heightened amygdala activation during emotion recognition in depressed youths compared to healthy controls [107, 205, 299, 377], others report decreased amygdala activity in depressed youths [28, 351]. These differences have been attributed to differences in the tasks: the first set explored amgydala responses to active viewing of emotional stimuli (facial recognition task) whereas in the second set, viewing was passive [377]. Beesdo and colleauges (2009) directly tested the extent to which amygdala hypoactivation during passive emotion processing is specific to adolescent depression [28]. Whereas anxious adolescents showed hyperactivation in the amygdala whilst passively viewing fearful faces, depressed adolescents showed hypoactivation in the amygdala in the same task.

Other studies suggest that the NA, which showed hypoactivation to positive stimuli in adolescents exposed to early-life stress, may be associated with emotional reactivity in depression [106, 119]. A recent study suggests that functional connectivity between the amygdala and VMPFC may also predict adolescent depressive symptoms, although these findings were restricted to females only, and the study sample were unselected individuals towards the end of adolescence (age 18) [48]. It should be noted that across all of these studies of the neural correlates of emotional reactivity, the valence of stimuli vary and therefore they may not reflect emotional reactivity to negative stimuli specifically.

Peer rejection

There is a growing literature on the neural correlates of adolescent peer rejection (for a review of studies of typically developing adolescents see Sebas-

tian et al. (2010) [313]). Adolescent peer rejection involves engagement of the amygdala [232], striatum [230, 232], caudate/putamen [138, 315], insula [230, 232], and DMPFC [230, 232], as proposed by adult emotion regulation models [270]. Additional activity has been seen in regions such as the ACC [230, 232, 315], precuneus [232], bi-lateral IFG/VLPFC [138, 230, 232, 315] and DLPFC [232].

Importantly, these studies suggest that neural activation during peer rejection modulates behavioural measures of emotional reactivity. For example, Masten and colleagues demonstrated that increased activation in the insula and subgenual ACC (subACC) during peer rejection correlated with self-reported ratings of distress [230]. The fact that activation of the VLPFC and DMPFC was negatively correlated with activation in the insula and distress during peer rejection tentatively implicates these regions in emotion processing (including possibly) regulation of peer rejection.

Preliminary evidence that neural responses to rejection are characteristic of adolescent depression comes from studies of typically developing adolescents, where neural activity in affective and regulatory regions correlates with depressogenic markers such as low self-worth [138], rejection sensitivity [230], and symptoms of depression [229]. However, to date no study has investigated the neural correlates of peer rejection in clinically depressed adolescents.

Peer acceptance

The representation of reward has been largely associated with the NA and striatum, however the DLPFC may also play a role in monitoring goal-related information [81]. Studies of depressed adults (compared to healthy adults) demonstrate reduced activity in regions associated with emotion

regulation such as the ventral striatum [101, 184] and VMPFC [184] in response to positive stimuli. One study in depressed youths found that, in contrast to adult studies, reward was associated with hypoactivation (rather than hyperaction) of the ACC [106].

Research has begun to investigate the neural networks involved in typically developing adolescents' responses to peer acceptance. Sebastian and colleagues found heightened right VLPFC activity to acceptance (versus rejection) in healthy adolescents compared to adults [315]. A study of healthy adolescents aged 9-17 found a number of cortical and subcortical regions which showed heightened activity during peer acceptance (versus rejection) [140]. These included the SFG, superior temporal gyrus, insula, fusiform gyrus, ACC, and anterior and ventral striatum (caudate, putamen). As far as I am aware, no previous studies report correlations between neural activity during peer acceptance and behavioural measures of emotional reactivity.

Davey and colleagues (2011) conducted the only neural study of depressed youths' (aged 15-24) responses to peer acceptance [80]. They contrasted positive versus neutral (rather than negative) feedback from peers. Behavioural data confirmed predictions that patients would experience less positive affectivity following peer acceptance, compared to healthy controls. Across the whole sample, there was heightened neural activity to positive feedback in anterior midline regions (VMPFC, pregenual ACC, dACC; BA10, 32, 24), the bilateral occipital/fusiform cortices, and the left amygdala [80]. However, patients showed greater activity in the left amygdala (18 voxels; peak Z score=2.47; x=-16, y=-4, z=-18) during peer acceptance than controls ($p < 0.05$; analysis corrected for multiple comparisons)[80]. Patients also showed heightened activity in regions including the VLPFC and right insula compared to controls, when positive and neutral feedback

were considered together, suggesting a neural sensitivity to peer feedback in general.

A second aim of the current study was therefore to investigate the neural correlates of peer rejection and acceptance, and whether depressed adolescents showed differential neural responses to peer feedback than healthy controls. Based on findings from studies of typically developing adolescents, I predicted that across the whole sample, peer rejection would elicit neural responses in regions associated with emotion regulation including the amygdala, insula, ventral striatum, as well as other neural correlates of peer rejection such as the NA, ACC, IFG, VLPFC, DLPFC and DMPFC. I predicted that these responses would correlate with behavioural measures of emotional reactivity. I predicted depressed adolescents to show heightened neural activity in these regions during peer rejection. I predicted that across the sample, regions such as the ventral striatum, NA, medial PFC and VLPFC would be engaged during peer acceptance. I also explored whether these regions correlated with behavioural measures of emotional reactivity. Based on Davey and colleagues' findings [80], I predicted depressed adolescents would show heightened neural activity in affective regions such as the amygdala, compared to healthy adolescents.

Peer desirability

In Chapter 4 I demonstrated that both depressed and non-depressed adolescents' responses to anticipated peer feedback varied as a function of peer desirability. Adolescents anticipated positive feedback from desirable peers and negative feedback from undesirable peers. I also found that activity in the left IFG (as well as the ACC and right frontal lobe) correlated pos-

itively with peer desirability during predicted peer feedback. That is, the more desirable peers yielded greater activation from this region. This finding did not vary across depressed or non-depressed adolescents. The extent to which participants are interested in talking to peers may also influence adolescents' emotional reactivity during the receipt of peer feedback.

A Chatroom study of socially anxious adolescents found more negative affective ratings in response to rejection (versus acceptance) from desirable (versus undesirable) peers [200], although the moderating effect of peer desirability did not extend to neuroimaging responses. In contrast, a Chatroom study of typically developing adolescents failed to find an effect of peer desirability on affective ratings of peer feedback [140], although peer desirability moderated neuroimaging responses peer feedback. Regardless of whether peers gave positive or negative feedback, activity in the IFG (BA47) was heightened during receipt of peer feedback from undesirable (versus desirable) peers [140].

Although the precise pattern of effects remains unclear, these data suggest an important role for peer desirability in behavioural and neural responses to peer rejection. This may be more so in the Chatroom paradigm than in other peer rejection paradigms, for example Cyberball, where the nature of rejection is less personal. In the current study I included peer desirability in my analysis of affective and neural responses to peer rejection and acceptance. I expected adolescents' affective ratings would be more negative in response to rejection from desirable (versus undesirable) peers, and more positive in response to acceptance from desirable (versus undesirable) peers. In my neural analysis of peer feedback, I explored which regions were responsive to feedback, when peer desirability was included as a covariate.

5.1.3 The current study

In this study, behavioural responses and brain activation patterns during peer feedback were compared in a group of adolescents with and without depression using functional neuroimaging.

In the first set of analyses, I compared affective ratings of peer rejection and peer acceptance in adolescents with and without depression. I predicted that rejection would elicit more negative responses than acceptance, and that depressed adolescents would respond more negatively to both peer rejection and peer acceptance, than healthy controls. I also investigated whether peer desirability amplified the effects of peer acceptance and peer rejection on affect. I predicted that desirable peers would elicit more negative responses to rejection and more positive responses to acceptance, compared to undesirable peers.

In the second set of analyses, I focused on brain activation patterns during receipt of i) negative feedback and ii) positive feedback. Based on previous studies of emotion regulation and peer rejection in typically developing adolescents, I expected a network of regions to be engaged during rejection feedback, such as the amygdala, NA, insula, ventral striatum, ACC, IFG, VMPFC, VLPFC, DLPFC and DMPFC. Studies of peer acceptance in typically developing adolescents have been few, however studies of the maturing reward circuitry implicates the ventral striatum and NA, medial PFC and VLPFC.

I explored whether activity in these regions correlated with affective ratings of peer feedback. I was particularly interested in identifying which of the regions engaged during peer feedback, varied between depressed adolescents and healthy controls. Guided by prior data on dysfunctional brain correlates associated with adolescent depression, between-group differences

were expected in the amygdala, insula, the IFG and VLPFC. Finally, I explored regions where neural activity during i) rejection and ii) acceptance, correlated with peer desirability, and regions which responded to peer feedback but were independent of peer desirability. In summary, I made the following predictions about the findings of the current study:

1. Affective ratings of peer rejection and peer acceptance would be lower in depressed adolescents than non-depressed adolescents. Across the group, desirable peers would elicit more negative responses to peer rejection than undesirable peers and more positive responses to peer acceptance than undesirable peers.

2. Peer rejection would engage regions such as the amygdala, NA, insula, ventral striatum, ACC, IFG, VMPFC, VLPFC, DLPFC and DMPFC. Neural activity in these regions would correlate with affective ratings of peer rejection and would be greater in depressed adolescents versus non-depressed adolescents. I explored how neural activity varied when peer desirability was included as a covariate and hypothesised that during receipt of rejection, neural activity in the IFG would correlate with peer desirability.

3. Peer acceptance would engage regions such as the ventral striatum and NA as well as the medial PFC and VLPFC. Neural activity in these regions may correlate with affective ratings of peer acceptance and would be greater in depressed adolescents versus non-depressed adolescents. I explored how neural activity varied when peer desirability was included as a covariate and hypothesised that during receipt of acceptance, neural activity in the IFG would correlate with peer desirability.

5.2 Methods

5.2.1 Participants

As reported in Chapter 4, participants were 15 medication-free adolescents aged 15-17 with a diagnosis of MDD according to DSM-IV and 15 adolescents with no current or previous psychiatric history (Table 4.1). Recruitment methods, participant characteristics and patient diagnoses are described in Chapter 4 (Section 4.2).

5.2.2 Procedure

The study procedure is described in more detail in Chapter 4 (Section 4.2). Data reported here are from phase 3 of the Chatroom task (Figure 5.1). In a previous session, participants had rated their desirability in talking to each of 60 peers in an online chatroom (phase 1). Once in the scanner, participants then rated how interested they expected each peer to be in talking to them (phase 2; Chapter 4). During phase 3 participants received positive or negative feedback from each peer (see Figure 5.2). Positive and negative feedback was delivered at random, and varied (randomly) between participants.

Prior to each peer's feedback, the peer face was presented alone for 2 seconds. The text 'INTERESTED' or 'NOT INTERESTED' appeared below the face for a further 3 seconds. Following each peer's feedback, participants provided an affective response by rating 'How do you feel about their decision? 0=very unhappy, 10=very happy'. This item was presented alongside the peer face and feedback text for 5 seconds.

Figure 5.2: Peer feedback task parameters (phase 3)

5.2.3 Data acquisition and analysis

Behavioural analysis

A 2 x 2 x 2 ANOVA test with feedback type (rejection, acceptance) and peer desirability (desirable, undesirable) as repeated-measures variables, and group (patient, control) as a between-group variable was conducted on affective ratings during receipt of peer feedback. Responses to peer rejection and acceptance were also correlated with symptoms of depression.

As reported in Chapter 4, 'peer desirability' scores were based on participants' phase 1 ratings of their interest in each peer. For behavioural analysis, a dichotomous variable was created based on these scores. For each participant, the 60 peers were ranked in terms of how interested the participant was in chatting to them. The 30 peers with the highest ratings

were labelled 'desirable peers'. The 30 peers with the lowest ratings were labelled 'undesirable peers'. For the purposes of neural analysis, the peer desirability scores (PD) were de-meaned and added to the GLM model as a covariate.

fMRI data acquisition, pre-processing and analysis

MRI data were acquired as described in Chapter 4 (Section 4.2.4). The first 26 volumes were removed to allow for T1 equilibrium effects. First-level analysis involved creating the following five explanatory variables (EVs): pre-feedback (photo alone), rejection (photo+rejection), acceptance (photo+acceptance), post-reject (behavioural rating of response to rejection), post-accept (behavioural rating of response to acceptance). ITI provided baseline BOLD activity. The onset of each EV was convolved with the hemodynamic response function using a variate of the gamma-function.

In line with the fMRI analysis methods in Chapter 4, I also added standardised de-meaned behavioural ratings of peer desirability as a covariate to the 'rejection' and 'acceptance' events ('rejection + PD' and 'acceptance + PD' respectively). To investigate the neural response to peer rejection (independent of peer desirability) my main contrast of interest was $(rejection > rejection + PD)$. A similar contrast addressed the main effect of peer acceptance on neural activity $(acceptance > acceptance + PD)$. The reverse contrasts identified regions where neural activity correlated with peer desirability (over and above regions which responded to the process of receiving peer feedback), during peer rejection $(rejection + PD > rejection)$, and peer acceptance $(acceptance + PD > acceptance)$. Finally, neural responses to peer rejection and peer acceptance were compared using the contrasts $((rejection > rejection + PD) > (acceptance > acceptance + PD))$ and

$((acceptance > acceptance + PD) > (rejection > rejection + PD))$.

Whole-brain higher-level analyses used a mixed-effects model to examine mean activation across the whole group to each of the lower-level contrasts. No subject demonstrated significant movement in the scanner (absolute movement $> 3mm$). Whilst patients and controls were modelled as separate EVs, a single-group variance was applied. Automatic outlier-deweighting was also applied. To begin with, each higher-level analysis was run at a (corrected) cluster-based threshold of Z=2.3, p<0.05 [111]. Given relatively subtle effects were expected, the same higher level analysis was run at an uncorrected threshold of Z=2.3, p<0.005. After extracting percent BOLD signal change within significant regions of interest, SPSS (v20.0, IBM Corporation, NY, USA) was used to perform appropriate between-group ANOVAs, t-tests and correlations. Corresponding Brodmann Areas (BA) were identified by transforming MNI coordinates into Talairach space.

5.3 Results

Since all participants appeared to believe the Chatroom task was real, no subject's data were excluded from the analysis. See Chapter 4 (Section 4.2.3) for a description of the manipulation check.

