Early effects of fluoxetine on emotional processing: implications for adolescent depression

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Abstract

Depression in adolescence is a major health problem, associated with poor psychological function and key risk factors both for later illness and suicidal behaviours. The antidepressant fluoxetine is commonly used in this population and it is shown to have a favourable benefit-to-risk profile. However, controversy still exists about the use of antidepressants in young people and there is little research focusing on underlying mechanisms of wanted and unwanted actions in this group.

This doctoral thesis aims to investigate, for the first time, the acute effects of fluoxetine on emotional processing, using a combination of behavioural and neuroimaging techniques. The aim is to achieve a greater understanding of the mechanisms underlying fluoxetine use in depressed adolescents, in light of differences seen in their clinical presentation and response to antidepressant drugs.

In the first study (Chapter Two), a single dose of fluoxetine was shown to decrease the recognition of anger in a sample of young adult volunteers, an effect not previously seen in acute studies of older participants. This effect may be particularly relevant for the treatment of adolescent depression, in which symptoms of anger and irritability are often prominent. Beyond this, fluoxetine was shown to increase the recognition of positive vs. negative facial information, and also exerted an anxiolytic-like influence, eliminating the emotion-potentiated startle effect. However, no influence was seen in measures of attentional vigilance to threat. In an attempt to overcome methodological limitations of this study, a paradigm was developed that is particularly sensitive to the detection of automatic biases towards threatening information (Chapter Three).

Chapter Four describes a neuroimaging study with depressed adolescents, in which a single dose of fluoxetine was found to reduce amygdala activity in response to anger. Early changes in amygdala activity to fear correlated with decreased symptoms of anxiety and depression in the first 7-10 days of treatment.

Chapter Five explores the effects of acute fluoxetine in a sample of high trait anger males. This study replicated the finding that fluoxetine acts to increase the recognition of positive information, whilst showing preliminary evidence for a reduction in attentional vigilance to angry faces.

Overall, fluoxetine was found to decrease the processing of anger across studies. This effect was seen alongside a broader influence on positive vs. negative information and anxiolytic-like properties. Together, these results indicate that fluoxetine has direct effects on processes that are especially relevant to adolescent depression and suggest a potential cognitive mechanism for the efficacy of this particular antidepressant in adolescent patients.

Declaration

I declare that the studies presented in this thesis represent my own work under the supervision of Prof. Catherine Harmer and Dr. Susannah Murphy.

A paper based on Chapter Two has been recently re-submitted to *Psychological Medicine*:

Acute fluoxetine decreases anger processing in young adult volunteers.

Liliana P. Capitão, Susannah E. Murphy, Michael Browning, Philip J. Cowen, Catherine J. Harmer. *Psychological Medicine.*

A paper based on Chapter Three has been published in *Emotion*:

Anxiety increases breakthrough of threat stimuli in Continuous Flash Suppression. Liliana P. Capitão, Stacey J. V. Underdown, Samuel Vile, Eunice Yang, Catherine J. Harmer, Susannah E. Murphy. *Emotion*, in press.

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This thesis is dedicated to all young people who struggle with depression.
May we find a better way to ease your pain.

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ASEC	Antidepressant Side-Effect Checklist
AR	Anger Recall
BDI	Beck Depression Inventory
BIS	Behavioural Impulsiveness Scale
BMI	Body Mass Index
BOLD	Blood-Oxygen-Level Dependent
BPAQ	Buss-Perry Aggression Questionnaire
BR	Binocular Rivalry
CBT	Cognitive Behavioural Therapy
CDI	Child Depression Inventory
CDRS-R	Children Depression Rating Scale Revised
CFS	Continuous flash suppression
CGI	Clinical Global Improvement
DLPFC	Dorsolateral Prefrontal Cortex
ECG	Electrocardiogram
EMG	Electromyography
EPI	Echo Planar Imaging
EPQ	Eysenck Personality Questionnaire
EPST	Emotion-Potentiated Startle Task
FERT	Facial Expression Recognition Task
GSK	GlaxoSmithKline
IFC	Inferior Prefrontal Cortex
IQ	Intelligence Quotient
FDA	Food and Drug Administration
fMRI	functional Magnetic Resonance Imaging
GM	Grey Matter
HTA	High Trait Anger
HRV	Heart Rate Variability
JRQ	Jitteriness Rating Questionnaire
MDD	Major Depressive Disorder
MHRA	Medicines and Healthcare products Regulatory Agency

MRI	Magnetic Resonance Imaging
NART	National Adult Reading Test
NICE	National Institute for Health and Clinical Excellence
OFC	Orbifrontal Cortices
PANAS	Positive And Negative Affective Schedule
PFC	Prefrontal Cortex
rACC	rostral Anterior Cingulate Cortex
ROI	Region Of Interest
RSVP	Rapid Serial Visual Presentation
RT	Reaction Time
SCID	The Structured Clinical Interview for DSM Disorders
SD	Standard Deviation
SEM	Standard Error of the Mean
SIQ-JR	Suicidal Ideation Questionnaire – Junior
sgACC	subgenual Anterior Cingulate Cortex
SMD	Severe Mood Dysregulation
SNRI	Serotonin-Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Re-uptake Inhibitors
STAI	State-Trait Anxiety Inventory
STAXI	State Trait Anger Expression Inventory
TADS	Treatment for Adolescents with Depression Study
TAS	Trait Anger Scale
US	United States
UK	United Kingdom
VAS	Visual Analogue Scales
VIF	Variance Inflation Factor
VLPFC	Ventrolateral Prefrontal Cortex
WM	White Matter
5-HT	Serotonin

Chapter 1:

Research background

1.1 General Introduction

Historically, the existence and treatment of depressive disorders in children and adolescents has been the subject of intense debate. Fifty-years years ago, prevailing views considered that depression in youth was either non-existent (Rie, 1966), not diagnosable using the same criteria as in adults (Glaser, 1967), or simply a transient phenomenon that was developmentally normal and self-limited (Lefkowitz & Burton, 1978).

Progressive advances in the field of Child and Adolescent Psychiatry have demystified these concepts and it is now widely accepted that paediatric depression is a legitimate diagnosis that deserves adequate treatment. Depression in youth is a common disorder, affecting 4-8% of adolescents (Birmaher et al., 1996). It is associated with poor psychological and academic functioning (Weissman et al., 1999) and represents a major risk factor for suicide, the third leading cause of death in adolescents aged 15 to 19 years (Cash & Bridge, 2009). Clinically, paediatric depression shares many of the symptoms that are seen in adulthood (Birmaher et al., 1996; Louters, 2004), but a key difference is that irritability is a cardinal symptom of depression in children and adolescents, but not adults (American Psychiatric Association, 2013).

After paediatric depression gained status as a legitimate psychiatric disorder (during the 1980s and 1990s), psychiatrists were faced with the difficult task of prescribing antidepressant drugs to young people in the absence of adequate knowledge regarding the pharmacokinetic properties of these medications and their risk-to-safety profile when administered in younger age groups (Zito et al., 2003). This knowledge was, at first, extrapolated from adult studies, but efforts were later made to conduct randomised clinical trials with children and adolescents.

With these advances, new debates soon emerged. The best example is the controversy initiated in the early 2000s following a series of studies showing that young people taking selective serotonin re-uptake inhibitors (SSRIs) exhibited more anxiety symptoms and suicidal behaviour than those taking placebo, especially early in treatment (Martinez et al., 2005; Hammad,

Laughren, & Racoosin, 2006). These concerns led to the UK prohibition of all antidepressant drugs in children and adolescents except fluoxetine in 2003, on the basis of evidence that this was the only medication sufficiently safe and efficacious to be used in this population (MHRA, 2003; Whittington et al., 2004). In 2004, a "black-box" warning regarding increased suicide risk was put in place in the US on all antidepressant drugs used to treat paediatric depression. However, of great concern is evidence that suicide rates have risen since then, suggesting that lack of treatment may also pose significant risks in this population and that the use of antidepressants may actually have an overall protective effect (Gibbons et al., 2007; Gibbons, Hur, Brown, Davis, & Mann, 2012).

The issues surrounding the use of antidepressants in youth are also part of a wider debate questioning if the plasticity seen during adolescence represents a window of opportunity for implementing drug interventions that may allow a more efficient recovery, or whether drug exposure in such a sensitive period may have long-lasting and unwanted effects (Andersen & Navalta, 2004). The brain continues to mature during childhood and adolescence into the early years of adulthood, and is therefore particularly susceptible to environmental and pharmacological insults during this sensitive period (Andersen & Teicher, 2008). Pre-clinical animal studies also highlight the variable effects of antidepressants throughout development, with more adverse side-effects seen earlier in life, and more beneficial effects in adulthood (Olivier, Blom, Arentsen & Homberg, 2011). This parallels evidence in humans suggesting that antidepressants have higher efficacy and tolerability in adult vs. young populations (see Garland, Kutcher, & Virani, 2009).

Despite the relatively high prevalence of paediatric depression and its devastating consequences for global functioning (the most serious of which suicide), the neurobiological and neuropsychological effects of antidepressants in children and adolescents remain poorly understood. This thesis uses a combination of neuroimaging and behavioural techniques in order to characterise the effects of acute fluoxetine on emotional processing. The aim is to gain a greater understanding of the mechanisms underlying antidepressant use in youth, in light of differences seen in their clinical presentation and response to antidepressant drugs. It is hoped that this knowledge will assist the development of more effective treatments for paediatric depression.

In the current chapter, the research background of this thesis will be presented in three separate sub-sections. Part A will review the main characteristics of depression in adolescence, the vulnerability associated with this developmental period, and the cognitive and neural

characteristics of depressive states in youth. Part B will provide a summary of the controversies surrounding the use of antidepressants in children and adolescents, as well as reviewing pre-clinical findings on SSRI effects throughout development. Part C will describe an approach that may be useful for understanding the effects of antidepressants in young people - the antidepressant model of drug action -, as well as presenting the specific study hypotheses.

1.2. PART A: Depression in adolescence

1.2.1. Clinical characteristics

The clinical presentation of early-onset Major Depressive Disorder (MDD) is largely similar to the phenomenology of adult MDD. A significant exception is that irritability is included as a cardinal symptom in the diagnosis of depression among children and adolescents, but not adults. Indeed, irritability is estimated to affect one third of depressed youth in community samples (Stringaris, Maughan, Copeland, Costello, & Angold, 2013) and up to 30-85% of clinically depressed populations (Ryan et al., 1987; Strober, Green, & Carlson, 1981).

Paediatric MDD is associated with high rates of comorbid disorders. Anxiety disorders represent the most common comorbid diagnosis, affecting between 20% and 75% of children and adolescents with depression, followed by disruptive disorders (affecting between 10% and 50%) and substance misuse (25%) (for a review see Birmaher et al., 1996).

The average age of onset for paediatric MDD is between 11 and 14 years (Lewinsohn, Rohde, Seeley, & Fischer, 1993) and the median length for an episode is 8 months (Birmaher et al., 2007). Early onset of symptoms, female sex and the presence of guilt and suicidal ideation predict longer episodes (Birmaher et al., 2004; Lewinsohn, Clarke, Seeley, & Rohde, 1994). Although most children and adolescents recover from their first depressive episode, there is an increased likelihood for recurrence (Lewinsohn, Rohde, Klein, & Seeley, 1999), with values reaching 20% to 60% by 1 to 2 years after remission, and 70% after 5 years (Birmaher et al., 2007). The probability of recurrence increases with the number of total episodes, and a substantial percentage of young people with depression will continue to suffer from this disorder during adulthood (Weissman et al., 1999).

1.2.2. Adolescence as a period of vulnerability for depression

Adolescence is a particularly vulnerable time for the development of depression. Hankin and colleagues (1998) reported a dramatic rise in one-year prevalence rates of depression during adolescence, from around 2% in early teenage years (ages 13 to 15), to 15% in middle adolescence (ages 15 to 18). It is also during adolescence that the risk of developing depression rises twofold in females, reaching the same female-to-male ratio of 2:1 reported in adult MDD (Birmaher et al., 1996).

Biological, cognitive and social changes

Substantial biological, cognitive and social changes during adolescence may explain the high incidence of depression in this age period (for a review see Lau, 2013). Adolescence is marked by the onset of puberty, which triggers a rise in hormonal levels and leads to noticeable alterations in physical appearance. The psychological implications of puberty, especially when occurring earlier than the average and in girls, may represent a vulnerability factor for depression (Hyde, Mezulis, & Abramson, 2008). The impact of hormonal changes on brain development and emotional processing has also received recent interest (Goddings, Burnett Heyes, Bird, Viner, & Blakemore, 2012; Spielberg, Olino, Forbes, & Dahl, 2014). The cognitive advances seen during adolescence allow the teenager to think more abstractly and to understand more complex problems about the environment (Marini & Case, 1994), which may also predispose the development of fears and worries (Laugesen, Dugas, & Bukowski, 2003). The teenager's gradual independency from his or her family is an important achievement, but as friendships become more salient, the impact of peer rejection also increases (Platt, Cohen Kadosh, & Lau, 2013; Stroud et al., 2009). These challenges, combined with genetic, environmental and psychological predisposing factors (e.g., Lau & Eley, 2006), place teenagers at increased risk for developing mood disturbances (Weir, Zakama, & Rao, 2012).

Neurobiological changes and associated risk

From a neurobiological perspective, adolescence is characterised by substantial changes in brain development (Giedd & Rapoport, 2010; Gogtay et al., 2004). This process of maturation parallels the social and cognitive advances that characterise this age period.

There is a linear increase in white matter (WM) during the adolescent years and more complex, non-linear and U-shaped alterations in grey matter (GM) (Giedd et al., 1999; Tamnes et al., 2010). These changes are the result of augmented myelination and/or synaptic pruning, which allow for increases in the speed of neutral transmission, higher neural efficiency and, ultimately, more specialised circuits (for review see Giedd, Keshavan, & Paus, 2008). Disturbances in these developmental pathways (such as disorganized or reduced integrity of WM; Serafini et al., 2014) can create a window of vulnerability for the development of depressive states (see Andersen & Teicher, 2008).

Adolescence is also characterised by changes in specific brain regions and in functional interconnectivity. Two heavily studied regions in relation to adolescence are the prefrontal cortex and limbic areas (including the amygdala and hippocampus). The prefrontal cortex is responsible for high-level cognitive abilities, including abstract reasoning, planning and cognitive control (Fuster, 2002). Structural neuroimaging studies suggest that this area undergoes progressive maturation during adolescence (e.g., Gogtay et al., 2004; Sowell et al., 2004), which parallels the cognitive advances seen during the transition between adolescence and adulthood. Changes in interconnectivity between the amygdala and prefrontal cortex also occur throughout development. Gee et al. (2013) suggested that early childhood is characterised by positive amygdala-prefrontal connectivity, which switches to negative functional connectivity during the transition to adolescence. This developmental switch is paralleled by a decline in amygdala activity, and is thought to underline the improvements in emotional regulation seen as a function of age (Gee et al., 2013).

It has been hypothesised that such developmental changes mediate the increased risk for experiencing mood disturbances during adolescence. Adolescents show increased amygdala activity in response to emotional stimuli (Guyer et al., 2008; Hare et al., 2008; Monk et al., 2003), along with decreased activity in the prefrontal cortex (Hare et al., 2008; Monk et al., 2003) when compared to adults. Together, these findings have led to the hypothesis that increased reactivity to emotional events, coupled with the poor regulatory abilities of an immature prefrontal cortex, underlies the increased emotional volatility and poor decision making that are characteristic of adolescence (Casey, Jones, & Somerville, 2011).

Consistent with this notion, traditional models of adolescent depression postulate that the increased vulnerability to MDD during adolescence results from an intensification of this imbalance or mismatch between the prefrontal cortex and limbic regions (e.g., Ernst, Pine, & Hardin, 2006; Nelson, Leibenluft, McClure, & Pine, 2005). According to this model, excessive reactivity of limbic structures - which fails to be down-regulated by an immature prefrontal cortex -, intensifies the negative experiences that are common in adolescence, such as peer rejection. This vulnerability may lead to the development of persistent negative states and ultimately to the development of full-blown depressive disorder, especially in those adolescents with premorbid psychological or genetic risk factors (for a review see Weir, Zakama, & Rao, 2012)

Recently, however, it has been suggested that this dual-system model may be too simplistic to account for the complex neurodevelopmental changes that occur during adolescence and depression more broadly (Pfeifer & Allen, 2012). The prefrontal cortex is not always underactive in healthy adolescents or even in clinical samples (when compared to adults), with both reductions and increases in activation being reported (e.g., Chantiluke et al., 2012; Lau et al., 2011; McRae et al., 2012; Pitskel, Bolling, Kaiser, Crowley, & Pelphrey, 2011; Tao et al., 2012;). Whilst these variations may result from methodological differences between studies, they could also reflect a complex, non-linear, pattern of prefrontal development during the transition from adolescence to adulthood.

1.2.3. Cognitive and emotional processing findings

MDD in childhood and adolescence is characterised by a pattern of negative thinking. Studies of youth with subclinical/clinical depression, or those at increased risk for developing MDD, report a negative bias in measures of emotion-related perception and stimulus appraisal, therefore corroborating the premises of cognitive models of depression, first developed and validated with adults (Beck, 1976). From a developmental perspective, however, these cognitive biases may become more prominent and stable in the transition from childhood to adolescence, possibly due to an increased capacity to think abstractly and reflect about accumulating life experiences (Abela & Hankin, 2008; Lau, 2013).

Depression in adulthood is associated with a bias towards the encoding and retrieval of negative information, as well as a decreased sensitivity towards positive material. Indeed,

depressed individuals show difficulties disengaging from negative cues, as reflected, for instance, by longer staring times at scenes depicting sadness (Eizenman et al. 2003). They also recall more negative self-descriptor words and less positive ones (see Mathews & MacLeod, 2005). This bias in negative processing is also reported in measures of facial expression recognition. Although there is variation in the direction and nature of this bias, consensus exist that depressed patients interpret facial stimuli as (more) negative than healthy controls (Bourke, Douglas, & Porter, 2010). Indeed, Gur et al. (1992) found that depressed patients were more prone than non-depressed controls to misclassify neutral faces as sad, and happy faces as neutral. Leppänen, Milders, Bell, Terriere and Hietanen (2004) found similar effects in a group of depressed patients, as in this study patients misclassified more neutral faces as being sad.

Similarly to what is seen in adulthood, adolescents suffering from depression show a tendency to process information more negatively than healthy controls. The literature is still incipient and the nature of these biases is not always consistent across studies, being sometimes dependent on negative mood inductions. Bishop, Dalgleish and Yule (2004) found that children with high levels of depressive symptoms showed enhanced recall of negative stories (relative to positive) when compared to a low depression group. In addition, Timbremont and Braet (2004) found that currently and remitted depressed youth endorsed more negative self-descriptors than healthy controls, but following a negative mood induction. Similarly, in a study by Kelvin, Goodyer, Teasdale and Brechin (1999), adolescents with high emotionality rated more negative words as self-descriptive after negative/dysphoric, but not after neutral, mood induction. The use of negative mood induction paradigms is especially relevant to measure biases in information processing that may be latent in at-risk individuals or in some depressed populations. Indeed, according to the diathesis-stress hypothesis, stressful situations or events can trigger a pre-existent psychological vulnerability, leading to stronger negative cognitions or reactions (Ingram & Luxton 2005). The cognitive biases seen after such priming procedures may therefore be more robust and more easily manifested.

Studies of attentional biases suggest that depressed youth have negative biases in attention when stimuli are presented at long exposure durations, although these findings are not always replicated. For instance, Joormann, Talbot, and Gotlib (2007) found that girls at familial risk of developing depression showed a bias to selectively attend to negative faces presented for

1500ms, again following a negative mood induction. Interestingly, Hankin, Gibb, Abela and Flory (2010) found that the pattern of attentional biases varied depending on whether depressed youth had comorbid anxiety or not. Purely depressed youth showed a bias to preferentially attend to sad faces, purely anxious youth to angry faces, and those comorbid for depression and anxiety preferentially attended to both. No information was provided regarding medication status. However, a study by Dalgleish et al. (2003) failed to support the presence of attentional biases in a sample of unmedicated depressed youth, even using stimuli presented for a duration as long as 1500ms.

Biases in the recognition of facial expressions have also been explored in young populations, although the number of studies available is still limited. Beek and Dubas (2008) found an association between the severity of subclinical depression and the recognition of anger and happiness in a community sample of children and adolescents. More specifically, higher depressive symptoms were associated with increased perception of anger in highly ambiguous (i.e., low intensity) faces. An inverse effect was found for the recognition of happiness, such that higher depressive symptoms were associated with a decreased perception of happiness in low intensity faces, but only in girls. Similarly, Schepman, Taylor, Collishaw and Fombonne (2012) found a decoding bias effect again in low intensity facial stimuli, but this time towards sadness. Indeed, in this study, adolescents with depression perceived low-intensity faces as sadder than controls. No group differences in the recognition of anger were seen.

Taken together, these findings are consistent with the notion established in the adult literature that depressed individuals are biased towards perceiving emotions negatively (for a review see Lakdawalla, Hankin, & Mermelstein, 2007). These findings corroborate the notion of continuity in the cognitive vulnerability factors associated with depression throughout development, and may illustrate a potential target for treatment across different ages.

1.2.4. Structural and functional brain abnormalities

Magnetic Resonance Imaging studies (MRI) have begun to elucidate the neural correlates of depression in adolescence. There is evidence indicating that depression in this age group is associated with similar structural and functional brain abnormalities as seen in adult MDD, but research in this area is still incipient.

Studies in adult MDD have identified both structural and functional abnormalities in the cortico-limbic circuitry, including the subgenual and rostral anterior cingulate cortex regions (sgACC and rACC), ventromedial (vmPFC), dorsolateral (dPFC) and orbifrontal (OFC) prefrontal cortices, insula, amygdala and hippocampus (for a review see Hamilton et al., 2012). These changes are thought to be linked to the experience of depression or vulnerability to develop this disorder, but moderating variables such as exposure to acute or chronic stress may also play a role. The experience of stress has pronounced effects in brain structure and connectivity (e.g., Chetty et al., 2014), which may be particularly relevant to adult depression given that a longer lifespan is associated with a higher probability to experience stressful events. Nonetheless, these findings have prompted researchers to examine similar structures in younger depressed samples.

Structural brain changes

There is evidence suggesting that the volume of the anterior cingulate cortex (ACC) is reduced in depressed youth compared with nondepressed age-matched controls. Botteron, Raichle, Drevets, Heath and Todd (2002) found a significant volume reduction in the subgenual ACC (sgACC) in young women with early-onset MDD versus healthy controls. Similarly, Boes, McCormick, Coryell and Nopoulos (2008) reported significantly lower ACC volumes in a sample of adolescent males with subclinical depressive symptoms, but in the rostral ACC (rACC). A recent study by Pannekoek and colleagues (2014) also replicated these findings in depressed youth.

Contrary to evidence suggesting a decreased volume in the orbitofrontal cortex (OFC) of depressed vs. nondepressed adults (Bremner et al., 2002; Lacerda et al., 2004; for a review see Drevets, 2007), Chen and colleagues (2008) failed to see significant group differences in either total OFC volume or grey matter OFC volume in medication-naïve depressed adolescents vs. controls.

Consistent with volumetric studies reported in adults (e.g., Sheline, Gado, & Price, 1998; Sheline, Sanghavi, Mintun, & Gado, 1999), there are reports of structural changes in the amygdala and hippocampus of depressed youth. Most findings suggest a decreased volume in the hippocampus (e.g., Caetano et al., 2007; MacMaster et al., 2008; MacMillan et al., 2003) and in the amygdala (Rosso et al., 2005) of depressed adolescents in comparison to healthy controls.

However, some studies failed to report volumetric differences in both these regions (Munn et al., 2007; Rosso et al., 2005).

An important limitation of most volumetric studies is the use of cross-sectional designs, which may not allow an accurate representation of brain volumes in adolescent depression given the dramatic neurodevelopmental changes seen throughout this period. In a recent study, Whittle et al. (2014) tackled this problem by using a longitudinal design. These authors found an association between the onset of depression in adolescence and abnormalities in the growth pattern of change in the hippocampus and amygdala. An attenuated growth of the hippocampus was associated with the onset of depression. Conversely, the pattern of changes in the amygdala was mediated by sex, such that opposing patterns of growth in this structure were both associated with the onset of depression but in different sexes - exaggerated growth of the amygdala was associated with depression in females, whereas the opposite was found in males.

Functional brain changes

The use of functional MRI in paediatric samples is at an early stage, lagging behind the research in adult depressed populations. Existing studies are generally similar to the findings obtained in adult neuroimaging studies, although experiments with young populations are less consistent and the direction of effects is sometimes different from that reported in adults (for a review see Kerestes, Davey, Stephanou, Whittle, & Harrison, 2014).

In line with findings seen in the adult literature, a number of studies report increased amygdala activation in depressed or at-risk adolescents vs. controls. For instance, Roberson-Nay et al. (2006) found heightened left amygdala activation during successful encoding of emotional faces in medication-free depressed adolescents when compared to healthy controls. Similarly, Yang et al. (2010) reported an increased activation of this structure in a sample of unmedicated depressed adolescents without comorbid psychiatric diagnoses (vs. healthy adolescents). It should be noted however, that in this study, the amygdala was hyperactive in response to both positive (happy) and negative (angry and fearful) faces. Interestingly, Monk and colleagues (2008) found a similar pattern in adolescents at high family-risk of depression, when compared to a low-risk group.

However, some studies report the opposite pattern of decreased amygdala activation in paediatric depressed patients compared to controls (Beesdo et al., 2009; Thomas et al., 2001) or

even an absence of group differences (van den Bulk et al., 2014). The reasons for this discrepancy are still unknown, but methodological variations in the tasks used could be responsible for such differences. For example, Beesdo et al. (2009) found that unmedicated adolescents with MDD had lower bilateral activation in the amygdala compared to both healthy and anxious comparison groups, but only in a condition involving passive viewing of fearful (vs. happy) faces. Indeed, when asked to rate the intensity of the emotion displayed in a likert scale, both the MDD and anxious groups showed the expected amygdala hyperactivity in comparison to controls, therefore highlighting the importance of attention-mediated processes in explaining this pattern. However, a separate study by Monk and colleagues (2008) in at-risk adolescents found a pattern of increased activation in a passive viewing condition (vs. low risk controls), although the stimuli used included varying levels of fear intensity, which may have increased the sensitivity of the task to the hyperactive effects of the amygdala in response to this emotion.

Functional studies also reveal changes in prefrontal regions that are important for the cognitive regulation of affective states. Halari and colleagues (2009) found reduced activation in a network of areas including the right dorsolateral (DLPFC) and inferior prefrontal (IFC) cortices in a sample of medication-free depressed adolescents when compared to healthy controls, which is consistent with the pattern of prefrontal hypoactivation reported in the adult literature. However, a study by Tao and colleagues (2012) reported increased activity in the orbitofrontal (OFC) and subgenual anterior cingulate (sgACC) cortices in a sample of depressed patients (vs. controls) before they initiated a course of antidepressant treatment. Increased activation of the sgACC in depressed vs. nondepressed adolescents has also been reported by Yang and colleagues (2009). These variable findings possibly reflect complex developmental trajectories in the prefrontal cortex, therefore highlighting the need to further investigate how depressive states may influence (or be influenced) by functional alterations in this region.

1.2.5. Summary

Paediatric depression is a common disorder that is similar in presentation to adult MDD, although irritability may be a more prominent symptom in children and adolescents. Similarly to adults, depressed youth are biased towards processing information more negatively. This cognitive pattern is associated with volumetric and functional changes in cortico-limbic areas known to be

involved in affective processing and regulation. Hyperactivity in the amygdala towards negative stimuli is often reported, along with changes in the prefrontal cortex, although studies are not always consistent regarding the direction of these effects (for a review see Kerestes et al., 2014).

1.3. Part B: Antidepressant usage in young people

The use of antidepressants in young people has been a topic of intense controversy in the past decade (Licinio & Wong, 2005). This started with a series of concerns expressed by regulatory health agencies both in the UK and US, culminating with all antidepressants except fluoxetine being contra-indicated[1] in the UK in 2003 and a FDA black-box label on all antidepressants regarding suicide risk in 2004. This FDA label stated that antidepressant use in young people aged less than 18 years carried an increased risk of suicidality. In 2006, the FDA extended this warning to young people aged 18 to 25 years. For a timeline of these events see Figure 1.

This section will briefly discuss the evidence leading to those concerns, as well as the implications of the 'black-box claim'. The studies supporting the efficacy of fluoxetine will also be reviewed. Finally, pre-clinical studies examining the effects of antidepressants during different ages periods will be assessed and some hypotheses will be raised regarding why antidepressants may have variable effects across development.

1.3.1. The SSRI controversy

The idea that antidepressant administration may paradoxically increase suicidal ideation is not new. In the early 1990s, anecdotal reports suggested that antidepressant administration could induce serious suicidality in both paediatric and adult patients (King et al., 1991; Teicher, Glod, & Cole, 1990). Subsequent investigations largely dismissed these safety concerns in adults, but fears regarding treatment emergent-suicidality in young people prevailed (see Mann et al., 2006).

During the 1990s, SSRIs were frequently used "off label" to treat paediatric depression (Birmaher, Brent, & Benson, 1998). However, given the limited evidence available, it was unclear whether these drugs were effective and sufficiently safe to be used in youth. Following a request by

[1] It should be noted that despite most antidepressants being contra-indicated for use in young depressed people, psychiatrists can still prescribe them "off-label" when judged clinically appropriate.

the FDA, multiple clinical trials were conducted in order to clearly establish the pharmacokinetic properties of antidepressants and their safety-to-risk profile in children and adolescents (e.g., Emslie et al., 1997; Emslie et al., 2002; Wagner et al. 2003; Wagner, Fisher, Pole, Borve, & Johnson, 2004). This new evidence ultimately led to fluoxetine being the first SSRI approved by the FDA for the treatment of paediatric depression in January of 2003. However, an association between suicidality and paroxetine use emerged in one of these trials, prompting regulatory agencies in the US and UK to further investigate the safety and efficacy of antidepressants in young people.

In 2003, the MHRA concluded that there was insufficient evidence from clinical trials to support the benefits of most SSRIs in treating paediatric depression. Drugs such as paroxetine, sertraline, citalopram, escitalopram and fluovoxamine failed to outweigh the risks of side-effects when used in patients under 18 years of age. Fluoxetine was the only drug found to have a favourable benefit-to-risk profile (MHRA, 2003). These findings were later supported by a meta-analysis of both published and unpublished data (Whittington et al., 2004). In response to these findings, the MHRA decided to contraindicate the use of all antidepressants apart from fluoxetine in the treatment of child and adolescent depression.

In the US, the FDA responded to these suicidality concerns by initiating its own analysis of data from pharmaceutical companies. This included an examination of more than 4540 patients involved in clinical trials, which revealed that antidepressant use was associated with double the risk of suicidality in comparison to placebo (4% vs. 2% respectively) (Hammad et al., 2006). The term suicidality was assessed using spontaneously reported occurrences of suicidal ideation or behaviour, as well measures of suicidal ideation in self-reported scales (although these were less frequently used). Despite the absence of completed suicides in any of the trials, the antidepressant-induced risk was modest but significantly different from placebo. This increased risk was considered a “class” effect, given the limited data available to analyse the risk of individual antidepressants.

The mechanisms underlying this increased suicidality remain elusive, but it has been suggested that the energizing effects of antidepressants may predispose children and adolescents to act on pre-existent suicidal thoughts (for a review see Goodman, Murphy, & Storch, 2007). A related explanation is that young people are more susceptible to the phenomenon of “behavioural

activation" induced by antidepressant use, which is characterized by agitation, anxiety, insomnia, irritability and other signs of increased arousal, often seen early in treatment (e.g., Walkup & Labellarte, 2001). These symptoms, if unrecognised or untreated, could potentially increase the risk for suicidality (Goodman et al., 2007).

Subsequent to this review, in September 2004, the FDA requested a 'black-box' warning to be added to all classes of antidepressants, regarding an elevated risk of suicidality in paediatric patients. The decision to add this label to all antidepressant classes resulted from concerns that those unstudied medications could be associated with a similar suicide risk. Later, the MHRA updated their guidelines regarding the use of antidepressants in children and adolescents, in order to advise against the use of SSRIs in the initial treatment of mild depression. Only if psychological therapy failed should fluoxetine be prescribed. This topic continued to be closely scrutinised by both agencies, and in 2007, the FDA extended the 'black-box' warning to young people up to 25 years-old. The phrasing of the previous warning was also updated, to highlight that depression and other psychiatric disorders are themselves associated with a risk of suicide. From the beginning of these events, the main intention of the FDA was not to completely discourage the use of antidepressants in the treatment of paediatric depression, but instead to raise clinicians' awareness regarding their risks, as well as promote a safe and regular monitoring especially at the beginning of treatment (Hammad et al., 2006). However, the consequences of this warning went beyond the adoption of careful and stricter clinical guidelines, to affect the population more widely.

As expected, there was a natural decline in the number of antidepressant prescriptions after this regulatory decision (Murray, Thompson, Santosh, & Wong, 2005). For instance, Busch, Frank, Martin and Barry (2011) reported a substantial 47% decrease between 2002 and 2006 in the use of antidepressants in a sample of young people aged 5 to 21 years who were new antidepressant users.

Somewhat surprisingly, there was also a reduction in the number of new formal diagnoses of depression given to young samples (Libby et al., 2007; Wijlaars, Nazareth, & Petersen, 2012). However, it should be noted that the number of referrals to psychological therapy for new onset cases of depression increased after the FDA warning (Bridge & Kelleher, 2011).

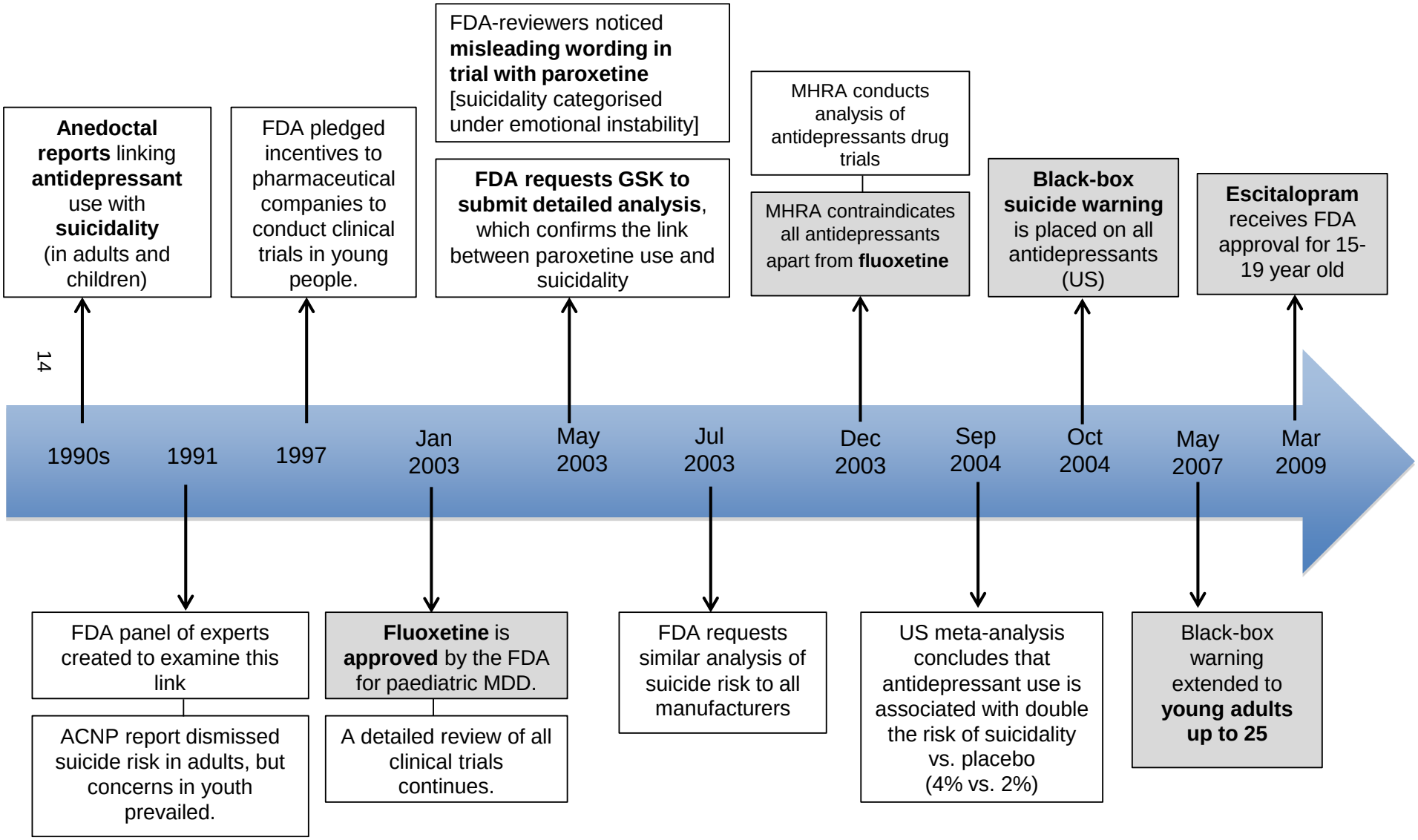

Figure 1. Timeline with the key regulatory events related to suicidality and antidepressant use in young people. Adapted from Licinio & Wong (2005).

Particularly concerning is the possible association between the decreased number of antidepressant prescriptions and increased rates of attempted and completed suicides (Gibbons, Hur, Bhaumik, & Mann, 2006; Gibbons et al., 2007; Lu et al., 2014; Olfson, Shaffer, Marcus, & Greenberg, 2003; Windfuhr et al., 2008). In a critical study, Gibbons et al. (2007) compared the prevalence of suicide in the periods before and after the release of the 'black-box' warning in the US and the Netherlands. The authors reported that the number of completed suicides increased by 14% in the US between 2003 and 2004, and by 22% in the Netherlands between 2003 and 2005, which coincides with the period in which a decrease in the number antidepressant prescriptions was seen. Remarkably, however, an additional study by Wolitzky-Taylor et al. (2010) found that, despite this increase in completed suicides, the frequency of suicidal ideation remained constant (after 2003), therefore highlighting the need for additional studies to investigate the factors predisposing suicide attempts. It should also be noted that the association between decreased antidepressant use and increased suicide rates is only correlational and might also be influenced by moderating variables that were not measured in these studies, such as availability of methods for committing suicide, access to psychological therapies, societal changes, among others.

The SSRI debate continues today and no robust conclusion has been obtained. Some authors have recently claimed that, based on newer findings, the 'black-box' claim should be withdrawn altogether (Gibbons, Hur, Brown, Davis, & Mann, 2012). However, there are others who continue to actively defend this regulatory decision, based on perceived methodological shortcomings in the trials demonstrating that antidepressants have higher benefits than risks (Jureidini et al., 2004; Sparks and Duncan, 2013).

Deciding whether to continue with the 'black-box' warning is an extremely complex topic. Recently, it has been suggested that the risk for self-harm in young people may be higher when SSRIs are prescribed at higher doses (Miller, Swanson, Azrael, Pate, & Stürmer, 2014), a finding that may help deconstruct the phenomenon of treatment-emergent suicidality. Additional studies need to be implemented, and regulatory agencies need to continuously assess the impact of "old" and "new" findings. Important progresses are already being made, including the 2009 FDA's approval of escitalopram for treating adolescent depression. Recent studies also highlight the potential efficacy of sertraline and citalopram in the treatment of adolescent depression (see Garland, Kutcher, & Virani, 2009; Hetrick, McKenzie, Cox, & Merry, 2012). These are key steps

towards broadening the access of young depressed people to additional pharmacological interventions, but much more needs to be done in order to clearly understand the effects of antidepressants in youth.

1.3.2. Developmental SSRIs effects: pre-clinical studies

Pre-clinical studies have proved invaluable in advancing our knowledge about the developmental effects of antidepressants. Overall, studies with rodents and other species show that antidepressants have age-dependent effects. Whilst adult exposure to antidepressants is associated with more consistent beneficial outcomes, administering this drug in earlier stages of development can be detrimental. The scenario is even more complex in adolescence, where both positive and negative findings have been reported (for review see Olivier, Blom, Arentsen, & Homberg, 2011).

In the following sub-sections, the effects of antidepressants across development will be briefly described. Studies using fluoxetine were selected as a model to understand the developmental effects of SSRIs, due to the relevance of this medication in the treatment of adolescent depression. It should also be noted that these studies are divided into chronic and acute administration, in which acute refers to the administration of a single dose of an antidepressant, whereas chronic refers to continued treatment (usually 4 weeks or more).

Adult SSRI exposure

The administration of SSRIs (including fluoxetine) in adulthood is confirmed to be generally beneficial in rodent studies, especially when considering chronic administration. Several experiments suggest that long courses of SSRI exposure produce both antidepressant and anxiolytic-like effects, well in line with the overall efficacy of antidepressants in adult human studies (for a review see Olivier et al., 2011).

For instance, Dulawa, Holick, Gundersen and Hen (2004) found that chronic fluoxetine treatment in mice increased the time spent in the open arms of the elevated plus maze as well food consumption in the hypophagia test, consistent with an anxiolytic effect. Similarly, Huang, Bannerman and Flint (2008) reported an increase in the time that mice spent eating in the predator odor cage test following chronic treatment with fluoxetine. These studies were performed with nonanxious animals, but similar findings have been obtained with anxious samples (David et al.,

2009).

Chronic SSRI administration is also associated with reduced aggressive behaviours. For instance, Wagner, Fisher, Pole, Borve and Johnson (1993) found that chronic treatment with fluoxetine decreased aggressive behaviour in male mice using the social intruder test. This finding was also confirmed in subsequent studies using different species (Perreault, Semsar, & Godwin, 2003). In addition, Datla, Mitra and Bhattacharya (1991) showed that chronic fluoxetine administration attenuated footshock-induced aggression, whereas Berzsenyi, Galateo and Valzelli (1983) and Kostowski, Valzelli, Kozak and Bernasconi (1984) found decreased muricidal (mouse-killing) aggression in rats after chronic fluoxetine treatment.