5.3.1 Behavioural data

Table 5.1 describes the behavioural responses to peer rejection and peer acceptance, which were analysed using SPSS (v20.0, IBM Corporation, NY, USA). Participants' mean response to rejection ranged from 0.47 to 7.73 and were slightly non-normally distributed ($D = 0.17, p < 0.05; skewness = -0.37; kurtosis = 1.55$). Participants' mean response to acceptance ranged from 5.60 to 8.20 and were normally distributed ($D = 0.11, p = 0.20$).

Feedback type	Whole group mean (sd)	Patient mean (sd)	Control mean (sd)
Peer rejection	4.53 (1.5)	4.16 (1.6)	4.90 (1.3)
Desirable peers	3.97 (1.6)	3.54 (1.7)	4.39 (1.3)
Undesirable peers	5.13 (1.6)	4.81 (1.7)	5.46 (1.5)
Peer acceptance	6.64 (0.7)	6.99 (0.7)	6.29 (0.5)
Desirable peers	7.13 (0.7)	7.45 (0.7)	6.80 (0.6)
Undesirable peers	6.19 (0.8)	6.54 (0.8)	5.85 (0.6)

Table 5.1: Affective ratings of peer feedback from desirable and undesirable peers

A 2 (feedback type) x 2 (peer desirability) x 2 (group) repeated-measures ANOVA was conducted on affective ratings following receipt of peer feedback. A main effect of feedback type revealed that affective ratings of rejection were significantly lower than affective ratings of acceptance ($F_{1,28} = 36.9, p < 0.001$; Table 5.1). There was no evidence of an interaction between feedback, group and peer desirability on affective ratings of feedback ($F_{1,28} = 0.1, p = ns$). However, there were two 2-way interactions.

There was an interaction between feedback type and group ($F_{1,28} = 4.2, p = 0.05$). Post-hoc t-tests revealed that patients showed more positive responses to acceptance than healthy controls ($t_{28} = 3.1, p < 0.01$), but the trend for patients to report more negative responses to rejection than healthy controls did not reach significance ($t_{28} = 1.4, p = ns$; Table 5.1). Furthermore, whereas depressive symptoms correlated with affective ratings of peer acceptance (Pearson's $R = 0.60, p < 0.001$), there was no evidence of a correlation between depressive symptoms and affective ratings of peer rejection (Pearson's $R = -0.29, p = ns$).

There was also an interaction between feedback type and peer desirability ($F_{1,28} = 78.8, p < 0.001$). Post-hoc t-tests revealed that rejection from desirable peers (compared to undesirable peers) was associated with more negative responses ($t_{29} = 7.4, p < 0.001$), and acceptance from desirable

peers (compared to undesirable peers) was associated with more positive responses ($t_{29} = 7.7, p < 0.001$; Table 5.1).

5.3.2 Neural analysis

Peer rejection

The hypothesis that regions involved in emotion processing and adolescents' responses to peer rejection (e.g. amygdala, NA, insula, ventral striatum, ACC, IFG, VMPFC, VLPFC, DLPFC and DMPFC), would be engaged during peer rejection was tested by examining mean BOLD activation (across all 30 participants) during rejection (compared to rejection when peer desirability was included as a covariate).

Corrected analysis of the whole sample identified three clusters of activation within the PFC which responded to rejection and were independent of peer desirability (Table 5.2; Figure 5.3, red colours). These clusters covered the 1) left insula, DLPFC, VLPFC, VMPFC, and frontal pole, 2) the right DMPFC and 3) the right VLPFC.

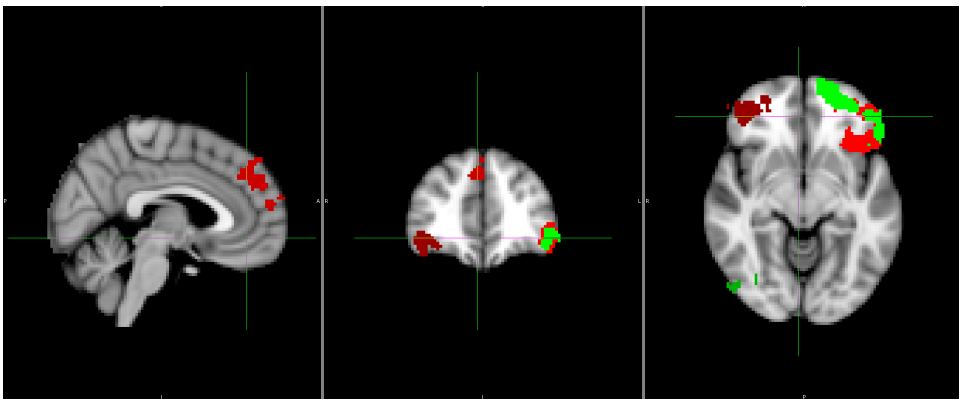

Figure 5.3: Significant clusters of activation during peer acceptance (green) and peer rejection (red) that were independent of peer desirability. Images thresholded at Z>2.3, p<0.05, corrected.

The extent to which neural activity in each of these clusters correlated

with affective ratings was then explored. One participant's BOLD responses in cluster 1 (mean=-289.19; group mean=120.59, sd=160.0), and cluster 3 (-1196.00; group mean=73.0, sd=274.2) were outliers. For the purposes of analysing correlations with behavioural responses and between-group differences in BOLD response, this participant's data were removed. It should be noted that results remained unchanged regardless of whether they were excluded or included. Participants' mean BOLD response in each of the clusters were normally distributed.

There was no evidence of a correlation between affective ratings of rejection and mean BOLD signal change during rejection in cluster 1 (Pearson's $R = 0.32, p = ns$), cluster 2 (Pearson's $R = 0.27, p = ns$), or cluster 3 (Pearson's $R = 0.27, p = ns$).

The extent to which neural activity during rejection (independent of peer desirability) varied between depressed and non-depressed adolescents was then explored. There was no evidence that mean BOLD response during rejection differed between patients and controls in cluster 1 ($t_{27} = 0.09, p = ns$), cluster 2 ($t_{23.5} = -0.18, p = ns$), or cluster 3 ($t_{27} = 1.48, p = ns$).

Feedback	**Brain region (Left/Right)**	**Brodmann Areas**	**Cluster size (voxels)**	**Z value**	**x**	**y**	**z**
Rejection[1]	1) Insula, DLPFC, VLPFC, VMPFC, frontal pole (L)	44, 45, 46, 47, 10	1664	4.22	-38	24	-4
	2) DMPFC (R)	6, 8, 9	518	3.64	4	48	30
	3) VLPFC (R)	-	469	4.25	42	44	-10
Acceptance[2]	1) VLPFC, VMPFC, frontal pole (L)	45, 10	688	3.88	-14	62	-10
	1) Fusiform gyrus (R)	-	439	4.42	36	-78	-18

Table 5.2: Brain regions where neural activity during peer feedback was independent of peer desirability

Notes: Coordinates represent the location (in MNI space) of the peak voxel in each cluster; All clusters significant at $Z>2.3$, $p<0.05$, corrected.

[1] $(rejection > rejection + PD)$
[2] $(accept > accept + PD)$

Peer acceptance

I hypothesised that regions associated with reward processing, such as the ventral striatum and NA, as well as regions reported in previous studies peer acceptance, such as the medial PFC and VLPFC, would be engaged when depressed and non-depressed adolescents received positive feedback from peers. To test this I examined mean BOLD activation (across all 30 participants) during peer acceptance (compared to acceptance when peer desirability was included as a covariate).

Corrected analysis revealed two clusters in 1) the left VLPFC, VMPFC, and frontal pole and 2) the right fusiform gyrus, which were engaged during peer acceptance (independent of peer desirability; Table 5.2; Figure 5.3, green colours). Of note, additional clusters in the right VLPFC (BA11; 189 voxels; Z value=3.52; x=32, y=45, z=-14) and right DMPFC (BA9; 131 voxels; z value=3.78; x=6, y=58, z=16 and 74 voxels; Z value=3.81; x=5, y=56, z=-16) were also identified when the same analysis was uncorrected for multiple comparisons.

The extent to which neural activity in the left PFC cluster correlated with affective ratings and varied between depressed and non-depressed adolescents was then explored. One participant's BOLD response was an outlier (mean=526.5; group mean=136.23, sd=146.9). For the purposes of analysing correlations with behavioural responses and between-group differences in BOLD response, this participant's data were removed. It should be noted that results remained unchanged regardless of whether they were excluded or included. Participants' mean BOLD response in the left PFC cluster were normally distributed.

Within the cluster in the left PFC (cluster 1), engaged during peer acceptance, there was no evidence of a correlation between affective rat-

ings of acceptance and mean BOLD signal change during acceptance (Pearson's $R = -0.21, p = ns$). The extent to which neural activity in this region varied between depressed and non-depressed adolescents was then explored. There was no evidence that mean BOLD response during acceptance (independent of peer desirability) differed between patients and controls ($t_{27} = 0.72, p = ns$).

Peer rejection vs peer acceptance

Figure 5.3 illustrates the similarity in regions engaged in peer rejection and peer acceptance, when peer desirability was partialled out. A final contrast directly tested whether there was a significant difference in neural activity during peer rejection (independent of peer desirability) and peer acceptance (independent of peer desirability). Uncorrected analysis identified small clusters where neural activity was greater during rejection compared to acceptance, in the left IFG (71 voxels; Z value=3.15; x=-36, y=28, z=0), right VLPFC (47 voxels; Z value=3.02; x=42, y=54, z=8), right DLPFC (46 voxels; Z value=3.25; x=38, y=20, z=44) and left DLPFC (26 voxels; Z value=3.07; x=-44, y=34, z=32 and 21 voxels; Z value=3.08; x=-30, y=48, z=32). However, none of these clusters survived when analysis was corrected for multiple comparisons. Neither were there any regions where neural activity during peer acceptance was greater than peer rejection.

Peer desirability

Finally, I explored which regions, engaged during peer feedback, were sensitive to peer desirability. At the corrected threshold, two clusters engaged during peer rejection correlated with peer desirability. These were located in the left parietal lobe (1039 voxels; Z value=4.4, x=-24, y=-48, z=58) and

right parietal lobe (775 voxels; Z value=3.82, x=26, y=-26, z=74). Also at the corrected threshold, five clusters engaged during peer acceptance, correlated with peer desirability. These were located in the right parietal lobe (BA40; 1686 voxels; Z value=3.68, x=40, y=-36, z=58 and 420 voxels; Z value=3.88, x=66, y=-22, z=26), left parietal lobe (766 voxels; Z value=4.00, x=-24, y=-48, z=58), and right occipital lobe (1224 voxels; Z value=3.72, x=14, y=-78, z=-8). One cluster was located in the left VLPFC (BA44; 500 voxels; Z value=3.37, x=-62, y=6, z=4).

5.4 Discussion

5.4.1 Summary of findings

Contrary to expectations, there was no evidence that affective ratings of peer rejection differed between depressed and non-depressed adolescents. However, depressed adolescents reported more positive affect from peer acceptance than non-depressed adolescents. In line with predictions, the effects of peer feedback on affective ratings were further exacerbated by peer desirability. Rejection from desirable peers elicited worse mood than rejection from undesirable peers. Similarly, acceptance from desirable peers elicited more positive mood than acceptance from undesirable peers.

Peer rejection (independent of peer desirability) engaged neural activity in three clusters: 1) the left insula, DLPFC, VLPFC, VMPFC, and frontal pole, 2) the right DMPFC and 3) the right VLPFC. There was no evidence that activity in these regions correlated with affective ratings of peer rejection. Neither was there any evidence that neural activity in any of these clusters, during peer rejection, varied between patients and controls. The only regions engaged during peer rejection, which correlated with peer

desirability, were in the left and right parietal lobe.

Peer acceptance (independent of peer desirability) engaged neural activity in two clusters: i) the left VLPFC, VMPFC and frontal pole and ii) the right fusiform gyrus. There was no evidence that activity in the left PFC cluster correlated with affective ratings of peer acceptance, or differed between patients and controls. Four regions in the parietal and occipital cortices, engaged during peer acceptance, correlated with peer desirability, as well as a cluster in the left VLPFC.

A contrast which compared peer rejection and peer acceptance (when peer desirability was partialled out) revealed only small clusters in the left IFG, right VLPFC, right DLPFC and left DLPFC where neural activity was greater during peer rejection than peer acceptance.

5.4.2 Interpretations

The findings of this study have implications for diathesis-stress models of adolescent depression, as well as models of emotion regulation, and its neural correlates, more generally. Previous studies suggest that peer rejection is a salient source of stress during adolescence, yet it is not clear why some adolescents exposed to rejection develop symptoms of depression, and others do not. Models of emotion regulation posit that heightened emotional reactivity to salient stimuli may underlie adolescent depression [51]. Although depressed adolescents report longer duration and greater frequency of negative affect compared to healthy controls [321, 322], it is not clear to what extent these findings reflect heightened emotional reactivity (versus explicit emotion regulation), or whether the findings extend to studies of peer rejection specifically. No previous studies have investigated depressed and non-depressed adolescents' responses to peer rejection, although studies

of typically developing adolescents have failed to demonstrate an association between sub-clinical depressive symptoms and emotional reactivity to peer rejection.

This current study is the first to test emotional reactivity following peer feedback, in depressed versus non-depressed adolescents. The first main finding of the study was that there was no significant difference in affective ratings of rejection between depressed and non-depressed adolescents. These findings fail to support the hypothesis that depressed adolescents show heightened emotional reactivity to peer rejection compared to non-depressed adolescents. In contrast, previous studies of peer rejection using the Chatroom suggest that socially anxious adolescents do show heightened emotional reactivity compared to healthy adolescents [200]. Although speculative, it is possible that depressed adolescents show similar emotional reactivity to healthy adolescents when faced with peer rejection, but that differences in the expectation of feedback and interpretation/attributions of peer rejection account for the negative effect of peer rejection on depression. Nevertheless some alternative explanations for the null-effects are first worth considering.

One possibility is that since the patients in this sample had relatively mild-moderate depression, the difference in symptomology between patients and controls was insufficient to find an effect of depressive symptoms at the group level.

A second possibility is that the failure to find differences between patients and controls in affective ratings of rejection is due to a researcher bias. For example, depressed adolescents may report how they think they *should* respond to peer rejection. However, the anonymity of the scanner environment is likely to have reduced the possibility of this bias, compared to the study being conducted with the experimenter in the room. A related

limitation of the experimental study design is that rejection from unknown peers may be less salient than from known peers. Partialling out peer desirability ratings may have controlled for this to a certain extent.

The lack of group differences in affective ratings of peer rejection extended to neuroimaging data. The study failed to find any differences in brain activity between depressed and non-depressed adolescents during peer rejection. This contrasts with findings from the adolescent emotional reactivity literature. Previous studies have found heightened neural responses in the amygdala during active emotional processing in depressed compared to non-depressed adolescents [377]. Studies of typically developing adolescents suggest that neural activity in affective and emotion regulation regions during rejection correlates with depressive symptoms [229] and other depressogenic markers [138, 230]. In the absence of group differences in response to rejection at the behavioural level, the failure to find between-group differences in neural activity during peer rejection is perhaps less surprising.

The second major finding of the current study was that depressed adolescents reported heightened emotional reactivity during peer acceptance, compared to controls. These data fit with the view that depression is associated with dysfunctional emotional reactivity to positive stimuli [135]. However, depression has generally been associated with blunted positive affect in response to positive stimuli, rather heightened emotional reactivity to positive stimuli. For example, depressed adolescents show shorter durations and frequencies of positive affect, compared to healthy controls [322]. Furthermore, a recent study showed that depressed adolescents reported less enjoyment from peer acceptance than controls [80]. A recent meta-analysis of 19 experimental studies of emotional reactivity in adult depression also found that depression was associated with reduced emotional reactivity to

positive stimuli [51]. The reasons for this effect are unclear.

Despite these group differences in affective ratings of peer acceptance, there was no evidence that depressed adolescents showed different neural activity to non-depressed adolescents. The only study of the neural correlates of peer feedback in clinically depressed adolescents, reported that depressed adolescents show heightened activity in the amygdala during peer acceptance (compared to neutral feedback), than healthy controls [80]. However, the effect is restricted to a cluster within the left amygdala which is just 18 voxels in size, with a relatively small (but significant) peak Z score (Z=2.47).

Together these fMRI results add further weight to the emerging conclusion of the current study, that adolescent depression is not associated with disrupted emotional reactivity. The findings fit with a meta-analysis conducted by Blackhart and colleagues, which found that although rejection elicits negative emotional responses, it does not cause significant distress or alter self-esteem [32]. Nevertheless, studies of peer rejection in depressed adolescents using other paradigms (such as Cyberball) may be useful in determining whether the null-effects reported here are a product of the Chatroom paradigm [140], or accurately detect the similarities in neural processing of peer rejection between depressed and non-depressed adolescents.

The neural activation of the group as a whole deserves some comment. Across the group peer rejection (independent of peer desirability) elicited activation in the left insula, DLPFC, VLPFC, VMPFC, and frontal pole, the right DMPFC and, the right VLPFC. This fits with social emotional models of adolescence [34, 256, 334] and previous studies of peer rejection in adolescent samples [138, 230, 232, 313, 315]. The findings also support models of emotion regulation more generally (e.g. Ochsner et al., 2012 [270]). For example, Ochsner and colleagues posit that emotion generation involves the

VMPFC (which integrates affective value with historical information/goals) and the insula (which is associated with viscerosensory inputs from body and awareness of affective states).

One unexpected finding was that during peer feedback, neural activity in emotion processing regions did not correlate with behavioural ratings of affect. A previous study of typically developnig adolescents demonstrated that during peer rejection, activity in the insula and subACC correlated with behavioural reports of distress [230]. This is consistent with adult data which also report correlations between self-reported distress and BOLD responses in regions associated with emotional processing, during exclusion (e.g. [93]). However, failure to find an association between self-reported distress and neural activity in adolescent peer rejection is not uncommon either [232, 315].

It it perhaps a surprise that there was no amygdala or ventral striatum activity in response to peer rejection or acceptance. These regions have been associated with emotional regulation in general [270], emotional reactivity in depressed adolescents (e.g. Yang et al., 2010 [377]), and have been reported in other studies of peer rejection in typically developing adolescents [232] and anxious adolescents [200]. As described in Chapter 4, one possibility is that the Chatroom was not provocative enough in the current sample. Although the Chatroom task has previously been found to elicit amygdala activity in anxious adolescents [141, 200], the same findings did not characterise typically developing adolescent samples [140, 142]. Studies of adolescents using other peer rejection paradigms, such as Cyberball [229–231, 315] and the Social Judgement Task [138] have failed to find evidence that the amygdala is involved in the neural processing of rejection.

Directly comparing peer rejection and peer acceptance demonstrated the

overlap in neural systems recruited when adolescents process positive and negative feedback (Figure 5.3). There were no regions which showed greater activity during acceptance than rejection. Uncorrected analysis revealed small clusters in the left IFG and bilateral PFC where activity was greater during rejection than acceptance. However, none of these regions survived the corrected threshold. Some adolescent studies have identified brain regions associated with emotional processing which respond more to peer rejection (than acceptance) [230–232], and others have observed heightened activity in emotion processing regions during peer acceptance (compared to rejection) [140]. However, the possibility that both forms of peer feedback engage similar brain regions means that alternative analysis methods may be needed to elucidate the subtle neural mechanisms involved in processing peer feedback.

More generally, behavioural and neural data in the current study demonstrate the value of including peer desirability as a covariate in analyses. An interaction between feedback type and peer desirability suggested that ratings of peer desirability during phase 1, predicted responses to rejection and acceptance during phase 3. As predicted, desirable peers elicited more positive feedback during acceptance, and more negative feedback during rejection, than undesirable peers. As previously highlighted, desirability to talk to peers may be an especially important variable to consider in the Chatroom paradigm, where interaction is more personal than in other peer rejection paradigms. In the current study, the occipital and parietal cortices were the main regions which showed an association between peer feedback and peer desirability. These regions are associated with visual processing primarily. However, there was also evidence that the left VLPFC (BA44), engaged during peer acceptance, correlated with peer desirability. This is more con-

sistent with the findings from the previous study (Chapter 4), where activity in the IFG, engaged during anticipation of peer feedback, correlated with peer desirability. Similarly, Guyer and colleagues (2012) found that activity in the IFG was heightened during the receipt of feedback from undesirable (versus desirable) peers, although this was the case for both positive and negative feedback [140].

One interesting finding is that peer rejection elicited activation in regions very similar to those elicited during the expectation of feedback (Chapter 4). These findings are in line with studies by Guyer and colleagues, who also found that brain regions engaged during expectation of peer feedback, were similar to those regions involved in the receipt of peer feedback [140, 142]. Lau and colleagues suggest that adolescent social anxiety may be associated with sustained amygdala activity in the anticipation of feedback and receipt of feedback [200]. Whereas both healthy and anxious adolescents showed heightened neural activity in the amygdala prior to peer feedback, this was sustained following feedback in anxious adolescents, but abated following feedback in healthy controls. The possibility that depressed adolescents also show sustained neural activity in affective regions between pre-feedback and feedback, compared to healthy controls was not investigated in the current study.

5.4.3 Strengths and limitations

The current study demonstrates the usefulness of the Chatroom paradigm for establishing causal relationships between peer rejection and negative emotional responses, and for investigating differences in emotional reactivity to peer rejection between depressed and non-depressed adolescents. First, there was no evidence that participants realised the Chatroom paradigm

was not real, which increases the validity of the results reported. Second, the paradigm was effective in eliciting distinct behavioural responses during peer rejection and peer acceptance. Specifically, participants felt significantly worse upon receiving negative versus positive feedback. This replicates findings from other studies which suggest experimental paradigms such as Chatroom [140] and Cyberball [313] are capable of evoking emotional reactions in typically developing adolescents, although the extent to which my study findings generalise to unknown peers remains to be tested.

One strength of the Chatroom paradigm in particular is that positive and negative feedback are delivered at random in an event-related design. This contrasts with the Cyberball task, where order effects may arise from the fact that rejection always proceeds peer acceptance. Adaptations of Cyberball have attempted to address these problems of order effects and expectancy violation [315].

As with all experimental measures of peer rejection, the Chatroom paradigm is limited to identifying the short-term effects of peer rejection on negative emotional responses. Ethical issues prevent the study of the long-term effects of peer rejection manipulations which are expected to elicit symptoms of depression or a clinical diagnosis of depression. For these purposes, longitudinal designs are more suitable, where the effects over time of naturally occurring peer rejection are measured. Of course these methods too are limited in that confounding variables such as the nature and type of rejection cannot be controlled for. Nevertheless, the combination of experimental and longitudinal studies provide the best way to examine the negative impact of peer rejection during adolescence.

As previously described, the patient group were a sample of mild-moderately depressed adolescents, due to the exclusion of participants who reported cur-

rent suicidal ideation or recent self-harm, or who were taking medication. Interpretations about the role of peer feedback in adolescent depression are restricted to non-severe cases of MDD.

Ethical issues surrounding the use of the Chatroom paradigm in this sample of depressed adolescents are also worth commenting on. Firstly, the study protocol, including the Chatroom paradigm, were stringently reviewed by the Oxfordshire NHS Research Ethics Committee, who provided a favourable opinion for the study. The exposure of participants to peer rejection was deemed to be no worse than they would be exposed to in everyday life. Furthermore, the potential benefits to scientific knowledge gained were deemed to be greater than the potential emotional costs. Parents were consulted about the nature of the Chatroom paradigm prior to adolescents taking part and no parent thought the study would have adverse effects on their child.

There was no evidence from the debrief at the end of the second session (Appendix A) that patients or controls were adversely affected by the deception or the peer rejection itself. These findings are in line with Nelson and colleagues who do not report adverse effects of the Chatroom paradigm on healthy [140, 142] or anxious adolescents [141, 200]. No formal long-term follow-up data were collected, although many of the study participants were invited to take part in an online questionnaire about their experiences of the MRI study. This study (which also included data from subjects with anxiety disorder who took part in another MRI study in our research group) found that contrary to expectations, participants with higher levels of anxiety actually showed more enjoyment from taking part in the MRI studies than did healthy controls [145]. The effects of depressive symptoms on enjoyment of the study were not investigated.

As previously described in Chapter 4, the use of session 1 ratings of peer desirability in the analysis of data at session 2 assumes that participants are able to recall their ratings of peer desirability, and that these ratings remain stable over time. Whilst future studies may consider either assessing the stability of peer desirability ratings, or repeating them at session 2, the fact that peer desirability had such significant effects on responses to both peer rejection and acceptance, suggests that they are valid measures of peer salience at least.

A limitation of the stimuli used in this phase of the task is that both positive and negative feedback was accompanied by a smiling (positive) face, rather than a neutral face, or an emotional face congruent with the feedback conveyed (i.e. negative feedback accompanied by a frowning face). This may have lead to the rejection trials having less of a negative effect on participants, or to participants being confused about the valence of the feedback. However, significant differences at the behavioural level between rejection and acceptance suggested that rejection trials elicited significantly more negative responses than peer acceptance.

Finally, as previously described, interpretations of fMRI data should be treated with caution. Since fMRI indirectly measures neural activity, its accuracy and temporal precision in detecting brain mechanisms are somewhat limited. Furthermore, the direction of group differences is not always consistent between studies, raising questions over whether greater or weaker activity is problematic. Neuroendocrine and cardiovascular measures provide information about how pathways in the autonomic nervous system may mediate the effects of peer rejection on neural responses and self-reported distress [345], enabling more elaborate, multi-level models of peer rejection to be developed. Complementary neurobiological methods such as MEG,

eye-tracking and pupil dilation, and neuroendocrine measures offer some advantages over fMRI and have begun to be used to study peer rejection in adolescence [8, 328, 345]. For example, Silk and colleagues found increased pupil dilation to peer rejection (versus acceptance), within the first two seconds following feedback [328].

5.4.4 Future research

Given the failure to find behavioural or neural differences in response to peer rejection, larger behavioural and psychophysiological studies may be helpful for identifying whether depressed adolescents really do react more negatively to peer rejection compared to healthy adolescents. FMRI is an expensive tool, and further investigation using less expensive methods to identify the effect of peer rejection in adolescent depression should be conducted before this study methodology is repeated. As previously described, behavioural studies using different experimental manipulations of peer rejection may also help to determine whether the null-effects reported here are the product of the Chatroom paradigm.

As described in Chapter 3, cognitive factors may moderate the effect of peer rejection on depression. For example, one previous study found no direct effect of peer rejection on depressive symptoms in a sample of clinically depressed adolescents [40]. However, adolescents aged 16.5 to 18.5 years (as opposed to younger adolescents) who experienced high levels of peer rejection and held negative cognitive schemas, reported significantly more symptoms of depression than those with low levels of rejection or positive cognitive schemas. Future studies might consider the extent to which cognitive factors moderate depressed adolescents' emotional reactivity to depression.

5.4.5 Summary

Peer rejection is a salient source of stress during adolescence, yet it is not clear why rejection elicits depressive symptoms in some individuals compared to others. The present study is the first to investigate whether heightened emotional reactivity to rejection characterises adolescent depression. Contrary to predictions, peer rejection did not elicit worse feelings in patients compared to healthy controls. Peer rejection (and peer acceptance) elicited activation in regions of the brain associated with emotional reactivity (e.g. insula and VLPFC). However, there was no evidence that neural responses to rejection differed between patients and healthy controls. The present study also reports data on the effects of peer acceptance in depressed and non-depressed adolescents. Contrary to predictions, and to findings from a previous study of depressed adolescents [80], depressed adolescents reported feeling better following peer acceptance than healthy adolescents. Based on the finding that peer desirability influenced participants' behavioural and neural responses to peer acceptance and rejection, I recommend that future studies include this confounding variable in their statistical models.