The acute effects of fluoxetine are not as clear (for review see Borsini, Podhorna, & Marazziti, 2002). Some studies suggest that acute administration of fluoxetine decreases anxiety (e.g., Brocco, Dekeyne, Gobert, Nanteuil, & Millan, 2000; Handley & McBlane, 1992; Nakamura and Kurasawa, 2001), while others show the opposite pattern (Salchner & Singewald, 2006) or absence of effects (Rodrigues-Filho & Takahashi, 1999).

Adolescent SSRI exposure

In mice, adolescence is defined as a distinct developmental period, encompassing post-natal day (P)28 to (P)60 (Spear, 2000; McCutcheon & Marinelly, 2009), therefore allowing researchers to compare the effects of antidepressants in adolescence vs. adulthood. However, antidepressant exposure in adolescence is more difficult to characterise. Pre-clinical studies in this age group have focused on the chronic effects of fluoxetine (in the short- and the long-term), and a mixture of both positive and negative effects is reported.

Studies supporting the adverse effects of fluoxetine show that this medication increases anxiogenic and depressogenic-like behaviours when administered in adolescence. For example, Oh, Zupan, Gross and Toth (2009) found that adolescent mice treated chronically with fluoxetine showed increased anxiety behaviours in the novelty-induced hypophagia test. These effects stopped after discontinuation of the drug. In contrast, Bhansali, Dunning, Singer, David and Schmauss (2007) reported a beneficial effect of chronic fluoxetine, as this SSRI prevented the exacerbation of depressogenic behaviour (as measured using the forced swimming test) following a maternal separation stress. In addition, Iñiguez, Warren and Bolaños-Guzmán (2010) examined

both the short and long-term effects of chronic exposure to fluoxetine in mice and found a mixture of both antidepressant and anxiogenic effects. Exposure to fluoxetine in adolescence was not only associated with a trend for shorter immobility times in the forced swimming test and increased preference to sucrose (suggestive of an antidepressant effect), but also with decreased times spent in the elevated plus maze (consistent with an anxiogenic effect). Importantly, these findings persisted when these same animals were tested later in adulthood.

More recently, Yoo and colleagues (2013) reported differential effects of chronic fluoxetine depending on prior exposure to adversity. In a maternal-separation group, fluoxetine exposure during adolescence reversed some of the depressogenic effects previously seen in the forced swimming test. However, in control adolescent mice, fluoxetine administration increased immobility during the forced swimming test, while decreasing time spent in open arms during the elevated plus maze test (suggestive of both depressogenic and anxiogenic-like effects). Fluoxetine may therefore have more beneficial effects in the presence of prior adversity or depressive-like symptoms, while inducing anxiogenic effects in normally developed mice.

Fetal and neonatal SSRI exposure

A number of pre-clinical studies have focused on the effects of fetal and neonatal SSRI exposure (for a review see Olivier et al., 2013). In line with studies conducted in humans, pre-clinical research shows that rodents administered with fluoxetine during pregnancy deliver smaller pups, which are also slower to gain weight over time (Vorhees, Acuff-Smith, Schilling, Fisher, Moran, & Buelke-Sam, 1994). Behaviourally, rodents exposed to antidepressants soon after birth show increased depressogenic and anxiogenic-like behaviour in adulthood, including increased immobility times in the forced swimming test (e.g., Hansen, Sánchez, & Meyer, 1997) and reduced time spent in the open field (e.g., Ansorge, Zhou, Lira, Hen, & Gingrich, 2004). Interestingly, however, early life fluoxetine exposure has been also associated with decreased aggression (Manhães de Castro et al., 2001) and impulsivity (Lisboa, Oliveira, Costa, Venâncio, & Moreira, 2007) in adulthood.

Mechanisms underlying the age-dependent effects of SSRIs

The studies above clearly demonstrate that antidepressants exert age-dependent effects, with more negative and mixed outcomes apparent early in development, and more beneficial effects seen in adulthood (particularly after chronic exposure). The mechanisms underlying these paradoxical effects are not fully understood, although some studies suggest that they may result from developmental influences on the serotonergic system, which is known to be altered in depression and also represents a major target of many antidepressant drugs (Cowen, 2008).

Beyond its role as a neurotransmitter in the mature brain, serotonin (5-HT) has important neurotrophic actions during many aspects of early neuronal development, including proliferation, migration, cell death and synaptogenesis (Gaspar, Cases, & Maroteaux, 2003). For that reason, administering a drug that could alter the balance of serotonin in the brain during a critical time in maturation could have detrimental consequences for the fine tuning of brain circuits, which are dependent on the preservation of normal neurodevelopmental responses mediated by 5-HT (Homberg, Schubert, & Gaspar, 2010).

The serotonergic system matures early, an aspect that may explain the higher efficacy of drugs acting on this system versus others that act preferentially on systems that mature later, such as the noradrenergic system (Murrin, Sanders, & Bylund, 2007). Indeed, it is well established that tricyclic antidepressants (which are not selective for serotonin, but rather also act on the noradrenergic and dopaminergic systems) are not effective for youth (Hazell, O'Connell, Heathcote, Robertson, & Henry, 1995). It should be noted, however, that some components of the serotonergic system are differently expressed in paediatric vs. adult samples, which may affect the response to SSRI drugs. For instance, compared to adult animals, children and adolescents have increased 5-HT synaptic activity, 5-HT receptors and 5-HTT density (Murrin et al., 2007).

The effects of early changes in the serotonergic system have been studied in animal models in which the serotonin transporter (5-HTT) gene is "inactivated" (5-HTT-/- mice). These animals exhibit high levels of 5-HT and therefore represent a model to explore the effects of enhanced serotonin levels early in development via the administration of antidepressants (Homberg, Schubert, & Gaspar, 2010). 5-HTT-/- mice show morphological abnormalities in pyramidal neurons in the prefrontal cortex and amygdala, and a number of behavioural dysfunctions, such as increased depressive-like behaviours after exposure to stress as well as a

significant deficit in extinction fear recall (Wellman et al., 2007). This demonstrates that early disturbances in the serotonergic system influence the morphological development of areas important for emotional regulation and their associated behavioural functions. While it is not possible to draw direct parallels between this model and the developmental effects seen across human studies, these findings highlight the importance of examining in more detail the involvement of the serotonergic system in normal and abnormal neurodevelopmental processes.

Finally, it should be noted that the regulation and functioning of serotonin is also influenced by genetic variations, such as the serotonin transporter polymorphism in humans. This could predispose the experience of adverse reactions across development, as shown by Kronenberg et al. (2007), in a study in which the short (S) allele of the serotonin transporter gene-linked polymorphic region (5HTTLPR) was associated with a poorer response to antidepressant treatment in youth. Similar findings have been found in adults (Perlis et al., 2003).

1.3.3. Efficacy of fluoxetine in treating paediatric depression

Of all antidepressants, only fluoxetine received FDA and MNRHA approval to treat depression in children and adolescents before or at the time of the regulatory warnings. More recently, escitalopram has also received FDA clearance to treat depression in 12 to 17 year-old adolescents. There are a number of studies investigating the efficacy of fluoxetine in paediatric depression, and these are summarised below.

Review of studies examining the efficacy of fluoxetine in paediatric depression

Emslie and colleagues (1997) conducted the first clinical trial showing higher efficacy of fluoxetine vs. placebo in the treatment of paediatric depression. In this single-site study, 96 children and adolescents were randomised to receive a 20mg daily dose of fluoxetine or placebo for 8 weeks. Fluoxetine was shown to be more effective in the two primary outcome measures used: the Clinical Global Improvement (CGI) scale and Children Depression Rating Scale-Revised (CDRS-R). Indeed, when beneficial clinical response was defined as an improvement rating of 1 or 2 on the CGI scale (much or very much improved), 57% of fluoxetine-treated patients showed improvement in comparison to 33% in the placebo group ($p=0.02$). The weekly ratings of the CDRS-R (which measures depression severity) also revealed significant differences between groups after 5 weeks (39.8 in the fluoxetine group and 46.8 in the placebo, $p=0.03$). However, no

differences were found in any of the self-report measures of depression or in the clinician's ratings of general psychiatric symptoms. When considering the percentage of patients achieving remission, there was a tendency for a higher number of fluoxetine-treated patients to reach the remission criterion (defined as a CDRS-R score of <28), but this difference was not significant (fluoxetine: 31% vs. placebo: 23%). No specific measures of suicidality were reported.

In 2002, Emslie and colleagues ran another (similar) study but this time in 15 trial units. Again, 9 weeks with fluoxetine showed higher efficacy than placebo in alleviating depressive symptoms. More specifically, the active treatment was associated with an increased improvement in the CDRS-R scale in comparison to placebo, a difference already evident after week 1 but which remained constant throughout the study ($p<0.5$). In addition, 52% of the fluoxetine-treated patients (vs. 37% on placebo) were considered much or very much improved according to the CGI scale ($p=0.028$). There was also an increased percentage of remitted patients (defined as a CDRS-R score of <28) in the fluoxetine group vs. placebo (41% vs. 20%; $p<0.01$). The number of adverse events did not seem to differ between groups, although again, no specific measures of suicidality were reported.

One of the most important studies investigating the effects of fluoxetine and psychological therapy in the treatment of paediatric depression was conducted in the US by the Treatment for Adolescents with Depression Study (TADS) team (March et al., 2004). This study was a large multi-site trial, in which 439 participants were randomised to receive one of 4 treatments: (1) fluoxetine monotherapy; (2) placebo; (3) Cognitive Behavioural Therapy (CBT), or (4) fluoxetine plus CBT. The administration of the fluoxetine and placebo as a monotherapy was double-blinded, whilst the remaining conditions were unblinded. Of note, participants at high risk for suicidality or self-harm were excluded. Consistent with other studies, the two main outcome measures for efficacy were the CGI and CDRS-R. Secondary measures included a self-report measure of depression (Reynolds Adolescent Depression Scale) and suicidality (Suicidal Ideation Questionnaire – Junior version, SIQ-JR). Fluoxetine was shown to be superior to placebo based on the CGI (61% vs. 35%; $p=0.001$), but not on the CDRS-R. However, monotherapy with fluoxetine was superior to CBT alone in both these measures (CGI and CDRS-R). Notably, fluoxetine combined with CBT was superior to the other conditions in all clinical measures of depression.

Given these findings, it seems that fluoxetine alone is relatively effective, but not as effective as fluoxetine with CBT.

Suicidality in this study was measured in a particularly robust way. The authors used, for the first time, a self-report measure of suicidal ideation (SIQ-JR). The combination of fluoxetine with CBT was associated with a higher decrease in the severity of suicidal ideation compared to fluoxetine ($p=0.002$) and CBT alone ($p=0.05$). Fluoxetine alone was not significantly different from CBT alone ($p=0.22$) or placebo ($p=0.36$). Therefore, the combination therapy was again the most beneficial treatment.

When considering self-harm related events, the fluoxetine conditions (fluoxetine alone and fluoxetine plus CBT pooled together) were associated with an increased number of events vs. placebo (odds ratio, 2.19; 95% CI, 1.03-4.62). However, when comparing each condition individually against placebo, no differences were seen ($p=0.15$). The pattern was similar for suicide-related events, but these differences did not reach statistical significance. Overall, this statistical analysis was also limited by the low number of actual suicide attempts, which is a common limitation in clinical trials measuring suicidality.

In the UK, a similar trial was conducted in 2007, but using slightly different criteria (Goodyer et al., 2007). The authors did not reject participants on the basis of suicidal ideation risk or self-harm, which was less problematic given the absence of a placebo condition. This decision therefore resulted in a sample with increased suicidality and depression severity in comparison to previous published studies. 208 children and adolescents were randomised to receive either (1) SSRI plus routine care or (2) combined SSRI, routine care and CBT for 12 weeks (plus 16 weeks of maintenance). Most participants received fluoxetine for the duration of the trial (82.2%), whilst the remainder received other SSRIs. In contrast with the findings seen in the TADS study, the combination therapy was not superior to SSRI plus routine care; and SSRI use was not associated with an increase in suicidal behaviour. Specifically, both after 12 and 28 weeks of treatment, patients receiving SSRI revealed similar clinical changes to the group receiving SSRI plus CBT. The main treatment outcome was the Health of the Nation Outcome scale (interview-rated). There was also a decrease in suicidal thoughts and self-harm over time, and, surprisingly, there was no evidence of a protective effect of CBT on suicidal ideation or behaviours.

In summary, these studies confirm the efficacy of fluoxetine (isolated or in combination with CBT) in treating childhood and adolescent depression. However, there is some inconsistency as to which measures are the most sensitive in detecting effects. Interview-rated measures seem to be more consistent, possibly due to the fact that adolescents who are moderately to severely depressed may find it harder to detect differences in their own symptoms over time. However, some studies failed to show effects even on these measures and such discrepancy in results has been subject to criticisms (Jureidini et al. 2004). Additional factors such as the presence of comorbidities and site characteristics may play a role, and should be further explored in future studies.

Why is fluoxetine beneficial in the treatment of adolescent depression?

An important question raised by the meta-analyses comparing the effects of fluoxetine vs. other antidepressants (e.g. Whittington et al., 2004) is why fluoxetine has a more favourable benefit-to-risk profile in this age group in comparison to other antidepressants. One answer could lie in the longer half-life of this medication in comparison to other antidepressant drugs (like paroxetine), which may help the patient attain steady-state levels and alleviate the effects of missed doses in a population known to have a faster metabolism (Smith, 2009). The mechanisms underlying fluoxetine vs. other antidepressants could also play a role, as fluoxetine is known to have different affinities towards specific 5-HT receptors (e.g., Hoffman, Mezey, & Brownstein, 1991). Another possible factor is that fluoxetine is reported to be helpful in decreasing irritability and anger in both depressed patients with anger symptoms and in people with intermittent explosive disorder (Coccaro & Kavoussi, 1997; Fava et al., 1991). This could be of relevance to the management of depression in young people where symptoms of irritability and anger are common and often prominent (Crowe, Ward, Dunnachie, & Roberts, 2006).

Nonetheless, the possibility that methodological variations between trials could have influenced the higher efficacy of fluoxetine should also be considered. The efficacy of different SSRIs has not been compared within the same study, so potential differences in the study design or quality could have affected the results. Some authors have greatly criticized the design of these experiments, arguing that the presence of methodological constraints may have overestimated the beneficial effects of SSRIs (see Jureidini et al., 2004). Others, however, simply recognise that doing clinical trials in young people entails important ethical challenges (such as deciding whether

to include high-suicidal patients, or a placebo condition; March et al., 2004). The increased placebo response in young people vs. adults could also represent an obstacle when examining drug effects (see Birmaher et al., 1998). Additional studies with more rigorous designs (e.g., in which different drugs are compared within the same experiment) are therefore needed.

1.3.4. Summary

Fluoxetine is the only antidepressant currently licensed for use in paediatric populations in the UK. This decision was made based on evidence showing that the benefits of this medication outweigh its risks, although some criticisms have been raised regarding the quality of the clinical trials conducted to support this claim. It is possible that the beneficial effects of fluoxetine result from its higher half-life and clinical utility in reducing irritability and anger symptoms, which are a prominent feature of adolescent depression. Developmentally, exposure to antidepressants (including of fluoxetine) is associated with mixed effects in adolescence, although the reasons behind this variability are unclear. Factors such as prior exposure to adversity or differences in 5-HTT polymorphisms may partly account for this variation, but more research is needed in order to explore the role of these elements.

1.4. Part C: The neuropsychological model of antidepressant drug action

This section describes an approach that holds the potential to investigate the effects of antidepressant treatment in youth: the *neuropsychological model of antidepressant drug action,* originally developed and validated in adults. This model is supported by evidence from behavioural and neuroimaging studies conducted in healthy populations, at-risk groups and clinical samples, and focuses on the critical role of negative biases in the development and treatment of psychological disorders (Harmer, Goodwin, & Cowen, 2009). This model forms the basis of the research presented in this thesis.

1.4.1. The rationale

According to the neuropsychological model of antidepressant drug action, antidepressant drugs work by remediating the negative biases that are characteristic of disorders such as depression and anxiety (Harmer, Goodwin et al., 2009). The model proposes that very soon after

initiating antidepressant treatment, patients begin to pay less attention to negative information in the environment, whilst positive information becomes increasingly more salient. Such changes are probably not immediately accessible to subjective report, but as the patient starts to interpret different situations under a more positive outlook, his/her mood gradually improves. This gradual process may therefore explain the delayed onset of action (of several weeks) associated with antidepressant use, which is well known in clinical practice but not yet fully understood (Harmer, Goodwin, et al., 2009; Harmer, Hill, Taylor, Cowen, Goodwin, 2003).

This model is also consistent with cognitive theories of depression which highlight the role of negative biases in the aetiology and maintenance of this disorder, as well as the importance of correcting such biases when successfully treating depressive states (Harmer, Goodwin et al., 2009). Indeed, rather than changing mood directly (as conceived in traditional theories, see Figure 2), antidepressants may work by targeting emotional biases in processes such as perception, attention, memory, etc. that are necessary for later improvements in mood (Harmer, Goodwin et al. 2009).

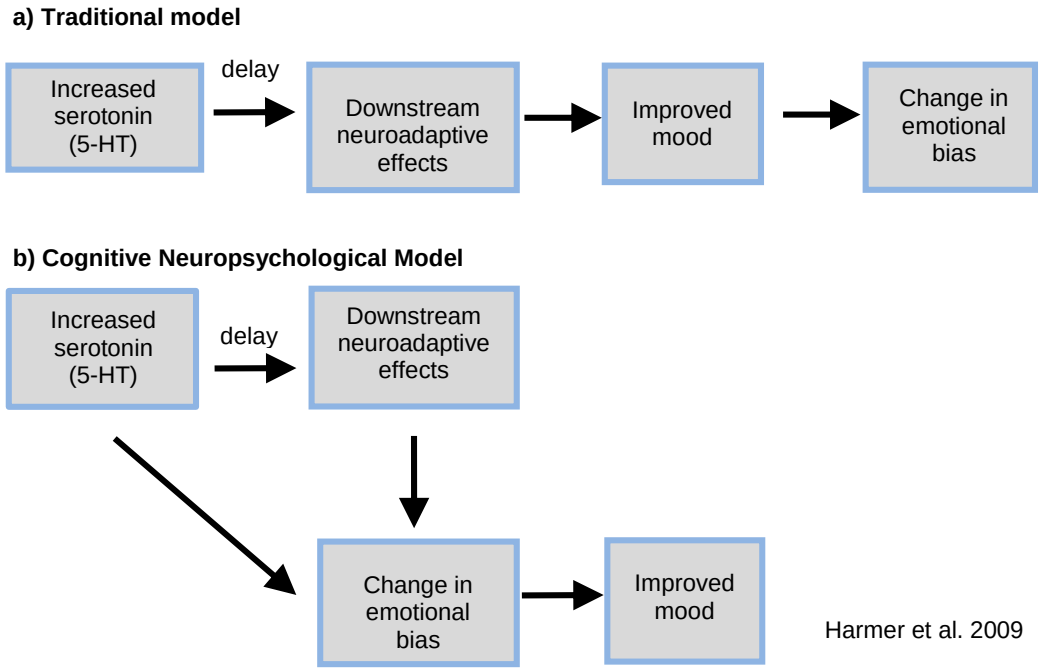

Figure 2. Neuropsychological model of antidepressant drug action. (a) According to the traditional theory of antidepressant drug action, the delayed onset of antidepressant action derives from the occurrence of downstream neuroadaptive changes (e.g., downregulation of post-synaptic 5-HT receptors). These changes contribute to gradual improvements in mood which ultimately help the patient change his bias in emotional processing. **(b)** In the more recent neuropsychological model of antidepressant drug action, the delay in clinical efficacy is largely mediated by the translation of changes in emotional bias into mood improvement, although some effects may also involve downstream neuroadaptive changes. Note: 5-HT, 5-hydroxytryptamine, serotonin.

1.4.2. Empirical evidence from healthy and clinical adult samples

The evidence leading to the formulation of the neuropsychological model of antidepressant drug action stems from studies using a wide range of tasks examining different elements of emotional processing, both at a behavioural and neuroimaging levels. Emotional processing is a type of cognitive processing of emotionally salient material that encompasses all stages of information analysis, including visual perception, attention, interpretation and memory (Harmer, Cowen, & Goodwin, 2011).

A number of behavioural tasks have been used in order to detect the subtle effects of antidepressants on emotional processing, as opposed to the effects induced by depression (for a review see Harmer, Cowen, et al., 2011). The *Facial Expression Recognition Task* assesses the perception and interpretation of key facial expressions. The *Categorization of Self-Referent Information* measures encoding of and memory for both positive and negative self-referent personality characteristics. The *Attentional Dot-Probe Paradigm* and *Rapid Visual Serial Presentation* measure biases in attention towards negative/threatening and positive stimuli. Finally, the *Emotion-Potentiated Startle Task* investigates affective modulation of the startle reflex. Many of these tasks are sensitive to both the effects of depression and of antidepressants, as they measure processes that are known to be altered in depressive disorders (such as facial expression recognition, attentional vigilance and memory) and which mediate the effects of antidepressant drugs. As mentioned previously, patients with depression show enhanced memory and attentional vigilance towards negative self-descriptive words and images, and are also biased towards perceiving ambiguous facial expressions as more negative (see Mathews & MacLeod, 2005). They also show reduced sensitivity to detect or recall positive information (Harmer, Goodwin, et al. 2009). As it will be discussed in the sub-sections below, antidepressants seem to revert these affective biases, a finding that corroborates the validity of using such paradigms in assessing depression and antidepressant effects.

It should be noted, however, that some of these tasks seem to be particularly sensitive to cognitive aspects which despite not being manifested as strongly in depressive states, reveal key mechanisms that underlie specific responses of antidepressant drugs. For instance, both the subliminal version of the Attentional Dot-Probe and the Emotion-Potentiated Startle are aimed at detecting the anxiogenic/anxiolytic effects of antidepressants, for which automatic cognitive

processes are more relevant. Indeed, whilst automatic fear processes are not as pertinent for characterising depression as a condition, they may reveal critical mechanisms underlying the action of antidepressant drugs.

Finally, it is worth noting that these behavioural paradigms have generally presented good reliability across studies, although methodological problems have been identified in some, particularly in the Attentional Dot-Probe (see Schmukle, 2005). There is, therefore, a need for continuous methodological refinement of these paradigms, in order to achieve a complete understanding of how antidepressant drugs influence different stages of information processing.

Neuroimaging tasks also include variations of the paradigms detailed above, but depending on the study aims, important methodological differences exist. Implicit tasks are often preferred, as it has been demonstrated that neural responses in the amygdala (a critical region involved in depression and antidepressant effects) are greater and more reliable in response to implicit than explicit stimuli (e.g., Critchley et al., 2000; Lange et al., 2003).

Behavioural effects

Using behavioural tasks, it has been shown that a single dose of the selective serotonin reuptake inhibitor (SSRI) citalopram increases the recognition of happy facial expressions (Harmer, Bhagwagar, et al., 2003) and attention towards positive words (Browning, Reid, Cowen, Goodwin, & Harmer, 2007). Similarly, short term (7 days) administration of this same drug increased the perception of and memory for positively valenced emotional material in healthy volunteers, whilst reducing the processing of fearful and angry faces (Harmer, Shelley, Cowen, & Goodwin, 2004). Other classes of antidepressants have shown analogous effects. A single dose of both the selective noradrenergic reuptake inhibitor (NRI) reboxetine and the serotonin-norepinephrine reuptake inhibitor (SNRI) duloxetine have been shown to increase the recognition of happiness (Harmer, Hill, et al., 2003; Harmer, Heinzen, O'Sullivan, Ayres, & Cowen, 2008). In addition, and similarly to the effects seen with citalopram, 7-day treatment with reboxetine decreased the identification of negative facial expressions of fear and anger, whilst increasing the recall of positive emotional words compared to placebo (Harmer et al., 2004). Across studies, these effects were independent of subjective changes in mood, which corroborates the hypothesis that such

emotional processing drug effects represent a direct mechanism of antidepressants rather than being dependent on mood changes.

Interestingly, these emotional processing measures are also relevant to the detection of adverse reactions seen early in the course of antidepressant treatment. For instance, acute administration of the SSRI citalopram has been shown to increase the processing of threatening information, in addition to its positive influence on emotional biases (Browning et al., 2007). Similar effects have also been reported in other studies involving a single administration of citalopram (Grillon, Levenson, & Pine, 2007; Harmer, Bhagwagar, et al., 2003). Despite being reversed with repeated treatment (Harmer et al., 2004), these anxiogenic-like effects are thought to reflect the mechanisms responsible for the adverse reactions seen in some patients at early stages of SSRI treatment, before anxiolytic effects are verified (Kent, Coplan, & Gorman, 1998).

Most of the studies that contributed for the development of this model were conducted initially with healthy volunteers, therefore allowing a precise understanding of drug effects in the absence of confounding changes in mood. However, their replication in clinically depressed samples is critical, in order to validate the hypothesis that such immediate changes on emotional processing biases contribute to later improvements in mood. Harmer and colleagues (2009) provided the first evidence in favour of this idea. In this study, a single dose of reboxetine was shown to increase the processing of positive affective biases (vs. placebo) in a sample of depressed patients. These effects were seen in the Facial Expression Recognition Task, Emotional Categorisation and Emotional Memory, and were of similar or even larger magnitude to those reported in healthy volunteers (see Pringle, Browning, Cowen, & Harmer, 2011). In addition, Tranter and colleagues (2009) found a positive correlation between the increased ability to recognise happy facial expressions two weeks after patients started treatment with citalopram and later clinical improvement measured at 6 weeks. Taken together, these studies confirm that emotional processing drug effects emerge early on in treatment (both acutely and within the first week) in both healthy and clinical samples, and suggest that they could represent an important mechanism for the emergence of therapeutic changes. The study by Tranter and colleagues (2009) also highlights the potential of using these measures to identify patients who are more likely to benefit from antidepressant medication.

Neuroimaging effects

The emotional processing changes seen with antidepressants are also associated with altered patterns of functional activity in a cortico-limbic network known to be involved in depression. This network includes structures responsible for threat processing (e.g. amygdala), attention and visual processes (e.g., frontoparietal cortex and extrastriate/fusiform gyrus), self-referential processes (e.g. medial prefrontal cortex), and cognitive control of emotional states (e.g., dorsolateral prefrontal cortex), among others (for review see Ma, 2014). For instance, the amygdala hyperactivity seen in depressed patients when they perceive negative information is normalised after 8-week treatment with antidepressants (Fu et al., 2004; Sheline et al., 2001). The activity of ventral striatum and frontoparietal cortex also decreases in response to sad faces after 8 weeks of antidepressant therapy, whereas the extrastriate cortex shows an increase in activation towards the presentation of happy facial expressions (Fu et al., 2004). A more recent study by Godlewska, Norbury, Selvaraj, Cowen, & Harmer (2012) found that the pattern of amygdala hyperactivity seen in depressed patients can be normalised as early as after 7-days of treatment with escitalopram, and in the absence of subjective changes in mood, therefore corroborating the notion that antidepressants act directly on key brain regions relevant to depression. This finding is especially important given that previous studies measured the neural effects of antidepressants at a treatment stage where mood improvement was already evident, and without a placebo control.

Similar effects have been reported in healthy samples. A single dose of citalopram vs. placebo decreased amygdala activity in response to fear in never-depressed volunteers (Murphy, Norbury, O'Sullivan, Cowen, & Harmer, 2009, but see Bigos et al., 2008). Likewise, 7-day treatment with both citalopram and reboxetine decreased amygdala response to masked fearful faces (Harmer, Mackay, Reid, Cowen, & Goodwin, 2006; Norbury, Mackay, Cowen, Goodwin, & Harmer, 2007). The activity of the medial prefrontal cortex (mPFC), an area involved in self-referential processing, is also decreased after antidepressant administration (Di Simplicio, Norbury, & Harmer 2012). In the experiment of Di Simplicio and colleagues (2012), 7 days of treatment with citalopram was found to decrease the activation of the mPFC in a sample of individuals at-risk for depression. In addition to these dampening effects within the amygdala and mPFC, 7-day treatment with antidepressants is also shown to increase the activity of the fusiform gyrus in response to positive information in healthy volunteers. Indeed, Norbury et al. (2007) found that 7

days of treatment with reboxetine increased the activation of the right fusiform gyrus in response to happy vs. neutral facial expressions.

Taken together, these findings from both clinical and healthy samples suggest that antidepressants may work by decreasing the activation of areas responsible for the excessive processing of negative information, whilst increasing the activity of brain regions implicated in the assessment of positive stimuli (for a review see Ma, 2014).

The use of neuroimaging to predict clinical therapeutic outcomes has also received growing attention in the past years, and the results are encouraging. Indeed, in 2011, Pizzagalli provided meta-analytic evidence showing that greater baseline activation in the anterior cingulate cortex is associated with faster rates of symptom improvement with antidepressants. A recent study in our laboratory has extended these findings to suggest that neural changes seen after one week of treatment with escitalopram (vs. placebo) can also help predict clinical responses in responders vs. non-responders (Godlewska, Browning, Norbury, Cowen, & Harmer, submitted for publication).

1.4.3. Implications for adolescent depression

Given the sensitivity of the neuropsychological model of antidepressant drug action to both positive and negative psychological effects of antidepressants, this approach holds great potential to investigate the behavioural and functional mechanisms underlying antidepressant use in youth. There is also initial evidence supporting the use of some of the paradigms detailed above when investigating affective processing (both at a behavioural and neural level) in paediatric depression. Indeed, facial expression recognition tasks have been used in depressed and at-risk children and adolescents (e.g., Schepman et al. 2012), and fMRI studies are increasingly being implemented to characterise the neural substrates underlying this disorder (e.g., Lau et al., 2009; Monk et al. 2008).

To the best of my knowledge, however, no study so far has looked at the effects of antidepressants on emotional processing biases measured behaviourally, and there is only one study that used fMRI to investigate the effects of antidepressants in a sample of depressed adolescents. In this study, Tao and colleagues (2012) found that 8 weeks of treatment with fluoxetine normalised the pattern of functional hyperactivity seen in areas such as the amygdala,

orbitofrontal cortex and subgenual anterior cingulate cortex. The activation within these areas was excessive in depressed adolescents in comparison to matched healthy controls at baseline, but these group differences disappeared after a long course of antidepressant treatment. This study is of critical importance, as it suggests that antidepressant administration has beneficial effects on brain regions relevant to the treatment of depression, which is in line with the findings in adults. However, given that after 8 weeks of treatment, 60% of patients showed significant therapeutic improvement, it remains to be established whether the neural changes described represent a direct mechanism of fluoxetine or a consequence of mood changes. Studies in adults are consistent with the first hypothesis (e.g., Godlewska et al., 2012), but in light of important differences in the antidepressant responses seen in youth vs. adults, it is vital to characterise the early effects of antidepressants drugs in depressed adolescents.

1.4.4. Summary, aims and hypotheses

The neuropsychological model of antidepressant drug action provides a useful approach to understand the neuropsychological mechanisms that are associated with antidepressant use. It has been previously demonstrated that antidepressants act early in treatment to remediate the negative biases that characterise depression in adults. These effects occur in the absence of noticeable changes in mood or anxiety and seem to predict subsequent responses to treatment.

Given the limited knowledge in the field of antidepressant research in youth, this thesis aims to investigate the effects of acute fluoxetine on emotional processing, using a combination of behavioural and neuroimaging measures that are sensitive to both the positive and adverse effects of antidepressants, as shown in the model of antidepressant drug action.

The introductory chapter has covered the research background behind the proposed field of study. The first experimental chapter, Chapter Two, examined the effect of a single dose of fluoxetine on emotional processing in a sample of young healthy volunteers (18-21 years-old). It was hypothesised that fluoxetine would increase the processing of threatening information, given previous evidence that acute administration of the SSRI citalopram induces anxiogenic-like effects in adults, and that young people may be more susceptible to the adverse effects of antidepressants. A second hypothesis was that fluoxetine would influence the processing of anger stimuli, given the prominence of anger and irritability in paediatric depression. Contrary to the first

hypothesis, fluoxetine failed to induce anxiogenic effects in this sample of participants. The emotion-potentiated startle effect was eliminated in participants receiving fluoxetine (vs. placebo), therefore showing a pattern more consistent with anxiolysis. No effects were seen in the measures used to assess attention to threatening vs. positive information. However, an important limitation of this study was the failure of the Attentional Dot-Probe to detect the basic fear/negative vigilance effect that was expected in the placebo group, which complicated the interpretation of the findings. In order to address this methodological limitation, the following chapter (Chapter Three) aimed to validate a new paradigm that is methodologically more robust and potentially more sensitive to anxiety-related automatic biases - Continuous Flash Suppression (CFS). Two studies were conducted. In the first, CFS proved sensitive in detecting automatic biases towards fearful (vs. happy faces) in healthy volunteers, an effect that was modulated by anxiety. The second study aimed to assess the sensitivity of CFS in assessing anxiolytic-like effects after 7-day treatment with the anti-anxiety drug diazepam.

Given the importance of directly investigating the effects of fluoxetine in clinically depressed adolescents, Chapter Four presents a study with adolescents aged 13 to 18 years old, who had been recently prescribed fluoxetine for depression. Participants were tested on a battery of neuroimaging and behavioural tasks after being administered with their first dose of fluoxetine vs. placebo. Following this session, participants started their fluoxetine treatment as prescribed by their Psychiatrist, and were clinically assessed after one and six weeks of treatment as part of the study. Given the previous findings with anger, it was hypothesised that fluoxetine would decrease amygdala activity in response to anger, and that this effect would be independent of subjective changes in mood or anxiety. Acute changes in the amygdala to fear and anger were also correlated with initial symptoms of anxiety, irritability and depression. In line with the study hypothesis, fluoxetine was shown to decrease amygdala activity in response to angry faces. Acute amygdala responses in response to fear were shown to correlate with reduced symptoms of anxiety/agitation and depression over the first 7-10 days of treatment.

In order to further explore the effects of fluoxetine on anger processing, the last experimental Chapter (Chapter V) includes a sample of male volunteers who are high in trait anger and therefore more susceptible to problems controlling this emotion. It was hypothesised that fluoxetine would decrease the recognition of, and attention towards, angry faces, as well as reduce

the subjective and autonomic experience of anger during the recollection of an anger-provoking situation. There was some evidence to suggest that acute fluoxetine modulated emotional processing, but this was not specific to anger. For a summary of the design and hypotheses of the experimental studies included in this thesis, see Table 1.

Table 1. Summary of the design and hypotheses of the experimental studies included in this thesis

II. Acute effects of fluoxetine in healthy volunteers	III. Validating the use of Continuous Flash Suppression (CFS) to detect automatic anxiety-related biases and anxiolytic effects		IV. Acute effects of fluoxetine in clinically depressed adolescents	V. Acute effects of fluoxetine in high-anger trait males
Study title – The effects of acute fluoxetine on emotional processing in young adult volunteers	**Study title** – The use of CFS to detect early biases towards threat	**Study title** – The use of CFS to detect anxiolytic effects	**Study title** – The effects of acute fluoxetine on emotional neural processing in depressed adolescents	**Study title** – The effects of acute fluoxetine on anger processing: an exploratory study with high trait anger males
Methodology: psychopharmacological manipulation (single 20mg dose of fluoxetine vs. placebo) and behavioural assessment of emotional processing biases	**Methodology:** use of Continuous Flash Suppression (CFS) to investigate the potency of emotional visual stimuli to gain access to awareness	**Methodology:** psychopharmacological manipulation (7-8 days treatment with 15mg diazepam vs. placebo) and use of CFS	**Methodology:** pharmacological manipulation (single 10mg dose fluoxetine vs. placebo) and fMRI and behavioural assessment of emotional processing biases	**Methodology:** psychopharmacological manipulation (single 20mg dose of fluoxetine vs. placebo) and behavioural assessment of emotional processing biases and anger processing
Design: Between-subjects, double-blind, placebo-controlled	**Design:** Within-subjects	**Design:** Between-subjects, double-blind, placebo-controlled	**Design:** Between-subjects, double-blind, placebo-controlled	**Design:** Between-subjects, double-blind, placebo-controlled
Sample: 35 young healthy adult volunteers (18-21 years old)	**Sample:** 49 healthy adult volunteers (18-29 years)	**Sample:** 35 healthy volunteers (18-29 years-old)	**Sample:** 26 depressed adolescents and 11 healthy controls (13-18 years)	**Sample:** 21 high-trait anger males (18-55 years-old)
Hypotheses: 1. Fluoxetine administration will increase the processing of threatening stimuli 2. Fluoxetine will influence the processing of anger 3. These effects will be verified in the absence of changes in subjective mood or anxiety	**Hypotheses:** 1. Fearful faces will reach awareness faster than happy faces 2. Anxiety, but not depression, will be associated with a higher speed to identify fearful faces	**Hypotheses:** 1. Diazepam will be associated with longer detection times towards fearful vs. happy faces	**Hypotheses:** 1. A single dose of fluoxetine will decrease amygdala activation in response to angry faces 2. These effects will be verified in the absence of overt changes in subjective mood or anxiety	**Hypotheses:** 1. Fluoxetine administration will decrease the recognition and attentional vigilance towards anger 2. Fluoxetine will decrease autonomic reactivity and subjective ratings of anger/irritability during the recall of an anger-provoking situation

Chapter 2:

Effects of acute fluoxetine on emotional processing in young adult volunteers

2.1. Introduction

The use of antidepressant medication in adolescents and young people has been controversial because many medications that are effective in older adults appear to have a less favourable risk-benefit ratio in young people (Whittington et al., 2004). Particular concern has been expressed about the risk of antidepressant-induced adverse behavioural reactions, such as anxiety/agitation, hostility and suicidal behaviour (Jureidini et al., 2004). Indeed it appears from controlled trials that young people taking selective serotonin re-uptake inhibitors (SSRIs) exhibit more suicidal behaviour than those taking placebo (Hammad et al., 2006; Martinez et al., 2005). However, the incidence of fatal suicide is not increased and, in fact, lowered rates of SSRI prescribing to adolescents following regulatory warnings in the United States was associated with an increased number of completed suicides at a population level (Gibbons et al., 2007; Lu et al., 2014).

Unlike many other antidepressants, the SSRI fluoxetine is thought to have a favourable risk-benefit ratio in children and adolescents with moderate to severe depression and is the only SSRI currently approved in the United Kingdom (National Institute for Health and Clinical Excellence, NICE, 2005). In the United States, fluoxetine was the only antidepressant approved for use in youth until 2009, when escitalopram also gained FDA clearance. Why fluoxetine might have a more favourable benefit-to-risk profile in this age is unclear (Hetrick, McKenzie, Cox, Simmons, & Merry, 2012; Whittington et al., 2004), although it has been suggested that its long half-life could be advantageous in less compliant patients (Smith, 2009). Another possible factor is that fluoxetine is reported to be helpful in decreasing irritability and anger in both depressed patients with anger symptoms and in people with intermittent explosive disorder (Coccaro and Kavoussi, 1997; Coccaro, Lee, & Kavoussi, 2009; Fava et al., 1991). This could be of relevance to the management

of depression in young people where symptoms of irritability and anger are common and often prominent (Crowe et al., 2006).

The neuropsychological effects of fluoxetine have been little investigated in humans. Our group has studied single doses of a wide range of antidepressants in adult healthy volunteers. As described earlier in this thesis, a single dose of both the selective noradrenergic reuptake inhibitor (NRI) reboxetine and the serotonin-norepinephrine reuptake inhibitor (SNRI) duloxetine has been shown to increase the recognition of happy facial expressions (Harmer, Bhagwagar, et al., 2003; Harmer et al., 2008). In contrast, acute administration of the SSRI citalopram increases not only the processing of positive information (as reflected in an enhanced recognition of happy faces and attentional vigilance to positive words) but also threat processes (increased recognition of fearful faces and startle responses) (Browning et al., 2007). The production of positive emotional bias has been suggested to underlie the antidepressant action of drugs effective in treating depression, whilst increased threat processing may be responsible for the worsening of anxiety seen in some patients at the initiation of SSRI treatment (Kent et al., 1998). The latter suggestion is supported by evidence that following repeated treatment with SSRIs, the acute effects on threat processing are reversed and an anxiolytic profile becomes apparent (Burghardt, Sullivan, McEwen, Gorman, & LeDoux, 2004).

Given the limited evidence available on the effects of antidepressants in young people, the present study aimed to investigate the acute effects of fluoxetine in a group of young healthy adults aged 18 to 21 years-old, using the same tests previously shown to be sensitive to both positive and negative effects of antidepressants on emotional processing (see Harmer, de Bodinat et al., 2011). More specifically, the *Facial Expression Recognition Task* was used to examine the perception and interpretation of key facial expressions. The Attentional Dot-Probe Paradigm and the Rapid Visual Search Presentation were used to measure biases of attention towards negative/threatening and positive stimuli. Finally, an Emotion-Potentiated Startle Task measured the affective modulation of the startle reflex (taken as an index of fear). By using these paradigms, the aim was to assess whether fluoxetine, like the SSRI citalopram, produced evidence of acute increases in threat processing, especially in this sample of young adult volunteers who may be more susceptible to the adverse effects of antidepressants. A second goal was to test if any effects on the processing of anger would be apparent given fluoxetine's efficacy in patients with anger problems.

2.2. Methods

2.2.1. Participants

Based on a power calculation computed a-priori, it was expected to recruit 30 to 35 participants to this study. Indeed, in a single dose drug study conducted in our laboratory, with 32 healthy volunteers in total - who were randomised to receive a single dose of another SSRI, citalopram, or placebo (Browning et al., 2007) -, the size of the effect of citalopram on fear recognition was 1.17. It was calculated that for an effect of this size, a minimum of 15 participants per group would be needed to obtain a power calculation of 0.95 at a α error probability of 0.05.

Thirty-five healthy volunteers aged between 18 and 21 were therefore recruited using posters and web-advertisements. Given NICE and FDA concerns that antidepressants may increase the risk of suicidal ideation and behaviour in young people up to 25 years-old, the 18 to 21 age range was chosen as it falls between the late adolescence and young adulthood period, and avoids the ethical constraints of administering drugs to healthy adolescents aged below 18.

All participants were given a standard medical screening and administered a psychiatric interview to determine suitability for participation in the study. Exclusion criteria included: current medical problems; personal history of psychiatric illness, including alcohol/substance abuse; first-degree family history of bipolar disorder; smoking frequency greater than 5 cigarettes per day; pregnancy or breast-feeding; BMI lower than 18 or higher than 30; usage of recreational drugs within the last 3 months and current use of psychotropic medication. To avoid retest effects, participants who had taken part in studies involving the same tasks were also excluded.

Ethical approval for this study was obtained from the Berkshire Research Ethics Committee (10/H0505/2). All participants gave written and verbal consent prior to their participation, and were reimbursed with £80 for their time.

2.2.2. Experimental design and procedures

Screening

Participants underwent a screening session lasting approximately one hour. This included the Structured Clinical Interview for DSM-IV Axis I (SCID-I; First, Spitzer, Gibbons & Williams, 1997) to determine suitability. From this interview, participants were determined to be free of current or past Axis I (psychological) disorders. Additionally, all participants were weighted and

required to have a brief physical exam with a medical doctor to ensure physical fitness. The doctor also reviewed participants' medical history.

The National Adult Reading Test (NART; Nelson, 1991) was administered in order to obtain an estimate of verbal IQ. Participants were also required to complete the Trait version of the STAI (STAI-T; Spielberger, Gorsuch, & Edward, 1970) and the Eysenck Personality Questionnaire (EPQ; Eysenck, & Eysenck, 1975). A detailed description of these measures is provided in Appendix 1.

Testing

Participants were asked to refrain from alcohol on the night before the testing and to limit caffeine consumption to one cup during breakfast. Participants arrived at the laboratory in the morning and were asked to fill the following questionnaires: the Beck Depression Inventory (BDI; Beck et al. 1961) as an indicator of mood; the state version of the STAI (STAI-S; Spielberger et al. 1970) as a measure of anxiety; Visual Analogue Scales (VAS) to assess alertness, contentedness and calmness symptoms (Bond & Lader, 1974); the Positive and Negative Affective Schedule (PANAS; Crawford & Henry 2004); the Jitteriness Rating Questionnaire (JRQ; Sinclair et al., 2009) to sensitively measure symptoms of anxiety and agitation; and finally an adapted version of the Antidepressant Side-Effect Checklist (ASEC; Uher et al., 2009). A detailed description of these measures is provided in Appendix 1.

Participants were then randomised to receive either 20 mg of fluoxetine or a matched placebo, in a double-blind procedure. Following drug/placebo administration, participants sat in a quiet room until the testing session began. During this time, participants were free to read, work and/or use their laptops, and were also carefully monitored for potential side-effects. In order to guarantee that fluoxetine had reached its peak level, which has been shown to take place after 6 to 8 hours (Rossi, Barraco, & Donda, 2004), the behavioural testing session started 6 hours after drug/placebo administration. The STAI-S, VAS, PANAS, JRQ, VAS and ASEC were administered again at the end of the testing.

Participants were not allowed to smoke or drink any additional caffeine for the duration of the study. For lunch, participants were offered a selection of light meals. The premenstrual week was also avoided for testing.

2.2.3. Experimental tasks

Facial Expression Recognition Task

The Facial Expression Recognition Task (FERT) measures the ability to identify and distinguish different facial expressions, being considered a probe of interpretation bias. Facial stimuli include different intensities of positive and negative facial expressions, therefore allowing the assessment of subtle facial discrimination in the face of complex and ambiguous emotions (Harmer, de Bodinat, et al., 2011). More specifically, the task stimuli included six basic emotions taken from the (Ekman & Friesen, 1976) Pictures of Affect Series: happiness, sadness, fear, anger, surprise and disgust. These facial expressions were morphed between each prototype and neutral, using techniques described by Young and colleagues (1997). Briefly, this procedure involves taking a variable percentage of the shape and texture differences between neutral (0%) and each emotional standard (100%) in 10% steps, leading to a range of emotional intensities. Four examples of each emotion at each intensity were given (total of 10 individuals). Each face was also given in a neutral expression, providing a total of 250 stimuli presentations. Facial stimuli were presented in a random order for 500ms, being immediately replaced by a blank screen. Participants were asked to make their response as accurately and as quickly as possible by pressing a labelled key on a response box. The outcome measures in this task include: 1) percentage of correct responses (i.e., the number of faces correctly identified as containing any intensity of a particular emotion), 2) percentage of misclassifications (false hits) and 3) reaction times (to emotions correctly identified).

Emotion-Potentiated Startle Task

The Emotion-Potentiated Startle Task (EPST) measures the eyeblink reaction to different emotional and neutral stimuli using electromyographic recordings. The eyeblink response is considered the first and fastest element of the startle response (Lang, Bradley, & Cuthbert, 1990), being an indicator of the fear reaction in both humans and animals. This version of the paradigm included pictures from the International Affective Picture System, designed to elicit positive, negative, or neutral emotions (Lang, Bradley, & Cuthbert, 2005). These stimuli had been rated and selected such that the negative and positive pictures were similar in terms of arousal but opposite in valence, whereas the neutral pictures were low on arousal and average on valence.

Stimuli were presented for 13 seconds, with an inter-trial interval of between 11-15 seconds. The eye-blink component of the startle response was recorded from the orbicularis oculi using electromyography (EMG-startle response system, San Diego Instruments, USA). Acoustic probes were 50ms, 95dB bursts of white noise with a nearly instantaneous rise time and were delivered binaurally through headphones (delivered at 1.5, 4.5, or 7.5 seconds following picture onset). Within each block of 21 pictures, probes were delivered on five of each trial type (neutral, positive or negative). To limit expectation of the noise, two trials per valence did not contain any startle probes and three probes per block were given within the inter-trial interval. To habituate participants to the startle probes and to orient them to the procedure, participants viewed an introductory set of nine neutral pictures and received nine startle probes (two of these within the inter-trial interval). Eye-blink reflex magnitudes were then calculated and z-transformed in order to normalise data and reduce inter-subject variability.

Attentional Dot-Probe Task

The Attentional Dot-Probe Task assesses attention to positive vs. negative stimuli using a reaction time measure. Two faces with different facial expressions are presented vertically on the computer screen and replaced by a cue (two dots) to which the volunteer has to respond. The rationale behind this task is that if participants have allocated their attention to the negative stimulus and the dot appears in the same place, they should be relatively faster to respond than if the dots appear in the opposite location. Pairs of photographs of 20 individuals displaying different emotions were taken from the JACFEE/JACNeuF sets of facial expressions (Matsumoto & Ekman, 1988). There were 3 types of face pairs: (1) neutral-neutral; (2) fearful-neutral and (3) happy-neutral. Each pair comprised facial expressions from the same individual. On each trial, one of the faces occupied the top half of the screen and the other the half the bottom, with a central fixation cue appearing in the middle. The emotional faces (i.e., fearful and happy) appeared in both locations with equal frequency.

There were two types of conditions: subliminal and supraliminal, in which the faces were presented for 100ms or 1000ms respectively. Participants were asked to report the orientation of the dots as quickly and as accurately as possible by pressing the corresponding labelled key on a keyboard. Consistent with Murphy, Downham, Cowen and Harmer (2008), attentional vigilance scores were calculated for each participant by subtracting the mean reaction time from trials when probes appeared in the same position as the emotional face (congruent trials) from trials when

probes appeared in the opposite position to the emotional word (incongruent trials). Therefore, positive scores suggest an attentional vigilance towards the emotional stimuli, whilst negative scores indicate attentional bias away from the emotional stimuli. Zero scores suggest no bias towards the emotional face.

Rapid Serial Visual Presentation

The Rapid Serial Visual Presentation (RSVP) measures the processing of different word valences under conditions of restricted attentional resources ("attentional blink effect"). Participants were asked to attend to a series of rapidly presented words and to focus on two word targets (T1 and T2), while the time between these two targets was varied. This task was adapted from a RSVP paradigm reported previously (Anderson & Phelps, 2001; Raymond et al., 1992) where each trial consisted of 15 words [2 targets (bright green) and 13 distractors (black)], each presented for 100ms and immediately followed by the subsequent stimulus. Participants were required to type the target words using the keyboard. The targets consisted of T1 and T2 words and were presented in bright green, as opposed to the black colour of the distractors. T1 stimuli were 56 neutral words averaging 4.38 letters in length. Distractor items were 79 neutral words of much longer length (average of 11.66 letters), in order to appropriately mask all target stimuli and also to maintain distinctiveness between the target and distractor stimuli. Six trial lags were introduced from lag 2 [1 distractor between the two targets (T1-T2) SOA=200ms] to lag 7 [6 distractors presented between the two targets (T1–T2) SOA=700ms]. T2 stimuli were neutral (e.g., arm, iron, owl), positive (e.g., gift, honour, cheer) and negative (e.g., abuse, rage, betray) words, selected from the ANEW database (Affective Norms for English Words; Bradley & Lang, 1999). The T2 stimuli were matched for average word length, written word frequency and familiarity (Coltheart 1981; Kučera & Francis 1967). Positive and negative words were also matched for arousal ratings. The outcome measure in this task was the percentage of T2 words correctly identified when preceded by a correct identification of the T1 word.

2.2.4. Statistical analyses

Data was analysed by using the IBM SPSS 22 software. Baseline characteristics (age, BMI, IQ, EPQ scales, STAI-T and BDI), excluding gender, were analysed by using independent t-tests. Gender was analysed using a chi-square test. The self-report scales used to assess the effects of fluoxetine (i.e., STAI-S, PANAS, ASEC and JRQ) were analysed using a split-plot

analysis of variance (ANOVA), with time (pre and post drug/placebo administration) and group (fluoxetine vs. placebo) as within- and between-subjects factors respectively.

The experimental tasks were analysed by using a mixed-design (split-plot) analysis of covariance (ANCOVA), with group (fluoxetine vs. placebo) as the between-subject factor and average STAI-S as a covariate. To explore the effects of gender on the FERT and Startle, the same analysis was repeated with gender as an additional between-subjects factor. Within-subject factors were emotion/valence (FERT, Attentional Dot-Probe, RVSP and EPST), stimulus presentation (Attentional Dot-Probe) and lag type (RVSP). Significant interactions in the ANCOVA were further explored using follow-up pairwise comparisons or separate repeated measures ANOVAs. When sphericity was violated, Greenhouse-Geisser-corrected p-values were used, but uncorrected degrees of freedom are reported for clarity. Due to skewness in distribution, the reaction times (RTs) for the Facial Expression Recognition Task (FERT) were submitted to a log-transformation that proved successful in ensuring normality. A p-value lower than 0.05 was used to denote statistical significance. Marginal differences with a p-value lower than 0.10 were also reported.

2.3. Results

2.3.1. Baseline characteristics

There were no significant differences between groups in terms of age, gender distribution, BMI, IQ (as measured using the NART), EPQ scales, trait anxiety (STAI-T) and depression (BDI) symptoms (all *p*s>0.17; see Table 2).

Table 2. Baseline characteristics.

	Placebo (N=18), mean (±SD)	**Fluoxetine (N=17), mean (±SD)**	**p**	**sig.**
Age	19.33 (1.19)	19.88 (0.86)	0.128	n.s.
Male:female ratio	9:9	8:9	0.862	n.s.
BMI	22.6 (2.75)	22.7 (2.89)	0.960	n.s.
Verbal IQ (NART)	117.16 (4.53)	117.81 (5.62)	0.706	n.s.
EPQ Neuroticism	6.44 (3.38)	4.76 (3.56)	0.162	n.s.
EPQ Psychoticism	2.94 (1.98)	2.24 (1.79)	0.276	n.s.
EPQ Extraversion	15.33 (3.29)	15.00 (4.33)	0.798	n.s.
Trait anxiety (STAI-T)	34.28 (6.29)	33.77 (6.96)	0.820	n.s.
Depression (BDI)	1.72 (1.99)	1.41 (1.91)	0.641	n.s.

Note: n.s. stands for non-significant.

2.3.2. Subjective ratings

There was no main effect of group or interaction with group when assessing jitteriness (JRQ), side effects (ASEC) or affect (PANAS) (all *p*s> 0.25). However, the placebo group showed increased state anxiety (STAI-S) across time points, i.e. even at baseline [main effect of group: F(1,31)= 6.970, p=0.013]. This measure was therefore controlled for in subsequent analyses.

When considering Contentedness Visual Analogue Scale (VAS) ratings, there was a trend for a main effect of group [F(1,29)= 2.901, p=0.099] and for an interaction between group and time [F(1,29)= 3.678, p=0.065]. Participants on placebo showed overall increased scores in this scale. The interaction with time was driven by group differences at timepoint 2, with the placebo group again revealing higher scores [F(1,29)= 4.493, p=0.043]. This may suggest that participants on fluoxetine were less content at the end of the session, but this finding needs to be interpreted with caution given that other measures of positive affect did not show the same pattern (e.g, positive scale of the PANAS). Fluoxetine did not modulate the ratings from the other VAS scales: Calmness [emotion x group: F(1,29)=0.038, p=0.847] or Alertness [emotion x group: F(1,29)=0.015, p=0.904].

A summary of the scores obtained in these measures is provided in Table 3.

Table 3. Subjective state changes after fluoxetine administration vs. placebo

	Pre		Post	
	Placebo (N=18), mean ±SD	**Fluoxetine (N=17), mean ±SD**	**Placebo (N=18), mean ±SD**	**Fluoxetine (N=17), mean ±SD**
State anxiety (STAI-S)	31.59 (6.76)	27.13 (5.86)	34.71 (7.79)	28.00 (5.84)
Jitteriness (JRQ)	5.06 (6.18)	2.18 (2.88)	5.56 (7.37)	3.59 (3.97)
Side-effects (ASEC)	1.67 (1.37)	1.18 (1.13)	2.44 (1.76)	2.12 (2.39)
PANAS positive (+)	27.94 (5.47)	28.29 (5.88)	26.94 (7.36)	26.24 (7.05)
PANAS negative (-)	11.50 (2.41)	10.71 (1.49)	13.72 (5.38)	11.24 (2.86)
VAS alertness	34.38 (14.91)	27.97 (9.50)	40.74 (16.90)	34.79 (11.09)
VAS contentedness	26.15 (17.81)	21.51 (4.95)	34.84 (17.55)	23.53 (11.77)
VAS calmness	31.11 (13.85)	26.13 (11.24)	37.68 (17.54)	31.45 (17.44)

2.3.3. Facial Expression Recognition Task

There was a significant interaction between emotion and group when considering accuracy levels [F(6,192)= 2.358, p=0.032]. Follow-up pairwise comparisons revealed that participants

receiving fluoxetine were less accurate at identifying anger [F(1,32)=5.011, p=0.032] and sadness [F(1,32)=5.022, p=0.032]. They also showed a trend to be more accurate at identifying happiness [F(1,32)=3.282, p=0.079]. No significant differences were seen in the remaining expressions (all *p*s>0.20) (see Figure 3a).

When analysing the reaction time taken to detect the facial expressions, a trend for an interaction effect between facial expression and group was seen [F(6,192)= 2.053, p=0.061]. Consistent with the decreased accuracy to detect anger, participants receiving fluoxetine showed a trend to be slower to detect this emotion [F(1,32)=3.169, p=0.085]. None of the other comparisons reached statistical significance (all *p*s>0.28) (see Figure 3b).

Finally, when considering the number of misclassifications, there was no interaction between emotion and group [F(6,192)=1.148, p=0.334] or main effect of group [F(1,32)<10.000, p>0.9]. However, due to the previous findings seen with anger accuracy, the effect on fluoxetine on anger misclassifications was further analysed. Consistent with the previous analysis, participants receiving fluoxetine misclassified less faces as angry [F(1,32)=4.759, p=0.037] (see Figure 3c).

Repeating this analysis with gender as an additional between-groups factor showed that this variable did not influence the effects of fluoxetine on any the measures [as revealed by a lack of emotion x group x gender interaction for accuracy: F(6,180)=0.644, p=0.695; misclassifications: F(6,180)=0.341, p=0.698 and RTs: F(6,180)=1.801, p=0.101].

2.3.4. Emotion Potentiated Startle Paradigm

Data from one participant was removed in the fluoxetine group due to excessive noise in the EMG trace. The analysis was therefore performed with 34 participants in total. A split-plot ANCOVA (with average STAI-S again as a covariate) revealed a significant interaction between valence and group treatment [F(2,62)=5.139, p=0.009]. There was no main effect of group [F(1,31)=0.370, p=0.547].

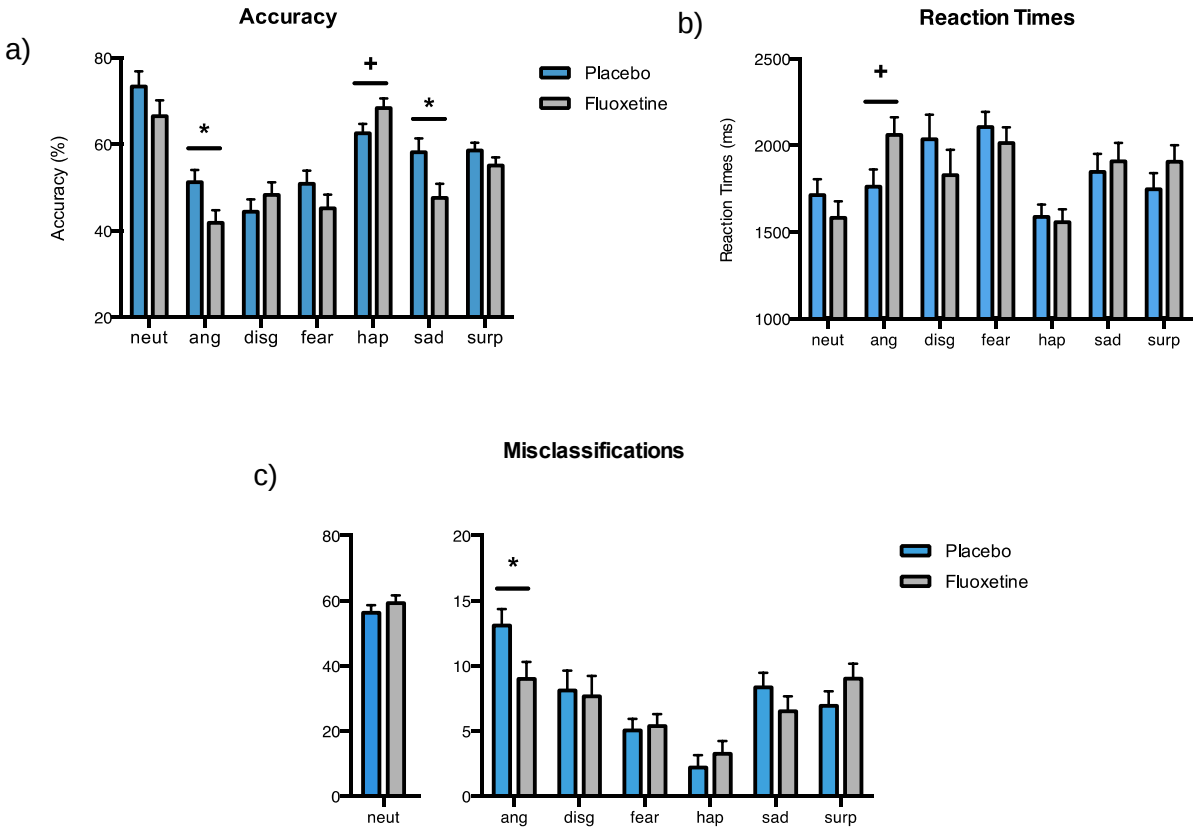

Figure 3. Facial Expression Recognition Task. (a) Accuracy of facial expression recognition in the fluoxetine and placebo groups. Values represent percentage of correct responses summed over the different intensity levels for that emotion. **(b)** Average Reactions Times (RTs) of facial expression recognition. Only correct responses were considered in the averaging of RTs. **c)** Misclassifications of each facial expression. Values represent the percentage of misclassifications for each facial expression, out of the total number of misclassifications given by each participant. For illustration purposes, the y axis was adjusted to display the percentage of misclassifications for neutral vs. the remaining facial expressions. Values represent adjusted means (ANCOVA analysis). Error bars represent SEM. *p <0.05 and + p < 0.10.

In order to explore the interaction between group and valence further, separate repeated measures ANOVAs were conducted for the two groups. This analysis revealed an effect of picture valence in the placebo group [F(2,32)=3.612, p=0.039], but not in the fluoxetine group [F(2,28)=2.500, p=0.100]. The participants on placebo showed the normal emotion-potentiated startle effect, with the z-transformed scores for the unpleasant pictures being significantly higher than those for neutral (p=0.002) and pleasant (p=0.008). However, this emotion potentiation of the startle response was eliminated in the fluoxetine group when compared to placebo (all *p*s>0.21; see Figure 4 a).

Repeating this analysis with gender as an additional between-groups factor showed that this variable did not influence the effects of fluoxetine on the z-transformed scores [as revealed by a lack of valence x group x gender interaction: F(2,58)=0.163, p=0.850].

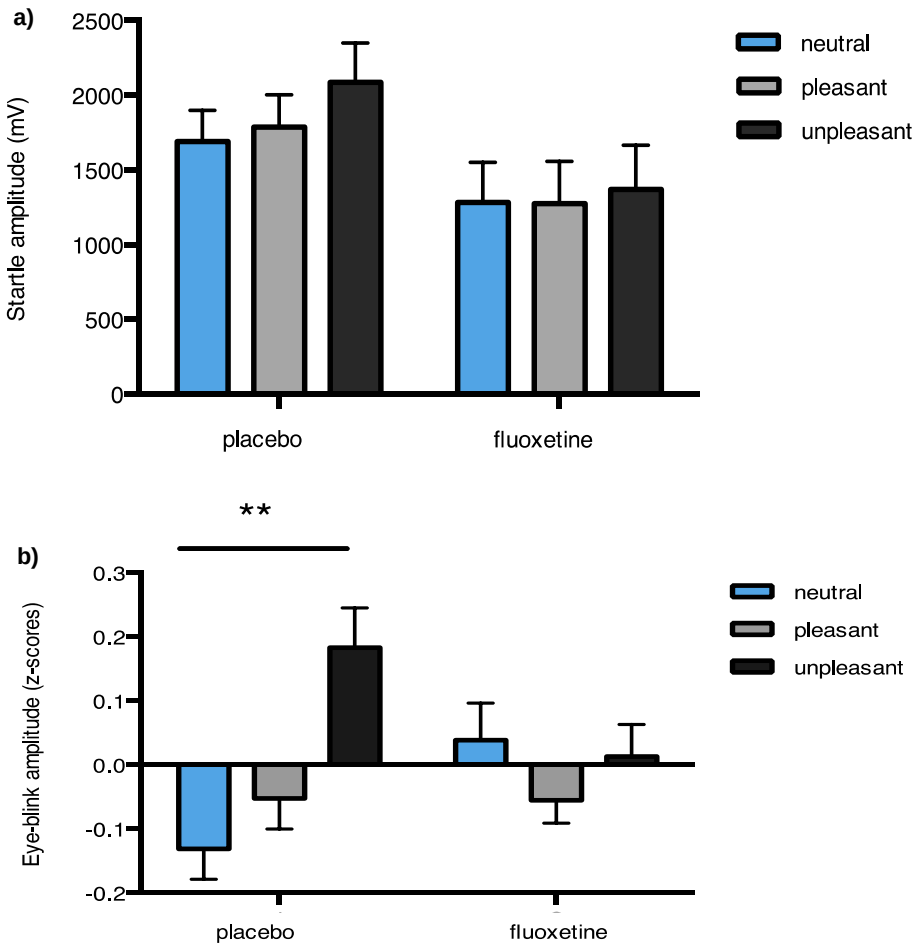

Figure 4. Emotion Potentiated Startle. Effect of fluoxetine vs. placebo on emotion-potentiated startle responses**.** Values are average z-transformed **(a)** or raw amplitude **(b)** eye-blink responses to the acoustic probes in the context of neutral, pleasant, and unpleasant pictures. Values represent adjusted means (ANCOVA analysis). Error bars represent SEM. **p<0.01

When considering raw scores, there was a trend for a significant interaction between emotion and group [F(2,62)=3.376, p=0.066] but no main effect of group [F(1,31)=0.416, p=0.524], reflecting the same pattern seen in the z-transformed scores (see Figure 4b).

2.3.5. Attentional Dot-Probe Task

There were no significant differences in accuracy rates between the two groups [placebo mean: 93.9%, fluoxetine: 93.8%; t(33)=0.160, p=0.874]. Incorrect trials were therefore not included in the reaction time analysis. In order to minimize the influence of outliers, trials with response times that were above 1200ms and below 200ms, as well as those that were 3 standard deviations above or below the mean RT (computed after the first cropping) were excluded (Browning, Holmes, Charles, Cowen, & Harmer, 2012).

A 2x2x2 split-plot ANCOVA was computed, with valence (happy vs. fearful) and stimulus presentation (short vs. long) as within-subject variables, group (placebo vs. fluoxetine) as a between-subjects factor and average STAI-S as a covariate. This analysis failed to reveal a significant interaction between valence and group [F(1,33)=2.718, p=0.109] or a main effect of group [F(1,33)=0.034, p=0.855].

2.3.6. Rapid Visual Serial Presentation

The analysis for this task was performed with 33 participants (16 in the fluoxetine group and 17 in the placebo) due to technical problems in data acquisition for two subjects. Consistent with previous studies using this task (Anderson & Phelps, 2001; De Martino et al., 2008), data were segregated into early (lags 2–3, 260–390ms) and late lag (lags 6–7, 780–910 ms). We performed a 3×2×2 drug split-plot ANCOVA: T2 stimulus valence (positive, negative, neutral) × lag (early vs. late) x group (fluoxetine vs. placebo).

The analysis revealed a trend for a main effect of lag [F(1,30)=3.105, p=0.088]. Follow-up pairwise comparisons revealed a higher number of correct responses in late vs. early lags (87.60% vs. 75.13%). There was no main effect of group, valence or any significant interactions with lag and valence (all *p*s>0.20).

2.4. Discussion

This study investigated the influence of acute fluoxetine administration on a battery of tasks previously shown to be sensitive to both the positive and negative effects of SSRIs on emotional processing (Harmer, Cowen, et al., 2011). The aim was to test whether fluoxetine would increase threat processing in this sample of young adult volunteers; and if any effects on the processing of anger would be apparent given fluoxetine's efficacy in treating patients with anger problems (Coccaro et al., 2009; Fava et al., 1991). Contrary to initial predictions, fluoxetine did not show an anxiogenic profile. Rather, the emotion-potentiated startle effect, a measure of fear processing, was eliminated after a single dose of fluoxetine (vs. placebo). The second hypothesis was nonetheless corroborated, as revealed by the decreased recognition of angry faces and the reduced number of anger misclassifications in the fluoxetine group. There was also a trend for participants on fluoxetine to be slower to respond to this emotion. Beyond these effects, fluoxetine

reduced the accuracy to detect sad facial expressions and produced a marginal increase in the recognition of happy faces. Finally, the influence of fluoxetine occurred in the absence of any pronounced overall changes in mood or anxiety, which suggests an immediate, direct effect of this medication on emotional processing.

Lack of acute anxiogenic effects of fluoxetine

Contrary to the initial prediction, fluoxetine did not reveal anxiogenic-like effects in this sample of young adult volunteers, showing instead a profile more consistent with anxiolysis. Indeed, participants on fluoxetine did not demonstrate the expected increase in the magnitude of startle responses during the presentation of unpleasant pictures. The startle paradigm is a well-established measure of fear and anxiety, and highly sensitive to the clinical effects of anxiolytic drugs (see Davis et al., 1993). An abolition of fear potentiated responses has been found previously with acute administration of the anti-anxiety drug diazepam (Murphy et al., 2008). Likewise, preclinical models using shock-induced vocalizations and conditioned freezing have reported anxiolytic effects after acute administration of fluoxetine (Nakamura & Kurasawa, 2001; Schreiber et al.,1998; but see Kurt et al., 2000).

This anxiolytic-like effect is nonetheless in contrast with previous studies showing that a single dose of the SSRI citalopram increases the processing of threat-related stimuli, as reflected by an increased recognition of fearful facial expressions (Browning et al., 2007) and higher emotional reactivity in the startle paradigm (Browning et al., 2007; Grillon et al., 2007). Such findings are thought to reflect the mechanisms responsible for the anxiogenic reactions seen with some patients at early stages of SSRI treatment (Kent et al., 1998). It is notable that fluoxetine failed to show the anxiogenic profile seen previously with citalopram, a difference that may be explained by the specific pharmacodynamic properties of fluoxetine, or by the characteristics of this population itself, given that previous studies using single doses of citalopram included participants who were, on average, slightly older (average of 24 years in the Browning et al., 2007 study; average age of 37-40 in the Harmer, Bhagwagar et al., 2003 study).

Despite belonging to the same class, citalopram and fluoxetine have a number of pharmacological differences that could underlie their contrasting acute effects on threat processing. For example, fluoxetine shows an affinity for the 5-HT_{2C} receptor that is close to its affinity for the 5HT transporter (Hoffman, Mezey, & Brownstein, 1991) and about five-fold greater than that of

citalopram (Pälvimäki, Kuoppamäki, Syvälahti, & Hietala, 1999). Together with evidence that fluoxetine acts functionally as a 5-HT_{2C} receptor antagonist, these findings have led to the proposal that some of its effects may be mediated via blockade of 5-HT_{2C} receptors, in addition to the well-characterised inhibition of the serotonin transporter (Ni & Miledi, 1997).

Notably, the anxiolytic-like effects reported in the current study are consistent with a $5HT_{2C}$ antagonism mechanism. Drugs that act as antagonists at this receptor have been shown to decrease anxiety-related responses in pre-clinical and human studies (e.g., Kennett et al., 1997; Martin et al., 2002). An example is the 5-HT_{2C} antagonist (and antidepressant) mirtazapine, which decreases the processing of threat-related information as well as startle responses in healthy volunteers (Arnone, Horder, Cowen, & Harmer, 2009).

The present study did not detect any robust effect of fluoxetine on the tasks used to measure attentional vigilance towards positive and threatening information (i.e., Attentional Dot-Probe and Rapid Visual Serial Presentation). Attentional bias towards threat is a key feature of anxiety and can be modulated by drug and psychological treatments which have clinical effects in these disorders (Murphy et al., 2008; Reinecke et al., 2013). This absence of effect therefore provides further evidence that acute fluoxetine does not induce an anxiogenic-like profile in young healthy people, although this interpretation is limited due to the absence of a basic fear vigilance effect in the placebo group. Given the findings from the Facial Expression Recognition Task, future research may wish to include anger cues in these measures, to assess the likely contribution of attention vs. perceptual changes in anger processing following fluoxetine administration.

Further studies should also explore the mechanisms underlying the adverse effects seen clinically in some patients. Although these findings propose an acute anxiolytic-like effect of fluoxetine, the possibility that this medication and other SSRIs may increase the processing of anxiety-related stimuli in vulnerable patients should not be excluded. Relevant to this is evidence suggesting that adverse reactions to SSRIs are influenced by variables such as drug metabolism (Brøsen, 2004) and, more broadly, genotypic differences (Kronenberg et al., 2007; Perlis et al., 2003). There is consequently a need to identify both genetic and psychological markers that can reliably predict which patients are most susceptible to encounter side effects after the initiation of antidepressant treatment.

Early effects of fluoxetine on anger processing

A prominent finding of the current study is the effect of fluoxetine on anger processing. Fluoxetine was shown to decrease the accuracy to correctly identify angry faces, as well as the percentage of emotions incorrectly labelled as angry, suggesting that, overall, participants on the active treatment were less likely than controls to "see" anger in this task. This highlights an important mechanism through which fluoxetine may act to alleviate symptoms in adolescent MDD.

This effect on anger could be considered a more specific effect of fluoxetine, given that other antidepressants do not seem to influence this emotion to the same extent (Pringle et al., 2011). Anger is a hallmark feature of irritability, which is one of the core symptoms of childhood and adolescent depression (American Psychiatric Association, & DSM-5 Task Force, 2013). Despite the limited research investigating emotional processing biases in young people, some studies suggest that depressive symptoms in adolescence are associated with an increased processing of anger (van Beek et al., 2006) and sadness (Schepman et al., 2012), as well as a decreased perception of happy faces (Beek & Dubas, 2008). This is consistent with the notion that depressed individuals are primed to perceive the world in a manner consistent with their internal feelings, given that sadness and irritability are two prominent symptoms of juvenile MDD. Fluoxetine could therefore act by reversing the biases that seem to characterise depression in young people.

The importance of anger/irritability in depression has been a focus of increasing interest (Fava et al., 2010; Judd, Schettler, Coryell, Akiskal, & Fiedorowicz, 2013; Stringaris, et al., 2013). Recent clinical studies suggest that irritability is common among depressed adults (Fava et al., 2010; Judd et al., 2013), indicative of earlier onset and increased disease severity (Judd et al., 2013; Stringaris et al., 2013), as well as a potential marker for a diagnostic subtyping symptom (Perlis et al., 2009). In adolescence, irritability is a cardinal symptom for establishing an MDD diagnosis and seems to affect one third of depressed youth in community samples (Stringaris et al., 2013) and up to 30-85% in clinically depressed populations (Ryan et al., 1987; Strober et al., 1981). There is growing evidence for the implications of irritability as a developmental symptom relevant for both the aetiology and the clinical presentation of depression. Stringaris and colleagues (2013) recently reported that irritability, when manifested during adolescent MDD, is more frequent in males, and is also associated with an increased risk for comorbid disruptive symptoms and sleep disturbances. Additional studies highlight the potential etiological role of this symptom in the development of depressive states, especially in light of evidence suggesting that irritability predicts

depression in early and late adulthood (Dougherty et al., 2013; Leibenluft, Cohen, Gorrindo, Brrok, & Pine, 2006; Stringaris & Goodman 2009; Stringaris, Cohen, Pine, & Leibenluft, 2009), and also shares common genetic underpinnings with this disorder (Stringaris, Zavos, Leibenluft, Maughan, & Eley, 2012).

This influence on anger processing is also of interest in view of the effects of serotonin on anger and aggressive behaviours more broadly. Fluoxetine increases 5-HT levels through inhibition of the serotonin transporters and has important effects in alleviating anger symptoms across different populations, including depressed patients with anger attacks (e.g., Fava et al*.,* 1991), patients with intermittent explosive disorder (Coccaro & Kavoussi, 1997) and females with premenstrual disorder suffering from irritability (Steinberg, Cardoso, Martinez, Rubinow, & Schmidt, 2012). In line with this, experimental manipulations that reduce serotonin levels via acute tryptophan depletion (ATD) exert the opposite effect of increasing aggression-related responses, particularly in individuals high in irritability (e.g., Bjork et al., 2000; Bond et al., 2001; Cleare & Bond, 1995; for a review see Young, 2013).

Beyond anger – effect on sadness and marginal influence on happiness

Beyond the effects seen on anger, fluoxetine was also shown to modulate the recognition of sadness, and, potentially, of happiness as well. The decreased processing of sad faces is consistent with a study by Harmer, de Bodinat et al. (2011), in which 7-day treatment with the 5-HT_{2C} antagonist agomelatine also impaired the processing of sad facial expressions in the same facial recognition paradigm. Similarly, the increased detection of happy faces parallels several studies involving single doses of different antidepressants. For instance, a single dose of the selective noradrenergic reuptake inhibitor (NRI) *reboxetine*, the selective serotonin reuptake inhibitor (SSRI) *citalopram* and the serotonin-norepinephrine reuptake inhibitor (SNRI) *duloxetine* have all been shown to increase the recognition of happy faces (Harmer, Bhagwagar, et al. 2003; Harmer et al. 2008). The production of a bias away from negative information and towards positive cues may therefore represent a critical mechanism underling a wide range of antidepressant drugs. Despite needing further replication, these effects may be particularly relevant for adolescent depression. Indeed, a bias to detect negative vs. positive information is a cognitive feature of this disorder (Lakdawalla et al., 2007), and sadness is also a prominent symptom of adolescent MDD (see Stringaris et al., 2013).

Limitations

It is important to consider some limitations to the present study. First, despite the fact that this study was of a randomised double-blind design, the groups differed in state anxiety. This discrepancy was resolved by including the scores as a covariate in the subsequent analyses.

It would have been useful to assess the effects of citalopram within the same study, in order to clearly dissociate the effects of fluoxetine and citalopram on emotional processing and the likely contribution of their pharmacological properties to these effects. Similarly, the inclusion of an older group would help determine whether the effects seen here are specific to fluoxetine and/or to young people.

The lack of effects of fluoxetine on the Attentional Dot-Probe and Rapid Visual Series Presentation was interpreted as further evidence that fluoxetine does not induce anxiogenic-like effects in this population. However, this interpretation was limited by a lack of replication of the threat vigilance effect that was expected in the placebo group, particularly in the Attentional Dot-Probe. It would be advantageous to develop a paradigm that is more robust in its assessment of attentional biases towards threatening information, in order to more clearly characterise the effects of fluoxetine on threat processes.

2.5. Conclusion

These findings suggest that a single dose of fluoxetine has direct effects on the way emotional information is processed in this sample of young healthy people. Given the critical role of anger in the clinical presentation of adolescent depression, this reduction in anger perception following fluoxetine administration (vs. placebo) highlights a potential mechanism through which this medication may exert its clinical action. The relative facilitation of positive (happy) vs. negative (anger and sadness) facial expression recognition more generally may represent a broad effect for reducing the affective symptoms that characterise depressive states. However, the influence on happiness was of marginal significance and therefore needs to be further replicated.

Fluoxetine also showed an anxiolytic-like profile, which was contrary to the initial study hypothesis. The mechanism of action of fluoxetine in this respect show potentially important differences to other antidepressants such as citalopram, which is pertinent in face of evidence that fluoxetine may have a more favourable benefit-to-risk profile in comparison to this and other

antidepressant drugs in young people with depression (Whittington et al., 2004). However, the interpretation of this effect was nonetheless limited by the methodological limitations seen in the attentional paradigms used to measure attentional vigilance to threat.

In light of this, the following Chapter (Chapter Three) is aimed at developing a more robust and sensitive paradigm to detect attentional vigilance mechanisms towards threat. In addition, and following on the need to clarify if the current profile of effects extend to clinically depressed and younger populations, the study presented in Chapter Four examines the influence of a single dose of fluoxetine (vs. placebo) on emotional neural processing in a group of depressed adolescents who have been prescribed this medication for depression.

Chapter 3:

Validating the use of Continuous Flash Suppression to detect automatic anxiety-related biases and anxiolytic effects

3.1. Introduction

The behavioural findings reported in Chapter Two add to the growing hypothesis that drugs used to treat depression and anxiety act early in administration to remediate the cognitive biases that characterise these disorders (Harmer & Cowen, 2013). Indeed, a single dose of fluoxetine was found to decrease the recognition of angry faces (vs. placebo), whilst having a broader influence on the processing of positive vs. negative information. A decrease in emotional reactivity was also verified in a paradigm measuring startle responses to unpleasant stimuli.

In contrast to previous studies investigating the effects of SSRIs on attention (e.g., Browning et al., 2007), fluoxetine failed to influence attentional biases to threat, as measured using the Attentional Dot-Probe and Rapid Visual Serial Presentation. One potential explanation for this lack of effects is that acute fluoxetine, unlike other SSRI drugs, does not influence attentional vigilance to threatening stimuli. However, such an interpretation is limited by the absence of a basic fear vigilance effect in the placebo group, particularly on the Attentional Dot Probe paradigm. The replication of this effect is critical to the validity of the task, especially when examining the effects of interventions that can modulate the magnitude and direction of attentional biases.

The Attentional Dot-Probe has been criticised for having very poor test-retest reliability and poor internal consistency, especially in non-clinical samples (Schmukle, 2005). Despite evidence suggesting a robust attentional bias towards threat in individuals with clinically diagnosed anxiety disorders (e.g., MacLeod, Mathews, & Tata, 1986; Mogg, Mathews, & Eysenck, 1992; Kroeze & van den Hout, 2000), this effect is much less consistent in healthy samples with varying levels of anxiety, where both positive and null findings have been reported (e.g., Bradley, Mogg, Falla, & Hamilton, 1998; Broadbent & Broadbent, 1988; Mogg, Bradley, de Bono, & Painter, 1997; Mogg et al., 2000). The reasons for this lack of reliability remain elusive, but it has been suggested that the Attentional-Dot Probe may show irregular or insufficient sensitivity to detect inter-individual

differences in attentional allocation to threat, a problem that may be more pronounced in healthy (i.e., non-clinical) samples (Schmukle, 2005).

The use of subliminal or masked techniques is often employed as an additional condition in the Attentional Dot-probe with the aim of measuring early, automatic biases towards threat. A comparable procedure is employed in the Rapid Visual Series Presentation, in which stimuli are presented under conditions of restricted attentional resources. Since these automatic biases operate at early stages of information processing, and are believed to be largely involuntary and outside of the participant's conscious control (Cisler & Koster, 2010), they could potentially represent a more sensitive measure of vigilance to threat. However, current procedures show methodological constraints in achieving successful masking or complete independence from top-down attentional responses. For instance, the use of subliminal stimulus presentation (as used in Chapter Two) does not guarantee a successful suppression of the visual stimulus on every trial or with every participant, given that some individuals can discriminate masked faces with stimulus onset asynchronies as short as 17–33ms (Pessoa, Japee, & Ungerleider, 2005). Likewise, the Rapid Visual Serial Presentation task does not allow the investigation of pure automatic processing given the high attentional demand of this paradigm, and the resulting influence of top-down strategic processes (see Kim & Blake, 2005). Together, these limitations have posed a significant constraint in measuring pure automatic or unconscious processes. It is therefore not entirely clear whether the lack of effects seen in some studies could reflect a "true" absence of effects on automatic or unconscious processes, or instead a limitation in assessment.