Work based on Chapters 4, 5 and 6 has been published in the following article: Haddad, A.D., Platt, B. James, A.C. and Lau, J.Y. (2012) Anxious and non-anxious adolescents' experiences of non-clinical magnetic resonance imaging research. *Child Psychiatry and Human Development*. The final publication is available at http://link.springer.com/article/10.1007%2Fs10578-012-0350-x

Chapter 6

Neural correlates of emotion regulation of peer rejection

Emotion regulation is the process of consciously modifying an emotional response to a salient stimulus. In contrast to emotional reactivity, it involves selective modification of an emotional response and monitoring of resulting emotional states. Self-report data suggest that when instructed, depressed adults are as capable of using emotion regulation strategies to down-regulate negative affect as non-depressed adults. However, neuroimaging studies have been able to identify subtle neural differences between depressed and non-depressed adults during emotion regulation. Regions such as the frontal pole and middle frontal gyrus, anterior cingulate, amygdala and insula respond differently during emotion regulation in depressed and non-depressed adults. Given the maturation of the regions (associated with emotional processing) during adolescence, I sought to investigate whether adolescents could effectively down-regulate negative responses to peer rejection, and whether this ability was impaired in depressed, compared to non-depressed, adolescents. The behavioural and neural correlates of cognitive reappraisal were

studied in the same depressed and non-depressed adolescents who had previously received positive and negative feedback from peers. Prior to MRI acquisition, participants received training in generating benign interpretations of ambiguous social scenarios. During MRI acquisition, participants were reminded of negative feedback they had previously received from peers, and instructed to either reappraise or attend to this feedback. Self-report measures suggested that all participants were able to down-regulate negative emotional responses to peer rejection, although this did not vary between depressed and non-depressed adolescents. However, activity in the right frontal pole was significantly reduced when depressed (versus non-depressed) adolescents reappraised rejection. This is the first study to investigate emotion regulation in clinically depressed adolescents, and to peer rejection specifically, therefore the findings make an important contribution to the literature. Nevertheless, some limitations associated with the study design mean that the data should be interpreted cautiously. Suggestions for future studies of emotion regulation in depressed adolescents are discussed.

6.1 Introduction

As highlighted in Chapter 1, peer rejection is a major source of psychosocial stress during adolescence [315], which experimental studies suggest elicits negative emotional responses and longitudinal studies suggest predicts later depressive symptoms (Figure 1.1A). The task of the preceding empirical chapters has been to investigate the biological and psychological factors which may explain why peer rejection elicits depressive responses in some adolescents but not others. In the previous chapter, I introduced the idea of adolescent depression as a disorder of emotion regulation. Emotion regulation is the process of modifying an emotional response, whether that be

in the magnitude, valence, duration or any other component of the emotion [135, 136]. I distinguished between two types of emotion regulation; i) emotional reactivity, and ii) explicit emotion regulation [143].

In Chapter 5 I described emotional reactivity as an automatic response to salient stimuli which has fleeting effects on behaviour and physiology. I investigated whether emotional reactivity, as measured by self-report and fMRI BOLD response, distinguished depressed and non-depressed adolescents' responses to peer rejection. Although the group as a whole showed heightened engagement of brain regions associated with emotional reactivity during peer rejection, there was no evidence that this differed between depressed adolescents and healthy controls. The findings did not support the view of adolescent depression as a disorder of emotional reactivity. The second type of emotional regulation, explicit emotion regulation, instead involves conscious attempts to modify emotions and operates over longer periods of time. In this chapter, I investigate whether dysfunctional emotion regulation may modify depresesd adolescents' responses to peer rejection (Figure 1.1B).

6.1.1 Explicit emotion regulation

As outlined by Gyurak and colleagues, explicit emotion regulation has three main characteristics [143]. First, it requires conscious effort to be initiated. Second, it requires monitoring during implementation. Third, it requires a degree of awareness and insight into emotional states [143]. Whereas emotional reactivity is an automatic response to any salient stimulus, the cognitive demands of explicit emotion regulation mean that it cannot be engaged in all the time [143]. Instead, is more selectively chosen depending on the scenario.

A standard method for measuring explicit emotion regulation is to expose participants to two distinct conditions. One which requires them to actively engage in emotion regulation, following some form of emotion regulation training, in which specific psychological strategies are taught and practiced. A control condition aims to capture natural emotional responses, without conscious emotion regulation, such as passively viewing an emotional stimulus without use of trained strategies. Using this basic paradigm, many different forms of explicit emotional regulation have been studied. Behavioural strategies such as emotional suppression control the extent to which emotional reactivity is expressed. For example, by asking participants to minimise the amount of face movement whilst watching short film clips of disgust-inducing scenarios [121]. Other strategies attempt to neutralise the negative attentional, attributional and interpretive biases, described in Chapter 3 [267]. These cognitive strategies can be distinguished as focusing on attentional change (e.g. selective attention or distraction, as mentioned in Chapter 3) or interpretive change (e.g. cognitive reappraisal or distancing). Whereas cognitive reappraisal involves generating alternative explanations for a negative emotional experience, distancing requires participants to mentally distance themselves from the emotional stimulus.

Cognitive reappraisal

Cognitive reappraisal is the most well-studied emotion regulation strategy. The classical cognitive reappraisal strategy developed by Ochsner and colleagues (2004) requires participants to 'decrease' responses to negative stimuli by reinterpreting the stimulus, for example by thinking "[the stimulus] is not real", "things will improve", or "[the stimulus] is not as bad as it looks" [269]. Responses to this condition are then compared to a 'look'

condition, where participants simply observe a negative or neutral stimulus. Prior to implementing the cognitive strategies, participants receive training in which they are provided with examples of reappraisal strategies and given the chance to practice reappraisal. During reappraisal, participants are reminded of the instructions, then view the stimulus (usually an emotionally charged photo), then make a rating of their negative affect.

As Ochsner and colleagues highlight, understanding emotion regulation processes in healthy participants is important for developing a normative model which can be used to understand how psychopathology, such as depression, might involve emotional dysfunction [267]. As reviewed by McRae and colleagues [239], instructed use of cognitive reappraisal improves self-reported mood [117, 135, 280, 329], affects physiological [329] and neural measures of emotional reactivity [280] in healthy adults.

Emotion dysregulation in adult depression

Cognitive reappraisal specifically address the negative cognitive biases thought to underlie depression, by consciously reframing or recontextualising a negative stimulus, such that they lessen the negative emotional experience [135, 239, 270]. A critical question, is whether depression is related to dysfunctional explicit emotion regulation. There are two key questions that need to be answered in order to understand this relationship. The first is whether depressed adults naturally use explicit emotion regulation strategies less often than non-depressed individuals. As far as I am aware, no study has investigated quantitative differences in spontaneous emotion regulation between depressed and non-depressed adults. However, several studies suggest that depressed adults use more ineffective emotion regulation strategies than non-depressed adults. For example, one study found that whilst watching a

sad film, recovered-depressed adults more frequently reported spontaneous use of suppression strategies than healthy adults [90]. In a second phase of the study, where all participants were instructed to use suppression or reappraisal, suppression was found to be more ineffective in down-regulating negative emotions than reappraisal.

The second question is whether depressed adults are able to use explicit emotion regulation strategies when instructed. Studies of depressed adults have also investigated whether they show deficits in the ability to use emotion regulation when instructed, compared to healthy adults [24, 90, 102, 170]. Several studies of cognitive reappraisal have shown that depressed adults [102] and adults recovered from depression [90], are as able to down-regulate negative emotions in response to photos as healthy controls. Depressed adults also appear to be able to use suppression to down-regulate negative emotions as well as healthy adults [24] and there is no evidence that suppression influences physiological measures differently in depressed than non-depressed adults [170], although depressed adults may find it harder to down-regulate emotions using suppression than healthy controls [24].

6.1.2 The neural correlates of emotion regulation

Emotion regulation can be difficult to study behaviourally because it relies on participants' introspections about their own (often brief) emotional experiences. Advances in neural imaging enable the study of how the brain mediates the emotion regulation process (for reviews see [15, 270]). Cognitive reappraisal is a complex strategy that involves representation of mental states, retrieving solutions from semantic memory, selecting the most appropriate solutions, monitoring the effectiveness of these strategies, whilst keeping the goal of reappraisal in mind, therefore it is associated with neural

activity in a wide range of brain regions [238, 268].

Ochsner and colleagues' neural model proposes the recruitment of three major regions during cognitive reappraisal; 1) the DLPFC, posterior PFC and inferior parietal lobe (involved in selective attention and working memory), which may be involved in holding in mind the reappraisal or attending to reappraisal-related information, 2) the dACC, which is likely to be involved in tracking how well reappraisal is modifying emotion, and 3) the VLPFC, which may play a role in selecting goal-appropriate responses from semantic memory [270]. The DMPFC may also be involved as it is involved in attributing mental states and the stimuli in emotion regulation paradigms are often photographs of people with differing emotional expressions. The recruitment of these regions is likely to regulate emotion by modifying responses in three major affective regions; 1) the amygdala (the most commonly modulated region), 2) the ventral striatum, 3) the insula and VMPFC (less commonly modulated) [270].

Several adult neuroimaging studies of cognitive reappraisal support Ochsners' model, using both the reinterpretation cognitive reappraisal strategy [239, 266, 280] and the distancing cognitive reappraisal strategy [23, 91, 121, 167, 186, 212, 269, 356]. Importantly, neural activity in the medial PFC and amygdala during emotion regulation appears to positively correlate with behavioural measures of how difficult depressed participants find the task [24]. The extent to which reinterpretation may use more left lateralised (VLPFC) regions whereas distancing may use more right lateralised and parietal regions has been discussed recently, although not tested systematically [270]. Whilst the Ochsner model is based on cognitive reappraisal data, similar regions may also apply to other emotion regulation tasks [121, 270].

fMRI studies of cognitive reappraisal in adult depression

FMRI studies suggest that although depressed adults are as able to use cognitive reappraisal to effectively regulate negative emotional experiences as non-depressed adults, they show subtle differences at the neural level [24, 102, 158, 170]. For example, Johnstone and colleagues (2007) found that depressed adults showed heightened activity in the right PFC (middle frontal gyrus; BA10; Talairach coordinates x=32, y=46, z=25) during regulation, compared to an 'attend' condition [170]. In contrast, controls showed reduced activity in this region during reappraisal, compared to the control condition. Furthermore, although there was no main effect of reappraisal on amygdala activity, controls showed a negative correlation between amygdala activity and VMPFC activity (BA11/32; Talairach coordinate x=7, y=39, z=-11) during regulation, whereas patients showed a positive correlation. In a suppression paradigm, Beauregard and colleagues showed that activity in the right dACC, anterior temporal pole, amygdala and insula during emotion regulation was heightened in depressed adults compared to healthy controls [24].

A more recent study of mild-moderately depressed and non-depressed adults found both groups showed effective down-regulation of amygdala activity during reappraisal [102]. However, there was a positive correlation between depressive symptoms and the extent of amygdala deactivation during reappraisal. Furthermore, in patients, deactivation of the amygdala was not sustained after a 15 minute delay, whereas it was in controls. Deactivation of the amygdala during reappraisal was accompanied by increased activation in the DLPFC and inferior parietal cortex (IPL), although to a lesser extent in patients compared to controls.

Interestingly, group differences at the neural level have also been ob-

served when depressed patients were asked to up-regulate positive affect [158]. Heller and colleagues found that depressed adults were unable to sustain neural activity in the NA (a region associated with reward-processing) during up-regulation (compared to down-regulation). In contrast, healthy controls demonstrated increased NA activity during up-regulation (compared to down-regulation) throughout the study session. These findings are consistent with the fact that anhedonia is a salient symptom of depression and suggest that neural factors may contribute to deficits in emotion regulation of positive and negative affect.

Together these neuroimaging studies of depressed adults suggest that although depressed adults are able to use cognitive reappraisal to effectively regulate negative emotional experiences, they show subtle differences at the neural level (compared to healthy adults). Regions which respond differently in depressed and non-depressed adults during emotion regulation include the middle frontal gyrus (BA10), dACC, amygdala and insula. These findings require further investigation to determine the extent to which neural differences during explicit emotion regulation may contribute to the maintenance of depression. Nevertheless, they highlight the value of neuroimaging studies for differentiating information processing in depressed and healthy subjects.

Given that regions within the PFC are known to continue to develop during adolescence [57, 120, 227, 282, 320, 353], and in particular that regions associated with mentalising continue to develop during adolescence [49], studying cognitive reappraisal in adolescent populations is of theoretical and clinical importance [238, 270].

6.1.3 Explicit emotion regulation in adolescence

Few studies have investigated spontaneous emotion regulation in adolescence. One study of a large community sample of 387 adolescents and 630 adults found that adolescents used significantly less cognitive emotion regulation strategies less often than adults [115]. A seminal developmental study of cognitive control suggested that poor cognitive control in pre-adolescent children was associated with reduced activity in the VLPFC, compared to adults [47]. However, only three studies have explored the ability of children and adolescents to use emotion regulation strategies under instruction, and the neural correlates of emotion regulation in non-adult populations [213, 238, 282].

Studies of typically developing samples

The first non-adult study of emotion regulation was of fourteen 8-10 year olds who were instructed to reduce their emotional response to sad film clips using distancing (rather than cognitive reappraisal) [213]. Participants received a 30 minute training session prior to an fMRI scan, in which they were asked to imagine sitting in a cinema watching themselves react emotionally to a series of films. During the scan session participants viewed emotionally neutral or sad films and were given instructions about when to reappraise and when not to. Behavioural data suggested that when viewing sad films, participants reported significantly lower mood during non-reappraisal than reappraisal. Emotion regulation engaged neural activity in a range of regions within the PFC, including the lateral PFC (BA9 and 10), OFC (BA11), mPFC (BA10), and right VLPFC (BA47).

These findings are consistent with the previously described models of explicit emotion regulation in adult populations [270]. Although the re-

searchers report that neural activity in the PFC during reappraisal was less than in an identical study of adults [212], this difference was not tested statistically. Heightened neural activity was also observed in the right ACC, a region associated with affective processing. Retrospective reports of success suggested that participants were successful in using the distancing strategy 84% of the time. In summary, this study provides support for the notion that pre-adolescent children are able to use cognitive emotion regulation strategies, and that emotion regulation appears to engage similar frontal regions in pre-adolescents, as it does for adults (though speculatively, to varying degrees).

A second study of 15 typically developing 7-17 year-olds measured behavioural and neural effects of up- and down-regulating disgust responses using cognitive reappraisal [282]. Participants were shown disgusting images and given example reappraisal strategies such as 'pretend it's in front of you' or 'pretend it's fake' and were asked to rate their disgust response after 'look' trials, 'positive reappraisal' trials and 'negative reappraisal' trials. Behavioural data suggested participants could effectively regulate emotion using the cognitive strategy given. Disgust ratings were lower after down-regulation and higher after up-regulation. Reducing emotional responses was associated with reduced activity in affective regions such as the insula, thalamus and dACC. The degree of reduced activity in these regions also correlated with increased activity in the VMPFC. Although there was no evidence at the behavioural level that down-regulation was associated with age, neural data suggested reduced PFC activity in older (compared to younger) participants.