Given the pivotal role of preconscious mechanisms in the generation of anxiety responses (e.g., Cisler & Koster, 2010; Ouimet, Gawronski, & Dozois, 2009; Williams, 1997), it would be useful to validate a paradigm that is effective in measuring early preconscious mechanisms associated with attentional biases to threat. The development of such paradigm could potentially resolve some of the methodological constraints seen with the Attentional Dot-Probe and other tasks, and therefore provide a more robust and sensitive measure of attentional vigilance effects.

Continuous Flash Suppression (CFS) is a recently developed variant of Binocular Rivalry (Tsuchiya & Koch, 2005) that has the potential of providing a more reliable measure of early, automatic biases (Yang, Zald, & Blake, 2007). In this paradigm, a strong perceptual mask renders the target stimuli completely invisible for extended durations (up to one minute or more; Tsuchiya &

Koch, 2005). Hence, the time that this stimulus takes to reach awareness is taken as a robust index of preconscious processing. Because the target stimulus is made completely invisible prior to breakthrough (an effect that occurs in every trial and with every participant), it has been argued that any variable that facilitates its detection suggests enhanced processing that occurs prior to conscious awareness (Mudrik, Breska, Lamy, & Deouell, 2011). As a result, this paradigm has methodological strengths not achieved with traditional measures of preconscious or subliminal processing.

Using this approach, it has been found that high-contrast stimuli break suppression faster (Tsuchiya & Koch, 2005), as well as stimuli that are more familiar (Jiang, Costello, & He, 2007) and which convey an emotional threatening content (Yang, Zald, & Blake, 2007). Certain characteristics, such as familiarity and negative valence, may therefore benefit from a privileged encoding during the suppression phase (or in other words, benefit from a strong unconscious processing), ultimately strengthening the neural signals associated with the stimulus so that it emerges quicker into awareness (Yang, Brascamp, Kang, & Blake, 2014).

Stimuli made invisible using CFS can guide attention to potentially relevant stimuli and influence behavioural choices, physiological responses and subsequent conscious experiences. For instance, Jiang and He (2006) reported robust amygdala activity in response to invisible fearful faces, which was of similar magnitude to the response elicited by visible faces conveying the same emotion. Also, emotionally arousing stimuli made invisible by CFS have been found to influence the distribution of spatial attention to subsequent images presented consciously (Jiang, Costello, Fang, Huang, & He 2006). Taken together, these findings highlight the potential of CFS to measure the processing of stimuli outside of conscious awareness.

In light of these methodological strengths, this chapter aims to validate the use of CFS in detecting early anxiety-related biases. In order to achieve this, two studies were completed: the first (Study A) investigated the potential of CFS in measuring automatic-biases to threat in individuals with varying levels of anxiety, whereas the second (Study B) investigated its potential to detect anxiolytic effects induced by a commonly used anti-anxiety drug (diazepam). These studies are described in the sections below.

Study A: The use of CFS to detect early biases towards threat

3.2. Introduction

The aim of this study was to test the potential of CFS to measure automatic-biases to threat associated with anxiety. The presence of pre-attentive biases has long been highlighted in cognitive models of anxiety (e.g., Beck & Clark, 1997; Clark & Beck, 2010; Öhman, 1993; Öhman & Mineka, 2001) These models argue that anxiety is characterised by a hypervigilance to various forms of threat, which begins at an early, preconscious, stage of processing. Given that these biases are believed to operate automatically, outside of the participant's conscious control and independently of strategic influences (Cisler & Koster, 2010), they could potentially represent a more sensitive measure of vigilance of threat. Of note, these automatic biases seem to be at least partly distinct from those seen in depression, which is typically associated with biases in later, more strategic stages of processing (Gotlib & Joormann, 2010).

This experiment therefore examined the influence of subclinical symptoms of anxiety on times to detect fearful, happy and neutral faces presented in a CFS paradigm. The relative selectivity of this paradigm in detecting the effects of anxiety was tested by including a measure of depressive symptoms. It was hypothesized that anxiety, but not depression, would be associated with a faster detection of fear, thus indicating an early (and potentially preconscious) processing bias towards threatening stimuli that results in preferential access to awareness. If confirmed, this finding would demonstrate the validity of CFS in detecting such early biases in information processing.

3.3. Methods

3.3.1. Participants

Forty-nine healthy volunteers aged 18-29, with no reported history of neurological or psychological problems, free of any psychotropic medication and with normal or corrected-to-normal acuity were included in this study. A sample size of 49 participants was considered adequate. Indeed, a previous study with a lower sample size (N=36), found that trait anxiety was positively correlated with vigilance scores to fear (Fox, 2002), and this result had an effect size of

0.53. It was calculated that for an effect of this size, 34 participants would be needed to obtain a power calculation of 0.95 at α error probability of 0.05. However, given that the current study is the first to use CFS to measure the influence of anxiety on the access of emotional stimuli to awareness, it was decided to increase the sample size. This is expected to increase the power of the task and minimise the chance of type II errors.

The University of Oxford Research Ethics Committee provided ethical approval (reference: MSD/IDREC/C1/2011/62). The study was advertised using posters, web-advertisements and via word-of-mouth. Written informed consent was obtained from all participants. Participants were not directly reimbursed for their participation in the study, but they were entered in a competition to win a £20 Amazon voucher.

3.3.2. Experimental design and task

Interested participants where invited for a testing session at the Warneford Hospital. At the beginning of this visit, participants were briefly screened for the presence of current or previous psychological and medical disorders and recreational or psychotropic drug use. If eligible, they were then administered the State-Trait Anxiety Inventory (STAI) and Beck Depression Inventory (BDI) in order to determine their self-reported levels of anxiety and depression, respectively. This was followed by the administration of a Continuous Flash Suppression (CFS) task.

The CFS task used in this study was adapted from Yang, Zald, and Blake (2007). Stimuli consisted of grayscale faces displaying 1 of 3 different expressions (neutral, happy and fear) and grayscale Mondrian-like patterns as the suppressor mask. The face stimuli (2 male and 2 female identities) were taken from the standard Ekman set of facial expressions (Ekman & Friesen, 1976). All images were cropped in a square shape to remove features outside of the faces and the edges were smoothed using a Gaussian filter. The suppressor mask consisted of random combinations of partly overlapping rectangles of varying sizes and luminance (Mondrian patterns, see Figure 5). To achieve an initial strong suppression, the faces were normalized to 20% contrast (root mean square), whereas the Mondrian patterns were normalized to 60% contrast (root mean square).

Stimuli were generated with the Psychtoolbox (http://psychtoolbox.org/HomePage) for Matlab version 2010a (Mathworks, Inc.) and presented at 60 Hz on an LCD display (resolution: 1280*1024 pixels, 37.5 width Dell monitor). Stimuli were viewed using a mirror stereoscope, which

was positioned in front of the monitor and connected to a chin rest. All stimuli were surrounded by rectangular borders that served to promote stable binocular eye alignment. A black cardboard divider of matte material was placed between the participant's eyes, extending from the stereoscope midline towards the centre of the display in order to block the line of vision to the other eye's stimulus. Viewing distance was 49cm.

The lights were switched off during the experiment. Before the task was initiated, the mirrors of the stereoscope were carefully adjusted so that the fusion contours surrounding the stimuli were combined to produce a square frame, which occurred only when the eyes were appropriately aligned.

In the initial 1000ms, one eye was presented with full contrast, dynamic Mondrian patterns (4° x 4°; refreshed at 10Hz), whilst the other eye viewed a low contrast face image (1.7° x 1.7°). The contrast of the face was ramped up at a rate of 2% every 20ms, hence avoiding abrupt transients. When the face reached full contrast (1000ms after presentation onset), the contrast of the Mondrian patterns linearly decreased at a rate of 2%/100ms for the next 5100ms. The face remained at full contrast until a response was made (see Figure 5). All stimuli, as well as the eye to which the Mondrian patterns or faces were presented, were randomised and counterbalanced across trials. Given the design of the paradigm, participants were unable to predict the emotional expression of the face on a given trial, which therefore prevented the occurrence of *criterion biases* in their responses.

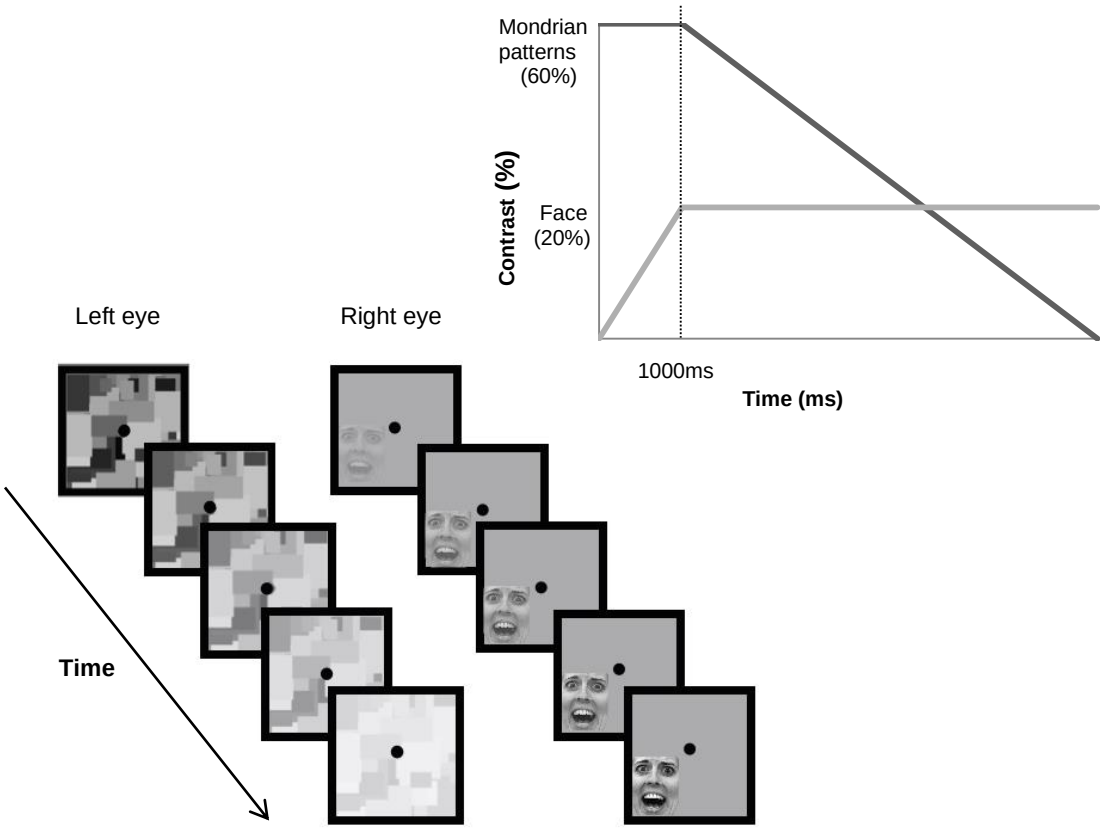

Figure 5. Example of a trial presentation in CFS. One eye was presented with flashing Mondrian-like pattern images, while the other viewed a facial image of 1 of 3 expressions (fearful, happy or neutral). The face contrast was ramped up to 20% by the end of the first 1000ms, point at which the contrast of the Mondrian patterns decreases from 60% until the observer indicated the location of the face via button press.

The face was presented within one of the four quadrants of the square. Participants were required to report its location via one of four corresponding buttons on a keyboard, as quickly and accurately as possible. Participants were instructed to press the button as soon as any part of the face (such as the eyes, mouth, etc.) emerged. Trials (n=180) terminated upon response and there was a self-timed break every ten trials. Visual and auditory feedback was given for incorrect responses.

3.3.3. Statistical analyses

The assumption of normality was assessed for all data. A logarithmic transformation was applied to both reaction times and depression scores to reduce the influence of positive skewness (Ratcliff, 1993). Transformation proved successful in assuring normality for reaction times but not for depressive symptoms.

Statistical analyses were performed on transformed reaction time data, but figures present original values for ease of interpretability. A repeated measures ANOVA was used to test the RT differences between fearful, neutral and happy faces. Any significant main effects were further explored using follow-up pairwise comparisons.

Two hierarchical multiple regressions were conducted in order to assess the predictive value of anxiety and depressive symptoms in explaining the differences in RTs to identify 1) happy vs. fearful and 2) happy vs. neutral faces. The relevant assumptions of this statistical analysis were tested. A final sample size of 46 was judged adequate given that three independent variables were included in each regression (15 participants per independent variable as rule of thumb; Stevens, 2009). The assumption of singularity was also met, as the independent variables (state and trait anxiety and depression) were not a combination of other independent variables. In order to examine the assumption of multicollinearity, the intercorrelation between the predictor variables were computed (see Table 4) and the collinearity statistics tests (i.e., Tolerance and Variance Inflation Factor, VIF) were analysed. This analysis revealed a high correlation between the predictor values, but the r-values were still below the suggested cut-off value of 0.8 (Hensher, Rose, & Greene, 2005). In addition, and most importantly, the tolerance and VIF values were well within accepted limits (Hair, Anderson, Tatham, & Black, 1995; Rovai, Baker, & Ponton, 2013). Therefore, the assumption of absence of high multicollinearity was considered to have been met. An examination of the Cook's distance score indicated no outliers. Finally, residual and scatter plots indicated the assumptions of normality, linearity and homoscedasticity were all satisfied (Hair, Anderson, Tatham, & Black, 1995).

Table 4. Intercorrelations among self-report measures

	State anxiety (STAI-S)	**Trait anxiety (STAI-T)**	**Depression (BDI)**
State anxiety (STAI-S)			
Trait anxiety (STAI-T)	0.680		
Depression (BDI)	0.491	0.682	

Note. All *r* values are significant at $p < 0.05$.

3.4. Results

3.4.1. Participants

Participants' trait anxiety scores ranged from 21 to 58 (mean=35.46; SD=8.15), and their state anxiety scores ranged from 20 to 52 (mean=32.20; SD=7.33). These scores are similar to the published norms for this age group (Spielberger et al., 1970). The mean for the depressive symptoms was 4.89 (SD=4.60), with values ranging from 0 to 19 (see Table 5).

Table 5. Anxiety and depression scores

	Mean (SD)	Range
Trait anxiety (STAI-T)	35.46 (8.15)	21 – 58
State anxiety (STAI-S)	32.20 (7.33)	20 – 52
Depression (BDI)	4.89 (4.60)	0 – 19

3.4.2. Effect of valence on the RTs taken to detect fear, happy and neutral

Reaction time (RTs) on correct trials was the primary outcome measure in this study. Data from 3 participants were excluded from the analysis because they made over 10% errors (consistent with Yang, Zald, & Blake, 2007), and therefore the final dataset consisted of 46 participants (24 female). The mean error rate was 2.17% (SD=2.02%). Incorrect trials were removed from the analysis, as well as trials in which the RT was +/- 2 standard deviations from the average RT. In total, 7.00% of the trials were removed (SD=2.25%). The percentage of trials removed did not differ across facial expressions (p=0.673).

Consistent with the work of Yang and colleagues (2007), the emotional expression of the faces influenced the rate at which they were detected. A repeated measures ANOVA showed a main effect of emotion [$F(2,90)=8.930$, $p<.0001$]. Follow-up pairwise comparison tests revealed that participants were faster to detect fearful faces than happy faces (means: 2.709 seconds vs. 2.819 respectively; p=0.001), but no significant differences were found between fearful and neutral faces (2.709s vs. 2.747s; p=0.251). Neutral faces were also detected faster than happy faces (2.747 vs. 2.819; p=0.003; see Figure 6).

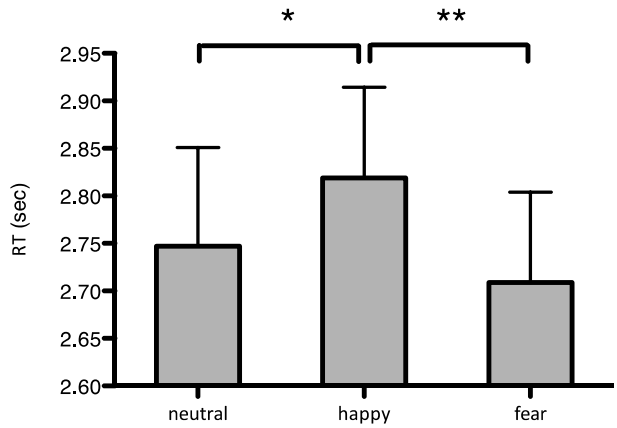

Figure 6. Continuous Flash Suppression. Average reaction times (RTs) for each of the facial expressions. Error bars represent standard error of the mean (SEM). ** $p < 0.01$. * $p < 0.05$.

3.4.3. Effects of Anxiety and Depression on RTs

Given that fearful and neutral faces were detected faster than happy faces, a three stage hierarchical multiple regression was performed in order to assess the predictive value of anxiety in explaining these differences in RTs. Depression was also included in order to compare the influence of these variables directly, given the hypothesis that anxiety but not depression would be associated with an increased bias towards threat.

A first hierarchical multiple regression model was therefore computed, in which the happy-fear RT difference was entered as the dependent variable, and STAI-T, STAI-S and depression BDI as the three independent (predictor) variables. STAI-T scores were included at stage one, STAI-S at stage two and BDI at stage three.

This hierarchical multiple regression revealed that at stage one, STAI-T symptoms contributed significantly to the model [$F(1,45)=5.639$, $p=0.022$], and accounted for 11.4% (R^2aj=9.3%) of the variance in the difference in RTs between happy and fear. STAI-T was significantly correlated with the dependent variable ($\beta=0.337$, $p=0.022$), suggesting that higher trait anxiety scores were associated with an increased relative speed to detect fear in comparison to happy faces (see Figure 7).

Introducing the variable STAI-S did not significantly improve prediction (R^2 change=0.002, $F=0.121$, $p=0.730$), nor did BDI (R^2 change=0.004, $F=0.214$, $p=0.646$). Consistent with this, both STAI-S and BDI failed to show a statistically significant correlation with the dependent variable in this model ($p>0.6$; Table 6).

A second hierarchical regression model was computed, in which the predictive value of both anxiety and depressive symptoms in explaining the RT differences between happy and neutral conditions was assessed. This multiple regression revealed that the models including trait/state anxiety and depressive symptoms failed to significantly predict this variable (all *p*s>0.1).

Table 6. Summary of hierarchical regression analysis for variables predicting happy-fear RTs difference

Steps	Variable	R^2 (R^2aj)	F (1,45), p value	ΔR^2	β	t	p-value
Step 1	**Trait anxiety (STAI-T)**	11.4% (9.3%)	5.639, p=0.022	0.114	0.337	2.375	0.022
Step 2	**Trait anxiety (STAI-T)**	11.6% (7.5%)	2.824, p=0.070	0.002	.383	1.961	0.056
	State anxiety (STAI-S)				-0.068	-0.348	0.730
Step 3	**Trait anxiety (STAI-T)**	12.1% (5.8%)	1.919, p=0.141	0.004	0.443	1.881	0.067
	State anxiety (STAI-S)				-0.063	-0.321	0.750
	Depression (BDI)				-0.092	-0.462	0.646

Note. Dependent variable – difference in Reaction Times (RTs) between happy and fear; Predictors (independent variables) – trait anxiety (step 1), state anxiety (step 2) and depressive symptoms (step 3).

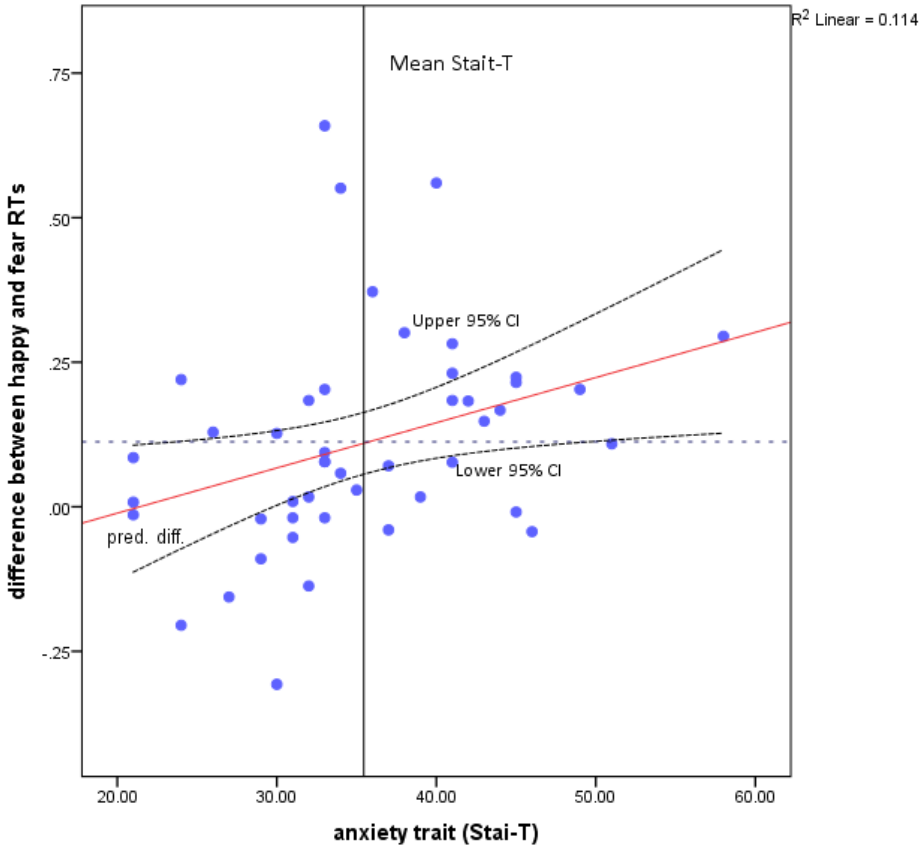

Figure 7. Scatterplot and regression line of happy-fear difference against trait anxiety scores. The points in the graph are the difference scores between the reaction times (RTs) for detecting happy and fearful faces. The mean difference of these scores is shown by the coarsely-dashed horizontal line. Vertical line represents the average of trait anxiety (STAI-T) scores. The sloping unbroken line between the curved lines is the regression line showing the relationship between the difference scores and STAI-T.

3.5. Discussion

The aim of the current study was to investigate the influence of anxiety symptoms on the access of emotional stimuli to awareness using CFS, a stringent paradigm of visual suppression. The finding of a faster detection of fearful faces in comparison to happy is consistent with the study by Yang, Zald and Blake (2007), and therefore provides additional evidence from a much larger sample in support of the view that threat-related stimuli are prioritised over positive stimuli under conditions of strong perceptual suppression. Consistent with the initial hypothesis, higher trait anxiety scores were associated with a faster detection of happiness vs. fear. This finding suggests a selective influence of anxiety on the relative speed to detect fearful expressions, which is further corroborated by the lack of predictive value of depression in this model (even though anxiety and depression scores were themselves highly correlated across individuals). In addition, and of

special relevance to the current thesis, this finding corroborates the validity of using CFS to detect early biases towards threatening information.

The strength of using CFS to measure early, automatic processing of emotion

The present study confirms the validity of a paradigm that can be used to more reliably investigate early, automatic processing mechanisms, in a way that is not achieved with standard paradigms of visual or attentional suppression. The superiority of CFS comes from a more precise control over the point at which a stimulus is consciously perceived, which is not ensured with the traditional method of binocular rivalry (BR). In BR, the perceptual alternation between stimuli is unpredictable and the suppression times are variable and usually very short. In CFS there is a similar competition between images, but in this paradigm the target stimulus is suppressed by a strong suppressor mask which, due to its dynamic nature, is more resistant to neural adaptation. CFS therefore ensures much longer suppression times (up to 13 times longer than for BR) that occur in every trial (Tsuchiya & Koch, 2005).

CFS also overcomes problems found in other paradigms such as backward visual masking and subliminal presentation. In these paradigms, it is extremely difficult to ensure that stimulus presentation is truly outside of awareness. For instance, the use of fixed presentation times does not account for the fact that visual awareness thresholds vary across time and between individuals (Matthews & Wells, 2000). Although including post-trial awareness 'checks' may aid in differentiating between those individuals in which masking was successfully ensured or not, these measures are not completely immune to priming effects or response biases. In contrast, the CFS paradigm used here directly measures the threshold at which different stimuli are consciously perceived after an initial period of rigorous visual suppression that is successfully ensured in every trial and with every individual. Furthermore, these detection thresholds were acquired with an objective location discrimination task that minimised the influence of potential response biases.

Taken together, these characteristics of CFS allow for a more rigorous and unbiased assessment of which features influence the transition of different cues from a preconscious to a conscious state of visual perception. The current finding that fearful faces are detected faster than happy lends weight, therefore, to the argument that attentional biases operate automatically, prioritising the detection of threat-related stimuli at a very early stage of information processing

(e.g. Gray, Adams, & Garner, 2009; Singer, Eapen, Grillon, Ungerleider, & Hendler, 2012).

The effect of anxiety on early, automatic processing

In this study, higher trait anxiety scores were associated with an increased speed to detect fearful in comparison to happy facial expressions, a finding that provides critical support to contemporary models of anxiety and sheds light on the mechanisms through which these emotional biases are first manifested and how they may ultimately lead to uncontrollable anxiety thoughts and symptoms.

It is interesting to note that only trait anxiety was found to be correlated with the happy-fear difference, with state anxiety failing to show an association with this measure. This finding is consistent with the study by Doty, Japee, Ingvar, and Ungerleider (2013), which found a correlation between trait – but not state – anxiety and the sensitivity to detect masked fearful faces. Overall, this suggests that the early processing bias found in the present study is related to a more stable and robust personality measure of anxiety, rather than to situational oscillations in this variable.

The presence of automatic biases in anxiety has long been highlighted in cognitive models of psychological dysfunction (e.g., Beck & Clark, 1997; Clark & Beck, 2010; Öhman, 1993; Öhman & Mineka, 2001). Overall, these models share the assumption that threatening stimuli are initially processed at a pre-attentive level, which is thought to be characterized by all features of automaticity – i.e. stimulus-driven, unconscious, involuntary and completely independent from strategic or more elaborate processes (Cisler & Koster, 2010; Ouimet, Gawronski, & Dozois, 2009). These features have an adaptive purpose, as they allow for fast responses to any potential sources of threat, without the need for slower high-level processes. Anxiety is conceptualized as being the result of an increased sensitization towards the automatic detection of threat. This initial orientation to threat increases autonomic arousal and sets the stage for the activation of more elaborative threat-related processing biases that further reinforce anxiety (Cisler & Koster, 2010).

These ideas have propelled the development of different experimental paradigms aimed at tapping into the automatic nature of emotional processing biases, such as visual masking and binocular rivalry (BR). Using BR, Gray and colleagues (2009) found that anxiety symptoms were associated with an increased dominance of threat-related faces. Similarly, in studies using

backward masking techniques, anxious participants were faster to detect probes replacing masked threatening faces compared to masked positive or neutral (Mogg & Bradley, 1999).

Such studies have provided critical evidence in support of the hypothesis that anxiety is associated with early processing biases. However, these techniques face the limitations of not ensuring complete visual suppression or complete independence from strategic processes. The current study used a more robust technique – CFS – to surpass these potential limitations and the current findings are consistent with the existing literature: in individuals with higher levels of trait anxiety, fearful faces were faster to break visual suppression than happy faces.

The effect of depression

This study was not designed to fully test the hypothesis that variations in depression would also affect CFS. However, the lack of correlation between depression and the early detection of emotional faces is consistent with the long-standing view that depressed individuals fail to show automatic biases that orient their attention towards negatively-valenced stimuli (e.g., Gotlib and Joormann, 2010; Mathews & MacLeod, 2005). According to this perspective, depressed patients are not drawn to negative stimuli during early stages of visual processing, showing difficulty disengaging their attention only after these cues become accessible to their conscious perception.

The argument that depression is a disorder of predominantly high-level processing has nonetheless been challenged in recent studies. Sterzer, Hilgenfeldt, Freudenberg, Bermpohl and Adli (2011), using a paradigm similar to the one implemented here, found that depressive symptoms were associated with a shorter suppression of sad faces and, to a lesser degree, with longer suppression of happy. It is important to note, however, that some of the participants in their study also had co-morbid anxiety disorders, making it difficult to determine whether the effects obtained were due to depression alone or to an interactive effect of depression and anxiety. Future studies are necessary to clearly characterise the nature of information-processing biases in pure depressive states.

Limitations

Despite providing critical insight into the influence of anxiety in the early detection of threatening information, the interpretation of the current findings should be viewed in light of some

limitations. Anxiety and depression symptoms were substantially correlated, which excludes the possibility of drawing any firm conclusions regarding their separate effects or causality. However, the lack of predictive value of depression in explaining the difference in RTs between fearful and happy faces suggests that anxiety may play a greater role in modulating preconscious processing of threatening stimuli.

It should also be noted that differences in stimulus characteristics other than emotional content might have influenced the transition of facial stimuli in reaching awareness. The stimuli here were fully equated for overall contrast and mean luminance but not for other low-level spatial features, such as focal contrast around the eye region and spatial frequency content. Some studies point to low-level configurational features being the element of the face that drives the prioritisation of emotional stimuli, rather than the emotion content itself. For example, Gray, Adams, Hedger, Newton and Garner (2013) argued that the prioritisation effect seen with fearful faces may be fully explained by the salience of their low-level features (such as the high-contrast around the eyes), rather than by the emotional meaning conveyed by the faces themselves.

However, the use of low-level information in the threat prioritisation effect may correspond to an adaptive mechanism of our visual system. Much of the visual display that we see contains a majority of low spatial frequency information (Field & Brady, 1997; Geisler, 2008). Hence, it seems sensible that the visual system has adapted to respond quickly to low-level features, and that the fear advantage effect relies on this predisposition to quickly detect low spatial frequency information with the intention of identifying threat. Indeed, the finding that trait anxiety - which represents a variable that is independent from the physical properties of the stimuli - lowered the threshold to detect fearful vs. happy faces suggests an involvement of psychological elements in the transition of threatening stimuli into awareness. Regardless of the precise mechanism (low- or high-level) through which the effect is achieved, these findings suggest a meaningful association between anxiety and the speed to detect fearful faces in CFS. However, future studies should nonetheless explore the influence of low-level features in the detection of threatening stimuli by highly anxious individuals so that the mechanism underlying such effect is clearly characterised.

Finally, it is important to note that additional studies investigating the presence of preconscious biases in anxiety using CFS should also include a binocular control condition not involving interocular suppression, but which replicates the perceptual characteristics of CFS. The

use of such condition would be important to clearly determine if the threat bias found in highly anxious individuals is caused exclusively by stronger unconscious processing, or by a lower threshold for conscious detection.

Study B: The use of CFS to detect anxiolytic effects

3.6. Introduction

Another way of validating the use of CFS as an anxiety-related measure is to test its sensitivity to pharmacological agents known to be effective in the treatment of anxiety disorders. If this paradigm proves to be sensitive to the effects of anxiolytic drugs in a manner consistent with their clinical action, such finding would corroborate the utility of using CFS to detect attentional biases that are relevant for anxiety.

Diazepam is commonly used in clinical practice to treat a wide range of anxiety disorders. It belongs to the class of benzodiazepines, and is known for its anxiolytic, sedative, amnesiant, muscle relaxant and antiepileptic properties (Mandrioli, Mercolini, & Raggi, 2008). Despite some of these effects being adverse and the focus of clinical concern, benzodiazepines are effective in reducing anxiety symptoms (Buffett-Jerrott & Stewart, 2002). Consistent with its clinically efficacy, a number of studies suggest that diazepam acts on emotional processes relevant to anxiety, including attention, facial recognition and startle responses. For instance, acute diazepam administration has been reported to decrease the recognition of both angry and fearful expressions (Blair & Curran, 1999; Zangara et al., 2002). In line with this, Murphy, Downham, Cowen and Harmer (2008) found that a single dose of diazepam modulated attentional bias to masked emotional stimuli.

The current investigation sought to use CFS in order to examine the effects of short-term (7-8 days) administration of diazepam treatment on preconscious processing. Based on research examining acute doses of the drug, it was predicted that diazepam would reduce the detection of threatening stimuli in comparison to positive. More specifically, participants receiving diazepam were expected to show longer detection times towards fearful vs. happy faces in the same CFS paradigm used in Study A.

3.7. Methods

3.7.1. Participants

Participants were recruited through adverts on local information websites, posters and emails to student bodies within colleges. Interested participants were invited to come in to the Department of Psychiatry for a screening session. The screening consisted in the administration of a standard psychiatric interview and a medical assessment conducted by a research Psychiatrist. Exclusion criteria included: current or past Axis-I psychiatric disorder (assessed via the Structured Clinical Interview for DSM-IV; First, 1997): current or recent (<1 month) use of psychotropic medication other than the oral contraceptive pill; current or recent (<3 months) use of recreational drugs; smoking frequency greater than five cigarettes per day; pregnancy or breast-feeding; and BMI less than 19 or greater than 30.

After screening, 35 participants were deemed suitable for participation in the study. However, due to technical problems (N=3) and difficulties in achieving fusion (N=1) during the testing session, only 31 participants were included in the final analysis. This sample size is in line with a power calculation based on a study conducted in our laboratory, in which emotional processing tasks were administered following a single dose of diazepam vs. placebo. In this study, which included 24 healthy volunteers in total, the size of the effect of diazepam on masked vigilance to happy-neutral faces was 1.13. It was subsequently calculated that for an effect of this size, a minimum of 15 participants per group would be needed to obtain a power calculation of 0.90 at α error probability of 0.05

3.7.2. Experimental design and procedures

Participants were randomly assigned, in a double-blind procedure, to a short-term treatment with either 15 mg of diazepam (n=18) or placebo (n=17). Treatment included 7 or 8 days of drug/placebo administration. The additional day was used in some instances for scheduling reasons. It should be noted that, initially, participants were only provided with half the drug/placebo pills, with the remainder being given at a second meeting in the middle of the week. This provided an opportunity to check the adherence to the drug (as participants were asked to return the empty pill boxes), monitor the presence of any side-effects as well as reduce the potential for abuse of the

drugs. Participants were instructed to abstain from alcohol during the treatment period, and not to cycle or drive when coming to the study sessions. The treatment course for females was scheduled such that the testing days did not fall within the premenstrual week.

Participants took 5mg of diazepam or placebo in the morning after breakfast, and 10mg in the evening after their evening meal. Before taking the medication each morning, participants completed a side-effects checklist, and a number of measures detailing subjective mood and energy for the previous 24 hours: Visual Analogue Scales (Bond & Lader, 1974), the Befindlichskeit Scale of Mood and Energy[2] (BFS; Von Zerssen, Strian, & Schwarz, 1974) and the Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988). Testing occurred on the last two days of treatment, and participants were instructed to take their morning dose one hour prior to the start of testing. On one of the days (depending on availability), participants completed the same Continuous Flash Suppression (CFS) paradigm used in study A and some questionnaires: the state anxiety questionnaire and Visual Analogue Scales measuring alertness, anxiety and mood[3]. This study was part of a larger fMRI experiment being conducted in our laboratory. After completing the final tests, participants were unblinded by a third party. Participants received £100 reimbursement for their participation.

Continuous Flash Suppression Task

The task was the same CFS paradigm described in study A. The only difference was that the facial expressions were presented at a size of 2º instead of 1.7º (2º x 2º).

3.7.3. Statistical analyses

The assumption of normality was assessed for all data. A logarithmic transformation was applied to reaction times to reduce the influence of positive skewness (Ratcliff, 1993). Transformation proved successful in assuring normality.

[2] The BFS is a self-rating scale consisting of 56 pairs of adjectives that characterise opposite states of mood. Participants are asked to indicate which pair most corresponds with their current state. A detailed description of the other measures used in this study can be seen in Appendix I, as these scales were also used in Chapter Two.

[3] A shorter version of the Visual Analogue Scales was used on the test day.

Subjective ratings were analysed using a split-plot ANOVA. The baseline characteristics of the two groups (i.e., EPQ, IQ, BDI, STAI-T) were compared using independent t-tests. Gender distribution and testing order were analysed by using a chi-square test.

Daily measures of subjective mood and side-effects were analysed using a split-plot ANOVA. Specifically, VAS and BFS scores were subjected to a split-plot ANOVA with day (1-7) as within-subjects factors, and treatment (diazepam vs. placebo) as the between-subjects variable. The PANAS was submitted to a split-plot ANOVA in which valence was added as an additional within-subjects factor. Daily side-effects questionnaires[4] were coded according to the intensity of the experienced side-effect (0=none, 1=mild, 2=moderate, 3=severe), and the resulting data submitted to a split-plot ANOVA with symptoms (7 in total) and days (1 to 7) as within-subjects factors.

The measures collected on the testing day (i.e., STAI-S and short version of the VAS) were analysed using independent t-tests.

Reaction times to detect happy, fearful and neutral faces were analysed using a split-plot ANOVA with emotion (3 levels) as the within-subjects factor, and group (diazepam vs. placebo) as the between-subjects variable. This analysis was repeated in separate additional ANCOVAs in which the performance measures shown to differ between groups were included as covariates.

When appropriate, statistically significant main effects or interactions were followed up with pairwise comparisons. When sphericity was violated, Greenhouse-Geisser-corrected p-values were used, but uncorrected degrees of freedom are reported for clarity. P-values lower than 0.05 were used to denote statistical significance. Marginal differences with a p-value lower than 0.10 were also reported.

3.8. Results

3.8.1. Baseline characteristics

The two groups did not differ significantly in age, scores on the Beck Depression Inventory (BDI; Beck et al., 1961), the Spielberger State and Trait Anxiety Inventories (Spielberger et al.,

[4] The Daily side-effects questionnaire is a non-validated checklist that includes common side effects of pharmacological drugs used for the treatment of depression and anxiety.

1983), or any of the subscales of the Eysenck Personality Questionnaire (EPQ; Eysenck & Eysenck, 1975). However, they differed in IQ scores (as measured with the NART), with the diazepam group showing higher values. This comparison is nonetheless limited by the fact that NART scores were missing for 5 participants in the diazepam group. Values are presented in Table 7.

Table 7. Mean scores on baseline scales for each group

	Placebo (n=15) mean (±SD)	**Diazepam (n=16) mean (±SD)**	***p*-value**	***Sig.***
Age	22.73 (4.09)	23.69 (3.96)	0.358	n.s.
Male:female ratio	6:9	9:7	0.366	n.s.
Verbal IQ (NART)	113.13 (5.72)	118.47 (4.12)	0.377	n.s.
Depression (BDI)	1.47 (1.99)	1.69 (1.81)	0.599	n.s.
Trait anxiety (STAI-T)	27.67 (5.35)	29.00 (8.41)	0.770	n.s.
State anxiety (STAI-S)	24.73 (3.99)	28.12 (8.06)	0.470	n.s.
EPQ Neuroticism	5.33 (3.24)	4.87 (3.30)	0.700	n.s.
EPQ Psychoticism	2.47 (1.77)	3.06 (2.20)	0.415	n.s.
EPQ Extraversion	16.47 (3.72)	15.13 (3.70)	0.118	n.s.

Note. n.s. stands for non-significant.

3.8.2. Daily questionnaires

The effects of diazepam on the daily VAS scales were assessed separately using a split-plot ANOVA. There was no interaction effect with group or a main effect of group, suggesting a lack of effect of diazepam on all scales: Alertness (all *p*s>0.11), Calmness (all *p*s>0.13) and Contentedness (all *p*s>0.51). A similar lack of effects was seen with the PANAS (all *p*s>0.15) and BFS (all *p*s>0.37).

The analysis with side-effects revealed an interaction between scales and group [F(6,174)=4.021, p=0.006]. Follow-up comparisons demonstrated that this interaction was driven by differences in four of the scales. Participants on diazepam reported higher overall drowsiness (p=0.015) and a higher impairment in coordination (p=0.002). There was also a trend for participants on diazepam to report increased forgetfulness (p=0.065) and decreased agitation symptoms (p=0.055). A summary of the subjective ratings is presented in Table 8.

Table 8. Subjective ratings before and after 7 days of diazepam vs. placebo

Scales		Before treatment		After treatment	
		Placebo (n=15) mean (±SD)	Diazepam (n=16) mean (±SD)	Placebo (n=15) mean (±SD)	Diazepam (n=16) mean (±SD)
Visual Analogue Scales	Alertness	28.59 (13.71)	34.98 (15.50)	32.37 (12.89)	39.13 (14.19)
	Calmness	23.30 (11.42)	38.47 (18.18)	34.02 (15.22)	37.41 (15.91)
	Contentedness	30.65 (15.24)	35.88 (19.99)	29.65 (13.09)	32.63 (11.66)
PANAS	PANAS +	32.71 (7.82)	29.47 (8.54)	29.38 (5.80)	27.92 (8.23)
	PANAS -	10.64 (1.15)	11.93 (3.58)	11.29 (1.99)	11.09 (1.09)
BFS		12.50 (10.98)	20.69 (20.03)	17.56 (15.47)	21.47 (18.19)
Side effects	Drowsiness	0.13 (0.52)	0.38 (0.50)	0.34 (0.48)	0.79 (0.55)
	Light-headedness	0.00 (0.00)	0.00 (0.00)	0.01 (1.30)	0.31 (0.39)
	Confusion	0.00 (0.00)	0.06 (2.50)	0.33 (0.13)	0.14 (0.29)
	Lack of coordination	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.43 (0.49)
	Forgetfulness	0.00 (0.00)	0.00 (0.00)	0.06 (0.17)	0.36 (0.60)
	Agitation	0.00 (0.00)	0.06 (0.25)	0.10 (0.34)	0.21 (0.06)
	Muscle weakness	0.00 (0.00)	0.19 (0.40)	0.08 (0.18)	0.22 (0.27)

Note. Ratings were made daily throughout the 7-day period. Values represent ratings before treatment and average ratings over days 2-7 (after treatment).

3.8.3. Testing Day Questionnaires

In order to assess the effects of diazepam vs. placebo on subjective measures administered on the test day, independent t-tests were conducted. No differences between groups were seen for STAI-S ($p=0.245$), or for most of the items of the VAS, including disgust, drowsiness, anxiety, happiness, nausea and sadness (all $ps>0.15$). However, there was a trend for participants receiving diazepam to be less alert on the day of the testing session ($p=0.088$) (see Table 9).

Table 9. Effects of diazepam vs. placebo on the testing day

	Placebo (n=15) mean (±SD)	Diazepam (n=16) mean (±SD)	p-value	Sig
STAI-S	27.6 (4.70)	30.69 (8.98)	0.245	n.s.
VAS alert	67.80 (15.56)	54.75 (24.38)	0.088	+
VAS disgust	4.93 (7.57)	4.56 (8.15)	0.861	n.s.
VAS drowsy	29.27 (22.16)	42.25 (26.42)	0.150	n.s.
VAS anxious	10.47 (12.39)	10.75 (14.01)	0.922	n.s.
VAS happy	70.87 (12.30)	65.31 (17.34)	0.379	n.s.
VAS nausea	5.73 (10.49)	11.19 (21.10)	0.654	n.s.
VAS sadness	6.07 (4.09)	16.87 (21.69)	0.358	n.s.

Note: +p<0.10; n.s. stands for non-significant.

3.8.4. Continuous Flash Suppression Task

As in study A, reaction times (RTs) for correct trials were the main outcome measured in this study. Data from 1 participant (diazepam group) was excluded from the analysis due to excessive error rates (>10% errors; consistent with Yang et al. 2007), and therefore the final dataset consisted of 31 participants (15 in the placebo group and 16 in the diazepam group).

The placebo group had a higher mean error rate than the diazepam group (2.44% vs. 1.00%, p=0.016). Incorrect trials were nonetheless removed from the analysis, as well as trials in which the RT was +/- 2 standard deviations away from the average RT. The percentage of total trials removed was also significantly higher in the placebo group (placebo: 7.15% vs. diazepam: 4.87%, p=0.007), as were the percentage of trials excluded in the happy condition (9.11% vs. 5.52%, p=0.007). Finally, there was a near-significant trend for the placebo group to have significantly more trials excluded in the fear condition (5.67% vs. 3.33%, p=0.057). There was no difference between groups when considering percentage of neutral trials removed (p=0.442) and raw reaction times (p=0.418). These values are presented in Table 10.

Table 10. Mean percentage of incorrect and excluded trials and raw reaction times

	Placebo (N=15) mean (±SD)	Diazepam (N=16) mean (±SD)	p-value	sig.
incorrect trials (%)	2.44 (2.03)	1.00 (0.91)	0.016	*
neutral trials excl. (%)	6.67 (2.60)	5.73 (2.85)	0.442	n.s.
happy trials excl. (%)	9.11 (3.67)	5.52 (3.59)	0.010	*
fearful trials excl. (%)	5.67 (3.72)	3.33 (2.72)	0.057	+
total trials removed (%)	7.15 (2.28)	4.87 (2.09)	0.007	**
raw reaction time (sec.)	3.16 (1.35)	2.79 (1.17)	0.418	n.s.

Note: **p<0.01, *p<0.05, +p<0.10, n.s. stands for non-significant.

The CFS task was completed on either the 7th or 8th day of treatment. However this distribution did not differ between groups [5:10 vs. 5:11; $x^2(1, 31)=0.015$, p=0.901].

As expected, there was a main effect of emotion [F(2,58)=20.828, p<.0001]. In line with Study A, follow-up pairwise comparisons revealed that fearful faces were detected faster than happy faces (means: 2.721 seconds vs. 2.936 seconds, respectively; p<.0001), but no significant differences were found between fearful and neutral faces (2.721 vs. 2.750; p=0.522). Neutral faces were also detected faster than happy faces (2.750 vs. 2.936; p<.0001).

Contrary to the study hypothesis, however, there was no effect of diazepam on the rate at which neutral, happy and fearful faces were detected [F(2,58)=0.899, p<0.412]. Also, no main effect of group was seen [F(1,29)=0.617, p=0.438]. See Figure 8.

Additional covariate analyses were conducted with those variables that were shown to differ between groups. Separate ANCOVAs were computed including the (i) percentage of incorrect responses, (ii) percentage of trials excluded for happy and fear and (iii) and total number of trials removed. These analyses revealed similar results to the original ANOVA.

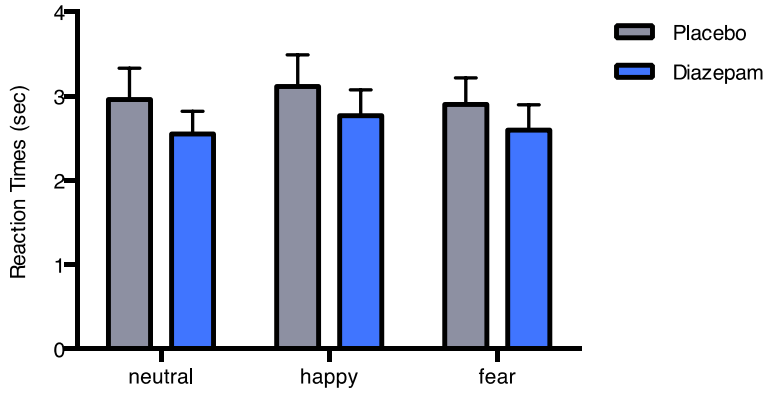

Figure 8. Average reaction times for each of the facial expressions in the placebo and diazepam groups.

3.9. Discussion

This study aimed to assess the effects of diazepam on the early detection of emotional and neutral information using CFS. Contrary to the study prediction, it was found that diazepam did not affect the rate at which happy, fearful and neutral faces were detected. Indeed, the performance seen in the diazepam and placebo group were comparable, with both groups being faster to detect threatening vs. happy faces, therefore replicating the finding obtained in Study A. However, participants in the diazepam group were more accurate than the placebo group in detecting the location of the face. At a subjective level, participants on diazepam reported increased drowsiness and impaired coordination over the 7 days of treatment, as well as a trend for reduced alertness on the testing day.

Lack of effects of diazepam on the early detection of threatening stimuli

In this study, diazepam did not have any effects on the detection of threatening stimuli, which was predicted to be involved in the anxiolytic response of this agent. Due to the paucity of studies using alternative masking or subliminal techniques when investigating the effects of diazepam on emotional processing, this finding is difficult to interpret. However, in a study conducted by Murphy and colleagues (2008), a single dose of diazepam was found to modulate attentional vigilance to facial stimuli, which were made subliminal using backward masking. Specifically, in this study, acute diazepam was found to increase attentional vigilance to masked happy vs. neutral faces. Given that neutral faces represent the more threatening element of the pair, this was interpreted as suggesting a reduced attentional vigilance to mildly threatening or ambiguous neutral faces induced by diazepam. Nonetheless, such interpretation was constrained by the lack of effects on threatening fearful faces, and by the absence of an absolute bias to fear in the placebo group.

The contrasting effects between the current study and the one conducted by Murphy et al. (2008) may be explained by differences in drug administration. It is possible that acute administration of diazepam produces effects that are different from those seen with longer treatment. Whilst acute administration may be associated with effects seen at a preconscious level, these effects may be attenuated with repeated treatment due to tolerance or habituation to the drug. However, this comparison between studies is difficult due to the use of different paradigms.

It should be noted that the fact that the placebo group showed the expected emotion modulation effect in reaction times (i.e., faster detection of fearful vs. happy faces) nonetheless corroborates the validity of CFS in investigating potential drug effects (or their absence). As noted in Chapter Two, it is essential to replicate the main effect of emotion in the placebo group in order to understand any potential effects of pharmacological interventions.

Effects of benzodiazepines on visual processing

Although the effects of diazepam on attentional vigilance have been little investigated, there are some studies looking at the effects of benzodiazepines (including diazepam and lorazepam) on visual processing more broadly. For example, in a "shine-through" backward-masking paradigm (in which the time between the presentation of the target and the mask - stimulus onset asynchrony - was varied), participants treated with acute lorazepam needed a much longer stimulus onset asynchrony (SOA) than participants receiving placebo in order to reach a comparable detection performance (Giersch & Herzog, 2004). In addition, acute lorazepam has been shown to impair performance in perceptual tasks involving matching objects to similar pictures (Pompéia, Pradella-Hallinan, Manzano, & Bueno, 2008) or to incomplete reference images (Giersch, Boucart, Speeg-Schatz, Muller-Kauffmann, & Danion, 1996), as well as in tasks involving the detection of discontinuities in random-shaped and meaningless outlines (Beckers, Wagemans, Boucart, & Giersch, 2001).

Notably, diazepam has been found to produce less detrimental effects on visual processing than those seen with lorazepam. In the studies of Beckers and colleagues (2001) and Wagemans, Notebaert and Boucart (1998), diazepam-treated participants failed to reveal the perceptual deficits seen with lorazepam, showing instead a performance comparable to findings seen in the placebo group. Similarly, Giersch and Herzog (2004) reported that, unlike lorazepam, diazepam administration did not affect stimulus onset asynchrony (SOA) between the stimuli and the mask, being therefore less susceptible to "masking" effects. Despite not being directly comparable with the current study, this pattern is somewhat consistent with the absence of a diazepam influence on overall reaction times seen here. Indeed, and in line with the study by Giersch and Herzog (2004), diazepam did not seem to affect the visual thresholds for facial detection, given the lack of group differences in raw reaction times. It is also interesting to note that lorazepam has been shown

previously to completely disrupt the binocular fusion effects necessary to adequately perform in a binocular rivalry paradigm (van Loon et al., 2013). The influence of diazepam on pure binocular rivalry has not been assessed previously, but given that participants who completed the current study did not show any problems in achieving fusion, it is tempting to speculate that diazepam is associated with preserved abilities in this domain. Why these drugs are associated with such contrasting effects remains elusive, but it has been suggested that these differences may be mediated by distinctive affinities for a variety of $GABA_A$ receptors (Möhler, Fritschy, & Rudolph, 2002). The clinical implications of such effects are nonetheless unclear, and future studies are needed in order to clarify this.

Effects of diazepam on cognitive performance

A surprising result found in this study was the superior performance seen in the diazepam group when considering accuracy rates. These effects cannot be explained by differences in subjective measures, given that participants receiving diazepam reported increased drowsiness over the 7 days of treatment and a trend to be less alert on the testing day, a finding that is at odds with their improved behavioural performance. However, it should be noted that participants receiving diazepam had higher IQ scores than the placebo group, which could account for such difference in performance. This explanation is nonetheless limited by the fact that IQ values were missing for 5 participants in the drug group.

It is also interesting to note that despite the fact that benzodiazepines have consistent sedative effects, as measured by subjective ratings, their influence on cognitive tasks varies across studies. Whilst some studies suggest that diazepam and other similar acting drugs induce deficits in performance, many show preserved abilities in spite of sedation (see Buffett-Jerrott & Stewart, 2002). One factor that may influence the interpretation of these results is that most studies investigating the effects of diazepam have used single doses. Clinically, it is known that continued use of diazepam and other benzodiazepines is associated with tolerance, especially towards sedation, and to a less extent towards the anxiolytic effects of these drugs (Bateson, 2002). Tolerance can develop as early as after a second dose administration (Rosenberg & Chiu, 1985). It is therefore possible that the tolerance effects seen with longer usage could be associated with differences that are more consistent with a preserved cognitive performance. For instance,

Liljequist, Linnoila and Mattila (1978) showed that the number of mistakes in a paired association-learning task was increased after acute administration of diazepam, but this effect was no longer seen after a 14-day treatment.

The reason for the improvement performance on the CFS task following diazepam administration therefore requires further investigation. In particular, it would be useful to investigate whether this improved performance is task-specific or seen across a number of different tasks. In the absence of such a comparison and replication, the interpretation of this finding is problematic, as it may represent a Type I error.

Limitations

The lack of diazepam effects on the early detection of emotional and neutral stimuli (as measured using CFS) suggests that this drug does not modulate the preconscious processing of emotional information. It would have been useful, however, to use additional paradigms aimed at measuring conscious and preconscious emotional processes - including different subliminal and masking techniques -, as well as tasks involving conscious and strategic processes. Having a variety of methods within the same study would allow a better investigation of the extent to which diazepam may (or not) affect different processes that are thought to underlie the automatic vs. strategic detection of emotional cues.

Based on the current data, it is not possible to determine whether the lack of reaction time differences between the placebo and diazepam groups is specific to this paradigm. It would be of high interest to include a binocular control condition task not involving interocular suppression, as well as a simple reaction time paradigm, in order to measure potential threshold and reaction time differences in the diazepam vs. placebo group.

CFS is thought to represent a robust measure of preconscious processing, but its validity in detecting pharmacological effects remains to be fully determined. Future studies are needed in order to explore the potential of this paradigm to detect the effects of drug interventions known to be effective in treating anxiety disorders.

Finally, this study did not use any direct measure of adherence to the drug/placebo pills administration (only a pill count was performed). However, the fact that the groups differed in their ratings of drowsiness, motor coordination and, to a less extent, alertness, suggests that

participants were being compliant to the protocol. Nevertheless, it would be useful for future studies to include measures of diazepam plasma concentration, especially on the testing day.

3.10. General Conclusion

The aim of this chapter was to validate the use of CFS in detecting automatic anxiety-related biases and anxiolytic effects. Previous paradigms used to investigate the presence of attentional vigilance biases in anxiety have shown poor re-test reliability (Schmukle, 2005). The reasons for this lack of reliability remain unclear, but it has been suggested that these tasks may show insufficient sensitivity to detect subtle inter-individual differences in attentional allocation to threat, especially in non-clinical samples.

The notion that anxiety is associated with an automatic bias towards the detection of threat has long been highlighted in cognitive models of psychological dysfunction. These early biases are believed to be largely involuntary and to occur outside of the participant's conscious control (Cisler & Koster, 2010), and could potentially represent a more sensitive measure of vigilance to threat. However, current procedures used to measure preconscious or automatic biases show methodological constraints in achieving successful masking or complete independence from top-down attentional responses, therefore compromising the validity of these tasks.

Due to its methodological strengths, CFS has the potential of providing a robust measure of preconscious or automatic processing. Indeed, the results from study A confirmed the validity of using CFS to detect the effects of anxiety on automatic biases towards threat, given that anxiety symptoms were associated with a faster detection of fearful vs. happy faces. This task may therefore be more robust than other paradigms due to its strong suppression effects, which are not seen with traditional measures of visual masking. This finding is of high interest for future studies that may wish to use CFS to measure early attentional biases to threat across various clinical and non-clinical populations, and under the influence of different pharmacological interventions.

The lack of effects of diazepam on the early detection of threatening stimuli using CFS was nonetheless surprising. Despite being sensitive to the effects of preconscious biases in anxiety, study B failed to validate the use of CFS in detecting the effects of an anti-anxiety drug, diazepam. The reasons behind this are unknown, but it is possible that acute diazepam does not modulate the presence of pure preconscious biases known to be involved in anxiety. Future studies should

include a wide range of techniques used to measure these processes, as well as different benzodiazepine drugs.

Chapter 4:

The effects of acute fluoxetine on emotional neural processing in depressed adolescents

4.1. Introduction

Fluoxetine is the first-line pharmacological treatment for adolescent depression (National Institute for Health and Clinical Excellence, NICE, 2005), but the neuropsychological mechanisms underlying its action in youth are poorly understood. The findings reported in Chapter Two suggest that fluoxetine has early effects on emotional processes that may be especially relevant for the treatment of this disorder. Fluoxetine (when compared to placebo) was found to decrease the processing of anger and sadness and also eliminate startle responses. The effect on anger was of particular interest as it confirmed the initial hypothesis that fluoxetine would have an effect on this key emotion for adolescent depression.

However, whilst the use of a healthy and relatively homogenous sample has the advantage of increasing power to detect drug-related differences - unconfounded by the severity of symptoms and other clinical characteristics -, it remains critical to investigate the effects of fluoxetine in a depressed adolescent sample. Only by investigating clinical populations directly can we achieve a comprehensive picture of how antidepressants exert their effects. This is especially important for paediatric samples, where the investigation of wanted and unwanted effects underlying antidepressant drug use has been little investigated (see Jureidini et al., 2004).

Functional Magnetic Resonance Imaging (fMRI) is a powerful method for investigating the mechanisms of drug action in both adult and paediatric populations. Several studies have now applied this technique to the study of antidepressant effects in adults, and an emerging finding is that antidepressant drugs act to normalise activity in cortico-limbic areas that are associated with depressive states. Indeed, long-term treatment with antidepressants has been shown to reduce the activation of areas such as the amygdala, medial prefrontal cortex and anterior cingulate in response to negative cues, whilst increasing the activation of these same regions towards positive

information (for review see Ma, 2014). In adolescents, there is only one study that examined the effects of treatment with fluoxetine on neural functioning. In this experiment, Tao and colleagues (2012) found that chronic fluoxetine administration (8 weeks) normalised the pattern of functional hyperactivity in response to fearful faces seen in the amygdala, orbitofrontal cortex and subgenual anterior cingulate. The activation within these areas was excessive in depressed adolescents relative to matched healthy controls prior to treatment, but this pattern was normalised with fluoxetine.

The study by Tao and colleagues (2012) represents an important first step towards a better understanding of the mechanisms of antidepressant treatment in young people, but critical questions remain unanswered. First, these authors did not include a placebo condition, therefore raising the possibility that the effects seen with fluoxetine could have been result (at least to some extent) of treatment expectations or repeated testing. Second, it is not possible to determine whether the neural changes seen with fluoxetine contributed to the improvement seen at a symptom level, or if they were instead a consequence of mood effects (given that 60% of the adolescents showed significant clinical improvement after 8 weeks). In order to clarify these issues, it is important to examine the effects of fluoxetine early in treatment and using a placebo-controlled condition.

A specific approach that has proved useful in characterising the initial effects of antidepressants is to combine fMRI with acute or short-term drug manipulations. Applying this method to the study of antidepressant effects in adults has highlighted that SSRIs and other similar drugs produce immediate changes on neural regions known to play a key role in the development and maintenance of depression, most notably the amygdala (e.g., Murphy et al., 2009; Rawlings, Norbury, Cowen, & Harmer, 2010). Critically, these neural changes are seen in the absence of any effects on subjective symptoms, and therefore they represent a direct effect of the drug. A single dose of mirtazapine, for instance, has been shown to decrease the activity of the amygdala in response to fear, whilst increasing its activation towards happy faces in healthy volunteers (Rawlings et al., 2010). Similarly, 7-day treatment with escitalopram (vs. placebo) decreases the activation of the amygdala towards fear in a sample of depressed adults, and in the absence of any changes in depressive symptoms (Godlewska et al., 2012). These findings represent a critical

component of the *neuropsychological model of antidepressant drug action* described earlier in this thesis.

A compelling aspect of this model is its potential to predict subsequent clinical responses to treatment. Indeed, in a study by Tranter and colleagues (2009), an increased ability to recognise happiness two weeks after patients started treatment with citalopram was associated with later clinical improvement measured at six weeks. Similarly, a study by Godlewska and colleagues (submitted) showed that early changes in amygdala activation seen after one week predicted later clinical responses at six weeks in those patients who benefited from treatment.

The purpose of the current study was to apply a similar approach to the investigation of antidepressants effects in clinically depressed adolescents, by combining pharmacological fMRI, behavioural testing and clinical assessments conducted prior to treatment and after one and six weeks. Participants were recruited immediately before they started a course of fluoxetine, therefore providing a unique opportunity to examine the early effects of this drug in a medication-free sample and with a placebo-controlled condition. On the first day of the treatment, participants were randomised to receive either 10mg of fluoxetine or placebo and were then asked to complete an fMRI scan along with emotional processing tasks. After this session, participants continued the antidepressant treatment as prescribed and monitored by their treating Psychiatrist.

In light of the previous findings seen with healthy volunteers in Chapter Two, the main study hypothesis was that a single dose of fluoxetine would decrease neural processing of angry facial expressions, in the absence of overt changes in mood or anxiety. A region-of-interest (ROI) approach was conducted in the amygdala, as this region is critically involved in the pathophysiology of depression and is also sensitive to early antidepressant effects in adult samples (e.g., Murphy et al., 2009; Rawlings et al., 2010). Additional brain areas affected by fluoxetine were identified using a whole-brain analysis. Finally, in order to explore if acute changes in amygdala activation could be associated with subsequent clinical outcomes, neural responses in this region to anger and fear were correlated with symptoms of irritability, anxiety and depression measured over the course of the treatment.

4.2. Methods

4.2.1 Participants and recruitment

Based on a power calculation computed a-priori, it was expected to recruit 34 patients to this study. Indeed, in a single dose fMRI study conducted in our laboratory, with 26 healthy volunteers in total - who were randomised to receive a single dose of another SSRI, citalopram, or placebo (Murphy et al. 2009) -, the size of the effect of citalopram on amygdala activity in response to fearful vs. neutral faces was 1.19. It was calculated that for an effect of this size, 13 participants per group would be needed to obtain a power calculation of 0.90 at α error probability of 0.05. Given that a clinical sample is used in this study, and therefore the measurements were anticipated to contain a higher level of variability, it was proposed that 17 patients per group (34 in total) would give sufficient power to detect an effect. However, due to difficulties in recruitment, only 13 patients per group were recruited (26 in total).

In addition to the patient groups, an additional sample of control participants was also included (but they were not administered any antidepressant/placebo). In total, 37 adolescents participated in this study: 26 adolescents with a DSM-IV diagnosis of Major Depressive Disorder (mean ± SD: 15.73 ± 1.4 years; range: 13-17 years); and 11 healthy controls (mean ± SD, 16.0 ± 1.5 years; range, 13-18 years). In the patient group, 13 participants were randomised to receive fluoxetine and 13 placebo (see Figure 9 for details). Adolescents with MDD were recruited from Child and Adolescent Mental Health Services (CAMHS) in Oxfordshire, Buckinghamshire, Berkshire and Central and Northwest London, and the healthy controls were recruited from the local community in Oxfordshire. The patient referral was made through a Child and Adolescent Psychiatrist, who prescribed antidepressant medication to the patients as part of their normal care. A risk assessment was made on an individual basis by the treating Psychiatrist to determine that it was safe for the patient to wait 2-7 days to initiate the antidepressant treatment (given that the first antidepressant dose was taken within the study). This delay was necessary to allow for the fMRI scan to be arranged. Exclusion criteria included: history of bipolar disorder or schizophrenia; substance abuse; recent use of a psychotropic medication that could affect the results; and MRI incompatibility (presence of metal implants, pregnancy or claustrophobia),

Participants aged 16 to 17 gave written informed consent. For participants younger than 16, written consent and assent were taken from the adolescent and their parent/guardian,

respectively. Participants were reimbursed with £30 for their time. Ethical approval for this study was obtained from the Southampton Research Ethics Committee (12/SC/0030).

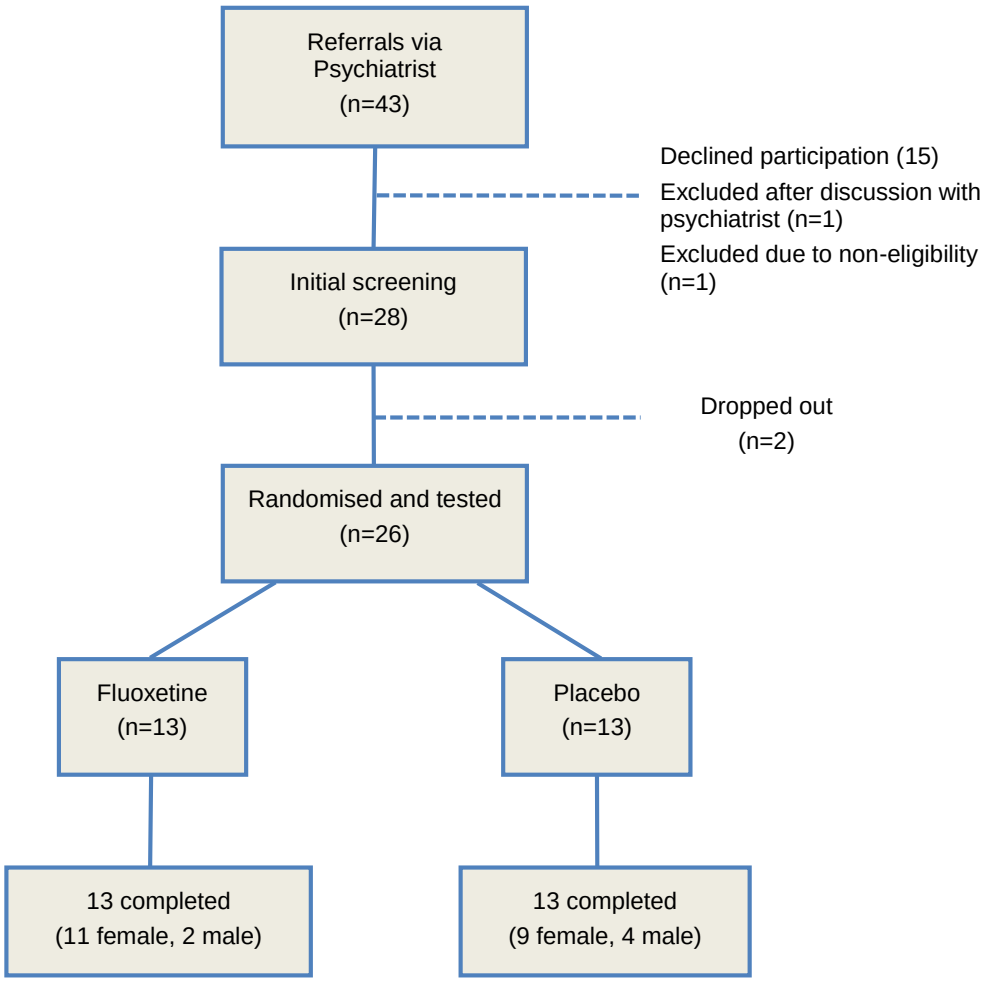

Figure 9. Flow chart of patient participation.

4.2.2. Experimental design and procedures

Patients

Initial contact

After being referred to the study by the treating Psychiatrist, the patient was contacted by telephone. During this initial telephone call, participants were given a brief description of the study and assessed for MRI compatibility. If suitable, they were then invited for an initial screening session that took place either in the CAMHS clinic or at the Warneford Hospital in Oxford.

Screening

The screening session included the administration of several measures aimed at determining clinical diagnoses and assessing the severity of the depressive episode. Additional scales were also administered in order to measure trait anxiety, suicidal ideation and general intellectual ability.

Specifically, psychiatric diagnoses were determined through the administration of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL; Kaufman, Birmaher, Brent, Ryan, & Rao, 2000) to the adolescent. This interview was administered either by a Clinical Psychologist (myself) or a Child and Adolescent Psychiatrist (Dr. Robert Chapman). I was present in all clinical interviews. Depression severity was measured using the Childhood Depression Rating Scale-Revised (CDRS-R; Poznanski & Mokros, 1996) and the Child Depression Inventory (CDI; Kovacs, 1992). Trait anxiety was measured using the Trait scale of the State-Trait Anxiety Inventory for children (STAI-C; Spielberger, 1973). Suicidal ideation was quantified using the Suicidal Ideation Questionnaire- Junior version (SIQ-JR; Reynolds, 1987). Finally, the 2-subscale version of the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) was used to determine Full IQ scores. The parent was also given the Child Behaviour Checklist (CBCL; Achenbach & Rescorla, 2001) for completion. A detailed description of these measures is provided in Appendix 2.

Testing

The testing session (~8 hours in total) took place as soon as possible after the screening visit. Patients and their parent (when available to join) were asked to start the session in the morning. The patient was then randomised to receive either 10mg of liquid fluoxetine or a matched placebo, in a double-blind procedure. Placebo was peppermint syrup, which was relatively close in taste to fluoxetine and measured to the equivalent volume (consistent with Chantiluke et al., 2014). Both fluoxetine and the placebo were mixed with water in a cup by a research Psychiatrist not involved in the study. Participants were asked to drink this mixture. Following drug/placebo administration, patients sat in a quiet room until the testing session began. During this time, participants were free to read, work and/or use a laptop, and were also carefully monitored for potential side-effects. When necessary, this period included the administration of some of the

measures that were not completed during the screening session due to time constraints. Care was taken in order to avoid an excessive burden for the patient (e.g., breaks and refreshments were offered regularly).

All participants were asked to refrain from alcohol on the night before and to limit caffeine consumption to one cup on the testing morning. Participants were not allowed to smoke or drink any additional caffeine for the duration of the study. For lunch, patients were offered a selection of light meals.

In order to guarantee that fluoxetine had reached its peak level, which has been shown to take place after 6 to 8 hours (Rossi et al., 2004), the testing session started 6 hours after drug/placebo administration. The testing included a 45-minute functional Magnetic Resonance Imaging (fMRI) scan and a behavioural task outside the scanner. Specifically, the fMRI scan included an emotional faces task, an emotional regulation paradigm, and structural and resting-state scans. After the scan, participants completed the Facial Expression Recognition Task (FERT). The emotional regulation paradigm and resting state data will not be presented in this thesis.

In order to measure subjective changes in mood and anxiety, as well as potential side-effects, patients completed self-report scales at three time points: (1) before the antidepressant/placebo administration, (2) before the scan and (3) immediately after the scan took place. A more detailed description of these measures is provided in Appendix 2.

Following this session, the patients started their antidepressant treatment as prescribed and monitored by their treating Psychiatrist.

Text monitoring and follow-up sessions

After the testing session, patients were sent a daily test message for 10 days (sent at 4.30 pm), aimed at assessing symptoms of agitation. The first message was sent on the first day of the treatment as prescribed by their Psychiatrist, which usually started the day immediately after the testing visit. The text message was the following*: "Hello_____. We hope it's ok to ask you a question about how you're feeling today. How agitated do you feel right now (e.g., shaky, irritable, restless?). Please reply with a number from 0 to 10 (0 meaning not at all and 10 meaning extremely). We'll be sending this text message every day at 4.30 pm for the next __days. Thank*

you". Participants were instructed to reply with a number ranging from 0 to 10 as soon as possible after seeing the text message.

Two additional follow-up sessions scheduled to take place one and six weeks after the testing session were also conducted with the patient group. These sessions were aimed at assessing any subjective changes in mood, anxiety, side-effects and suicidal ideation during the antidepressant treatment. The first follow-up included the following measures: Childhood Depression Rating Scale-Revised (CDRS-R); Children's Depression Inventory (CDI); State scale (A-State) from the State-Trait Anxiety Inventory for children (STAI-C); adapted version of the Visual Analogue Scale (VAS); Side-effects Checklist; and an adapted version of the Suicidal Ideation Questionnaire- Junior version[5] (SIQ-JR). The Global Improvement (CGI-I) scale was used to quantify clinical improvement. The second follow-up included all these measures, with the exception that the SIQ-JR was the original 15 items version and the Trait scale (A-Trait) from the STAI-C was also added.

All these questionnaires (apart from the Side-effects Checklist) have been frequently used in both behavioural and fMRI studies of adolescent depression, and show good consistency and test-retest reliability.

Healthy controls

Healthy adolescent controls were sent an email with detailed information about the study and then contacted by telephone to confirm initial eligibility. If suitable, they were also invited for a screening session taking place at the Warneford Hospital. This session involved the administration of the same questionnaires included in the visit with patients. The K-SADS-PL was administered in order to confirm that healthy controls were free from current and previous psychological disorders.

Eligible participants were then invited to come for the testing session. This session involved an fMRI scan, self-report questionnaires and the Facial Expression Recognition Task (FERT). The following questionnaires were administered immediately before and after the scan: State scale (A-State) of the State-Trait Anxiety Inventory (STAI-C), Visual Analogue Scale (VAS), and Antidepressant Side-effects Checklist.

[5] This instrument represents an adapted and non-validated version of the full questionnaire and was only used to monitor the occurrence of suicidal thoughts within the first week of treatment.

Healthy controls were not administered any pharmacological intervention, and therefore their testing session was of a shorter duration (approximately 2 hours).

4.2.3. fMRI and behavioural task designs

Emotional faces task

The emotional faces task included happy, fearful and angry facial expressions. The participant was instructed to classify the gender of the face (male or female) by pressing a keyboard button as quickly and accurately as possible. This covert/implicit task has proved sensitive to the acute effects of antidepressant administration on neural processing (e.g., Rawlings et al., 2010).

A single trial consisted of the presentation of a central fixation cross for 2900ms, followed by presentation of a facial expression for 100ms. A block design was used, with each block consisting of ten trials of the same valence of expression, and lasting a total of 30s. Every block was followed by 30s of fixation cross, before the next block began. Blocks alternated in valence: the first consisted of fearful faces, the second of happy faces and the third of angry faces. This sequence of blocks was repeated four times, resulting in a total of 12 blocks.

The task ran on E-prime software (version 2.0; Psychology Software Tools Inc., Pittsburgh, PA, USA). Participants completed this paradigm while in the MRI scanner, using a button box resting on their leg. Stimuli were projected onto a screen behind the scanner and displayed to participants via an angled mirror positioned above the head. Accuracy and reaction times were recorded by E-prime.

Facial Expression Recognition Task

Following the fMRI scan, participants were administered the Facial Expression Recognition Task (FERT), which included the presentation of six emotions: fear, happy, sad, surprise, anger and disgust. This task was the same as the one used in Chapter Two.

4.2.4. fMRI data acquisition and analyses

Blood-oxygenation-level-dependent (BOLD) fMRI and T1-weighted anatomical images were acquired using a Siemens 3 Tesla TIM MAGNETOM Trio scanner, equipped with a 32-

channel head matrix coil (Siemens, Erlangen, Germany) and located at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR). Functional imaging consisted of 45 T2-weighted echoplanar imaging (EPI) slices (TR=3000ms, TE=30ms, matrix 64 x 64, slice thickness=3mm), $3mm^3$ voxels. An anatomical image (TR=2040ms, TE=4.7ms), $1mm^3$ voxels, was also acquired to allow later registration of the fMRI data into standard space.

fMRI data were pre-processed and analysed using FEAT (FMRI Expert Analysis Tool), version 6.00, part of FSL (FMRIB's Software Library; www.fmrib.ox.ac.uk/fsl). Pre-processing involved a number of steps designed to reduce unwanted variability in the data and to improve the validity of statistical analyses. The following steps were therefore implemented in each subject's fMRI volume time series: motion correction using FMRIB's Linear Image Registration Tool (MCFLIRT; Jenkinson, Bannister, Brady, & Smith, 2002); deletion of non-brain tissue using the Brain Extraction Tool (BET; Smith, 2002); spatial smoothing with a Gaussian kernel of 5 mm full-width-half-maximum; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma of 70s). In addition, registration to high-resolution image and to a standard template [Montreal Neurological Institute (MNI)] was implemented using FNIRT nonlinear registration (Andersson, 2007).

In the first-level analysis, individual activation maps were computed using the general linear model with local autocorrelation correction (Woolrich, Ripley, Brady, & Smith, 2001). Three explanatory variables were modelled: "fear", "happy" and "angry" faces. Temporal derivatives were included in the model as covariates of no interest to increase statistical sensitivity. Variables were modelled by convolving each block with a haemodynamic response function, using a variant of a gamma function (i.e. a normalisation of the probability density function of the gamma function) with a standard deviation of 3s and a mean lag of 6s.

In the second-level analysis, whole-brain individual data were combined at a group level (healthy controls, placebo and fluoxetine) using a mixed-effects group cluster analysis across the whole brain corrected for multiple comparisons (Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004). Such a mixed-effects approach accounts for intra-subject variability and allows general population inferences to be drawn. Significant activations were identified using cluster-based thresholding of statistical images with a height threshold of Z=2.0 and a (corrected) spatial

extent threshold of $p<0.05$ (Friston, Worsley, Frackowiak, Mazziotta, & Evans, 1994). This threshold has been used in previous fMRI research and is more stringent than the uncorrected whole-brain findings reported in some published fMRI studies of child and adolescent MDD (Gaffrey et al., 2011; Tao et al., 2012).

The main effect of the task was initially assessed by exploring activation in the happy, fearful and anger conditions compared to fixation, with all participants combined.

Given the a-priori hypothesis that fluoxetine would influence the activation of the amygdala, an anatomical ROI analysis was conducted. The ROI masks for the left and right amygdala were defined anatomically using the Harvard-Oxford atlas, part of FSL. This is a more targeted approach to analysis and constrains the search for active voxels to a particular area of interest. A visual inspection of the amygdala ROI, as overlaid on an average EPI image generated from all participants, ensured that it did not fall into regions of susceptibility artefact. BOLD percentage signal change extracted from the amygdala ROI mask (left and right hemispheres) was subsequently analysed using a split-plot ANOVA (within-subjects variables=valence and hemisphere; between-subjects variable=group). Significant interactions were investigated further with follow-up pairwise comparisons to determine the directionality of the effect.

At the whole-brain level, fearful and angry faces were contrasted with happy between groups (healthy controls, depressed patients on placebo and depressed patients on fluoxetine), therefore resulting in two emotion contrasts of interest: (1) anger > happy and (2) fear > happy, and four group contrasts: placebo > healthy controls; healthy controls > placebo; fluoxetine > placebo; placebo > fluoxetine. Significant interactions from whole-brain analyses were further explored by extracting percentage BOLD signal change, which was analysed using appropriate follow-up t-tests to determine the directionality of the effect.

4.2.5. Statistical analyses

Socio-demographic characteristics (of the three groups) were analysed using one-way ANOVAs (age and IQ) and chi-square tests (gender, handedness, ethnicity, family income level and family composition). Baseline clinical characteristics were compared between the patients receiving placebo vs. fluoxetine using chi-square tests (illness duration, mean age at onset of depression, mean number of MDD episodes, presence of psychotic features/low mood/irritability,

naivety to antidepressant treatment, current psychological treatment, presence of comorbidities, CGI-severity) or independent-t tests (CDI, CDRS-R, A-Trait, SIQ-JR, CBCL scales and CGAS).

Self-report measures collected on the testing day (A-State, VAS and Side-effects) were analysed by using split-plot ANOVAs. First, a split-plot analysis with two groups (patients receiving placebo vs. fluoxetine) was conducted, in which all three timepoints (before drug/placebo administration, before scan and after scan) were considered. A subsequent analysis was conducted with all 3 groups (healthy controls, patients receiving fluoxetine and patients receiving placebo) but only considering with two timepoints, due to the fact that healthy controls were not administered the pharmacological intervention/placebo.

Behavioural performance on the emotional faces task was analysed using a split-plot ANOVA with group (fluoxetine, placebo and healthy controls) as the between-subjects factor and RTs or accuracy as the within-subjects factors (three emotions: happy, fear and anger).

In order to investigate if acute changes in amygdala activity were associated with subsequent clinical responses to treatment, several Pearson correlation coefficients were computed. This analysis was only conducted in the fluoxetine group. Percentage signal change in the amygdala ROI mask (right hemisphere) in response to anger and fear was correlated with the change from baseline to follow-up 1 (computed as a difference score) in state anxiety (A-state) and irritability symptoms (CDRS-R item 13 and VAS irritability), respectively. Amygdala activity to fear was also correlated with agitation symptoms measured using the daily text messages (symptoms averaged across the first 3, 5 and 10 days of treatment). Change from baseline to follow-up 1 in depressive symptoms (as measured using the CDRS-R raw score and CDI) was correlated with amygdala responses to both anger and fear. Data from follow-up 2 was not included in this analysis because of data attrition.

The Facial Expression Recognition Task (FERT) was analysed by using a split-plot ANOVA with group (fluoxetine, placebo and healthy controls) as the between-subjects factor.

Changes in clinical symptoms over the three sessions (baseline, follow-up 1 and follow-up-2) were measured using repeated measures ANOVA. Clinical scales included the CDI, CDRS-R (total score), CDRS-R suicidality, CDRS-R irritability, A-State, A-Trait, SIQ-JR, VAS scales and Side-effects checklist. The CGI-I was analysed using a Fisher test due to its ordinal nature. Three timepoints were considered for all measures, expect for the SIQ, STAI-T and CGI-I. The SIQ and

STAI-T were only administered at the baseline and follow-up 2. An adjusted measure of the SIQ was collected in follow-up 1 but it cannot be used for statistical comparisons. The CGI-I was determined in follow-up sessions 1 and 2.

When sphericity was violated, Greenhouse-Geisser-corrected p-values were used, but uncorrected degrees of freedom are reported for clarity. A p-value lower than 0.05 was used to denote statistical significance. Marginal differences with a p-value lower than 0.10 were also reported.

4.3. Results

4.3.1. Demographic and clinic characteristics

Demographic and clinical characteristics of the three participant groups are presented in Table 11. There were no significant group differences in mean age, gender distribution, handedness, BMI, ethnicity, IQ, family income or family composition (all *p*s>0.14).

The mean total CDRS-R score at baseline was moderately severe for both the placebo and fluoxetine groups (placebo: mean=62.85, SD=8.08; fluoxetine: 62.46, SD=11.46). Suicidal ideation was also high in both groups (placebo: mean=52.23, SD=24.84; fluoxetine: 49.62, SD=19.37). The patients were matched for depression severity (both measured using the CDI and CDRS-R), trait anxiety (A-trait), suicidal ideation (SIQ-JR) and CBCL scales (anxiety-depression; internalization; externalization and total scores) (all *p*s>0.11). The percentage of patients presenting with psychosis/irritability/low mood/psychiatric comorbidity, who were receiving concomitant psychological therapy and who were naïve to antidepressant medication was comparable between groups (all *p*s>0.43). The mean age of depression onset, MDD episode duration and total number of MDD episodes was also similar between groups (all *p*s>0.29).