A final study directly compared neural responses to reappraisal (of negative and neutral pictures) in children, adolescents and young adults (age

range 10-22). As per the previous studies, cognitive reappraisal was effective in reducing negative affect in the child, adolescent and adult groups [238]. A linear increase across age was observed in activation of left VLPFC (BA45) during reappraisal compared to just attending to the photos [238]. Importantly, adolescents used regions associated with mental state attribution (mPFC, posterior cingulate cortex, anterior temporal cortex) less than both adults and children during exposure to negative stimuli, and more than both adults and children during reappraisal [238]. These quadratic changes across developmental-time contrast with other cognitive control tasks, which improve linearly [238].

Together then, these data suggest that children and adolescents are able to effectively use emotional regulation to reduce emotional responses to negative films and photos. As has been found in adult studies, regions of the PFC including the orbital PFC (BA11), medial PFC (BA10), lateral PFC (BA9), VLPFC (BA47; BA45), and VMPFC appear to be involved in reappraisal. Affective regions such as the ACC, insula, and thalamus show reduced activity during reappraisal. Preliminary evidence suggests that compared to adults, adolescents may show increased activity in the PFC during reappraisal [238].

Explicit emotion regulation in adolescent depression

One unanswered question from the developmental literature, is whether deficits in explicit emotion regulation characterise adolescent depression. Previous studies suggest that adolescents with more depressive symptoms report using emotion regulation strategies less often than adolescents with fewer depressive symptoms [113]. However, to date no experimental studies have investigated whether explicit emotion regulation is disrupted in

adolescents with depression. Current treatments for adolescent depression such as cognitive behavioural therapy involve training explicit emotion regulation through cognitive reappraisal. However, although they appear to be effective in reducing symptoms of depression in around 60% of cases of paediatric depression [214], relatively little is known about the mechanisms behind cognitive reappraisal.

As described in Chapter 1 and explored further in Chapter 3, rejection-related cognitive biases may underlie the development and maintenance of adolescent depression. For example, negative cognitive self-schemas have been shown to moderate the effect of peer rejection on depressive symptoms in adolescent psychiatric patients [40]. Similarly, negative interpretive biases are likely to play a causal role in adolescent depression [221]. Despite peer rejection being a salient source of stress during adolescence, to date the only experimental studies of explicit emotion regulation in children and adolescents have measured responses to sad or generally negative stimuli [213, 238, 282], rather than rejection-specific stimuli.

Furthermore, previous studies of emotion regulation in adults have typically relied on reappraisal of relatively simple emotional stimuli. As Ochsner and colleagues (2012) report [270], 33/43 reappraisal studies use pictures from the international affective picture system database (IAPS) [197]. These pictures depict normatively positive or negative stimuli [270], however they may not be useful for investigating reappraisal of a specific emotion or emotional experience (e.g. peer rejection) [270].

The first aim of the current study was to investigate whether a cognitive reappraisal task developed to measure explicit emotion regulation of peer rejection in adolescents, would effectively reduce rejection-related negative mood and belief in negative thoughts.

In a sample of depressed and non-depressed adolescents who had recently been exposed to peer rejection (data reported in Chapter 5), the ability of adolescents to use a cognitive reappraisal (reinterpretation) strategy to down-regulate their negative emotional response to peer rejection was tested. In a control block of trials, participants were asked simply to attend to negative feedback from peers. I predicted that across the whole sample, post-reappraisal measures of negative mood and belief in negative thoughts would be significantly lower than pre-reappraisal measures. Based on findings from the adult literature, I did not expect any behavioural differences between depressed and non-depressed adolescents in the efficacy of reappraisal.

Studies of adults suggest that although depressed adults do not show behavioural differences from non-depressed adults during emotion regulation, there are subtle neural differences between depressed and non-depressed adults. Regions such as the middle frontal gyrus (BA10), dACC, amygdala and insula show differential activation in depressed versus non-depressed adults [24, 102, 158, 170]. **The second aim of the present study was therefore to investigate the neural correlates of emotion regulation in adolescents, and whether neural activity during reappraisal differed between depressed and non-depressed adolescents.** Based on previous studies of typically developing adolescents, I predicted that regions such as the orbital PFC (BA11), medial PFC (BA10), lateral PFC (BA9), VLPFC (BA47; BA45), and VMPFC would to be involved in reappraisal. I hypothesised that affective regions such as the ACC, insula, and thalamus would show reduced activity during reappraisal. Based on adult studies, I predicted that neural activity in these regions would correlate with behavioural measures of reappraisal efficacy. Finally, I expected that depressed and non-depressed adolescents would show differential neural activity in re-

gions such as the middle frontal gyrus (BA10), dACC, amygdala and insula during reappraisal. Based on the mixed adult literature, and the lack of developmental studies, I made no predictions about the direction of these effects.

6.1.4 The current study

In this study, behavioural responses and brain activation patterns during explicit emotion regulation of rejection were compared in a group of adolescents with and without depression using functional neuroimaging (phase 4; Figure 6.1). As part of the same study, participants' responses to predicted peer feedback (phase 2; Chapter 4) and actual peer rejection (phase 3; Chapter 5) had previously been investigated.

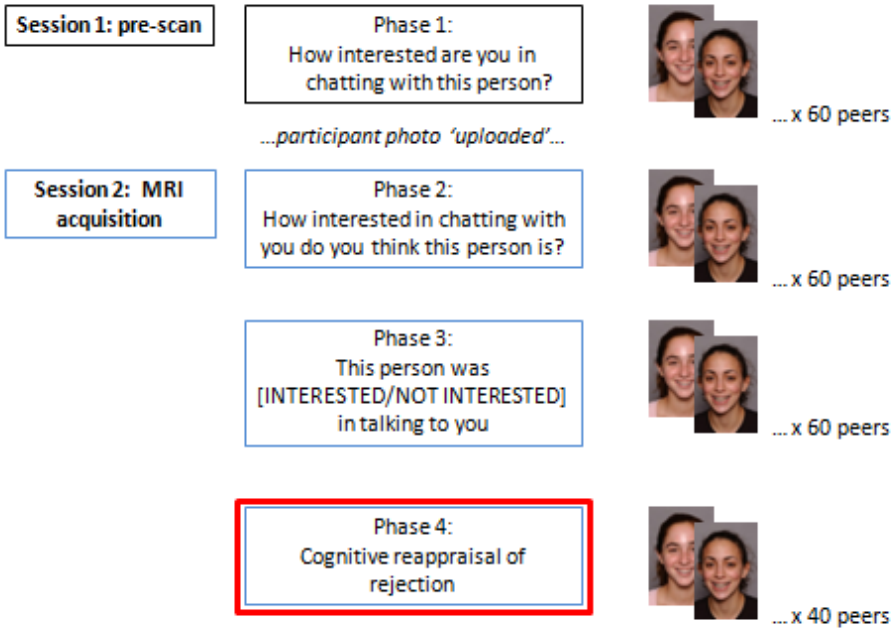

Figure 6.1: The Chatroom paradigm (phase 4).

In the first set of analyses, I compared ratings of rejection-related i) neg-

ative mood and ii) belief in negative thoughts, before and after cognitive reappraisal (although these were collected retrospectively). I investigated whether these changes varied between depressed and non-depressed adolescents. I predicted that both negative mood and belief in negative thoughts would be significantly less following cognitive reappraisal, compared to before reappraisal. Based on previous studies of adults, I did not expect cognitive reappraisal to affect mood and belief in negative thoughts differently between depressed and non-depressed adolescents.

In the second set of analyses, I focused on brain activation patterns during cognitive reappraisal ('reappraise' trials) and non-regulation ('attend' trials). I explored regions where neural activity was greater during cognitive reappraisal, compared to non-regulation. These regions were then used to compare neural activation between depressed and non-depressed adolescents. Based on previous studies of typically developing adolescents, I expected a network of regions to be associated with cognitive reappraisal, including the orbital PFC (BA11), medial PFC (BA10), lateral PFC (BA9), VLPFC (BA47; BA45), and VMPFC. Guided by prior data on dysfunctional brain correlates associated with adolescent depression, between-group differences were expected in these regions, although I refrained from making predictions about the directions of these effects. In contrast, I expected occipital areas to show greater activation during 'attend' trials, compared to reappraisal.

A final set of analyses explored whether neural responses during emotion regulation of rejection correlated with behavioural measures of training success. I hypothesised that across the sample, neural activity in brain regions engaged in reappraisal would correlate with rejection-related reduction in negative mood and reduction in belief in negative thoughts. I made no predictions about the direction of this correlation.

In summary, I made the following predictions about the findings of the current study:

1. Across the group, reappraisal would reduce negative mood and belief in negative thoughts.

2. Reappraisal would be associated with increased activity in the orbital PFC (BA11), medial PFC (BA10), lateral PFC (BA9), VLPFC (BA47; BA45), and VMPFC. Patients would show differential neural responses to controls.

3. Across the group, neural activity in regions engaged in reappraisal would correlate with behavioural ratings of the effectiveness of reappraisal training.

6.2 Methods

6.2.1 Participants

As reported in Chapter 4, participants were 15 medication-free adolescents aged 15-17 with a diagnosis of MDD according to DSM-IV and 15 adolescents with no current or previous psychiatric history (Table 4.1). Recruitment methods, participant characteristics and patient diagnoses are described in Chapter 4 (Section 4.2).

6.2.2 Procedure

The study procedure is described in more detail in Chapter 4 (Section 4.2). Data reported here are from phase 4 of the Chatroom task (Figure 6.1). In a previous session, participants had rated their desirability in talking to each of 60 peers in an online chatroom (phase 1). Once in the scanner,

participants rated how interested they expected each peer to be in talking to them (phase 2; Chapter 4). Participants then received positive and negative feedback from each peer (phase 3; Chapter 5). During the phase reported here, participants reviewed negative feedback from peers and were asked to implement their reappraisal training. After scanning, behavioural data assessed their ability to use the reappraisal training.

6.2.3 Reappraisal training

Reappraisal training was developed by Catherine Campbell (CC; Trainee Clinical Psychologist) and Jennifer Lau and piloted on a sample of typically developing adolescents. Appendix B provides a description of the training, and Appendix C describes the pilot study, which is reported elsewhere [54, 283]. The training aimed to increase the use of a benign interpretational style when faced with ambiguous social information. In brief, the pilot data support the effectiveness of the training in improving interpretational style and reducing negative mood.

The training lasted roughly 15 minutes and was delivered by myself and CC using a standardised script (see Appendix B). Researchers referred to the cognitive reappraisal as a 'new skill'. Reappraisal training introduced participants to association of thoughts and emotional responses using example scenarios of ambiguous social scenarios. The researcher described how negative thoughts about a social scenario might elicit negative mood states, and how generating positive thoughts could improve mood. For example, the researcher described a scenario in which the participant hears some classmates laughing behind their back, and how negative interpretations of this might lead to a negative mood state. They were given examples of thoughts that could lead to more positive mood such as 'They might have been laughing at

someone else' and 'I have plenty of friends anyway'. During a final scenario, which described peer rejection in an online environment, participants were asked to generate as many reappraisal strategies as possible. They were then given additional strategies by the researcher.

Following cognitive reappraisal training, the researchers explained how and when to use it in the scanner. Participants were specifically instructed that they should not use the reappraisal training during the anticipation or receipt of feedback. They were told that they should wait until the final stage of the scan session, when they would be explicitly asked to use it. Phase 4 of the scan session was administered in a mixed design, with reappraisal and attend trials delivered in separate blocks. This design was explained to participants, so that they understood they should just let their natural emotions occur during the first set of photos, and to use their new skill in the second set. Participants were informed that when prompted to use their new skill, they should generate as many 'helpful' thoughts as possible in the 8 seconds given.

6.2.4 Task parameters

Following the peer feedback phase (phase 3; Chapter 5), participants were verbally reminded that they were about to be asked to use their new skill. The reappraisal phase lasted around 11 minutes and was administered in two blocks of 20 trials. In each block, participants were told that the upcoming photos were of peers who were 'less interested' in chatting with them. To ensure that the stimuli for reappraisal were standardised across participants, the 40 peers from the original Nelson dataset described in Chapter 4 were used (i.e. the young adult stimuli were removed). As a result, the peers were not the same peers who had actually given peers 'not interested' feedback.

The vague feedback 'less interested' was intended to minimise the threat to believability. In the debrief, none of the participants reported noticing this.

In the first block (attend), participants were instructed to allow their natural emotional response to peer rejection using the prompt 'How are you feeling?'. In the second block (reappraise), participants were instructed to use their new skill with the prompt 'Unhelpful → Helpful'. Prior to each block, participants read instructions about how to respond to the upcoming peer rejection and spoke to the researcher to confirm they understood the task requirements. In each trial the peer face and the text 'Less interested' were presented together for 2 seconds. The prompt 'How are you feeling?' or 'Unhelpful → Helpful' was then added for a further 8 seconds.

6.2.5 Rejection-related negative mood and belief in negative thoughts

In order to assess whether cognitive reappraisal had affected participants' rejection-related negative mood and belief in negative thoughts, self-report data were collected after MRI scanning. Retrospective rating of pre-reappraisal negative mood was measured using the following item: "How did you FEEL when other people said they were not interested in chatting to you?", which was scored from 0-10, with 10 reflecting 'very sad'. Retrospective pre-reappraisal belief in negative thoughts was measured using the following item: "How much did you believe the [previously reported] UNHELPFUL thought to be true?", which was scored from 0-100%, with 100% reflecting complete belief in the negative thought. Post-reappraisal mood was assessed using the following item: "How did you FEEL after using your new skill?", and post-reappraisal belief in negative thoughts using the following item: "Think about your initial UNHELPFUL thoughts when people said they

were not interested in chatting with you. After you had used your new skill, how much did you believe these to be true?". Both post-reappraisal measures were scored on the same scale as the respective pre-reappraisal measures.