All patients were antidepressant-naïve apart from 3. Two of these patients had received treatment with fluoxetine in the past (due to depression) and another patient was taking amitriptyline for the treatment of fibromyalgia immediately before starting fluoxetine. This patient stopped taking amitriptyline for a period of 4 days before the testing session. This washout period was considered appropriate given that amitriptyline has a mean elimination half-life of 20 hours (ranging from 9 to 46 hours; Pagliaro, 1999).

Table 11. *Demographic and clinical psychopathology characteristics*

	Placebo (N=13)	Fluoxetine (N=13)	Controls (N=11)
SOCIO-DEMOGRAPHICS			
Age	15.38 (1.45)	16.08 (1.32)	16.27 (1.91)
Female:male ratio	11:2	9:4	10:1
Ethnicity (caucasian), N (%)	12 (92.3%)	13 (100%)	9 (81.82%)
IQ	111.08 (12.38)	109.85 (10.19)	117.09 (8.54)
Left:Right Handedness (ratio)	0:13	2:11	0:11
BMI	23.56 (6.04)	24.08 (4.33)	21.50 (2.22)
Family income level (range)	2.92 (1-6)	3.08 (1-6)	4.4 (2-6)
Family composition (intact), N (%)	6 (46.2%)	6 (46.2%)	8 (72.7%)
CLINICAL CHARACTERISTICS			
Duration of illness (months)	14.1 (8.58)	13.8 (11.57)	N/A
Mean age at onset of depression (SD)	13.81 (2.21)	14.42 (1.55)	N/A
Mean number of MDD episodes (range)	1.15 (1-2)	1.08 (1-2)	N/A
Psychotic features, N (%)	2 (15.28%)	3 (23.1%)	N/A
Low mood, N (%)	13 (100%)	13 (100%)	N/A
Irritability★, N (%)			
Threshold	7 (53.85%)	7 (53.85%)	N/A
Sub-threshold	5 (38.46%)	5 (38.46%)	N/A
Antidepressant naïve, N (%)	11 (84.6%)	12 (92.3)	N/A
Current psych. therapy/counselling, N (%)	8 (61.54%)	9 (69.23%)	N/A
Comorbid disorders Φ, N (%)			
None	8 (61.5%)	6 (46.2%)	N/A
Anxiety			N/A
GAD	2 (15.4%)		N/A
PTSD	2 (15.4%)		N/A
OCD		1 (7.7%)	N/A
Social phobia	1 (7.7%)	2 (15.4%)	N/A
Specific phobia		3 (23.1%)	N/A
Eating			N/A
Anorexia		1 (7.7%)	N/A
EDNOS	1 (7.7%)	1 (7.7%)	N/A
ASD			N/A
Diagnosed	1 (7.7%)	1 (7.7%)	N/A
Probable	1 (7.7%)	1 (7.7%)	N/A
Fibromyalgia	1 (7.7%)		
CDI	32.00 (6.42)	29.92 (6.97)	4.36 (4.41)
CDRS-R	62.85 (8.08)	62.46 (11.46)	18.18 (1.99)
A-Trait	50.69 (4.52)	48.08 (5.37)	26.45 (3.08
SIQ-JR	52.23 (24.84)	49.62 (19.37)	3.55 (3.30)
CBCL			
Anxiety-depression	13.75 (4.61)	14.67 (2.23)	1.36 (1.91)
Internalizing scores	28.00 (8.62)	27.25 (6.36)	2.36 (2.29)
Externalizing scores	12.17 (9.57)	11.08 (7.33)	3.00 (4.67)
Total scores	67.08 (22.56)	65.42 (17.58)	8.00 (7.96)
CGAS	44.77 (3.06)	45.23 (3.24)	99.36 (1.57)
CGI-severity	4.08 (3-6)	4.00 (3-6)	N/A

Notes: ★As measured using the K-SADS. **Φ**According to DSM-IV criteria. Note: 2 patients in the placebo group had 2 comorbid disorders. 1 patient in the fluoxetine group had 2 comorbid disorders and another one 3. GAD stands for Generalised Anxiety Disorder. PTSD stands for Post-Traumatic Stress Disorder. OCD for Obsessive Compulsive Disorder. EDNOS for Eating Disorder not Otherwise Specified. ASD for Asperger Syndrome. Family income per year was obtained using the following categories: 1=Under £14,999; 2=£15,000-£30,000; 3=£30,000-£45,000; 4=£45,000-£60,000; 5=£60,000-£75,000; 6=Above £75,000.

4.3.2. Subjective ratings on the testing day

A 3x2 split-plot ANOVA with timepoint (before drug/placebo, before scan and after scan) as the within subjects-factor, and group (placebo vs. fluoxetine) as the between-subjects factor was computed in order to investigate the effects of fluoxetine on subjective ratings administered on the testing day. No significant effects were seen on state anxiety (A-State) or on any of the VAS scales (all *p*s>0.36). However, participants receiving fluoxetine reported a significantly lower number of side-effects across time points, i.e., even at baseline [main effect of group: F(1,21)=6.199, p=0.021]. See Table 12.

An additional 2x3 split-plot ANOVA was conducted, with only 2 timepoints now being considered (before and after scan) and all 3 groups (patients on placebo, patients on fluoxetine and healthy controls). There was no significant interaction between timepoint and group (all *p*s>0.29), but a significant main effect of group was seen for all measures (all *p*s<0.05). Healthy adolescent controls revealed significantly lower values than both the fluoxetine and placebo groups when considering A-State, VAS tired, VAS sad and VAS irritable (all *p*s<0.05). Healthy controls were also happier overall (p<0.05). Interestingly, when considering the VAS item "thinking about death", healthy controls revealed scores that were significantly lower than the patient group (p=0.005), but comparable to those seen in the fluoxetine group (p=0.151). See Table 12.

Table 12. Subjective ratings on the testing day.

	Baseline		***Before testing***			***After testing***		
	Plac. (N=13) mean ± SD	**Fluox.** (N=13) mean ± SD	**Plac.** (N=13) mean ± SD	**Fluox.** (N=13) mean ± SD	**Controls** (N=11) mean ± SD	**Plac.** (N=13) mean ± SD	**Fluox.** (N=13) mean ± SD	**Controls** (N=11) mean ± SD
A-State	40.00 (4.18)	39.31 (4.40)	38.85 (5.40)	37.31 (3.97)	29.00 (2.72)	37.61 (6.37)	36.54 (5.14)	28.45 (3.56)
VAS tired	62.54 (32.98)	62.54 (19.23)	65.85 (26.42)	67.69 (20.11)	42.09 (17.34)	72.23 (26.83)	71.08 (16.61)	47.73 (16.88)
VAS happy	35.15 (19.62)	31.15 (23.39)	40.46 (20.43)	37.92 (23.08)	58.27 (18.53)	39.23 (24.11)	36.69 (23.29)	57.27 (19.79)
VAS sad	57.69 (24.30)	43.54 (23.44)	54.00 (22.17)	35.00 (26.52)	13.82 (16.19)	48.64 (24.28)	36.46 (26.86)	11.18 (15.55)
VAS tense	59.62 (23.75)	51.00 (20.15)	55.85 (15.68)	51.92 (25.63)	12.00 (12.93)	44.69 (30.83)	31.92 (25.96)	9.64 (14.20)
VAS irritable	50.46 (31.47)	41.84 (22.07)	42.38 (31.44)	30.85 (15.94)	11.82 (18.35)	35.15 (31.77)	33.85 (24.82)	10.09 (15.44)
VAS death	36.54 (30.51)	19.69 (21.58)	28.31 (29.89)	15.08 (20.21)	3.18 (7.22)	29.62 (28.34)	16.54 (20.77)	3.36 (8.19)
Side-effects	4.62 (2.50)	3.08 (1.44)	4.54 (2.57)	2.62 (2.02)	0.82 (0.60)	4.62 (2.53)	2.77 (1.59)	1.09 (0.94)

4.3.3. Functional MRI behavioural performance

Two participants did not complete the scan due to unforeseen MRI incompatibly (N=1) and unexpected lack of operator (N=1). In addition, the data from 1 participant had to be removed due to image artefacts (N=1). The final analysis for the fMRI task therefore consisted of 12 subjects in the fluoxetine group, 11 in the placebo group and 11 in the healthy adolescents group.

Subjects were highly accurate in their performance (>94.6%). A split-plot ANOVA revealed a significant interaction between emotion and group when considering accuracy levels [$F(4,62)=2.553$, $p=0.048$]. However, follow-up pairwise comparisons failed to reveal any significant differences between groups (*p*s>0.11). No main effect of group was seen [$F(2,31)=0.412$, $p=0.666$]. When considering RTs, no main effects or interaction between emotion and group were seen (*p*s>0.6). Values are presented in Table 13.

Table 13. Behavioural performance on the Emotional faces task.

	Placebo (N=11), mean ± SD	**Fluoxetine** (N=12), mean ± SD	**Controls** (N=11), mean ± SD
Accuracy (%)			
Happy	95.68 (5.93)	96.25 (3.29)	98.64 (3.03)
Fear	94.55 (5.90)	97.08 (3.43)	95.23 (3.25)
Anger	97.50 (5.24)	96.25 (5.06)	98.18 (3.18)
Reaction Times (ms)			
Happy	705.51 (158.06)	686.97 (82.57)	733.12 (144.21)
Fear	703.73 (148.67)	691.88 (86.83)	738.69 (138.72)
Anger	716.49 (146.94)	690.23 (97.03)	738.70 (150.87)

4.3.4. Functional MRI Results

Main effect of Task

Presentations of fearful, happy and angry facial expressions produced activation in a broad network of brain regions across groups (fluoxetine, placebo and healthy controls combined). These regions include the occipital cortex, anterior cingulate and parietal lobe (see Table 14), and are overall consistent with previously published reports using the same task (e.g., Rawlings et al., 2010).

Table 14. Peak cluster activation in brain regions significantly activated during presentation of happy, fearful and angry faces.

	Number of voxels	Brain regions	Maximum Z	Peak MNI coordinates (x, y, z)
Happy	20946	R Occipital pole; lateral occipital cortex	7.02	26, -92, 10
	5865	L Postcentral gyrus; superior parietal lobe	6.22	-46, -32, 52
	5093	Central opercular cortex	5.44	-44, -4, 14
	3005	Supplementary Motor area; anterior cingulate	6.07	-6, -4, 54
	10009	R Lateral occipital cortex; superior parietal lobe,	4.15	34, -60, 44
Fear	65140	L Postcentral gyrus; Supramarginal gyrus, anterior division; superior parietal lobe	7.16	-42, -30, 46
	2166	R Lateral occipital cortex; superior parietal lobe	5.04	32, -58, 46
Anger	49841	Occipital pole	7.57	14, -102, 8
	1707	R Lateral occipital cortex; Superior Parietal Lobe	5.12	32, -60, 44

Note. These findings correspond to data combined across groups from three separate contrasts: happy vs. fixation, fear vs. fixation and anger vs. fixation.

1. Effect of depression (depressed patients on placebo vs. healthy controls)

Hypothesis-driven ROI analysis of amygdala activation

In order to explore the effects of depression on amygdala activation towards emotional faces, the percentage signal change values extracted from the anatomical ROI masks were submitted to a split-plot ANOVA. Within-subjects variables were emotion (i.e., percentage signal change for happy, fearful and angry faces) and hemisphere (right and left), and the between-subjects variable was group (depressed patients receiving placebo vs. healthy controls). This revealed a significant interaction between emotion, hemisphere and group [$F(2,40)=5.410$, $p=0.008$]. Follow-up comparisons showed that this effect was being driven by a trend for significant group differences in the right amygdala for fearful and angry faces. Specifically, depressed participants revealed a trend for a significantly lower activation relative to controls in response to

fear [F(1,20)=3.912, p=0.062] and for an increased activation in response to anger [F(1,20)=4.143, p=0.055] in the right hemisphere. No differences were seen for the left hemisphere (all *p*s>0.28). See Figure 10.

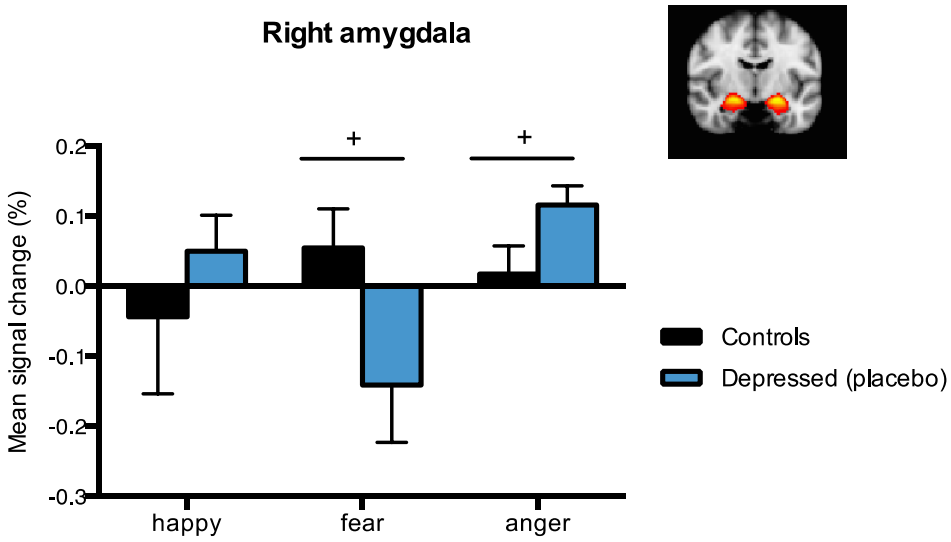

Figure 10. Mean percentage signal change from the anatomical mask in the amygdala, based in the Harvard-Oxford Atlas (placebo vs. healthy controls). Bars represent the mean percentage of signal change (%). Error bars show the standard error of the mean. +p<0.10.

Exploratory whole-brain analysis

Fear > happy contrast

A whole-brain analysis (threshold *Z*>2.0, *p*<0.05, corrected) revealed a decreased response in depressed adolescents (on placebo) relative to healthy controls for the fear > happy contrast in regions implicated in affective processing. These regions include 3 clusters in the left hemisphere: *middle frontal gyrus* (x=-56, z=32, y=28; *Z*=3.95, voxel size=1412, *p*<0.05), *inferior frontal gyrus* (x=-52, z=16, y=4; *Z*=3.5, voxel size=970, *p*<0.05) and *supramarginal gyrus* (x=-60, z=-44, y=44; *Z*=3.61, voxel size=640, *p*<0.05). Of note, the cluster including the middle frontal gyrus corresponds to Broadmann Area (BA 9), which includes the dorsolateral prefrontal cortex (DLPFC). The inferior frontal gyrus corresponds to Broadmann Area 44 (BA 44), which includes the posterior region of the ventrolateral prefrontal cortex (VLPFC).

A post-hoc analysis of percentage BOLD signal change for the cluster containing the *middle frontal gyrus* revealed a significantly decreased activation in the depressed group to fearful facial expressions [t(20)=2.238, p=0.037], and a trend for an increased activation to happy [t(20)=-1.753, p=0.095]. See Figure 11 a). Similarly, the analysis with the *inferior frontal gyrus* revealed

decreased responses in the depressed group towards fear [t(20)=2.341, p=0.030], but no group differences for happy [t(20)=-0.751, p=0.462]. See Figure 11 b).

Finally, the analysis with the cluster containing the *supramarginal gyrus* revealed no significant group differences for happy [t(20)=-1.053, p=0.305] or fearful faces [t(20)=1.081, p=0.293].

These whole-brain analyses are consistent with the reduced activation seen in the ROI analysis of the right amygdala in response to fear. Of note, however, no significant effects were seen in the contrast comparing anger to happy.

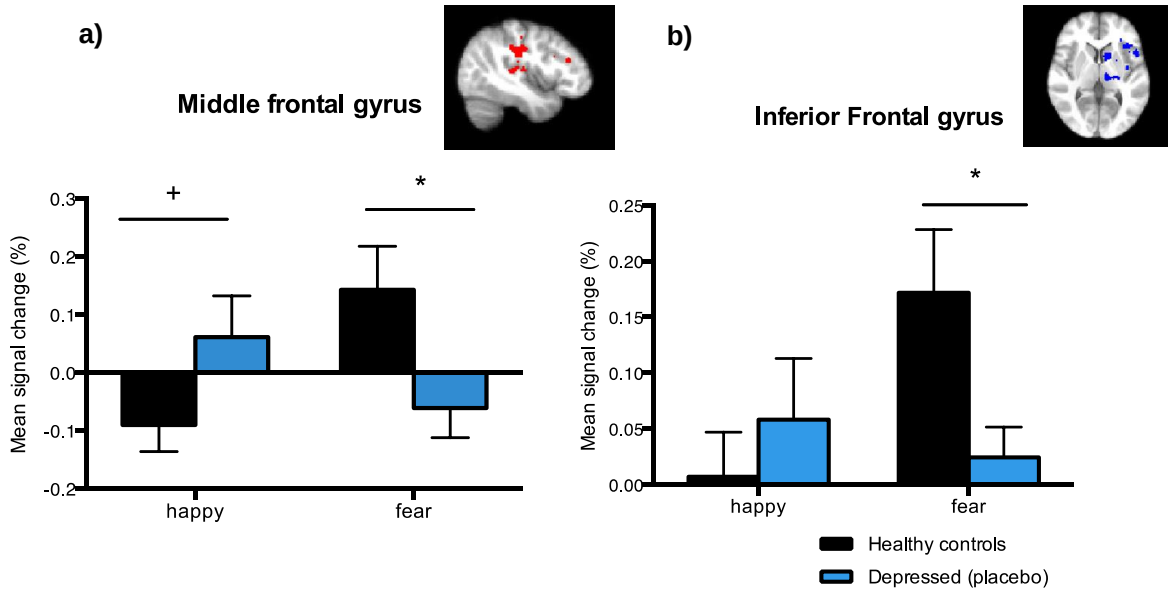

Figure 11. Significant clusters of activation in the fear>happy contrast, placebo>controls (a) Significant cluster of activation in the middle frontal gyrus (including BA 9, dorsolateral prefrontal cortex, DLPFC). **(b)** Significant cluster of activation in the Inferior Frontal gyrus (BA 44, which includes the posterior region of the ventrolateral prefrontal cortex, VLPFC). Images thresholded at Z>2.0, p<0.05, corrected. +p<0.10, *p<0.05.

2. Effect of fluoxetine (drug vs. placebo)

Hypothesis-driven ROI analysis of amygdala activation

A split-plot ANOVA with percentage signal change in the amygdala ROI masks for fear, happy and anger revealed a significant interaction between emotion, hemisphere and group [F(2,42)= 7.640, p=0.001]. Follow-up comparisons showed that this effect was being driven by differences in the right amygdala for fearful and angry faces. Specifically, participants in the fluoxetine group showed a trend for a higher activation in the right amygdala in response to fear [F(1,21)= 3.741, p=0.067], and a lower activation in the same hemisphere in response to anger

[F(1,21)= 7.747, p=0.011]. These effects are opposite to those seen in the comparison between placebo-treated participants and healthy controls. See Figure 12.

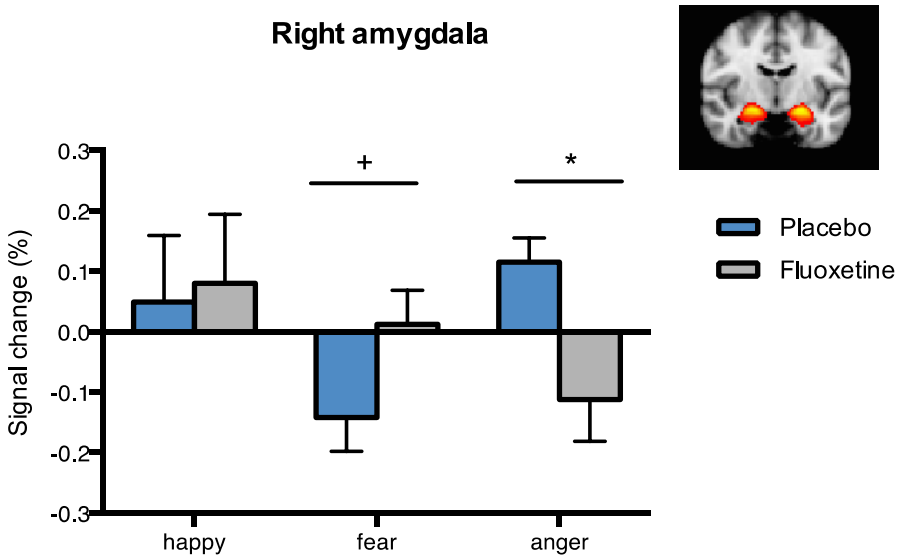

Figure 12. Mean percentage signal change from the anatomical mask in the amygdala, based in the Harvard-Oxford Atlas (fluoxetine vs. placebo). Bars represent the mean percentage of signal change (%). Error bars shown the standard error of the mean. +p<0.10, *p<0.05.

Exploratory whole-brain analysis

Angry > happy contrast

A whole-brain analysis (threshold *Z*>2.0, *p*<0.05, corrected) revealed decreased response in the fluoxetine group relative to placebo for the angry > happy contrast in three clusters in the occipital, temporal and frontal cortices. Fluoxetine attenuated the neural responses in the *left superior temporal gyrus* (x=-46, y=22, z=0; *Z*=3.44; voxels size: 1114), *occipital pole* (x=2, y=-98, z=12; *Z*=3.67; voxel size: 1002), *middle temporal gyrus* (x=-18, y=-40, z=12; *Z*=3.63; voxel size: 684) and *left superior frontal gyrus* (x=-14, y=-12, z=70; *Z*=3.32; voxel size: 574 voxels). Of note, the cluster encompassing the superior temporal gyrus extended into the hippocampus and insular cortex, whereas the cluster containing the middle temporal gyrus included the posterior cingulate. The cluster containing the left superior frontal gyrus included the Pre-Supplementary Motor area.

Post-hoc analysis of percentage BOLD signal change for the *superior temporal gyrus* revealed a pattern of reduced activation in responses to angry faces in the fluoxetine group [t(21)=2.499), p=0.021]. No differences were seen in response to happy [t(21)=-1.353, p=0.190]. See Figure 13 a).

Similarly, the analysis with the *occipital pole* showed a decreased activation towards anger in the fluoxetine group [t(21)=2.256, p=0.035], but, again, no differences were seen for happy [t(21)=-0.579), p=0.569]. See Figure 13 b).

The analysis with the *middle temporal gyrus* revealed an increased activation towards happy faces [t(21)=-2.649), p=0.015]. No differences were seen in response to anger [t(21)=1.480), p=0.154]. See Figure 13 c). This same pattern was seen in the *superior frontal gyrus*, in which increased activation towards happy faces was seen [t(21)=-3.277), p=0.004], but no group differences for anger [t(21)=-0.941), p=0.357]. See Figure 13 d).

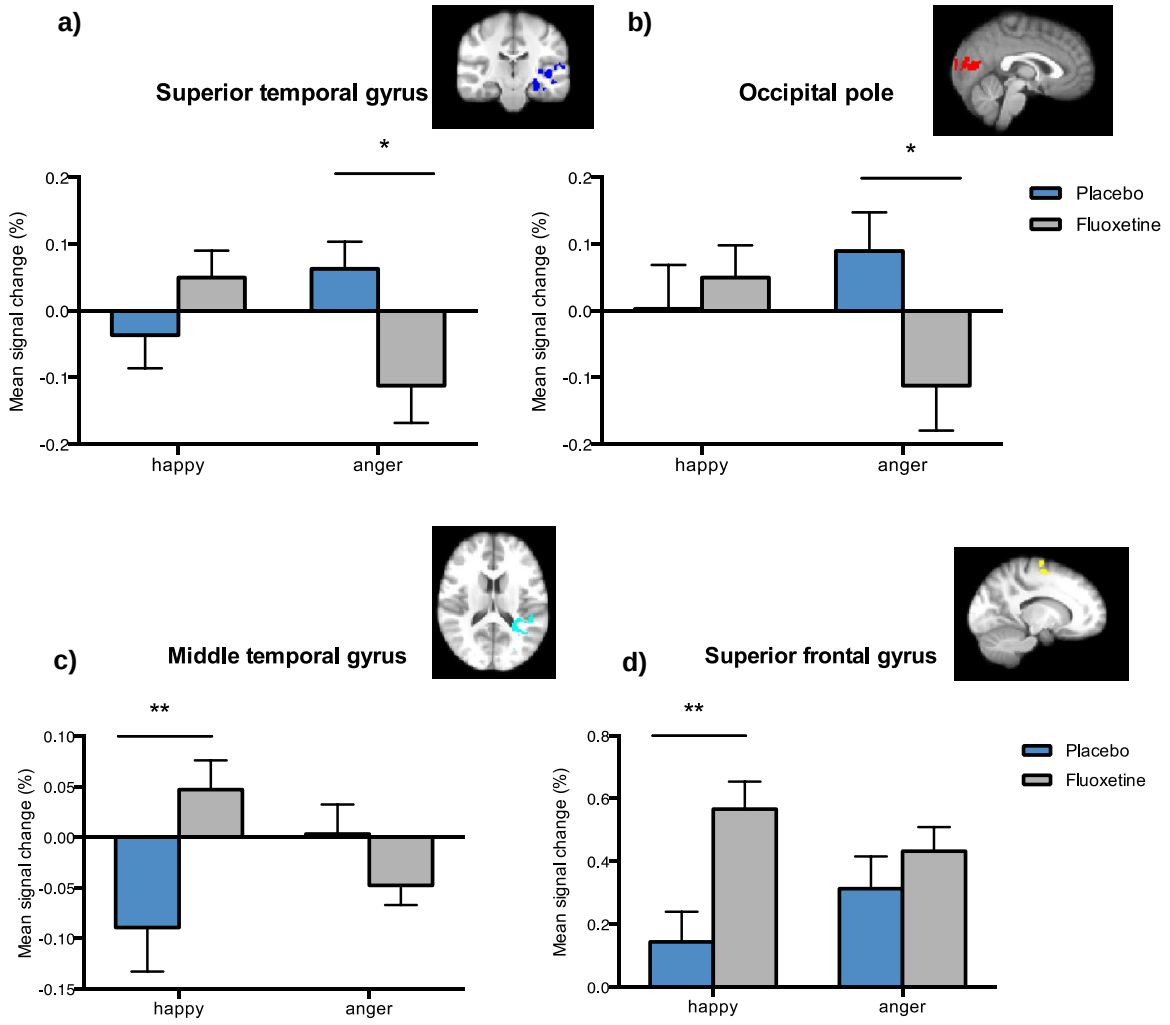

Figure 13. Significant clusters of activation in the angry>happy contrast, fluoxetine>placebo (a) Significant cluster of activation in the superior temporal gyrus (extending into the hippocampus and insular cortex). **(b)** Significant cluster of activation in the occipital pole. **(c)** Significant cluster of activation in the middle temporal gyrus (including the posterior cingulate). **(d)** Significant cluster of activation in the superior frontal gyrus (including the Pre-Supplementary Motor Area). Images thresholded at Z>2, p<0.05, corrected. Bars show the mean percentage of signal change (%), error bars shown the standard error of the mean. *p<0.05. **p<0.01.

Fear > happy contrast

A whole-brain analysis (threshold *Z*>2.0, *p*<0.05, corrected) revealed increased response under fluoxetine relative to placebo for the fear > happy contrast in two clusters: *frontal pole* (x=38, y=42, z=-14; *Z*=3.44; voxel size: 777), which includes the orbitofrontal cortex and insula; and *superior and middle temporal gyrus*, including the Wernicke area (*Z*=3.44, x=-60, y=-38, *Z*=3.3; voxel size: 527).

Post-hoc analysis of percentage BOLD signal change in the *frontal pole* revealed increased responses in the fluoxetine group towards fear [t(21)=-2.652, p=0.015], but no differences were seen for happy [t(21)=1.062, p=0.300]. See Figure 14 a).

When considering the *superior and middle temporal gyrus*, there was increased activation in the fluoxetine group towards fear [t(21)=-2.761, p=0.012], but again, no differences were seen for happy [t(21)=0.213), p=0.833]. See Figure 14 b).

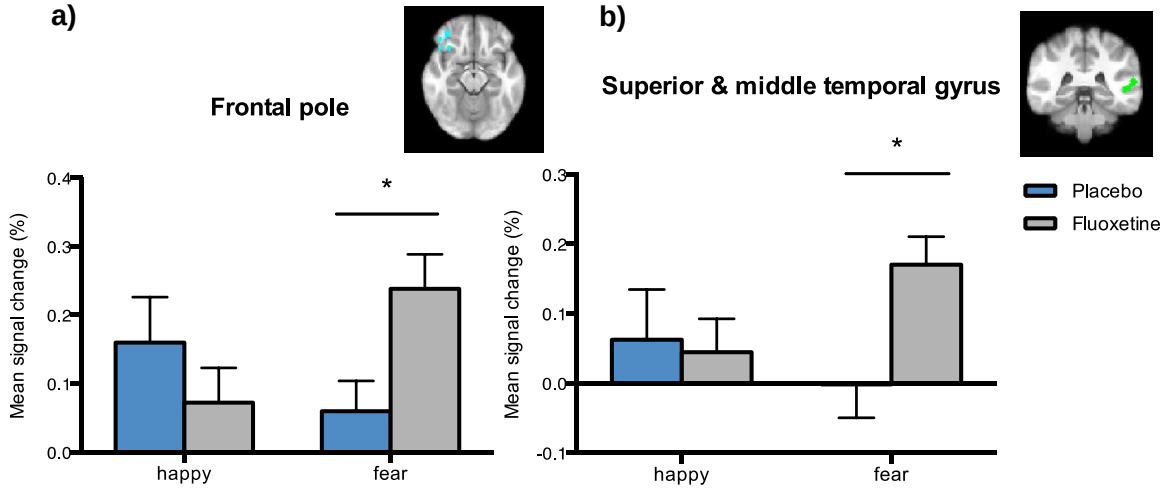

Figure 14. Significant clusters of activation in the fear>happy contrast, fluoxetine>placebo (a) Significant cluster of activation in the frontal pole (including the orbitofrontal cortex and insula). **(b)** Significant cluster of activation in the superior and middle temporal gyrus (including the Wernicke area). Images thresholded at z>2, p<0.05, corrected. Bars show the mean percentage of signal change (%), error bars shown the standard error of the mean. *p<0.05.

4.3.5. Facial Expression Recognition Task

In order to assess if the modification of neural responses by fluoxetine could relate to potential group differences in emotional perception abilities, a facial expression recognition task (FERT) was administered after the fMRI scan. Due to time constraints, two patients were not administered this paradigm. The final analysis therefore included 12 patients in the placebo group, 12 patients in the fluoxetine group and 11 healthy control adolescents. The analysis of the FERT

failed to show a significant interaction between emotion and group when considering accuracy [F(12,192)=0.676, p=0.712], reaction times [F(12,192)=1.120, p=0.346] or percentage of misclassifications given [F(12,192)=0.728, p=0.563]. However, a main effect was seen for all these measures (all *p*s<0.0001)

Accuracy values are presented in Figure 15 for illustrative purposes. The accuracy levels for neutral and happy faces were the highest overall. Angry faces were detected more accurately than fearful faces (p=0.002), but at a comparable level to sadness (p=0.295).

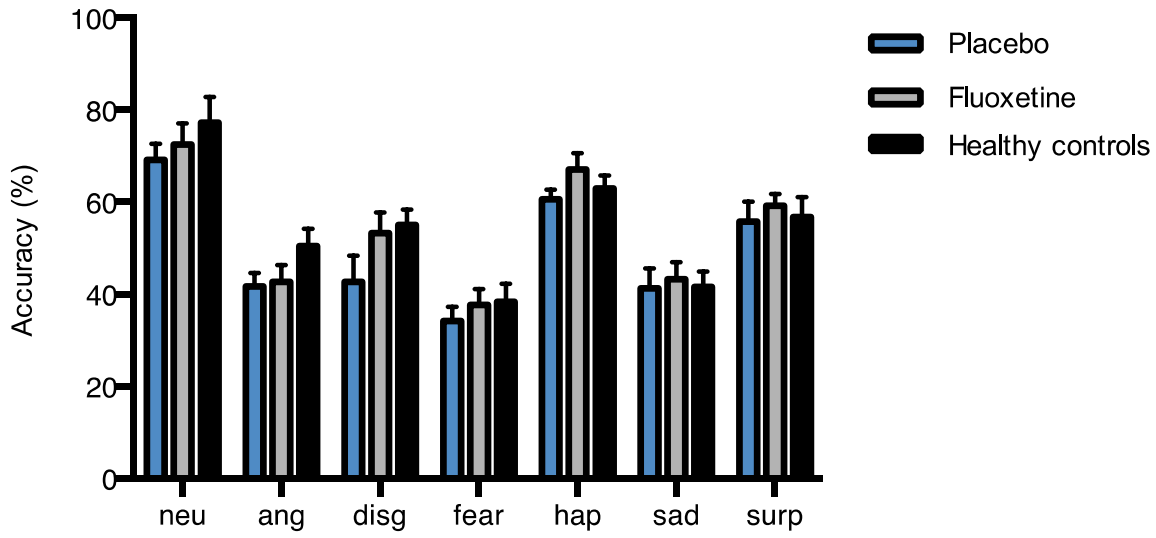

Figure 15. Accuracy of facial expression recognition in the placebo, fluoxetine and healthy control groups. Values represent percentage of correct responses summed over the different intensity levels for that emotion. Error bars represent standard error of the mean.

4.3.6. Clinical changes over time

A repeated measures ANOVA with timepoint (baseline, follow-up 1 and follow-up 2) was conducted to assess any potential changes in the clinical measures collected throughout the study. There was a significant main effect of time when considering most of the measures. Indeed, measures of depression severity (as quantified using the CDRS-R and CDI), irritability symptoms (item 13 from the CDRS-R and VAS irritability), suicidal ideation (SIQ-JR), trait anxiety (A-Trait) and tension (VAS item) were significantly reduced over time (all *p*s<0.05). The CGI-I showed a trend for a significant reduction in severity between follow-up sessions 1 and 2. In contrast, side-effects symptoms showed a significant increase with time (p<0.01). The suicide ideation item from the CDRS-R, state anxiety (A-State), VAS items “sad”, “thinking about death” and “tired” did not change significantly across timepoints (all *p*s>0.13). See Table 15.

A repeated measures ANOVA with the responses given to the daily text messages (10 days in total) failed to reveal a main effect of time (p>0.2).

Table 15. Subjective Ratings at baseline, follow-up 1 and follow-up 2.

	Baseline (N=26) mean ± SD	**Follow-up I** (N=26) mean ± SD	**Follow-up II** (N=20) mean ± SD
CDI	30.96 (6.64)	24.73 (10.97)	20.27 (12.98)
CDRS-R (raw total)	62.36 (9.79)	58.42 (13.87)	45.09 (15.04)
CDRS-R suicidality	3.85 (1.57)	3.50 (1.86)	2.86 (1.59)
CDRS-R irritabiliy	3.27 (1.31)	2.81 (1.27)	2.40 (1.31)
A-Trait	49.44 (5.02)	N/A	42.64 (9.04)
A-State	39.65 (4.22)	39.67 (5.54)	37.55 (7.72)
SIQ-JR	50.92 (21.86)	27.23 (14.43)	35.00 (22.61)
CGI-I	N/A	3.62 (1.02)	3.13 (1.14)
VAS tired	62.54 (26.45)	64.84 (28.62)	58.14 (28.68)
VAS happy	33.15 (21.25)	30.20 (23.44)	41.19 (27.63)
VAS sad	50.62 (24.48)	53.36 (28.58)	38.29 (31.26)
VAS tense	55.31 (22.03)	47.04 (27.61)	32.28 (31.78)
VAS irritable	46.15 (26.99)	40.24 (28.80)	20.57 (24.88)
VAS death	28.12 (27.28)	29.96 (28.87)	18.81 (27.03)
Side-effects	3.85 (2.15)	5.04 (3.36)	6.14 (4.10)

Note: These values correspond to the patient groups combined. *Baseline* corresponds to the measures collected during the screening session or immediately before the drug/placebo being administered. The *follow-up 1* was conducted after one week of treatment and the *follow-up 2* after six weeks.

4.3.7. Correlations between amygdala activity and clinical symptoms

In the fluoxetine group (N=12), activity in the right amygdala in response to anger and fear was correlated with clinical symptoms of anxiety, irritability and depression. There was a trend for a significant correlation between activation in the right amygdala in response to fear and the A-State difference score from baseline to follow up 1 (r=-0.589, p=0.057). Higher activation in this area was associated with a lower change in symptoms. There was also a negative correlation between amygdala response to fear and change from baseline in the CDRS-R to follow-up 1 (r=-0.679, p=0.016), but not in the CDI (r=0.078, p=0.821). Finally, increased responses in the right amygdala towards fear were associated with significantly lower symptoms of anxiety over the first 10 days of treatment (as measured using the daily text messages) (r=-0.678, p=0.045). A similar trend was

seen when only the first 5 initial days were considered (r=-0.542, p=0.085), but not when considering the first 3 (r=-0.285, p=0.369).

There was no significant correlation between activation in the right amygdala in response to anger and change in irritability or depressive symptoms (all *p*s>0.72).

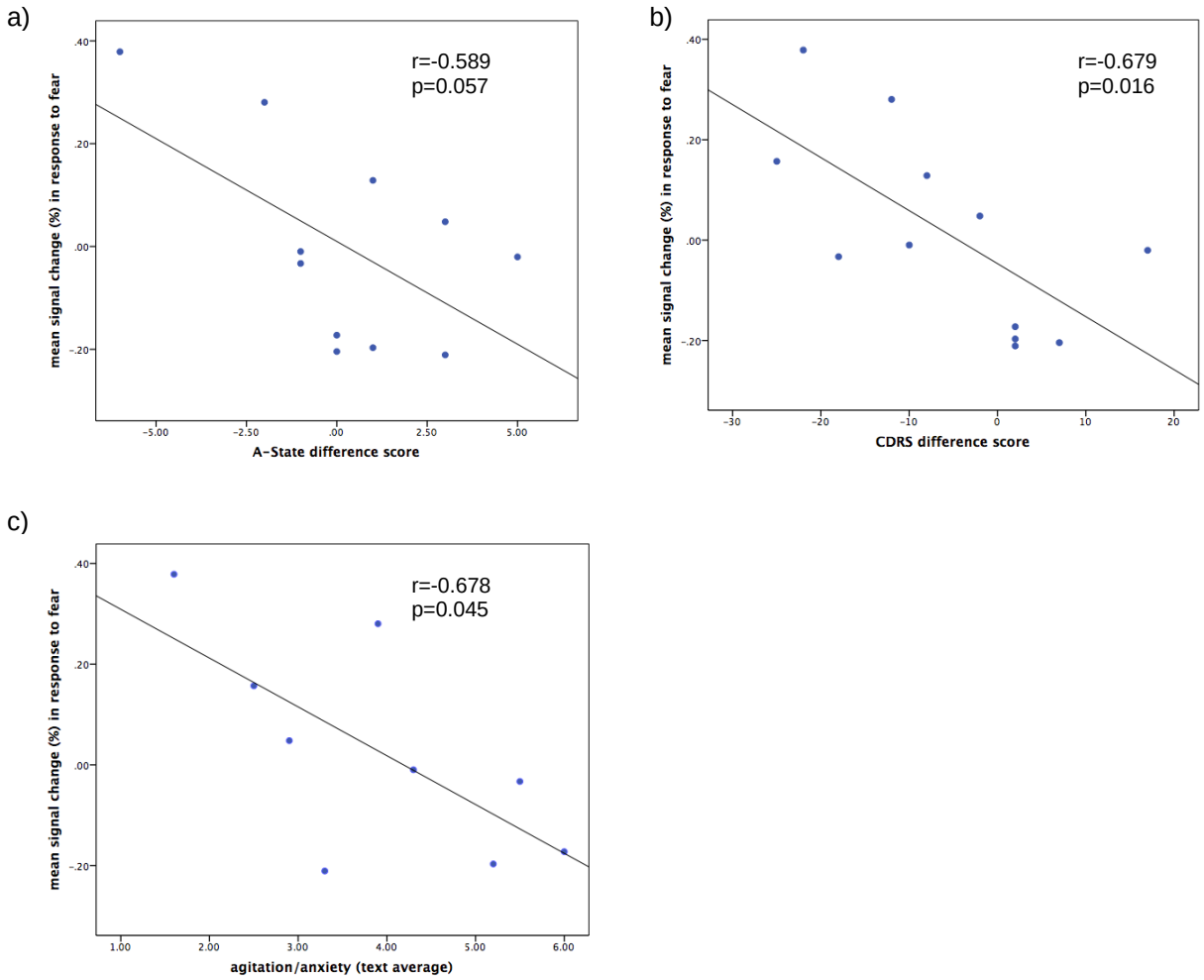

Figure 16: Graphs a) and b): Scatterplot of the correlation between percentage signal change (%) in the right amygdala and the difference score from baseline to follow-up I in state anxiety (A-State) and depression (CDRS-R), respectively. Graph c): Scatterplot of the correlation between percentage signal change (%) in the right amygdala and text message scores measuring agitation/anxiety, averaged across the first 10 days of treatment.

4.4. Discussion

This study sought to investigate, for the first time, neural responses to emotional stimuli following a single dose of fluoxetine (vs. placebo) in a group of depressed adolescents. The placebo-treated participants were initially compared with a group of healthy adolescents in order to explore the presence of emotional processing biases in amygdala responses to anger, happiness

and fear. Following this analysis, the effects of fluoxetine were subsequently compared to placebo. The activity of the amygdala in response to anger and fear was also correlated with initial symptoms of anxiety, irritability and depression. The main study prediction was that a single dose of fluoxetine would decrease the processing of angry faces in the amygdala (vs. placebo), the a-priori region of interest, and in the absence of any changes in subjective mood or anxiety.

Two main findings emerged from this study. First, when compared to healthy adolescents, depressed patients on placebo showed a trend for increased activation in the amygdala towards angry facial expressions, and a trend for decreased activation in response to fear. Second, this pattern was reversed in the fluoxetine vs. placebo groups. Indeed, in line with the initial hypothesis, a single dose of fluoxetine vs. placebo decreased activation in the amygdala in response to angry faces. Fluoxetine was also shown to increase amygdala activity in response to fear. Whole-brain responses in occipital, temporal and frontal regions known to be involved in emotional processing revealed similar patterns of activation. These neural changes were seen in the absence of overall effects of fluoxetine on mood or anxiety symptoms.

Effects of depression

Depression in adolescence was associated, in this study, with a trend for increased amygdala activation in response to angry facial expressions. This finding is in line with studies showing that anger is a key emotion in adolescent depression (Stringaris et al., 2013). There is nonetheless very limited research using fMRI to explore the processing of anger during adolescent MDD episodes, which complicates the interpretation of this borderline effect. One of the studies that measured the neural processing of anger in addition to other facial expressions was conducted by Yang and colleagues (2010) and included a facial-emotion matching paradigm. The left amygdala was found to be increased in a sample of unmedicated adolescents with pure MDD relative to matched healthy controls. This hyperactivity was seen in response to anger, but also to fear and happiness, therefore contrasting with the findings seen here. The reasons for this difference remain elusive, but variations in genotypic/psychological sample characteristics or in the functional paradigms used could be partially responsible (see Hariri, Bookheimer, & Mazziotta, 2000; Lau et al., 2009). For instance, there is some suggestion that emotional matching paradigms induce greater amygdala activation relative to tasks involving verbal labelling (Hariri, Bookheimer,

& Mazziotta, 2000), which could have influenced the reactivity of this structure - irrespective of the valence being presented - in the study by Yang and colleagues (2010).

Of special relevance to the effect seen here with anger, are a few studies suggesting a link between anger perception and depressive disorders in young people. Beek and Dubas (2008) found that depressive symptoms in adolescence were associated with an increased perception of angry facial expressions along with decreased perception of happiness. Similarly, in a study by McClure, Pope, Hoberman, Pine and Leibenluft (2003), bipolar children and adolescents were more likely than healthy controls to misinterpret happy, sad, and fearful expressions of children - but not of adults - as angry. The clinical implications of this finding are yet to be determined, but it is possible that this bias to overperceive anger could relate to the irritability symptoms and interpersonal difficulties that are associated with both depression and bipolar disorder.

A pattern of increased amygdala activation in response to angry facial expressions has been previously reported in paediatric samples - including adolescents with depression (Yang et al., 2010), bipolar disorder (Kim et al., 2012) and severe mood dysregulation (Thomas et al., 2013) -, as well as in adults with intermittent explosive disorder (Coccaro, McCloskey, Fitzgerald, & Phan, 2007). In the study by Coccaro and colleagues (2007), amygdala activity was also positively correlated with symptoms of lifetime aggression. Given the prominent role of anger and irritability in all these conditions, it is possible that an increased reactivity towards anger represents a neural substrate underpinning these behavioural symptoms. Indeed, irritability was a symptom commonly reported in the current study, being evident at a threshold level in slightly more than half of the participants (53.85%), and at a sub-threshold level in in 38.46%, which is line with previous evidence from clinical samples (e.g., Strober et al., 1981).

Another potential explanation for the pattern of increased activity towards anger is that this emotion may be especially salient in adolescent MDD, due to an increased sensitivity to negative interpersonal cues. Depressed teenagers are especially sensitive to the possibility of rejection from peers and to negative feedback in general (for a review see Platt, Cohen Kadosh, & Lau, 2013). In light of these concerns, anger may signal the possibility of rejection or social/physical harm, therefore inducing a pattern of increased vigilance. This hypothesis is merely speculative given that participants in this study were not specifically asked to rate the faces in terms of hostility or valence. Future studies may wish to include subjective ratings in order to clarify the judgements

ascribed to different emotions. Indeed, such approach has proved useful in previous adolescent studies involving emotional processing tasks (e.g., McClure et al. 2007).

In the current study, participants with depression showed an equivalent performance relative to healthy controls in a facial expression recognition task administered outside the scanner. It is therefore not possible to equate the abnormal pattern of neural activation towards anger with an increased behavioural bias to perceive this emotion. However, the facial expression recognition task (FERT) used in this study may have shown reduced sensitivity to detect differences in anger processing between groups since it was administered after the fMRI scan, during which participants were repeatedly exposed to angry faces. In support of this idea is a previous study showing a lack of group differences in the recognition of negative stimuli when the FERT was administered following an fMRI scan involving faces (Murphy et al., 2009).

It is intriguing that depressed adolescents showed a trend for the opposite pattern (i.e., decreased activation in the amygdala) with respect to fearful faces. This activation was accompanied by reduced activation in the middle and inferior frontal gyrus, which correspond to the dorsolateral (BA 9) and ventrolateral (BA 44) prefrontal cortices, respectively. A similar pattern of parallel activation between the amygdala and ventrolateral prefrontal cortex (VLPFC) has been seen in paediatric patients with bipolar disorder (Rich et al., 2006; but see Pavuluri, O'Connor, Harral, & Sweeney, 2007). The amygdala and VLPFC have both been implicated in threat monitoring (Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003; Phillips, Drevets, Rauch, & Lane, 2003). Specifically, the VLPFC is thought to modulate the amygdala engagement in order to facilitate the flexible allocation of attentional resources when responding to threatening cues (Hariri et al., 2003). The DLPFC is an area of cognitive control, which has also been implicated in the regulation of affective responses originated within the amygdala and other limbic structures (Phillips, 2003). Of note, there was a trend for a significantly increased activation of the DLPFC towards happiness vs. fear. These results may be in line with a deficient regulation of responses to threat-related vs. positive cues, but such finding is difficult to interpret.

A pattern of decreased activation in the amygdala along with decreased prefrontal recruitment during the processing of fearful faces is nonetheless inconsistent with current theories of emotional regulation (e.g., Mayberg, 2003). It is thought that deactivation in the amygdala in response to emotionally salient stimuli results from increased prefrontal engagement, and not the

opposite. This theory has arisen from studies in the adult neuroimaging literature, but evidence from young samples is much more variable (Lau, 2013). For instance, whilst reduced connectivity between the prefrontal cortex and limbic structures is often reported in adult patient studies, the relationship between these structures is much more complex in adolescence (see McClure et al., 2003). Such variability has led some authors to suggest that dual-system models may be inadequate to fully account for the intricate interplay that occurs between limbic and higher-level cognitive systems during adolescence (Pfeifer & Allen, 2012).

Previous studies with depressed adolescents have also reported inconsistent findings regarding amygdala reactivity in response to fear in adolescent depression. Whereas several studies suggest that young depressed people show an increased activation in this structure in response to fearful faces (Hall et al., 2014; Tao et al., 2012; Yang et al., 2010), others report an opposite pattern of deactivation (Beesdo et al., 2009; Thomas et al., 2001) or lack of group differences (van den Bulk et al., 2014). The reasons for this discrepancy remain uncertain, but methodological variations in the fMRI paradigms used could be partly responsible, given cumulative evidence showing that activation in the amygdala and other structures is strongly affected by task parameters across development (see Guyer et al., 2008; McClure et al., 2007; Nelson et al., 2003). Thomas and colleagues (2001) reported decreased activation in the amygdala in a small sample of 5 currently unmedicated depressed female adolescents vs. matched healthy controls. In this blocked-design study, participants were required to passively view neutral and fearful faces for 200ms each. The group differences were seen in a contrast involving fearful faces vs. fixation. Similarly, Beesdo and colleagues (2009) found that unmedicated adolescents with MDD had lower bilateral activation in the amygdala compared to both healthy and anxious comparison groups, but only in a condition involving passive viewing of fearful (vs. happy) faces for 4000ms. Indeed, when asked to rate the intensity of the fearful emotion displayed on a likert scale, both the MDD and anxious groups showed amygdala hyperactivity in comparison to controls, therefore highlighting the potential role of attention-mediated processes in explaining this pattern. In contrast, a separate study by Monk and colleagues (2008) reported increased amygdala activation in a passive viewing condition with varying levels of fear intensity but in a group of adolescents at risk for developing MDD. This pattern was not seen during constrained attention. A study by van den Bulk and colleagues (2014), on the other hand, failed to see any group

differences between unmedicated adolescents and matched healthy-controls in amygdala activity towards fear. However, in the study by Bulk and colleagues (2014), not all participants presented with depression as the main diagnosis.

In the current thesis, participants were required to identify the gender of the face, which was presented for 100ms. It is notable that the study by Thomas and colleagues (2001) also found a decreased amygdala activity in response to fearful faces that were also viewed for a short duration (200ms). The task reported by Thomas and colleagues (2001) and the one used here contrast with studies in which participants are asked to focus on the intensity of the emotion, and in response to faces that are usually presented for longer durations (3000ms or 4000ms). Interestingly, a study by Beesdo and colleagues (2009) reported opposite patterns of amygdala activity depending on whether the task involved passive viewing or explicit emotion assessment. Given these differences in task design and stimuli presentation, it is possible that the pattern of amygdala hyperactivity in response to fear in adolescents is more consistently seen in tasks involving explicit assessment. These differences possibly reflect distinctive patterns underlying automatic vs. strategic processes. Future studies including both types of conditions are necessary in order to clarify how task parameters may affect amygdala activity in adolescent depression.

The contrasting pattern seen in response to angry vs. fearful faces (when compared to happy) could be partially explained by the divergent meanings conveyed by these two emotional categories, which may be especially salient during adolescence. Fearful and angry faces seem to be equally negative and anxiety-provoking, but hold a different communicative signal. Specifically, fearful faces convey the message that there is the possibility of danger, but with an ambiguous source; whereas angry faces represent themselves the source of threat, therefore communicating the possibility of direct aggression towards the observer (Whalen, 1998). In line with this, it has been found that despite angry faces being rated as equally unpleasant, threatening, and anxiety provoking as fearful faces (relative to neutral and happy), they are perceived by adults as being significantly more likely to cause harm (Strauss et al., 2005), and also elicit higher eye-blink responses (Springer, Rosas, McGetrick, & Bowers, 2007).

It is tempting to speculate that these differential meanings could be especially salient to young people. Indeed, adolescents undergo strong changes in terms of social awareness and, as mentioned previously, this age group is especially sensitive to the possibility of rejection in social

interactions (Harper, Dickson, & Welsh, 2006; Kloep, 1999). Anger, in particular, is generally regarded as a social and self-conscious emotion (Berkowitz, 1999), which conveys the possibility of physical or social harm. This emotion could therefore become especially prominent during adolescence and, in particular, during a depressive episode, where excessive self-awareness and social concerns lie at the core of the disorder. The increased ambiguity associated with fear, on the other hand, may lead to a more variable pattern of appraisal during adolescent depression and adolescence more broadly. In the context of the present study, it is possible that the hyperactivity in the amygdala towards anger reflects a pattern of hypervigilance in response to hostility or social rejecting cues, whereas the decreased activity in response to fear reflects a pattern of increased avoidance or disengagement. However, these hypotheses are speculative and require further investigation.

There is limited evidence exploring the development of fear and anger recognition during adolescence, which further constrains the interpretation of the current findings. However, a study by Thomas and colleagues (2007) suggest that the ability to recognize fear improves gradually from childhood through adolescence and then adulthood, whereas the ability to recognise anger shows a sharp increase in sensitivity from teenage to adult years. In the view of the authors, this increased salience of anger could result from the enhanced learning of social rules during adolescence. Whilst this data is limited to healthy populations, it raises the question as to whether a different developmental trajectory for the detection of anger relative to fear could underlie, to some extent, the differential pattern of amygdala reactivity seen here (see Mackie, Devos, & Smith, 2000).

Effects of fluoxetine administration

A single dose of fluoxetine was shown in this study to decrease neural responses to angry faces whilst increasing activation towards fear. These effects are opposite to those seen in the placebo group vs. healthy controls, which could suggest that fluoxetine is reversing the borderline pattern of hyper- and hypo-response seen in response to anger and fear (respectively) prior to treatment. The increased activation towards fearful faces was also associated with a reduced experience of anxiety/agitation and depressive symptoms in the first 10 days of treatment.

The reduced engagement of the amygdala in response to anger is line with the initial study prediction, built from the finding seen in Chapter Two, in which a single dose of fluoxetine was shown to decrease the recognition of angry facial expressions in a group of young adult volunteers. Together, these findings highlight an important mechanism through which fluoxetine may act to exert its clinical action.

The replication of such an immediate effect of fluoxetine on the processing of anger is of critical importance. To my knowledge, only one study has looked at the influence of this medication on neural responses, but following a long course of fluoxetine treatment (8 weeks) and in the absence of a placebo-controlled condition (Tao et al., 2012). The current findings therefore extend the study by Tao and colleagues (2012), by demonstrating that fluoxetine produces effects that can be seen as early as after a single dose, and independently of any overall changes on mood or anxiety. This dissociation between the effects of fluoxetine on emotional processing vs. subjective mood is important to clearly delineate the mechanisms that may precede (and contribute to) subsequent changes in clinical symptoms.

Increased amygdala activation towards anger has been previously reported in disorders were irritability is a key symptom (e.g., Coccaro et al., 2007; Kim et al., 2012; Thomas et al., 2013). The effect of fluoxetine on this emotion could therefore represent an important mechanism underlying the alleviation of these symptoms. However, despite irritability levels showing a reduction over time in this study, amygdala activation failed to correlate with this measure. The current study was limited by a very modest sample size, especially in the correlation analysis where only twelve participants (from the fluoxetine group) were included. Larger studies should be conducted in order to explore the neural substrates underlying irritability and how it could be modulated during pharmacological or psychological treatments. Such studies should also include improved measures of irritability, such as the Affective Reactive Index (ARI). This is a recently developed tool that has the potential to provide a more comprehensive measure of irritability (Stringaris, Goodman, et al., 2012). The scale focuses on three aspects of irritability: i) threshold for an angry reaction, i) frequency and ii) duration of anger feelings or behaviours, and takes into consideration the perspective from both the child/adolescent and the parent.

An alternative - but not necessarily incompatible - explanation for the pattern of reduced amygdala activation in response to anger (following fluoxetine administration) is that this

medication acts to remediate a cognitive bias that may predispose adolescents to feel more self-conscious in social interactions and particularly sensitive to peer rejection. As mentioned previously, social rejection is indeed particular prominent in adolescent depression (Platt et al., 2013), and the amygdala is thought to play a key role in the anticipation of negative feedback from peers (Lau et al., 2012). Facial displays of anger, *per se*, are not a sign of peer rejection specifically, but they may evoke similar reactions during a developmental period that is particularly sensitive to the possibility of social harm – e.g., the teenager could interpret the anger emotion as an indirect sign of hostility and rejection, especially within the context of enhanced social fears. It would be of particular high interest to assess how fluoxetine may influence the perception of more complex affiliative cues, both in the context of positive/rewarding vs. negative/punitive social interactions. Ecologically valid paradigms have been used in order to explore neural responses to negative peer feedback and signs of disapproval in the context of depression and anxiety in youth (e.g., Belli, Rogers, & Lau, 2012; Lau et al., 2012), and the results show that depression and anxiety symptoms are indeed associated with heightened neural responses towards social rejecting cues (for review see Platt et al., 2013). To investigate if fluoxetine administration could reverse the pattern of hypervigilance in response to negative feedback is a topic of research that holds great potential.

An increased activation in the middle temporal gyrus (including the posterior cingulate) and superior frontal gyrus in response to happy faces (vs. anger) was seen in the fluoxetine group versus placebo. This is consistent with the antidepressant effect of fluoxetine reported in Chapter Two, in which a trend for an increased recognition of happy faces was seen along with a decreased processing of anger. This finding is also in line with the study by Rawlings and colleagues (2010) in which a single dose of mirtazapine vs. placebo was found to increase limbic and prefrontal responses in response to happy faces.

It is possible that fluoxetine acts to normalise the hypo-activation seen in the placebo group in response to fearful faces. A similar pattern of normalisation to fear has been found in a group of highly neurotic adults who were administered 7-day treatment with the SSRI citalopram (Simplicio, Norbury, Reinecke, & Harmer, 2014). The increased activation towards fear could therefore reflect decreased avoidance towards an emotion that represents a more benign threatening cue. While fear is naturally ambiguous and not always associated with negative consequences for the

individual, anger represents a more immediately harmful sign, for which a pattern of avoidance may be more normative. There is some recent evidence suggesting that accurate responses to fear predicts pro-social behaviour (Marsh, Kozak, & Ambady, 2007), therefore highlighting the ambiguous nature of this emotion and the fact that it may not always be associated with threat *per se.*

The reasons behind the differential pattern of activation in response to anger vs. fearful faces (when each was compared to happy) are merely speculative and future studies are needed in order to clarify how these emotions are processed across development and especially during episodes of adolescent depression. For instance, experiments combining both fMRI and eye-tracking methodologies could help determine whether differential patterns of brain activation to fearful vs. angry faces in adolescent MDD implicate distinctive perceptual and/or attentional processes. Specifically, if eye-tracking was to reveal an increased pattern of ocular exploration to fearful faces following fluoxetine administration, this would suggest that fluoxetine acts to reduce ocular avoidance of fear which might be seen in the depressed state. Of relevance, a previous study by Di Simplicio and colleagues (2014) implemented this approach in a study with highly neurotic participants and found that 7-day treatment with citalopram increased ocular exploration of facial stimuli. Another aspect that is worth noting is the fact that amygdala activity towards fear has been shown to be modulated by genetic polymorphisms in the serotonin transporter gene. In a study by Lau and colleagues (2009), patients with two copies of the LA allele exhibited greater amygdala activity than patients with S or LG alleles in response to fear. Similar genetic variations in the participants included in the current study could have affected the results in an unpredictable fashion. Future imaging genetic studies are therefore needed in order to clarify how genetic variables may modulate the activity of the amygdala towards various threat-related cues, including fear and anger.

In the current study, the effects of both depression and fluoxetine were seen in the right amygdala rather than bilaterally. Previous studies point to a similar pattern of right lateralisation in adolescents with mood disorders (Lau et al., 2009; Pavuluri, O'Connor, Harral, & Sweeney, 2007) and in participants receiving SSRI administration (e.g., Di Simplicio et al., 2014; Godlewska et al., 2012; Murphy et al., 2009; Rawlings et al., 2010). Interestingly, it has been suggested that the right amygdala is involved in the automatic processing of briefly presented stimuli, whereas the left

amygdala is involved in sustained emotional processing of longer cues (Gläscher & Adolphs, 2003; Morris et al., 1998). The fact that the studies reporting a right lateralisation effect included implicit tasks is indeed consistent with the involvement of the right hemisphere in automatic processing. However, the detection of laterality is not always consistent in the literature (see Zald, 2003) and, therefore, future research should clarify the role of hemispheric lateralisation when investigating drug effects using fMRI.

In addition to the effects within the amygdala, fluoxetine was shown to influence activation in a broad network of temporal, occipital and frontal regions. Specifically, fluoxetine decreased activation in response to anger in the superior and middle temporal gyrus (including the hippocampus and the insular cortex), occipital pole and superior frontal gyrus. A parallel increase in the frontal pole (including the orbital cortex) and superior and middle frontal gyrus was seen in response to fear. These exploratory results were highly consistent with the primary analysis focused on the ROI amygdala mask, and should be further examined in forthcoming experiments.

The involvement of the insula and orbitofrontal cortex is consistent with the role of these regions in emotional processing. Despite being more traditionally linked with disgust, there is growing evidence suggesting that the insula plays a broader role in emotion processing. The hippocampus and orbitofrontal cortex are also thought to integrate a broad network of regions involved in the processing of emotionally salient stimuli (Bechara, Damasio, & Damasio, 2000; Immordino-Yang & Singh, 2013). It is notable that the study by Tao and colleagues (2012) also verified a modulation effect on the orbitofrontal cortex, although the activation of this structure was reduced after 8 weeks treatment with fluoxetine.

While the understanding of how limbic and prefrontal regions interact in adolescence is still incipient, this study provides an important insight into how antidepressant treatment may act to modulate critical regions involved in affective processing. Future research is needed in order to fully explore such complex connections within the brain and across development, in both healthy adolescents and depressed youth.

Limitations

The results of this study should be interpreted in light of several limitations. First, the effects of depression were measured in a placebo condition, therefore constraining any

comparison with previous non-pharmacological research in depressed adolescent samples. Participants were not aware of which group they belonged to, and therefore, any expectations regarding treatment could have affected the results in an unpredictable fashion. The fact that the findings produced by fluoxetine were opposite to those of the placebo group argues in favour of using this approach, but future replication is needed.

Second, the findings seen in the amygdala did not emerge within the whole-brain analysis. There is nonetheless a scientific rationale for using an ROI approach to investigate percentage signal change in this brain region (Poldrack, 2007). Due to its small size, it is possible that any BOLD activity occurring in the amygdala failed to survive multiple comparison corrections underlying the whole-brain analysis, which makes the targeted ROI approach particularly useful. Notably, the finding seen with the amygdala ROI mask was consistent with the whole-brain results that emerged in other regions.

A third limitation pertains to the limited sample size. Due to the many challenges imposed by recruiting depressed adolescents immediately before they started antidepressant treatment (and within a short period of time), the sample size fell short of initial expectations. Future studies with larger samples are warranted.

Fourth, additional characteristics of the clinical sample complicate interpretations. The inclusion of subjects with various comorbid conditions, despite reflecting a more naturalistic approach, may have influenced the results in unpredictable ways. The study inclusion criteria were relatively broad and patients were only excluded if they had current or previous history of bipolar disorder, schizophrenia or substance misuse. Patients with an Asperger Syndrome diagnosis (or with Asperger traits) were not excluded, nor were patients who presented themselves with psychotic symptoms as part of the depressive episode. Despite the groups being relatively well matched for disorder comorbidities, the current study lacks enough power to examine the potential confounding effects of these comorbid disorders. Replication of these findings in a more diagnostically homogenous sample is therefore important, despite being much more difficult to attain.

The same principle applies to the inclusion of patients who were receiving concomitant psychological therapy. Despite this distribution being equivalent in the placebo and fluoxetine

groups, it is not possible to examine any potential effects of therapy on the emotional processing task used here due to the small sample size.

The gender distribution was not significantly different between groups, but there was a higher proportion of males in the fluoxetine group. Again, this could have affected the pattern of results seen in the current study.

Finally, the emotion contrasts of interest in this study included two comparisons: anger > happy; fear > happy. However, given the opposite pattern of neural activity seen with these conditions, it would be useful to perform an analysis in which fear and anger are directly compared. Future studies may also wish to include sadness as an additional negative expression. Whilst this emotion does not engage amygdala activation as robustly as fearful faces (e.g., Phan, Wager, Taylor, & Liberzon, 2002), it could still offer important insights into the mechanisms underlying fluoxetine, as well as help clarify any differences between anger, fear and sadness. Of relevance, fluoxetine was shown to decrease the recognition of sadness in Chapter Two. Low mood/sadness is a critical feature of adolescent depression that is seen in a significant percentage of adolescents suffering from MDD, having equal or stronger status than anger in most cases (Stringaris et al., 2013). Additional studies are needed in order to explore how this emotion may be affected by fluoxetine treatment.

4.5. Conclusion

To the extent of my knowledge, this was the first study to investigate the effects of acute fluoxetine administration on neural processing in clinically depressed adolescents. As predicted, fluoxetine was shown to decrease amygdala activity to angry faces in comparison to placebo. This pattern was opposite to that seen when patients on placebo were compared with healthy controls, therefore suggesting that a potential mechanism of fluoxetine is to decrease amygdala hyperactivity towards this emotion. An effect on fear was also apparent. Participants receiving fluoxetine showed an increased activation in response to fear. Again, this effect was opposite to that seen in the placebo group vs. healthy controls. Of interest, amygdala activity in response to fear was negatively correlated with initial symptoms of agitation/anxiety and depression.

These findings are important, as they extend the only study that has been conducted so far with depressed adolescents, in which fluoxetine was administered for 8 weeks. Fluoxetine is shown

here to have immediate effects on emotional processing, independently of changes in mood. The effects on anger may be particularly relevant for adolescent depression, whilst the changes in amygdala reactivity in response to fear may represent an early biomarker of therapeutic response. Despite being very preliminary, this latter finding highlights a useful approach to detect early indicators of clinical response, a much-anticipated advancement in the field of Child and Adolescent Psychiatry.

Chapter 5:

The effects of acute fluoxetine on anger processing: an exploratory study with high trait anger males

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5.1. Introduction

The results from the studies reported in Chapter Two and Four showed that a single dose of fluoxetine (vs. placebo) decreased the behavioural and neural processing of angry faces. Given the efficacy of fluoxetine in treating disorders where anger is a key symptom (Coccaro & Kavoussi, 1997; Fava et al., 1991), these findings highlight a potential mechanism through which fluoxetine may exert its clinical action. However, following on from these effects, it would be useful to establish whether fluoxetine modulates emotional processes, and particularly anger processing, in individuals with high levels of trait anger (HTA). Such findings would be directly applicable to the pharmacological treatment of disorders where irritability, anger and aggression are especially prominent.

A transdiagnostic perspective in the characterisation and treatment of psychological disorders - supported by evidence showing the benefits of targeting shared processes that seem to overlap across conditions (e.g., Fairburn, Cooper, & Shafran, 2003; Norton, 2012) -, has been receiving growing interest within both the psychiatric and academic communities (e.g., Etkin & Cuthbert, 2014). Anger symptoms and irritability are common in a wide range of conditions, including adolescent and adult depression, bipolar disorder, anxiety and intermittent explosive disorder (e.g., Fava et al., 2010; Stringaris et al., 2013). Within this framework, understanding the mechanisms through which fluoxetine influences anger-related processes may shed light on the clinical applicability of this medication and help refine future drug treatments.

The propensity to react with anger across different situations is a risk factor for the development of more serious anger-related problems (Deffenbacher, Oetting, Lynch, & Morris, 1996), and can be quantified along the dimensions of *state* and *trait anger* (Spielberger, 1988). *State anger* describes the angry feelings experienced by an individual at a particular point in time,

and can vary in intensity from “mild irritation or annoyance to strong fury or rage” (Spielberger, Reheiser, & Sydeman, 1995). It is typically associated with autonomic arousal, including increased heart rate, blood pressure and perspiration (Berkowitz, 1999; Taggart, Boyett, Logantha, & Lambiase, 2011). *Trait anger*, on the other hand, corresponds to the general tendency to feel angry. Spielberger (1996) suggested that individuals high in trait anger tend to perceive more situations as frustrating or irritating, which consequently leads to frequent experiences of state anger and its associated components, and to a poorer psychological functioning (see Anderson, Linden, & Habra, 2005; Deffenbacher et al., 1996).

Few studies have investigated the role of perceptual and attentional mechanisms in the expression of trait and state anger. However, there is evidence suggesting that a propensity to attend to hostile information may contribute to the experience of anger-related feelings (for a review see Owen, 2011). For instance, Honk, Tuiten, de Haan, vann de Hout and Stam (2001) found that male and female individuals scoring high on trait anger (HTA) showed an attentional bias for angry faces in a pictorial emotional Stroop paradigm. Males with high self-reported aggression are also biased to perceive aggression-related words in the Attentional Dot-Probe (Smith & Waterman, 2004). Such enhanced attention towards anger-related stimuli might predispose the individual to encounter situations that are likely to trigger anger experiences; e.g., an HTA individual could interpret a criticism from a work colleague more negatively than a person low in trait anger, which predisposes him or her to feel more “angry”. These experiences may further reinforce the pattern of hypervigilance towards signs of hostility.

In the study reported in Chapter Two, fluoxetine was associated with a decreased recognition of angry facial expressions and with a reduced emotional reactivity towards unpleasant stimuli. Given the potential role of increased vigilance to hostile information in the development and maintenance of anger, it is of high interest to examine if fluoxetine is capable of decreasing perceptual, attentional and autonomic effects associated with the experience of this emotion in HTA individuals who are susceptible to experience anger frequently in their daily lives. If confirmed, these findings would strengthen the hypothesis that fluoxetine acts to reduce the processing of anger cues, an effect that may help alleviate behavioural symptoms of anger and irritability.

The current study therefore assessed, for the first time, the influence of a single dose of fluoxetine vs. placebo in a sample of volunteers scoring high in trait anger. To control for gender,

only male subjects were included in the present study. A battery of emotional tasks was carefully designed to investigate information processing in response to this key emotion. Following the findings seen in Chapter Two and Four, this study hypothesised that fluoxetine would decrease the processing of (and vigilance towards) angry facial expressions, whilst enhancing the processing of happy faces. It was also predicted that fluoxetine would decrease participant's autonomic reactivity and subjective ratings of anger and irritability in response to recalling a situation where they had become very angry or lost his temper.

5.2. Methods

5.2.1. Participants

Forty male healthy volunteers who described themselves as being "hot-headed" or as having a "fiery" or "quick" temper, and aged between 18 and 55, were recruited from the community using posters and web/radio-based advertisements. Following the eligibility screening, only twenty-one participants were tested (see Figure 16). This sample size is lower than anticipated, as the initial plan was to test thirty-five participants in total, given the results seen in Chapter 2.

Interested participants were emailed a brief description of the study along with the Trait Anger Scale (TAS) of the State Trait Anger Expression Inventory (STAXI; Spielberger, Krasner, & Solomon, 1988). Those scoring at or above the 75th percentile on the TAS (i.e., a score of 21 or higher) were then invited to participate, in accordance with cut-offs used in previous studies (e.g., Eckhardt & Cohen, 1997; Lopez & Thurman, 1993; Tafrate, Kassinove, & Dundin, 2002).

All participants were given a standard medical screening and administered Structured Clinical Interviews for DSM-IV Axis I (First et al. 1997) and Axis II Disorders (First, 1997) to determine eligibility for the study. The Attention Deficit Hyperactivity Disorder (ADHD) Module from the Mini-International Neuropsychiatric Interview version 5.0 (M.I.N.I. 5.0) (Sheehan et al., 1998) was also administered in order to exclude participants with ADHD which may be present in a HTA population (Ramirez et al., 1997). Exclusion criteria were current significant medical problems; personal history of psychiatric illness, including alcohol/substance abuse; first-degree family history

of bipolar disorder; smoking more than 5 cigarettes per day; recreational drug use within the last 1 month; BMI below 18 or greater than 35 and current use of psychotropic medication.

Ethical approval for this study was obtained from the Oxford University Medical Sciences Inter-divisional Research Ethics Committee (MSD-IDREC-C3-2013-001). All participants gave written and verbal informed consent prior to their participation, and were reimbursed with £80 for their time. At the end of the study, participants who were interested in learning strategies to overcome their anger issues were given a brochure detailing specialised services offered in Oxford, online resources, and books.

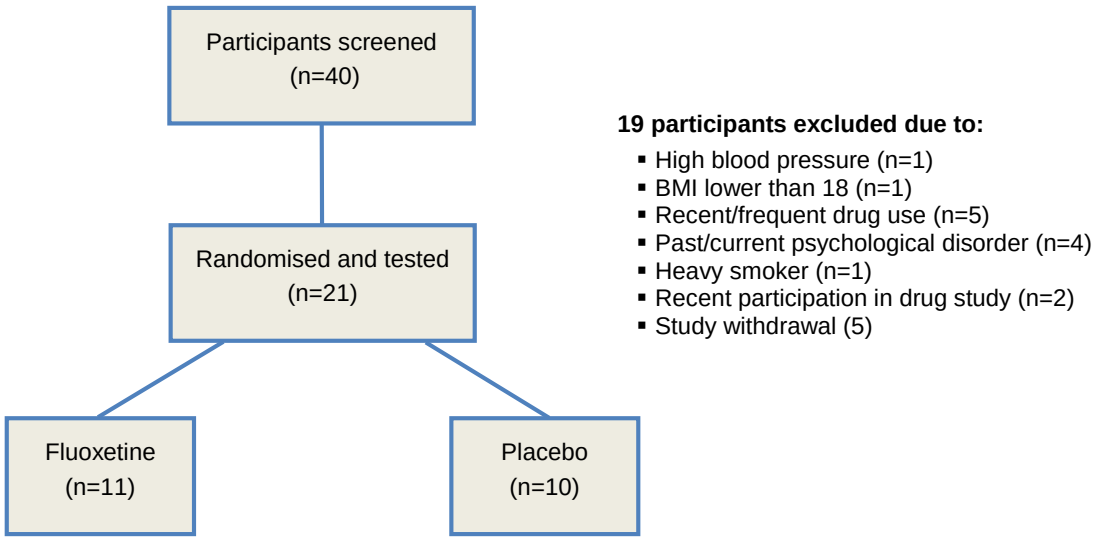

Figure 17. Flow chart of patient participation.

5.2.2. Experimental design and procedures

Screening

Participants underwent a screening session lasting ~90-120 minutes. This appointment included administration of the Structured Clinical Interviews for DSM-IV Axis I (SCID-I; First et al., 1997) and Axis II Disorders (SCID-II; First, 1997) and a pre-screening questionnaire that covered all items of the SCID-II which served to determine which sections should be explored in more detail during the interview. From the SCID-I interview, participants were determined to be free of current or past Axis I (psychological) disorders. The presence of Axis II (personality) disorders was not an exclusion criterion.

All participants were then weighed and underwent a brief physical exam by a medical doctor to ensure physical fitness. The National Adult Reading Test (NART; Nelson, 1991) was administered in order to obtain an estimate of verbal IQ. Participants also completed the Barratt Impulsiveness Scale (BIS; Patton, Stanford, & Barratt, 1995), Buss-Perry Aggression Questionnaire (BPAQ; Buss & Perry, 1992), the trait version of the STAI (STAI-T; Spielberger, Gorsuch, & Edward, 1970) and the Eysenck Personality Questionnaire (EPQ; Eysenck & Eysenck, 1975). In order to quantify the impact of high trait anger on their daily lives, participants were asked to rate how their experiences with anger affected or interfered with their general functioning, including work and relationships, on a scale from 0 to 10 (0 being "not at all", and 10 "extremely").

A comprehensive description of the measures used during the screening visit can be found in Appendix 3.

Testing session

The design of the testing session closely mirrored the study described in Chapter Two. Participants were asked to refrain from alcohol on the night before of the testing and to limit caffeine consumption during breakfast to one cup. Participants arrived at the laboratory for a morning appointment and were asked to fill in a series of questionnaires before the drug/placebo administration: the *Beck Depression Inventory* (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) as an indicator of mood; the state version of the STAI (Spielberger et al., 1970) as a measure of anxiety; *Visual Analogue Scales* (VAS) to assess alertness, contentedness and calmness symptoms (Bond & Lader, 1974); the *Positive and Negative Affective Schedule* (PANAS; Crawford & Henry, 2004); and finally an adapted version of the *Antidepressant Side-Effect Checklist* (ASEC; Uher et al., 2009). These measures are described in more detail in Appendix 3.

Participants were then randomised to receive either 20mg of fluoxetine or a matched placebo, in a double-blind procedure. After medication/placebo administration, participants sat in a quiet room until the testing session began. During this time, participants were free to read, work and/or use their laptops, and were carefully monitored for potential side-effects. For lunch, participants were offered a selection of light meals. Participants were not allowed to smoke or drink any additional caffeine for the duration of the study.

Six hours after drug/placebo intake, participants were asked to complete the STAI-S, VAS, PANAS, JRQ, VAS and ASEC again. This was followed by the administration of a battery of emotional processing tasks, as described below.

5.2.3. Experimental tasks

Facial expression recognition task

See Chapter Two for experimental details.

Emotional Categorisation and Memory

The emotional categorisation task assesses speed to respond to positive and negative self-referent personality descriptors, and can be considered a probe for measuring emotional biases during interpretation and encoding. Stimuli included 30 disagreeable (e.g. domineering, hostile) and agreeable (e.g. honest, original) words (from Anderson, 1968), which were matched for length and ratings of frequency and meaningfulness (Anderson, 1968; Francis & Kučera, 1982). Each trial began with a fixation cross lasting 500ms followed by the word (also presented for 500ms) and ending with a post-trial interval of 3s. Participants were required to categorise these personality characteristics as *likeable* or *dislikeable* as quickly and as accurately as possible. Specifically, they were asked to imagine whether they would like or dislike overhearing someone referring to them as possessing this characteristic, so that the judgment was in part self-referential. The outcome measures of this task were: 1) percentage of words correctly identified (i.e., percentage of words correctly identified as agreeable and disagreeable) and 2) reaction times taken to identify each word.

About 10 minutes after the emotional categorisation task was completed (in which the Attentional Dot-probe was administered), participants were given a surprise free recall and recognition test. Specifically, participants were asked to freely recall and write down as many of the personality characteristics as they could remember in 2 minutes. The outcome measures for the free recall were: 1) percentage of positive and negative words correctly recalled, and 2) number of false positives (words falsely recalled that were not originally presented in the task). The recognition task comprised of 60 target words plus 60 matched distractors (30 positive, 30

negative) (Anderson 1968; Francis & Kučera, 1982). Each trial consisted of a 500ms fixation cross, followed by the word presentation for 500ms, and a post-trial interval of 3s. Participants were asked to indicate whether each word presented on the screen was one of the personality characteristics presented during the categorisation task ('familiar') or not ('novel'). The outcome measures were: 1) percentage of positive and negative words correctly recognised, 2) percentage of correct "rejections", and 3) reaction times.

Attentional Dot-Probe Task

The Attentional Dot-Probe Task assesses attention to different emotional stimuli using a reaction time measure, as described in Chapter Two. However, in this version of the task, backward masking was used in order to create a subliminal condition. Additionally, the task was modified to include angry faces due to the relevance of anger for the present study.

Pairs of photographs of 30 individuals (15 women and 15 men) displaying different facial expressions were taken from the JACFEE/JACNeuF sets of facial expressions (Matsumoto & Ekman, 1988). Each face pair included one emotional expression and one neutral or two neutral expressions, from the same individual. Therefore, there were 4 types of face pairs: (1) neutral-neutral; (2) fearful-neutral, 3) happy-neutral and 4) anger-neutral. On each trial, one of the faces occupied the top half of the screen and the other the half bottom, with a central fixation cue in the middle. The emotional faces (i.e., fearful, happy or anger) appeared in both locations with equal frequency.

There were two types of conditions: masked and unmasked. In the unmasked condition, the face pair was presented for 100ms and then a probe appeared in the location of one of the preceding faces. The sequence of events was the same in the masked condition, except that the face pair was displayed for a short period of time (16ms) and was immediately replaced by a mask consisting of a jumbled face and displayed for 84ms, before being replaced by a probe. Probes consisted of two dots presented either vertically (:) or horizontally (..).

On half of the emotional-neutral face trials, the probe appeared in the same position as the emotional face and on the other half, the probe appeared in the same position as the neutral face. There were 258 trials in total - 2 practice trials, 128 masked trials (32 happy-neutral, 32 fear-neutral, 32 angry-neutral, 32 neutral-neutral) and 128 unmasked trials (32 happy-neutral, 32 fear-

neutral, 32 angry-neutral, 32 neutral-neutral). There were 8 blocks each of unmasked and masked trials (16 trials per block), which were presented in an alternating order with an ABAB design. The task was fully counterbalanced for emotion location, probe location and probe type. Stimuli were presented using the E-prime software.

Participants were asked to respond to the orientation of the dots as quickly and as accurately as possible by pressing correspondingly labelled keys on a keyboard. The outcome measures in this task were vigilance scores towards happy, fearful and angry faces. A description of how vigilance scores are calculated is provided in Chapter Two.

Continuous Flash Suppression

The Continuous Flash Suppression (CFS) paradigm described in Chapter Two was modified to include angry faces. There were 240 trials in total (60 trials for each of the 4 conditions: neutral, happy, fear and angry faces). The outcome measures were: 1) reaction times to detect each of the emotional expressions and 2) percentage of correct responses (i.e. trials in which the location of the face was correctly identified).

Anger recall paradigm

Participants underwent an anger recall (AR) paradigm, which has been adapted from previous published research (e.g., Burg, Soufer, Lampert, Collins, & Soufer, 2011; Why & Johnston, 2008). This task aims to generate an intense anger reaction by asking the participant to recall an event in which he had become very angry or lost his temper, while measuring electrocardiogram (ECG) responses. Participants are asked to recall an event that occurred during the past year, and that was still upsetting for the participant to think about. If participants were able to think of more than one event, they were then instructed to select the most intense. This AR paradigm consisted of a 5-min baseline period, a 7-min anger recall task (including 2 minutes of imagery), and a 5-min recovery period. Figure 17 provides a summary of all the procedures involved in this paradigm.

After the participant confirmed that he was able to identify an incident, the connection to the ECG monitoring equipment was established. To avoid movement artefacts, participants were asked to keep as still as possible throughout the task. Participants were seated in a comfortable

chair, and subsequently instructed to clear their mind and relax as much as possible during a 5-minutes baseline (rest) period. After completion of the baseline, participants were asked to rate their subjective state by using an adapted computerised version of the *Anger Attack Questionnaire* by Fava, Anderson, & Rosenbaum (1990). Example items are: accelerated heart rate, hot flashes/face reddening, tightness of the chest, sweating, feeling out of control, etc. Items related to mood and state anxiety (such as “irritable/angry”, “happy”, “sad”, etc.) were also added. Each item was scored on a 10cm horizontal visual analog scale, from “not at all” to “extremely”.