Complete data (pre- and post-measures) on negative mood were available for 28 participants (93%). Missing mood data were distributed evenly between patients (N=1) and controls (N=1). Complete data on belief in negative thoughts were available for 22 participants (73%). There was slightly more missing data on belief in negative thoughts from patients (N=6) than controls (N=2), although this difference was not statistically significant ($\chi^2 = 2.73, p = ns$).

6.2.6 Data acquisition and analysis

Behavioural analysis

The effect of reappraisal on change in participants' retrospective reports of negative mood and belief in negative thoughts, was analysed in a repeated measures ANOVA with group (patient, control) as a between-groups factor and change in mood (pre- and post-reappraisal) and change in negative thoughts (pre- and post-reappraisal) as repeated-measures variables.

fMRI analysis

MRI data were acquired as described in Chapter 4 (Section 4.2.4). The first 14 volumes were removed to allow for T1 equilibrium effects. This included the instructions for the attend block, therefore instructions for the reappraise block were included as an EV of no interest in the model. First-level analysis involved creating the following five explanatory variables (EVs): i) pre-attend (reviewing rejection), ii) attend, iii) pre-reappraise

(reviewing rejection), iv) reappraise, and v) instructions for the reappraise block (an EV of no interest). ITI provided baseline BOLD activity. The onset of each EV was convolved with the hemodynamic response function using a variate of the gamma-function.

To investigate neural responses to cognitive reappraisal of rejection my main contrast of interest was (reappraise>attend). No subjects demonstrated significant movement in the scanner (absolute movement>3mm). Motion outliers were partialled out in one subject's data (absolute motion=2.2mm). Whole-brain higher-level analyses used a mixed-effects model to examine mean activation across the whole group to this lower-level contrast. Whilst patients and controls were modelled separately, a single-group variance was applied. Automatic outlier-deweighting was also applied. To begin with, the higher-level analysis was run at a (corrected) cluster-based threshold of Z=2.3, $p<0.05$ [111]. Given relatively subtle effects were expected, the same higher level analysis was run at an uncorrected threshold of Z=2.3, $p<0.005$. After extracting percent BOLD signal change within significant regions of interest, SPSS (v20.0, IBM Corporation, NY, USA) was used to perform appropriate between-group ANOVAs, t-tests and correlations. Corresponding Brodmann Areas (BA) were identified by transforming MNI coordinates into Talairach space.

6.3 Results

As previously reported, all participants appeared to believe the Chatroom task was real. Furthermore, all of the participants were able to perform the reappraisal training and each participant was able to generate at least one cognitive reappraisal strategy used. Therefore, data from all 30 participants were included in the analysis. See Chapter 4 (Section 4.2.3) for a description

		Group mean (sd)	Patient mean (sd)	Control mean (sd)
Negative mood	Pre-reappraisal	4.3 (2.0)	4.8 (1.8)	4.1 (2.2)
	Post-reappraisal	2.5 (1.9)	3.0 (2.0)	2.0 (1.8)
Belief in negative thoughts	Pre-reappraisal	55.6 (27.2)	66.7 (29.2)	46.2 (22.4)
	Post-reappraisal	29.3 (22.3)	38.3 (26.8)	23.1 (16.9)

Table 6.1: Effect of reappraisal training on rejection-related negative mood and belief in negative thoughts

of the manipulation check.

6.3.1 Behavioural effects of reappraisal

Table 6.1 describes behavioural data from phase 4, describing retrospective reports of rejection-related negative mood and belief in negative thoughts, before and after training. All measures were distributed normally. A repeated measures ANOVA revealed a significant reduction in negative mood following reappraisal ($F_{1,26} = 31.7, p = 0.001$) but no interaction with group ($F_{1,26} = 0.1, p = 0.8$). Similarly, there was a significant reduction in participants' belief in negative thoughts following reappraisal ($F_{1,20} = 22.7, p = 0.001$) but no interaction with group ($F_{1,20} = 0.05, p = 0.8$).

6.3.2 Neural correlates of reappraisal

Reappraisal versus attend

The hypothesis that regions of the PFC and insula would be engaged during cognitive reappraisal was tested by analysing mean activation across the whole sample to reappraisal (versus attend) trials. Uncorrected analysis identified one cluster in the right frontal pole (BA9/BA10; 199 voxels; Z value=3.84, x=20, y=64, z=18; see Figure 6.2), although this cluster did not survive corrected analysis. A number of other small clusters were engaged during reappraisal (compared to attend) (see Figure 6.2), including one in

the left frontal pole (51 voxels; Z value=3.00; x=-10, y=64, z=12) and one in the left insula (11 voxels; Z value=2.87; x=-28, y=22, z=-2).

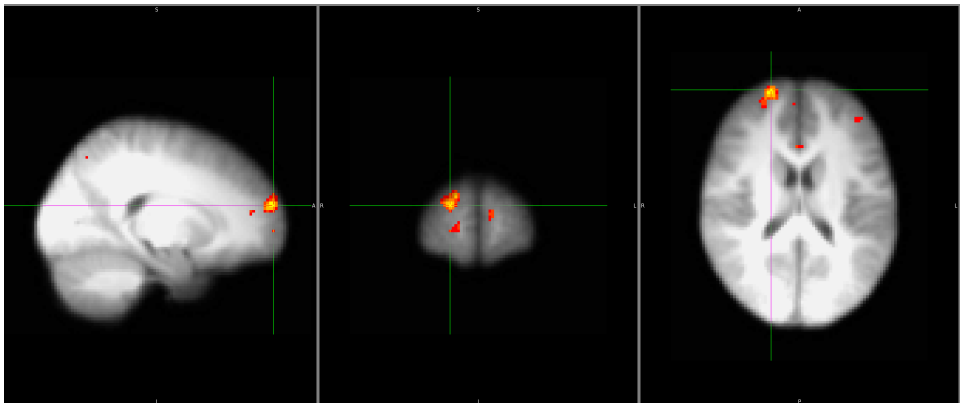

Figure 6.2: Right frontal pole activity in whole-brain analysis of reappraisal (versus attend) trials. Images thresholded at Z>2.3, p<0.05, uncorrected.

Percent BOLD signal change from the cluster in the right frontal pole was extracted for reappraise (versus baseline) and attend (versus baseline) to investigate any between-group differences. T-tests revealed that neural activity in the right frontal pole during reappraise was significantly less in patients (mean=0.12, sd=0.05) compared to controls (mean=0.24, sd=0.06; $t_{28} = 5.95, p < 0.0001$). During attend trials, percent signal change was negative in controls (mean=-0.09, sd=0.03), but significantly more negative in patients (mean=-0.2, sd=0.03; $t_{28} = 10.04, p < 0.0001$; Figure 6.3). However, a repeated measures ANOVA with group as a between subjects variable (patient, control) and % signal change in each condition (reappraise, attend) failed to find an interaction between group and condition ($F_{1,28} = 0.01, p = ns$).

There was no evidence that neural activity in this region of the right frontal pole during reappraisal correlated with change in negative mood following reappraisal (Pearson's $R = 0.02, p = ns$), or change in negative beliefs (Pearson's $R = 0.07, p = ns$). Neither did they correlate with pre-

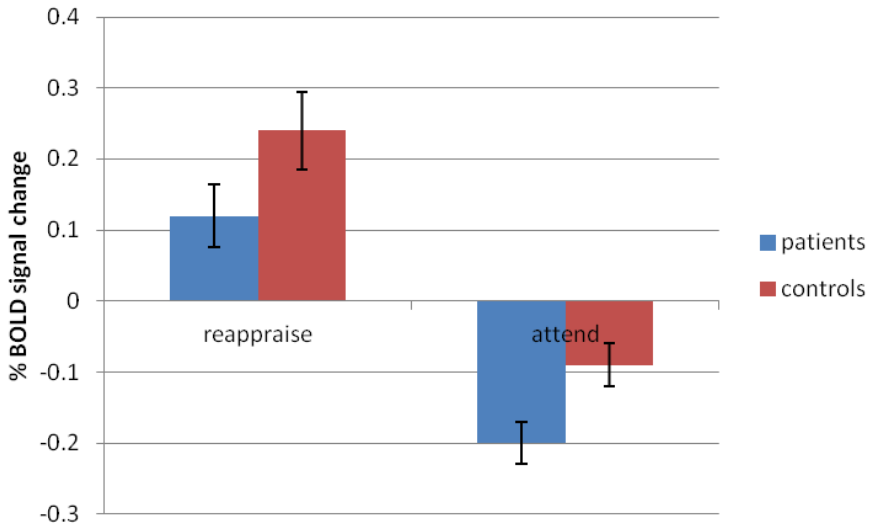

Figure 6.3: Between-group differences in right frontal pole activity during reappraise and attend trials

reappraisal negative mood (Pearson's $R = -0.27, p = ns$) or negative beliefs (Pearson's $R = -0.20, p = ns$), or post-reappraisal mood (Pearson's $R = -0.26, p = ns$) or negative beliefs (Pearson's $R = -0.23, p = ns$).

In order to explore regions where neural activity was heightened during attend trials, mean activation during attend (versus reappraise) across the whole sample was analysed. At the corrected level a large cluster of activation in the occipital lobe was identified (1407 voxels; Z value=3.92; x=2, y=-80, z=32). Since this region has not been associated with vulnerability for depression, and was not hypothesised to differ between patients and controls, between-groups analysis of activity in this region was not explored.

6.4 Discussion

6.4.1 Summary of findings

Across the sample, reappraisal improved rejection-related negative mood and belief in negative thoughts. There was no evidence that this varied between patients and controls. Analysing the neural correlates of reappraisal (versus non-regulation) revealed engagement of the right frontal pole (BA9/BA10 regions). Neural activity in this region was significantly less in patients (compared to controls) during reappraisal. During attend trials, patients showed significantly more deactivation of the right frontal pole compared to controls. Across the sample, activity in the right frontal pole was not associated with retrospective reports of change in negative mood or belief in negative thoughts.

6.4.2 Interpretations

The present study sought to investigate whether the relationship between peer rejection and adolescent depression might be driven by emotion dysregulation (Figure 1.1B). Whereas a previous chapter tested whether depressed adolescents show heightened emotional reactivity to peer rejection (Chapter 5), the current study concerned explicit (conscious) emotion regulation of peer rejection. Explicit emotion regulation is the process of using effortful control to initiate, monitor and reflect on changes in emotional state. Studies of adults suggest that depressed adults may use explicit emotion regulation less often than non-depressed adults, although experimental studies suggest that when instructed, depressed adults are just as able to regulate their emotional response to negative stimuli as are non-depressed adults.

Although depressed adolescents may also use less cognitive emotion reg-

ulation strategies than non-depressed adolescents, few studies have investigated whether adolescents are able to effectively regulate negative emotional responses. Ongoing development of the brain regions which underlie emotion regulation may render adolescents less capable of using cognitive emotion regulation strategies. However, data from the current study suggest that both depressed and non-depressed adolescents were successful at down-regulating their negative responses to peer rejection (though I do not have adult data to compare this with). These data replicate findings from other studies of emotion regulation in typically developing paediatric samples. Furthermore, they demonstrate that adolescents are able to regulate their emotional response to peer rejection specifically. This is the first study to measure emotion regulation in response to a relatively complex social situation. As highlighted by Ochsner and colleagues, studies of simple emotional stimuli lack ecological validity. Together, the findings provide support for the use of cognitive reappraisal of peer rejection in adolescents. Although these results are promising, some limitations of the measures of reappraisal success are first worth noting.

Firstly, data were collected retrospectively, once participants were out of the scanner. A limitation of retrospective self-reports is that they are less reliable than concurrent data collection. Previous studies of explicit emotion regulation in adolescent samples have measured affect during the reappraisal paradigm [213, 238, 282], I had concerns that this could lead to demand characteristics, distract from the main reappraisal task and increased length of time needed to collect MRI data. A recent study of the neural correlates of peer rejection also used a retrospective measure to assess distress [315]. Another limitation of the current study was that reappraisal training administered before scanning may have affected responses during

the attend block. Since I have no behavioural measures of whether participants were simply looking at the emotional stimuli, versus reappraising, interpretations about the validity of the reappraisal and control events are limited.

The second main finding was that the right frontal pole was engaged when adolescents engaged in reappraisal of rejection, compared to simply attending to rejection. The heightened activity of the frontal pole in reappraisal replicates findings from adult [270] and adolescent [213, 238, 282] studies of emotion regulation, although in this study it should be interpreted with caution since activity in this region did not survive corrected thresholding.

There were other regions which I predicted to be involved in emotion regulation which were not. These include the VLPFC, which I demonstrated was engaged when adolescents viewed peer rejection (Chapter 5), and affective regions such as the dACC, insula, and amygdala. Based on the adult literature I would have expected reappraisal to down-regulate activity in these affective regions. Although the amygdala is the most common region to be modulated by reappraisal [270], the previous chapter demonstrated that peer rejection failed to elicit amygdala activity during the receipt of rejection. This has been attributed to issues associated with the Chatroom paradigm. Therefore it is likely that when reviewing previously received rejection, insufficient amygdala activity was engaged to be able to demonstrate down-regulation of this region during reappraisal. The fact that amygdala activity was not seen during the attend trials also supports this explanation.

A third finding was that despite a lack of behavioural differences between depressed and non-depressed adolescents in the effects of reappraisal on negative emotional responses to rejection, fMRI data identified subtle neural

differences between depressed and non-depressed adolescents. Specifically, the increase in activity in the right frontal pole was greater in controls, compared to patients, although this difference was not apparent at the whole-brain level. Numerous adult studies have suggested that neural differences during emotion regulation may persist in the absence of behavioural effects [24, 102, 158, 170]. However, this is the first study to investigate neural differences during emotion regulation between depressed and non-depressed adolescents. Although the finding that depressed adolescents show less engagement of the right frontal pole during emotion regulation makes intuitive sense, given that they are expected to be less effective in emotion regulation, it contrasts with previous studies of emotion regulation in depressed adults. For example, Johnstone and colleagues (2007) found that depressed adolescents showed heightened activity in the right PFC during reappraisal, compared to healthy controls [170]. Interpreting the direction of effects in neuroimaging studies of emotional processing is notoriously difficult, because it often depends on reverse inferencing and it is not clear whether hyperactivation represents increased effort, or ease, of processing. Finally, since depressed adolescents also showed reduced activity during the attend block (compared to healthy adolescents), the extent to which blunted responses can be attributed to group differences during reappraisal, rather than differences in emotional processing per se, or due to patients having lower baseline BOLD signal than controls, is limited.

6.4.3 Strengths and limitations

Some strengths and limitations of the study methodology are worth mentioning. Firstly, although a pilot study demonstrated that the reappraisal training developed for the current study was effective (Appendix C), and

preliminary behavioural data support this, the effectiveness of the training for depressed and non-depressed adolescents has not been rigorously tested. Other reappraisal strategies reported in the literature have been repeatedly used and demonstrate efficacy in multiple samples (e.g. Ochsner et al., 2004 [269]). The reappraisal strategy reported here provides a unique addition to the reappraisal literature, because it is the first to train emotion regulation of peer rejection specifically and in adolescents with psychopathology. Indeed, the need for reappraisal strategies to target responses to specific social emotional stimuli is echoed by [270] and [238]. However, further evaluation and development of the current reappraisal training script could be extremely useful for future studies of adolescent emotion regulation.

The study was designed to maximise statistical power. Given that this was the first study to test the neural correlates of reappraisal training in adolescents with depression, it was unclear what the strength of the neural activation during reappraise and attend trials would be. Therefore a block of control trials preceded the block of regulation trials. However, future studies should consider interspersing reappraisal and control trials in order to control for the effects of fatigue and practice on behavioural and neural responses. Indeed, post-hoc exploration of neural data during the pre-attend and pre-reappraisal trials (which theoretically should involve identical review of rejection) identified distinct differences. Specifically, whilst pre-attend trials were associated with similar neural activation to that found during rejection in phase 3, pre-reappraisal trials showed minimal neural activation, compared to baseline. Speculatively, this could be because during the second block of trials the effects of rejection on neural activity were less, and therefore that reappraisal had a different effect than it would have done if administered in the first block.

Finally, the depressed adolescents included in the current study were recruited from clinical settings, as well as the community. There was no evidence that participants from the two setting differed in symptom severity (Chapter 4, Section 4.2). However, patients had more experience of psychological therapy than depressed adolescents recruited from the community (Chapter 4, Section 4.2). The heterogeneity of the sample is a somewhat inevitable limitation of clinical studies in adolescent populations. Nevertheless, this may have had implications for the findings of this phase of the MRI study in particular. One possibility is that patients were more effective in using the reappraisal strategy than depressed adolescents recruited from the community because they had more experience of cognitive bias modification. I was unable to test this directly due to the small number of depressed participants recruited from the community (N=4), and a lack of detail about the nature of treatment administered to patients. Although differences in experience of psychological therapy varied significantly between the patient and control group, there was no evidence that this influenced self-report ratings of reappraisal effectiveness. Furthermore, the fact that the groups were evenly matched for IQ suggests that differences in concentration and task perseveration which might have varied between the two groups, were unlikely to confound the results.

6.4.4 Future studies

The previous chapter demonstrated the salience of peer acceptance in adolescence, and for depressed adolescents specifically. Contrary to predictions, depressed adolescents showed more enjoyment from peer acceptance than non-depressed adolescents. Studies of emotion regulation of peer acceptance may help to elucidate whether depressed adolescents are more proficient in

up-regulating regions associated with positive mood states. Indeed, a study of adults probed the neural correlates of positive emotion regulation in depressed adults [158]. Although depressed adults showed similar patterns of heightened activity in the NA to non-depressed adults, this activity was not sustained in a second block of emotion regulation. The extent to which positive emotional responses to peer acceptance can be sustained by depressed adolescents is a worthwhile avenue of future research.

The previous chapters also highlighted the moderating role peer desirability on behavioural and neural responses during anticipation and receipt of peer feedback. In the current study it was not possible to investigate this, since peer desirability was not controlled between the two conditions. Previous studies of emotion regulation in adolescents have not included the salience of emotional stimuli in their analysis, therefore predictions about the direction of effects are difficult to make. One possibility is that across all participants, rejection from desirable peers may result in greater engagement in cognitive reappraisal because of the value of being liked by these peers. An alternative, but not mutually exclusive possibility, is that emotion regulation of rejection from desirable peers may be more difficult if these peers elicit a stronger affective response compared to undesirable peers. These speculations would be worthy of investigation in future studies of emotion regulation of adolescent peer rejection.

6.4.5 Summary

The present study addresses several limitations in the existing cognitive reappraisal literature. It is the first to examine the behavioural and neural correlates of a cognitive reappraisal strategy for emotion regulation related to peer rejection. Furthermore, it is the first study to examine cognitive

reappraisal in depressed adolescents. Preliminary data suggest that the cognitive reappraisal training is effective in reducing negative mood and belief in negative thoughts, and that reappraisal is associated with brain frontal regions, possibly to a greater extent in patients compared to controls. However, replications with changes to the study design could provide more robust data.

Conclusion

Adolescence is a vulnerable time for depression. One-year point prevalence rates rise dramatically around the age of 15 [148], which may account for the fact that suicide is the leading cause of death in adolescence [31, 131]. However, models of adolescent depression lag behind those of adults.

Adolescence is by definition a time of immense biological and social change. Social upheaval during adolescence [43, 74, 199, 240, 341] and these changes may precipitate the onset of mood disorders. Indeed, psychosocial stress is a powerful predictor of adolescent depression [350] and peer rejection may be a particularly salient source of stress during adolescence. At the same time, the brain undergoes substantial structural and functional development during adolescence, particularly in regions associated with social emotional processing [34]. Numerous models propose that imbalances between heightened affective regions, perhaps driven by a surge in gonadal hormones during adolescence [256], and immature regulatory regions underlie adolescent depression [58, 270].

This thesis has been an attempt to investigate how biological and social changes during adolescence may compound each other in the development of adolescent depression. The concept that neural factors are correlated with behavioural susceptibility to the effects of psychosocial stress (and peer rejection in particular) is not new. Existing data suggest that the development

of the brain during adolescence may explain why peer rejection elicits greater distress in typically developing adolescents, compared to adults or children [315]. However, what remains relatively under-investigated, is the extent to which biological and psychological factors may explain why some adolescents are more vulnerable to the effects of peer rejection than others.

In Chapter 1 I proposed a framework for investigating the role of peer rejection in adolescent depression. I summarised complementary evidence from longitudinal and experimental studies which support a bi-directional relationship between peer rejection and adolescent depression (Figure 1.1A). For example, longitudinal studies suggest that peer rejection predicts later symptoms of depression and experimental studies confirm that peer rejection is causally associated with negative emotional responses. In addition, depressed adolescents are more often rejected than non-depressed adolescents. Across five empirical chapters, I investigated how biological and psychological factors might explain the destructive cycle between peer rejection and adolescent depression (Figure 1.1B).

In Chapter 2 I explored the extent to which genetic factors may explain the negative impact of psychosocial stress on adolescent depression. Specifically, I tested whether the 5HTTLPR polymorphism might modify the effects of socioeconomic status on adolescent mood disorder. I found that the high expressing 5HTTLPR genotype modified the effects of high SES on symptoms of anxiety (but not depression) in patients diagnosed with a mood disorder. Whilst this study provided preliminary support for the moderating role of biological factors in explaining the relationship between psychosocial stress and adolescent mood disorder, it also highlighted the limitations of molecular genetic approaches to understanding adolescent depression. Because large sample sizes are needed to observe significant

effects, relatively specific or complex stressors (such as peer rejection) are more difficult to measure than more general stressors such as SES. Recruiting adolescent samples can be challenging enough, but is made even greater when adolescents with a clinical diagnosis of depression are required. As such, studies such as this one often involve pooling data from adolescents with anxiety as well as depression. Moreover molecular genetic studies have been plagued with conflicting findings, with a large risk of finding false positive results.

In Chapter 3 I reviewed how underlying cognitive factors may explain why some adolescents are more vulnerable to the effects of peer rejection than others. I reviewed studies which suggest that negative interpretations and maladaptive attributional styles may modify the effects of peer rejection on adolescent depression. Whilst adult models of depression suggest that negative attentional biases may also contribute to depression, few studies have investigated this possibility in adolescent depression. I demonstrated that adolescents with low self-esteem and more symptoms of depression show evidence of an attention bias towards social threat (rejecting faces), compared to adolescents with high self-esteem and fewer symptoms of depression. Contrary to adult studies of depression, an attention bias was seen at a relatively short stimulus exposure (500ms). Using a novel cognitive bias modification task I failed to find any evidence that these biases were causally related to negative mood states. One possibility is that negative biases exert their influence by moderating the effects of psychosocial stress (such as peer rejection) on depression. However, adult data suggest that the positive effects of CBM-A training on mood require greater numbers of trials and sessions than I used. Future studies which investigate the effects of manipulating CBM-A task parameters on training effects (e.g. number

of trials, nature of stimuli) may be useful. Nevertheless, this study lays the foundations for future studies of the moderating role of negative attention biases in peer rejection and adolescent depression.

A final set of neuroimaging studies investigated the neural correlates of peer rejection in clinically depressed and psychiatrically healthy adolescents, using the Chatroom paradigm [141]. In Chapter 4 I speculated that negative expectations of peer feedback may explain why depressed adolescents are exposed to more peer rejection experiences. I investigated the possibility that depressed adolescents hold more negative expectations of peer feedback than non-depressed adolescents, and that functional neural differences in emotional processing brain regions may underlie this effect. During fMRI acquisition, participants predicted how interested 60 peers would be in talking to them in an online chatroom. Depressed adolescents expected peers to be less interested in chatting to them than healthy controls. Furthermore, compared to controls, depressed adolescents showed heightened neural activity in the right caudate, a region engaged during anticipation of peer feedback which was independent of peer desirability. Although these data do not speak to the causal role of negative expectations in explaining depressed adolescents' peer rejection experiences, they tentatively suggest subtle cognitive and neural substrates which characterise the way that depressed adolescents anticipate peer rejection. Across the whole sample, neural activity in regions associated with emotional processing such as the ACC and IFG, correlated with peer desirability during anticipation of feedback. This supports previous data which highlight the importance of considering peer salience when investigating adolescent social interactions.

The possibility that depressed adolescents experience heightened emotional reactivity during the receipt of peer rejection was investigated in

Chapter 5. In the same scan session reported above, depressed and non-depressed adolescents received positive and negative feedback from fictitious peers, and rated their affective response to each peer's feedback. Contrary to expectations, depressed adolescents did not report significantly more negative affect when rejected by peers, than non-depressed adolescents. Peer rejection elicited neural activity in regions such as the insula, VLPFC and frontal poles, associated with emotional reactivity, in all participants. However, there was no evidence that this differed between depressed and non-depressed adolescents. Although behavioural reports suggested that depressed adolescents experienced heightened emotional reactivity to peer acceptance, this was not reflected in the neuroimaging data. Furthermore, given the inconsistency of this finding with the existing literature on positive emotional reactivity in depression, this effect is cautiously interpreted. Overall, the findings from this study suggest that individual differences in response to peer rejection are unlikely to be explained by heightened emotional reactivity.

A final study investigated whether the negative impact of peer rejection on adolescent depression might be explained by impaired emotion regulation (Chapter 6). In contrast to emotional reactivity, emotion regulation is the strategic and explicit modification of an emotional experience [143]. Although depressed adolescents are thought to use emotional regulation strategies less effectively than non-depressed adolescents, no study to date has investigated group differences during instructed use of emotion regulation, or the neural correlates of emotion regulation in depressed adolescents. During a final stage of MRI acquisition, depressed and non-depressed adolescents used cognitive reappraisal to down-regulate negative responses to peer rejection. Behavioural data suggested that all participants were successfully able

to down-regulate negative affect, although there was no evidence that this differed between depressed and non-depressed adolescents. Neuroimaging data suggested heightened activity in the right frontal pole when participants engaged in reappraisal of rejection (compared to simply attending to rejection). In contrast to previous studies of adults, which have found increased activity in PFC during emotion regulation in depressed (compared to non-depressed) adults, depressed adolescents showed reduced activity in the right frontal pole compared to non-depressed adolescents. These data require further examination, particularly since many depressed adolescents were engaged in psychological therapy at the time of testing, which may have influenced their ability to perform reappraisal. Nevertheless, they demonstrate the efficacy of a novel cognitive reappraisal strategy in modifying adolescents' behavioural and neural responses to peer rejection.

This thesis demonstrates the benefit of peer rejection paradigms for investigating adolescent psychopathology. Although the complexity of peer rejection is simplified in these studies [185], they provide the opportunity to standardise peer rejection experiences, thus minimising the effect of confounding variables on negative emotional responses. A recently modified version of the Chatroom task ('Chatroom Interact') exposes participants to rejection and acceptance through simulated online interaction, thus combining the dynamic nature of rejection addressed by Cyberball, and the personal and adolescent-relevant nature of Chatroom [328]. Developments in peer rejection tasks such as this may go a long way towards heightening the ecological validity of experimental paradigms, although prospective studies should continue to investigate the influence of peer rejection experiences on clinically significant depression.

This thesis demonstrates the value of using differing methodological ap-

proaches to understand adolescent psychopathology. Molecular genetic approaches offer insights into the heritability of adolescent depression, but are limited in their ability to measure the effects of specific social stressors, such as peer rejection, on behavioural outcomes. Future molecular genetic studies might consider whether the 5HTTLPR polymorphism modifies less distal outcome measures, such as neural responses to peer rejection. A study by Gregory and colleagues reports that genetic factors may have a relatively small effect on interpersonal cognitive vulnerability for depression [132], although this study was of pre-adolescent children rather than adolescents. Similarly, biological measures may be better able to detect subtle effects of emotion processing than behavioural or self-report measures. Particularly in CBM-A studies, where cognitive training targets fleeting processes, psychophysiological measures may be better able to disentangle the processes such as engagement and disengagement.

A final area for future research is to consider how the effect of peer rejection on depressive symptoms varies across adolescence. Although there is a dramatic rise in the prevalence of depression around the age of 15 [148], the extent to which this reflects the increasing influence negative interpersonal interactions is unknown. Experimental studies of peer rejection in more specific age groups, and studies of the presence of cognitive biases across adolescence may help to elucidate this issue.

In my fMRI studies I identified differences between depressed and non-depressed adolescents in their responses during the anticipation of feedback, and reappraisal of feedback, but not during the actual receipt of peer rejection. In contrast, data from anxious adolescents suggests that they do show heightened emotional reactivity immediately following negative peer feedback [200]. These data fit with models which suggest that whilst anxi-

ety is characterised by heightened stress reactivity, depression may be more associated with strategic or elaborative cognitive biases, although it was not possible to completely separate the effects of anxious and depressive symptoms in my fMRI studies. Indeed, disentangling the effects of anxious and depressive symptomology was also a challenge in my molecular genetic study. Adolescent depression and anxiety show strong comorbidity and identifying depressed adolescents without anxious symptoms is difficult. Although there are statistical methods for partialling out the effects of anxiety, more homogenous samples of depressed adolescents may inform the extent to which peer rejection experiences contribute to depression specifically.

The data reported in this thesis, which suggest specific genetic, cognitive and neural differences between depressed and non-depressed adolescents may have implications for the treatment and prevention of adolescent depression. Given the infancy of research into the role of peer rejection in adolescent depression, one has to be cautious about such implications. Nevertheless, future studies which test the extent to which genetic, cognitive and neural factors moderate depressive responses to peer rejection may provide insights into how adolescent treatment can be improved. The finding that depressed adolescents anticipate more negative feedback from peers than non-depressed adolescents may be of particular interest, since it not only explains why rejection elicits negative emotional responses, but potentially explains why depressed adolescents are then exposed to more rejection experiences. This possibility requires further investigation, for which CBM paradigms reported in Chapter 3 may be useful. The findings of a neuroimaging study of cognitive reappraisal (reported in Chapter 6) also provide preliminary evidence to suggest that depressed adolescents are able to successfully down-regulate negative emotional responses to peer rejection.

Figure 6.4 illustrates how CBM and cognitive reappraisal paradigms may provide useful tools for reducing symptoms of depression that result from peer rejection.

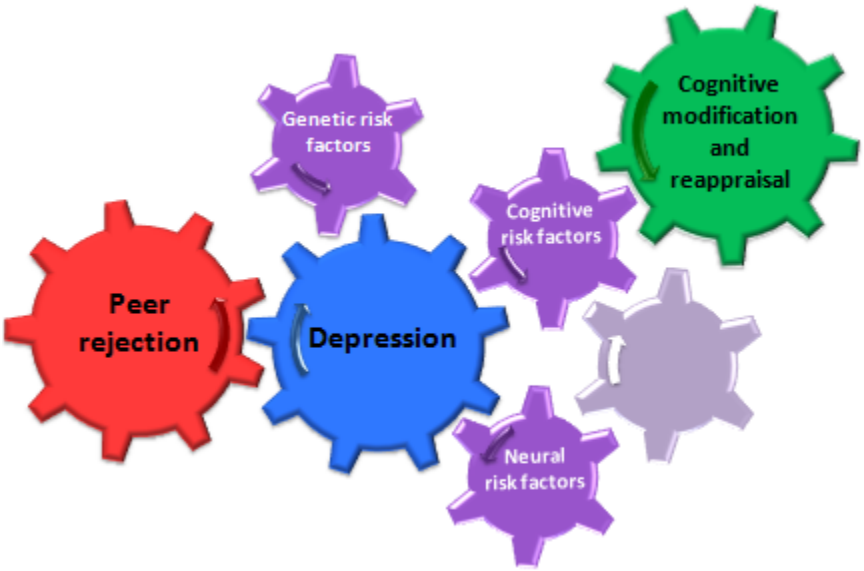

Figure 6.4: Paradigms that target cognitive processes may reduce depressive symptoms which result from peer rejection, alter neural processing, and reduce future peer rejection experiences

In summary, peer rejection is a salient source of stress during adolescence, which may explain why rates of depression increase during this period. This thesis contributes to models of adolescent depression, by outlining genetic, cognitive and neural factors which are characteristic of adolescent depression, and pending further investigation, may modify the effects of peer rejection on adolescent depression.

Appendices

Appendix A: FMRI study debrief

- We mentioned before that you might be chatting to these children in the chat room but actually that's not going to happen now, this is the end of the experiment. We will tell you a bit more about the study in a moment, but firstly, how did you find it?

- As I mentioned before, the purpose of the task was to examine how we form impressions of other people and how we socialise in online environments. We told you that you that this study would be about how young people meet and chat in online chat rooms but in fact, the photos were of child actors from the USA and there is no online chat room. The other children never saw your photo and the computer decided at random whether each child would be 'interested' or 'not interested' in chatting with you.

- We told you that this was an online chat room because people often react differently if they think they are being asked about a fake situation.

- We are interested in how it makes young people feel when they are told that someone else is interested in chatting to them or not. By

doing this experiment in the scanner we can also see how your brain reacts to these situations.

- We have designed these tasks so that they will be helpful to others in the future, and in order for us to do this, we needed you to believe that these were real people you were watching.
- We wanted to tell you about this immediately, and this is the only task that involved deception.
- Do you have any questions or worries following the task?
- If you find this task upsetting, it is important that you let us know by ringing, emailing or writing to us. We would like to do what we can to make sure you are comfortable with everything we've done today.

Appendix B: Reappraisal training

- Over the next fifteen minutes we are going to explain a new skill to you, and then get you to have a few practices at trying the new skill out.
- When we are in different situations we have different THOUGHTS going through our minds, in other words we tend to say different things to ourselves. Different THOUGHTS can make us have different FEELINGS. Some thoughts can make us feel positive, and some thoughts can make us feel negative.
- An example is if we look out of the window and see it's pouring with rain we might think or say to ourselves: "Oh no it's raining outside I won't be able to wear my new shoes" Having this THOUGHT, or saying this to ourselves might make us FEEL sad.
- If we have a negative thought like this then we tend to feel negative. Thoughts that make us feel negative, like sad or angry, can be called UNHELPFUL thoughts. Does that make sense?
- Suppose you look out of the window and see it's pouring with rain, BUT this time you think. "Oh good I get to wear my brand new wellies". How would you feel then? Prompt: Happy, excited. Ok, so you'd feel positive?
- This is because this time you have a more HELPFUL thought. HELPFUL thoughts make you feel positive, like happy or excited. They can also make bad things seem better, or less bad. Does that make sense?
- The new skill we are going to teach you is how to change UNHELPFUL thoughts into more HELPFUL thoughts. Does that make sense?

- Example: **Scenario One**. Imagine you are at school. You have heard that the most popular girl in your year is having a party at the weekend. You see the girl and some of her friends talking about the party in the playground. As you walk over to them, they laugh and run off.

- Your first thought is an UNHELPFUL thought, you say to yourself "They have just laughed at me and run off, they obviously don't like me". This makes you FEEL sad. This is because that is an UNHELPFUL thought. Does that make sense?

- To turn this UNHELPFUL thought into a more HELPFUL thought you might THINK to yourself "They may not have been laughing at me, but at someone else" "There are lots of other people in the playground they could have been laughing at" "I wasn't that close to them, I don't think they saw me". "Even if they were laughing at me, I'm not bothered". "I don't like them, anyway I don't care what they think"

- If you have any of these more helpful thoughts then you would FEEL better. What do you think? Does that seem to make sense to you?

- Let's practice.

- Practice: **Scenario Two**. Imagine you are sitting at your desk in school and you hear whispering behind you and people laughing. Your first THOUGHT is "People are saying nasty things behind my back, and this makes you feel sad. Would you say this is a helpful or unhelpful thought?

- This an UNHELPFUL thought.

- What could you THINK, or say to yourself to turn this unhelpful thought into a more HELPFUL thought?
- a) Yes, that's a very good idea. Can you think of more things you might think or say to yourself?
- Here are some more suggestions. "They are probably laughing at the teacher because he is wearing yellow trousers" "They may not be laughing at me at all, they could be whispering about anything" "Maybe they are just bored with the lesson" "Even if they are talking about me, I don't care it's stupid to act like that in class" "They're not my sort of people I've got lots of nice friends"
- b) That's a good try, and you might also say, "They are probably laughing at the teacher because he is wearing yellow trousers" "They may not be laughing at me, they could be whispering about anything" "Maybe they are just bored with the lesson" "Even if they are talking about me, I don't care it's stupid to act like that in class" "They're not my sort of people I've got lots of nice friends"
- Ok let's try another one
- Practice: **Scenario Three**. It's break time and a group of people are choosing teams for rounders. You don't get picked until right at the end, even though you are good at rounders. You first thought is an UNHELPFUL thought "They must really hate me"
- How could you turn that UNHELPFUL thought into a more HELPFUL thought?
- a) Yes, that's a very good idea. Can you think of some more things you might think or say to yourself?

- Here are some more suggestions. “Well they didn’t pick me first because I said I didn’t feel like playing” “It’s not important to get picked first, I know people will always include me in the end” “I got picked first last week so it’s good to give other people a chance” “They’re being kind to people who aren’t such good players” “They just picked the people who got there first, and I was late” “Being first isn’t a big deal”

- b) Yes, that’s a good try, and you might also say

- “Well he didn’t pick me first because he chose his best friend first” “You don’t always have to get picked first, I know people will pick me” “I got picked first last week so it’s good to give other people a chance” “They’re being kind to people who aren’t such good players” “They just picked the people who got there first, and I was late” “Being first isn’t a big deal”

- Last time

- Practice: **Scenario four**. You are in a chat room online. You see a picture of an older girl in the chat room who looks really friendly. You send a friend request to them so you can talk to them. It comes back that they have rejected your request. Your first thought is an UNHELPFUL thought “They are not interested in talking to me, they obviously don’t want to be my friend.”

- How would you FEEL if you had that thought?

- How could you turn this UNHELPFUL thought into a more helpful thought?

- a) Yes, that's a very good idea. Can you think of some more things you might think or say to yourself?

- Here are some more suggestions: "Well she was older, she probably wants to talk to people her age" "She doesn't know me so why would she want to talk to me" "She might not have time to talk now" "It doesn't matter its only one person, I can see if anyone else wants to talk" "It's not important, actually looking at her again I am glad she didn't want to talk to me"

- b) Yes that's a good try, and you might also say "Well she was older, she probably wants to talk to people her age" "She doesn't know me so why would she want to talk to me" "She might not have time to talk now"

- Have another try "It doesn't matter its only one person, I can see if anyone else wants to talk" "Its not important, actually looking at her again I am glad she didn't want to talk to me"

- How confident are you from 0% to 100% that you will be able to do use this new skill?

Appendix C: Reappraisal pilot study

The following text has been taken directly from Campbell (2012) [54].

Twenty eight participants (68% female), aged between 12 and 17 years (mean=14.14, SD=1.43) who attended an Oxfordshire school were randomly allocated to the reappraisal or a control training condition. During the control training, participants learnt how to how to convert degrees Fahrenheit to degrees Celsius. The training matched the length and difficulty of reappraisal training but contained no emotional impact.

Training effects were measured through changes on a pre and post interpretation bias measure [360] which assessed whether individuals selected benign or negative interpretations of ambiguous scenarios. Self-reported mood ratings were also taken. The interpretation bias measure presented sixteen ambiguous scenarios which reflected events that commonly occur for young people. Following each scenario participants saw two statements, one reflecting a benign interpretation and one reflecting a negative interpretation. Participants rated these on five point response scales (ranging from 1 = "I would not think that at all" to 5 = "I would immediately think that"). A score was obtained by summing the selected responses for the benign interpretations and the same method was used to obtain a score for the negative interpretations. Eight scenarios were presented prior to training (baseline interpretation measure) and eight were presented after (post-training interpretation measure). Subjective mood ratings were also taken on a 10-point scale, where 1 was the worst they could feel and 10 was the best.

Testing took place in school and participants completed the Children's Depression Inventory [192], the baseline interpretation bias measure and gave a mood rating. Participants were read the reappraisal or control train-

ing script depending on their group allocation. Following the training participants completed the post-interpretation measure and gave another mood rating. Participants were asked if they had any questions or concerns about what they had done and were then thanked for their time. The session took twenty five minutes to complete.

Using skewness and kurtosis there was no evidence to suggest that the data collected from the pilot were not normally distributed. A 2 x 2 x 2 mixed measures ANOVA, with Group (Experimental v Control) as the between-subjects factor and Time (Pre v Post) and Item (Benign v Negative) as the within-subjects factors, found a significant three-way interaction ($F_{1,26}$=6.57, p=0.016). This interaction was decomposed by exploring Time and Item effects in each Group separately. In the reappraisal group a significant 2-way Time-by-Item interaction was found ($F_{1,13}$=16.78, p=0.001) which did not characterise the control training condition ($F_{1,13}$=3.87, p=0.071). Clarified further, those who had received the reappraisal training showed a significant increase in benign interpretations (t_{13}=3.258, p=0.003), as well as a significant decrease in negative interpretations (t_{13}=3.259, p=0.003) from pre to post-training. In contrast, in the control group, no significant changes were found on responses to the benign items of the interpretation measure (t_{13}=0.983, p=0.172), however a reduction in negative interpretations did reach significance (t_{13}=1.770, p=0.05).

A 2 x 2 mixed measures ANOVA with Group (Experimental v Control) as the betweengroup factor and Time (Before v After) as the within-subjects factor was conducted on the mood ratings. A significant 2-way interaction ($F_{1,26}$=7.166, p=0.013) was found. Decomposing this further a significant effect of time was found for the reappraisal group ($F_{1,13}$=13.08, p=0.003)

[1]Pre v post-training means differ significantly $p < 0.003$

	Reappraisal	(n=14)	Control	(n=14)
	Pre	Post	Pre	Post
Benign interpretations	26.07 (5.47)	31.14 (5.59)[1]	28.00 (3.78)	28.79 (4.89)
Negative interpretations	20.29 (3.97)	16.86 (4.50)[1]	21.57 (6.07)	20.00 (6.52)
Mood Rating	7.60 (1.44)	8.50 (1.19)[1]	7.54 (0.99)	7.64 (1.10)

Table 6.2: Mean (standard deviation) of interpretation measures and mood for each group pre and post training from the pilot data

which did not characterise the control group ($F_{1,13}$=0.455, p=0.512). For example, those in the reappraisal group showed a significant improvement in mood from before to after training (t_{13}=3.617, p=0.003). Table 6.2 outlines the change in mean scores on both the interpretation measure and the mood ratings.

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