The experimenter initiated the AR condition by asking participants to focus on the previously selected anger incident and to indicate, on a 10cm scale, how angry they felt at the time. They were also asked to rate the outcome of the situation, in terms of it having been neutral, negative or positive (-10 to +10). These ratings were done on a computer.

This was followed by a 2-minute imagery period in which participants prepared silently and with their eyes closed for the upcoming recall. They were specifically asked to mentally place themselves back in the situation and recall, as vividly as possible, the sequence of events experienced, as well as any associated thoughts, feelings and physical sensations. Participants were also prompted to imagine that the situation was happening *again*, in the present. At the end of these 2 minutes of imagery, participants were asked to verbally rate the mental image in terms of *vividness* and *level of detail* on a scale from 0 to 10 (from “not at all” to “extremely vivid/detailed”), and the experimenter recorded these ratings on a scoring sheet.

The imagery period was followed by a 5-minute recall of the event. Participants were asked to verbally describe the incident in detail, trying to recreate it as best they could, and – if comfortable – with their eyes closed. They were specifically asked to include details such as *what they said and did*, *how the other person responded* (if anyone else was involved), and *what they were thinking and feeling*. The experimenter provided prompts during the recall in order to increase the reliving of the incident (e.g., paraphrasing back what the subject just said, or focusing the subject on feelings by asking "How did that make you feel?"), especially if pauses of more than 3 seconds occurred. The aim was to keep the participant engaged in talking about the incident, while promoting the vivid re-experience of anger throughout the condition. This verbal account was audio-recorded, conditional to consent from the participant.

Participants were then asked to complete the Anger Attack Questionnaire and additional subjective ratings on a computer. They were specifically asked to: 1) rate *how angry* they felt compared to the time of the incident (from “not at all” to “just as much”); 2) verbally describe the “worst thought” that they had during the anger incident, and then rate: *how much they believed the thought was true at the time* (from “not at all” to “completely convinced that the thought was true”); 3) *how much distressed they felt by having such thought* (from “not at all” to “as distressed as I ever felt”) and 4) *how angry this thought had made them feel* (from “not at all” to “extremely”). These ratings were completed on a computer, using 10cm horizontal graphic lines.

At the end, there was a standard 5-minute recovery period with identical instructions as for the initial baseline. This was also followed by the completion of the Anger Attack Questionnaire.

Electrocardiogram (ECG) analysis

During the anger recall paradigm, an ECG was recorded using a Spacelabs Healthcare CardioNavigator System (USA) in order to measure heart rate variability (HRV). HRV is defined as the fluctuation over time of the period between consecutive heartbeats.

Heart beat-to-beat intervals were quantified using a custom automated analysis program. The low frequency (LF: 0.04-0.15Hz) and high frequency (HF: 0.15-0.4 Hz) components of the heart rate were identified by fitting an optimal Auto-Regressive (AR) estimation technique to the data and assessing its frequency spectrum (Parati et al., 1995). This technique is preferable to the use of a Fourier Transform as it can characterise oscillations at any frequency instead of restraining the spectrum to pre-selected values. An additional advantage is that it allows the quantification of frequency content to shorter recording intervals (Parati et al., 1995). The magnitudes of the LF and HF are often taken as an index of sympathetic and parasympathetic activity (respectively), despite not being completely independent: the HF power is usually taken as a measure of baseline parasympathetic activity (Katona, Poitras, Barnett, & Terry, 1970; TFESCNASPE, 1996), whilst the LF index is more ambiguous, receiving contributions from both the sympathetic and the parasympathetic nervous system (Akselrod et al., 1981; Appel, Berger, Saul, Smith, & Cohen, 1989). The ratio of the two powers (LF/HF) represents a gold standard approach for measuring changes in sympathovagal (i.e., sympathetic vs. parasympathetic) balance. This LF/HF ratio was therefore used in the analysis to measure HRV, using normalised units (TFESCNASPE, 1996).

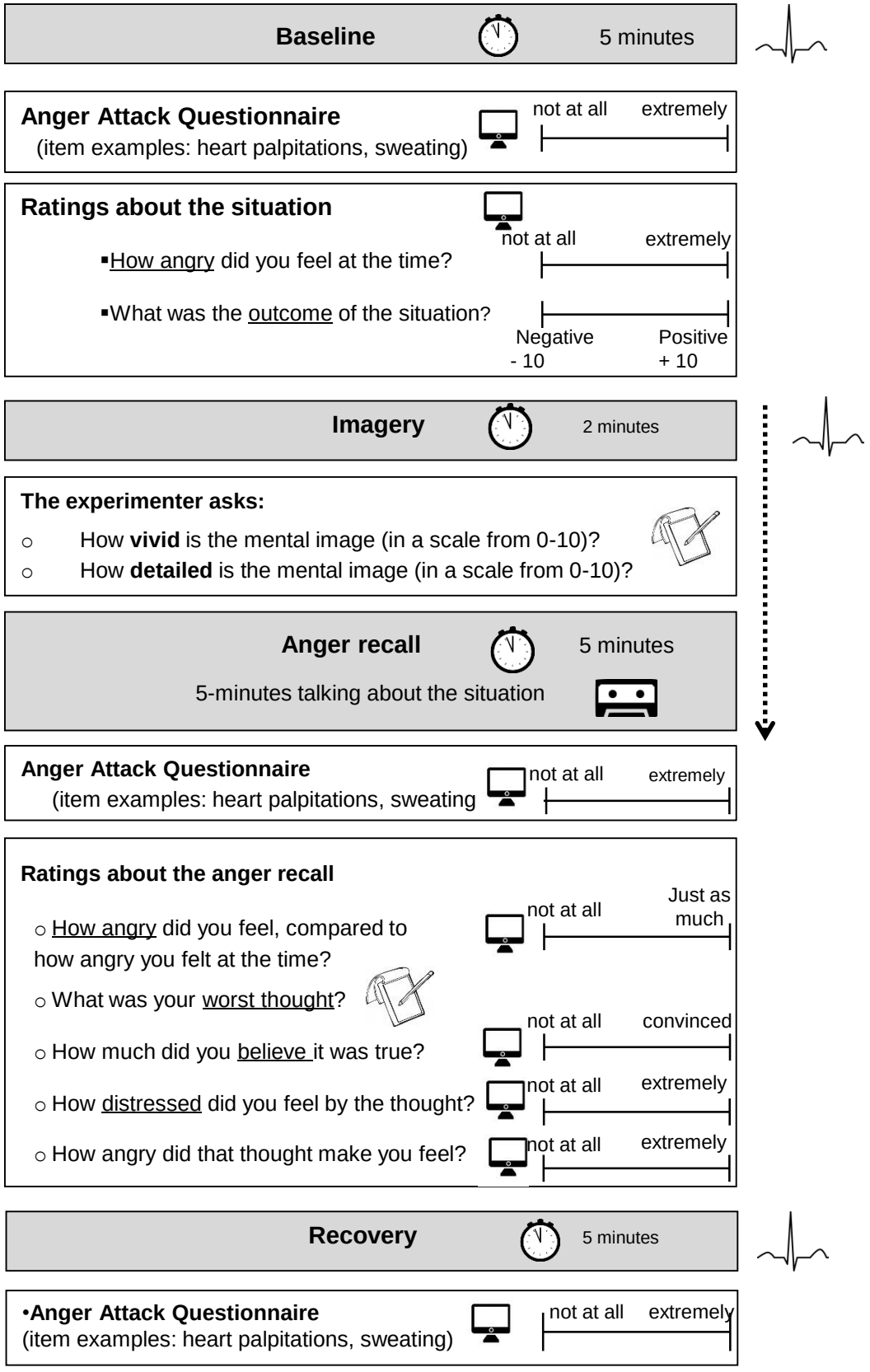

Figure 18. Conditions involved in the Anger Recall paradigm and respective ratings.

For each participant, a time-series was constructed to reveal HRV (using the LF/HF ratio) over the course of the three blocks: (1) baseline, (2) anger recall and (3) recovery.

5.2.4. Statistical analyses

Baseline characteristics (age, BMI, IQ, STAXI, BP, BIS, STAI-T, EQP, BDI and anger impact on functioning), excluding gender, were analysed by using independent t-tests. Gender was analysed using a chi-square test. The self-report scales used to assess the effects of fluoxetine on subjective ratings (STAXI-S, STAI-S, VAS Alertness, VAS Contentedness, VAS Calmness, PANAS and ASEC) were analysed by using a split-plot ANOVA, with time (pre and post drug/placebo administration) and group (fluoxetine vs. placebo) as within- and between-subjects factors respectively.

The experimental tasks were also analysed by using a split-plot ANOVA with group (fluoxetine vs. placebo) as the between-subjects factor. Within-subjects factors were emotion/valence (FERT, Emotional Categorisation and Recall, Attentional Dot-Probe, CFS) and stimulus presentation/masking (Attentional Dot-Probe). Accuracy responses in the Attentional Dot-Probe were analysed using a Mann-Whitney test due to absence of normality in the data. Accuracy responses in the CFS task were analysed using an independent t-test. When sphericity was violated, Greenhouse-Geisser-corrected p-values were used, but uncorrected degrees of freedom are reported for clarity.

In order to assess the effects of fluoxetine on Heart Rate Variability (HRV), split-plot ANOVAs were conducted with condition (baseline, anger recall and recovery) and timepoint (seven 2-minute windows, from 0 to 300s) as the within-subjects factors. The Anger Attacks Questionnaire was assessed using a similar split-plot ANOVA but with condition (baseline, anger recall and recovery) and scales (16 items in total) as the within-subjects factors. The effects of fluoxetine on the imagery condition HRV, anger recall/baseline HRV ratio and VAS anger recall ratings were analysed by using independent t-tests.

A p-value lower than 0.05 was used to denote statistical significance. Marginal differences with a p-value lower than 0.10 were also reported.

5.3. Results

5.3.1. Baseline characteristics

There were no significant differences between groups in terms of age, BMI and IQ (all *p*s>0.18). The scales from the Buss-Perry Aggression Questionnaire (BPAQ) and Behavioural Impulsiveness Scale (BIS-11) also yielded comparable scores in the placebo and fluoxetine groups, as did the Trait anger and the EPQ scales of Extraversion and Psychoticism (all *p*s>0.12). See Table 16.

Table 16. Baseline characteristics.

	Placebo (N=11), mean (±SD)	**Fluoxetine (N=10), mean (±SD)**	**p**	**sig.**
Age	32.09 (13.20)	29.80 (10.02)	0.662	n.s.
BMI	24.42 (2.48)	25.80 (4.20)	0.389	n.s.
Verbal IQ (NART)	115.05 (4.84)	104.00 (25.93)	0.181	n.s.
STAXI Trait anger	27.09 (4.13)	25.78 (4.32)	0.498	n.s.
STAXI Anger-in	20.36 (4.63)	15.80 (4.16)	0.019	*
STAXI Anger-out	20.00 (5.06)	16.20 (4.16)	0.077	+
STAXI Anger Control	23.64 (3.75)	23.50 (4.70)	0.942	n.s.
STAXI Anger Expression	32.72 (10.87)	24.50 (9.38)	0.080	+
BP-Physical aggression	26.64 (8.30)	27.20 (12.45)	0.903	n.s.
BP-Verbal aggression	23.18 (7.73)	21.50 (6.96)	0.608	n.s.
BP-Anger	29.55 (9.57)	25.40 (8.90)	0.318	n.s.
BP-Hostility	22.27 (8.22)	14.20 (7.52)	0.030	*
BIS-Cognitive	17.91 (3.27)	14.80 (5.37)	0.122	n.s.
BIS-Motor	23.36 (3.88)	23.20 (5.94)	0.941	n.s.
BIS-Nonplanning	23.36 (4.29)	23.70 (7.32)	0.902	n.s.
Trait anxiety (STAI-T)	47.55 (6.41)	42.20 (5.03)	0.048	*
EPQ-Neuroticism	9.91 (4.66)	6.25 (2.82)	0.066	+
EPQ-Psychoticism	4.82 (2.18)	4.00 (2.73)	0.477	n.s
EPQ-Extraversion	12.18 (5.96)	15.63 (5.73)	0.224	n.s.
Depression (BDI)	6.82 (5.49)	2.40 (3.24)	0.058	+
Anger impact on functioning**Φ**	5.45 (2.03)	5.00 (2.46)	0.668	n.s.

Note: **Φ**Measured by asking the participant "*In a scale from 0 to 10, how much you think your anger experiences affect/interfere with your general functioning, including work and relationships? [with 0 being "not at all", and 10 "extremely"]*; *p<0.05, +p<0.10; n.s. stands for non-significant.

However, the groups showed significant (or near significant) differences when considering Trait anxiety (STAI-T) (p=0.048), EPQ Neuroticism (p=0.066), BPAQ Hostility (p=0.030), STAXI Anger-in (p=0.019), STAXI Anger-out (p=0.077), STAXI Anger Expression (p=0.080) and BDI (p=0.058). The placebo group revealed slightly higher scores in all these measures. These values are presented in Table 16.

5.3.2. Subjective changes

Separate split-plot ANOVAs with time (pre and post) and group (fluoxetine vs. placebo) were conducted in order to test the effects of fluoxetine on state anger (STAXI-S), state anxiety (STAI-S) and side-effects (ASEC). These analyses revealed no interaction between time and group in any of these measures (all *p*s>0.16).

A split-plot ANOVA with time (pre and post) and valence (positive and negative) as within-subject factors revealed a trend for an interaction between valence and group for the PANAS [F(1,18)= 3.594, p=0.074]. Participants on fluoxetine showed a trend to score lower overall than the placebo group in the negative scale (p=0.068).

Finally, a split-plot ANOVA was conducted to assess the effect of fluoxetine on the VAS scales (Alertness, Contentedness and Calmness) separately. This analysis revealed a significant (or near-significant) main effect of group when assessing each of these scales. The placebo group scored higher than the fluoxetine group on the Alertness [F(1,17)=13.522, p=0.002] and Contentedness [F(1,17)=10.194, p=0.005] scales, irrespective of timepoint. There was also a trend for the placebo group to score higher on Calmness scores [F(1,17)=3.361, p=0.084] (see Table 17). No interaction effects between time and group were apparent (all *p*s>0.118)

Given the exploratory nature of this study, these variables were not included as covariates in the subsequent analyses.

Table 17. Subjective Ratings before and after a single dose of 20mg. fluoxetine or placebo

Scales	Pre		Post	
	Plac. (n=11) mean (±SD)	**Fluox. (n=10) mean (±SD)**	**Plac. (n=11) mean (±SD)**	**Fluox. (n=10) mean (±SD)**
State anger (STAXI-S)	10.73 (1.49)	11.50 (2.68)	10.82 (1.54)	10.10 (0.32)
State anxiety (STAI-S)	47.36 (5.22)	47.90 (4.36)	45.91 (4.80)	48.90 (2.13)
VAS Alertness	26.69 (11.88)	13.97 (9.05)	39.82 (15.67)	17.08 (11.68)
VAS Contentedness	26.78 (10.77)	13.43 (9.15)	29.72 (11.42)	15.39 (11.09)
VAS Calmness	25.34 (12.39)	22.94 (16.33)	32.97 (14.16)	17.27 (19.95)
PANAS positive (+)	35.55 (5.80)	37.11 (5.71)	27.73 (6.80)	33.30 (7.72)
PANAS negative (-)	14.82 (3.82)	12.11 (2.62)	12.55 (3.33)	10.60 (0.97)
Side effects (ASEC)	0.90 (1.37)	0.80 (1.40)	4.00 (2.05)	2.90 (3.57)

5.3.3. Facial Expression Recognition Task

A split-plot ANOVA with emotion as the within subject factor (seven levels: neutral, anger, disgust, fear, happiness, sadness and surprise) revealed a trend for a significant interaction between emotion and group when considering accuracy levels [F(6,114)=2.035, p=0.067]. Follow-up pairwise comparisons revealed that participants receiving fluoxetine were more accurate at identifying happiness [F(1,19)=8.524, p=0.009] and also showed a trend to be more accurate at identifying surprise [F(1,19)=3.498, p=0.077]. No significant differences were seen in the other expressions (all *p*s>0.16) (see Figure 18).

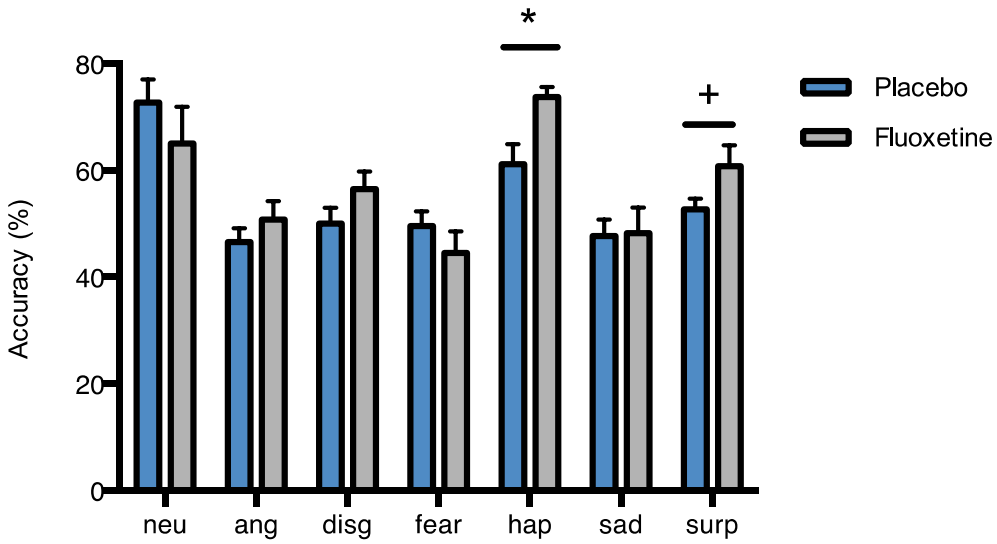

Figure 19. Accuracy of facial expression recognition in the fluoxetine and placebo groups. Values represent percentage of correct responses summed over the different intensity levels for that emotion. Error bars represent standard error of the mean.

When analysing reaction times, there was no significant interaction between facial expression and group [$F(6,114)=0.746$, $p=0.570$] and the main effect of group was also non-significant [$F(1,19)=1.327$, $p=0.264$]. A similar lack of significant effects was seen for the percentage of misclassifications [emotion x group: $F(6,114)=1.350$, $p=0.271$, main effect of group: $F(1,19)<0.001$, $p>0.99$].

5.3.4. Emotion categorisation and memory

In the emotion categorisation task, there was no significant interaction between word valence and group [$F(1,19)=0.204$, $p=0.657$] or main effect of group [$F(1,19)=0.772$, $p=0.391$] when considering the percentage of personality characteristic words correctly categorised as likeable or dislikeable. The reaction times were also not significantly affected by the treatment [group x emotion: $F(1,19)=0.241$, $p=0.629$; main effect of group: $F(1,19)=0.961$, $p=0.339$].

The percentage of words correctly recalled in the free recall memory test was not significantly affected by treatment (recall: group x emotion: $F(1,19)=1.670$, $p=0.212$; main effect of group: $F(1,19)<0.1$, $p>1.000$], nor were the percentage of false positives [group x emotion: $F(1,19)=0.139$, $p=0.713$; group: $F(1,19)=0.010$, $p=0.921$].

A similar lack of significant effects was seen for the percentage of words correctly recognised [group x emotion: $F(1,19)=0.020$, $p=0.889$; main effect of group: $F(1,19)=0.257$,

p=0.618], percentage of false alarms [group x emotion: F(1,19)=0.022, p=0.883; group: F(1,19)=0.430, p=0.520], and reaction times [group x emotion: F(1,19)=0.046, p=0.833; group: F(1,19)=0.069, p=0.795].

A significant (or near-significant) main effect of valence was seen in all these analyses, with likeable words being more easily categorised and recalled/recognised (all *p*s<0.10).

5.3.5. Continuous Flash Suppression (CFS)

Data from one participant in the placebo group was not recorded due to technical problems, so the final analysis of this paradigm consisted of 20 participants in total (10 in each group).

A split-plot ANOVA with emotion (4 levels: neutral, happy, fear and angry) as a within-subjects factor revealed no significant main effect of emotion [F(3,54)=1.285, p=0.289], group [F(1,18)= 0.085, p=0.774], and no significant interaction between emotion and group [F(3,54)=0.839, p=0.444]. Similarly, there were no differences between groups when considering total accuracy levels [t(18)=-0.734, p=0.473].

5.3.6. Attentional Dot-Probe Task

A Mann-Whitney test revealed that both groups showed similar accuracy levels overall in the Attentional Dot-Probe Task (placebo median: 88.28%, fluoxetine: 85.16%; U=34, p=0.138). Incorrect trials were therefore excluded from the reaction time analysis. In order to minimise the influence of outliers, trials with response times that were above 1200 and below 200ms, as well as those that were 3 standard deviations above or below the mean RT (computed after the first cropping) were excluded (Browning, Holmes, Charles, Cowen, & Harmer, 2012).

A 3x2x2 split-plot ANOVA with valence (happy, fearful and anger) and stimulus presentation (masked vs. unmasked) as within-subjects variables revealed no significant main effects or interactions [group x valence: F(2,38)=0.901, p=0.404; group x masking: F(1,19)=0.212, p=0.650; group x valence x masking: F(2,38)=0.816, p=0.424; group: F(1,19)=0.000, p=0.997].

However, due to the exploratory nature of this study, it was examined whether either group exhibited an absolute biases towards any of the three emotions (see Gotlib, McLachlan, & Katz, 1988). One sample t-tests were therefore used to compare attentional bias scores against a

hypothetical mean of zero (indicating no bias) within each group. Masked and unmasked trials were grouped together in order to increase power. This revealed that participants in the placebo group showed an attentional vigilance towards angry faces only [t(10)=2.591, p=0.027], but this effect was absent in the fluoxetine group [t(9)=0.225, p=0.827]. No effects were seen for happy or fearful faces in either group (all ps> 0.3). See Figures 19 a) and b).

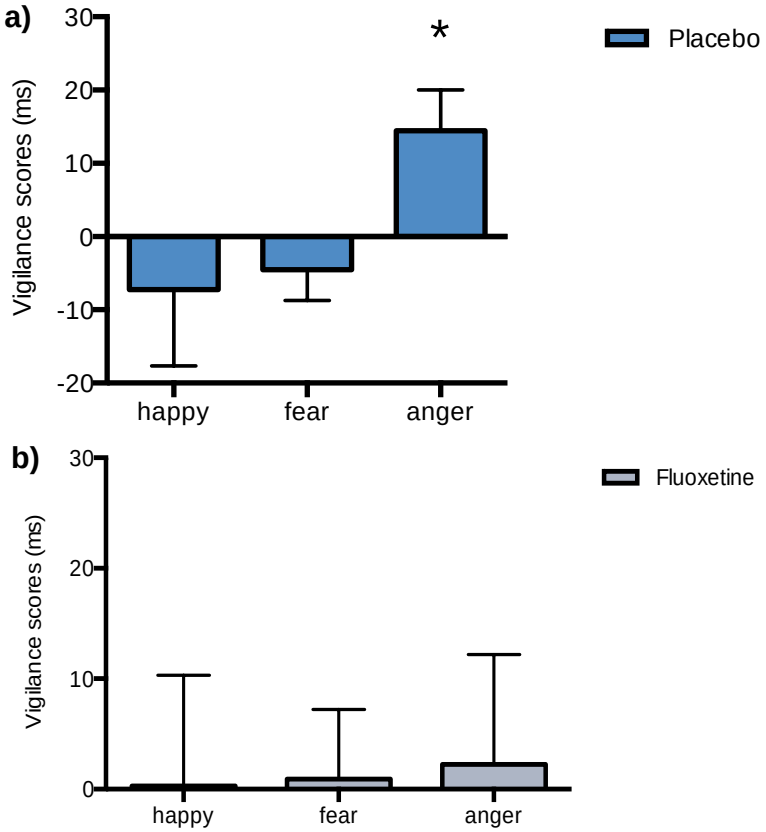

Figure 20. Attentional vigilance in the Attentional Dot-Probe task in the masked (a) and unmasked (b) conditions. Vigilance is calculated by subtracting mean reaction time to respond when probe replaces the emotional face (happy, fear or anger) from the reaction time when the probe replaces the neutral face. Thus the higher the vigilance score, the greater the attentional bias towards the emotional face. Error bars represent standard error of mean. *<p<0.05

5.3.7. Anger recall paradigm

Heart Rate Variability (HRV) analysis

A split-plot ANOVA with condition (baseline, anger recall and recovery) and timepoint (seven 2-minute windows, from 0 to 300s) as within-subjects factors failed to reveal a significant main effect of *condition* [F(2,34)=1.916, p=0.176], *timepoint* [F(6,102)=1.181, p=0.320], *group* [F(1,17)=0.011, p=0.917], or *interaction with group* [condition x group: F(2,34)=0.160, p=0.786; timepoint x group: F(6,102)=0.177, p=0.848; condition x timepoint x group: F(12,204)=1.438,

p=0.235]. The HRV during the 2-minute imagery period was also not significantly affected by treatment group [t(18)= -0.247, p=0.808]. See Figures 20 a) and b).

An additional analysis was conducted, in which a ratio score between the *anger recall condition* (averaged from 120 to 300s) and *baseline* (60 to 120s) were compared between groups. These specific timepoints were considered in order to obtain a stable measure of the baseline and the peak of the anger recall. No group differences were seen in this measure [t(19)=-1.065, p=0.300].

Subjective ratings

In order to investigate the effects of fluoxetine on the subjective report experienced during the anger recall, a split-plot ANOVA with *time* (baseline, anger recall and recovery), *scale* (16 items from the Anger Attacks Questionnaires in total) and *group* (fluoxetine vs. placebo) was conducted. The ratings were taken immediately after each of the three conditions.

There was a main effect of time [F(2,32)=32.170, p<0.001], with significantly higher ratings after the anger recall in comparison to ratings at baseline (p<0.001) and after the recovery (p<0.001) conditions. This suggests that the anger recall paradigm was effective in increasing the subjective experience of anger. See Figure 20 c).

An interaction between scale and group was also seen [F(2,15)=2.490, p=0.042]. The decomposition of this interaction revealed that the fluoxetine group reported feeling less nervous/tense [F(1,16)=5.954, p=0.027], less sad [F(1,16)=7.222, p=0.016] and happier [F(1,16)=4.769, p=0.044] overall than the placebo group. There was also a trend for participants receiving fluoxetine reporting to be less tired [F(1,16)=3.756, p=0.070], less dizzy [F(1,16)=4.089, p=0.060], and to have fewer heart palpitations [F(1,16)=3.427, p=0.083]. A visual inspection of the values across timepoints revealed that these group differences were more prominent in the baseline and recovery conditions (see Table 18).

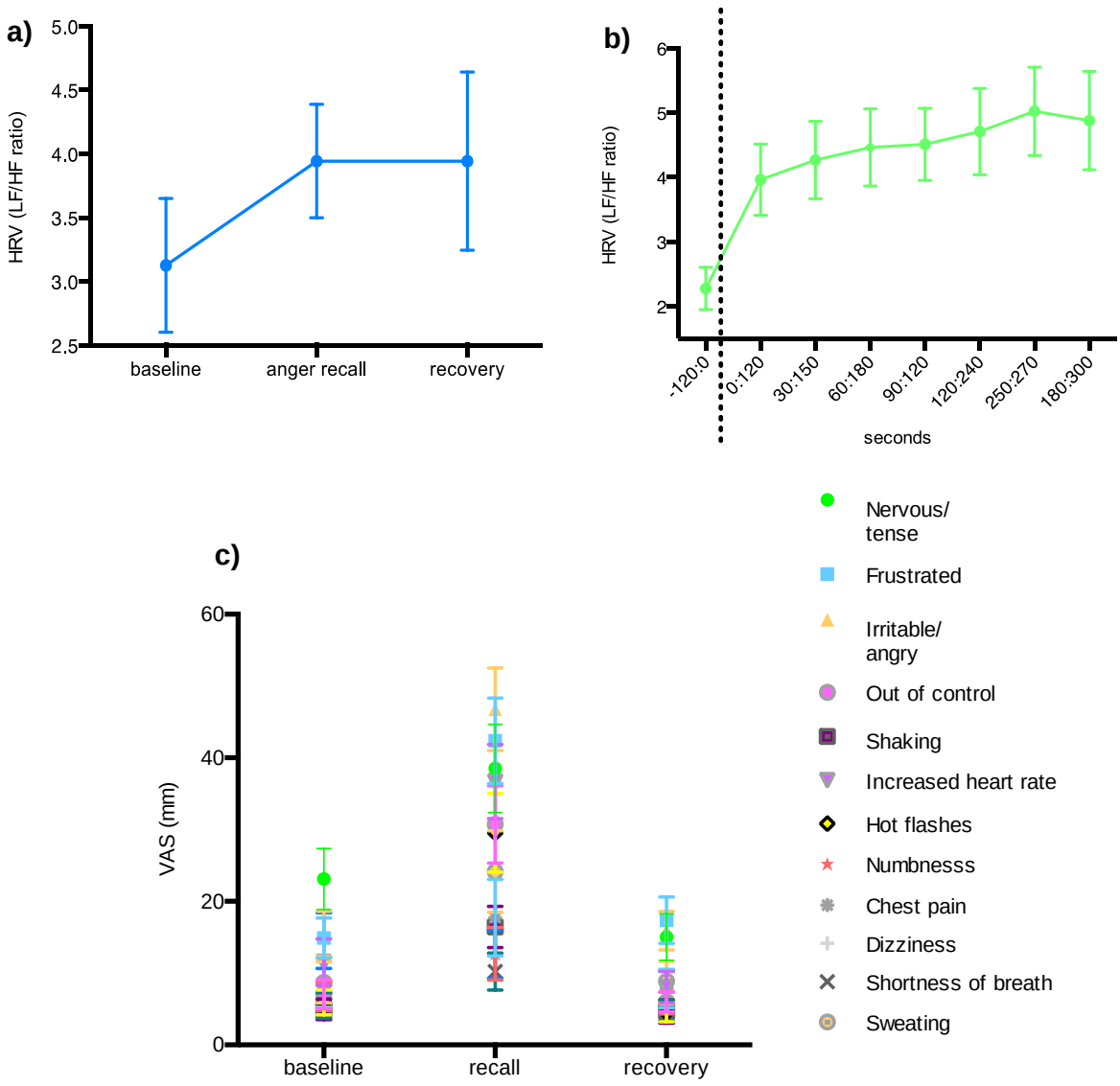

Figure 21. Average Heart Rate Variability (HRV) and subjective ratings during the anger recall paradigm (a) HRV for each of the conditions: baseline, anger recall and recovery. **(b)** HRV during first two minutes of imagery (-120:0) and 5 minutes of anger recall (0-300). The recordings have been cropped into 120-second windows, and then moved along by 30 seconds at a time. **(c)** Average subjective ratings for each of the three conditions. Only the physiological and psychological symptoms more directly associated with anger are presented here. The items “happy”, “sad”, “alert” and “tired” are only presented in Table 18 for ease of interpretability. Values represent average and standard error of the mean (SEM).

Table 18. Subjective Ratings During the three conditions of the Anger Recall Paradigm

Scales	Baseline		Anger recall		Recovery	
	Placebo (N=11) mean (±SD)	Fluoxetine (N=10) mean (±SD)	Placebo (N=11) mean (±SD)	Fluoxetine (N=10) mean (±SD)	Placebo (N=11) mean (±SD)	Fluoxetine (N=10) mean (±SD)
Nervous/ Tense	30.85 (16.24)	13.44 (16.07)	46.50 (25.09)	28.53 (25.32)	21.43 (14.92)	7.03 (6.21)
Frustrated	18.75 (12.99)	10.09 (8.42)	43.33 (26.20)	41.19 (25.67)	19.00 (14.02)	15.34 (14.01)
Irritable/ Angry	18.43 (15.52)	11.81 (9.83)	51.63 (25.48)	40.69 (23.09)	19.85 (9.58)	11.00 (12.16)
Alert	67.08 (14.26)	75.53 (28.66)	69.80 (17.07)	77.72 (22.58)	56.28 (23.61)	72.44 (27.82)
Tired	45.30 (19.09)	27.78 (15.05)	33.78 (21.18)	22.50 (23.42)	46.88 (17.37)	33.41 (22.01)
Out of control	9.58 (9.67)	3.94 (5.97)	37.30 (26.13)	22.50 (15.87)	6.65 (6.70)	5.19 (4.73)
Happy	58.38 (16.47)	72.69 (11.79)	37.38 (23.13)	51.12 (27.18)	55.58 (12.19)	71.81 (16.28)
Sad	24.83 (20.11)	7.00 (4.30)	35.85 (19.29)	21.81 (24.56)	32.70 (21.23)	10.25 (15.70)
Increased heart rate	14.30 (16.40)	8.06 (9.44)	46.15 (21.09)	24.84 (17.49)	9.60 (11.36)	6.09 (5.97)
Hot flashes	7.70 (8.91)	3.69 (4.03)	33.28 (23.75)	24.91 (23.23)	4.83 (6.83)	4.13 (3.12)
Chest pain	13.18 (18.42)	3.19 (5.28)	25.30 (28.17)	8.28 (6.53)	10.75 (14.72)	4.28 (2.92)
Numbness	7.08 (9.39)	6.40 (7.27)	15.13 (16.64)	9.66 (14.55)	7.66 (8.09)	3.59 (2.83)
Dizziness	20.40 (19.55)	7.19 (7.53)	17.40 (16.98)	6.63 (5.95)	11.68 (13.44)	3.03 (2.66)
Shortness of breath	6.58 (4.16)	8.83 (8.59)	10.63 (11.32)	9.72 (11.07)	5.95 (7.83)	3.41 (2.36)
Sweating	8.83 (10.94)	8.59 (14.50)	24.60 (25.67)	23.60 (23.62)	7.63 (10.78)	10.44 (13.56)
Shaking	5.53 (6.03)	4.25 (6.65)	18.73 (11.75)	13.56 (13.01)	5.78 (8.89)	3.25 (2.44)

Note: These ratings were taken immediately after each of the three conditions.

Separate independent t-tests were performed in order to explore the effects of fluoxetine on how participants rated the severity of the anger incident and associated consequences, cognitions and feelings. There were no differences between group on any of the measures: *anger during incident* [t(17)=1.085, p=0.293], *outcomes/consequences to the participant* [t(17)=0.106, p=0.916); *anger describing event* [t(17)=0.666, p=0.514], *worst thought belief* [t(16)=-0.959, p=0.352], *thought distress* [t(17)=0.206, p=0.839] and *anger while having worst thought* [t(17)=-1.645, p=0.118]. See Table 19.

Table 19. Anger Recall ratings

	Placebo (N=11), mean (±SD)	Fluoxetine (N=8) mean ±SD)	p	sig.
Anger during incident	79.50 (12.69)	70.72 (22.53)	0.293	n.s.
Outcome/consequences	49.84 (35.50)	48.16 (34.85)	0.916	n.s.
Anger describing event	49.66 (18.67)	44.67 (13.61)	0.514	n.s.
Worst thought belief	80.00 (20.32)	87.75 (11.52)	0.352	n.s.
Thought distress	65.32 (28.46)	62.97 (17.62)	0.839	n.s.
Anger associated with thought	61.20 (28.73)	78.69 (9.57)	0.118	n.s.

Note: Due to technical problems, there was only data available from 8 participants in the fluoxetine group.

5.4. Discussion

This exploratory study investigated, for the first time, the influence of a single dose of fluoxetine vs. placebo on emotional processing in a sample of adult male volunteers with high trait anger. The findings should be treated as preliminary given the modest sample size and the baseline differences between groups. Indeed, despite being matched for trait anger, anger control, aggression traits, impulsivity and extraversion, the groups revealed significant or near-significant differences in depression, hostility and in some measures of anger expression. The scores for the placebo group were higher in these measures, hence the results should be interpreted in light of this limitation.

Participants receiving fluoxetine showed an increased accuracy to detect happiness, and a trend for an increased recognition of surprise. No significant differences were seen, however, on the accuracy to detect angry facial expressions, or on the categorisation and memory of personality characteristic words. The analysis of the Attentional Dot-Probe provided some tentative evidence for a bias towards anger in the placebo group, which was absent in the participants receiving fluoxetine. However, this effect failed to emerge as an interaction in the overall ANOVA and was only evidenced in a further exploratory analysis. Finally, cardiovascular activity during the anger recall paradigm was not modulated by fluoxetine, despite some preliminary suggestion that fluoxetine reduced the subjective experience of anger-related emotions, particularly nervousness, dizziness and heart palpitations. Overall, these results are partially consistent with the initial study hypotheses that fluoxetine would increase the recognition of happiness, whilst reducing attentional

vigilance towards anger. However, the absence of fluoxetine effects on anger recognition is in contrast with the findings seen in Chapter Two.

Effects of fluoxetine on anger processing

There was some indication that fluoxetine reduced the attentional vigilance towards anger seen in the placebo group. This effect should be interpreted cautiously given the baseline group differences and the fact that it failed to emerge in the analysis of the Attentional Dot-Probe as a whole. It could therefore represent a Type I error. If replicated in a larger sample, this finding would strengthen the hypothesis that fluoxetine acts to decrease the processing of anger across different populations.

An increased attentional vigilance towards anger-related stimuli has been documented previously in individuals scoring high on trait anger (HTA) and/or aggression measures (for a review see Owen, 2011). For instance, Honk et al. (2001) found that HTA individuals were slower to name the colour of both masked and unmasked angry faces in comparison to a group scoring low in trait anger (LTA). Putman, Hermans and van Honk (2004) found a similar increase in the response latency for aggression-related words in a sample of HTA individuals, but only for masked faces. Importantly, this effect was not accounted by trait anxiety. Finally, a study by Smith and Waterman (2004) found that both violent offenders and aggressive undergraduates showed increased vigilance towards aggression-related words in the Attentional Dot-Probe, as well as increased interference when naming the colour of aggressive words in the Emotional Stroop paradigm. Together, these studies suggest that anger-related stimuli are especially salient to individuals who are susceptible to feel angry or who present with high levels of aggression, therefore increasing interference in the Emotional Stroop and capturing spatial attention in the Attentional Dot-Probe. In light of these findings, the decreased attentional vigilance towards anger seen in the fluoxetine group could speculatively reflect a mechanism that helps reduce the detection of hostile information in the environment and ultimately reduce the subjective experience of anger.

There is some evidence, however, that the attentional biases associated with HTA are sometimes dependent on the elevation of state anger, rather than representing a stable predisposition independent from context. Indeed, Eckhardt and Cohen (1997) found that HTA

individuals showed longer latencies when naming the colour of anger-related words, but only after being exposed to a verbal provocation in the laboratory. Similarly, Cohen, Eckhardt and Schagat (1998) found an analogous pattern in a study involving a visual search task. HTA participants showed increased response latencies towards anger-related words, but again this effect was only observed following an anger-provoking insult. It would be useful to include both high and low state anger conditions in future studies involving fluoxetine in order to investigate if the effects of this medication could be more robust or more easily detected following the experience of acute anger.

Contrary to the finding seen in Chapter Two, fluoxetine did not decrease the recognition of facial displays of anger in this sample of participants. This may be due to a single dose of fluoxetine not being sufficient to modulate the perception of angry facial expressions in the Facial Expression Recognition Task (FERT). However, fluoxetine instead influenced the recognition of more positive information, which is consistent with the findings seen in Chapters Two and Four. This interpretation, however, is limited by the absence of a comparison group with low levels of trait anger. Based on the data from this study, it is not possible to determine if participants receiving placebo showed an increased or decreased bias towards anger in this task (or none at all).

An alternative approach to explore the presence of potential biases in anger recognition is to perform a cross-study comparison with the data from Chapter Two. Male participants who received placebo (N=9, 18-21 years-old) were therefore selected from that study, and compared with the placebo-treated HTA males (N=11,18-55 years-old). When considering accuracy, both samples revealed comparable performance levels (angry males: 46.59% vs. young males: 47.67%, n.s.). However, HTA males gave significantly fewer anger misclassifications when compared to young healthy males (9.18% vs. 15.34%, respectively, $p<0.05$). A possible explanation for this is that HTA males are less likely to misclassify non-anger expressions as angry, but due to differences in age these findings are difficult to interpret. An additional analysis was therefore conducted with a dataset from a separate study in our laboratory, composed by participants within a wider age range. Again, only male participants were selected (N=10, 20-45 years-old). This analysis revealed slightly lower values for both anger accuracy and misclassifications in HTA males, but these differences did not reach statistical significance (accuracy for HTA and healthy males, respectively: 46.59% vs. 49.72; misclassifications: 9.18% vs. 13.11%, n.s.). Values from previously published and unpublished studies from our laboratory also show accuracy and

misclassification levels in healthy adult samples that fall within a similar range (accuracy: 40% - 55%; e.g., Harmer, Bhagwagar et al., 2003; Harmer et al., 2008; misclassifications: 10%-15%, unpublished observations), therefore supporting the preliminary notion that HTA males possess facial recognition abilities that are overall equivalent to healthy adults in the same age range. Additional research is needed in other to further characterise facial emotional recognition skills in this population.

Indeed, the interpretation of these results is made more difficult by the lack of existing studies in the literature exploring the facial emotion recognition abilities of HTA males. However, studies with related populations suggest that individuals with aggressive or delinquency symptoms are biased to perceive non-angry faces as angry. For instance, a study by Hall (2006) found that undergraduate students with high levels of aggression misclassified more faces as being angry in a facial expression recognition task. This finding has been replicated in additional studies with adult populations scoring high in measures of hostility (Larkin, Martin, & McClain, 2002), as well as in younger populations with conduct disorders (Cadesky, Mota, & Schachar, 2000) or with more severe delinquency problems (Sato, Uono, Matsuura, & Toichi, 2009). This increased processing of anger may reflect a tendency to perceive the environment as hostile, as emphasised by traditional models of aggression (Crick & Dodge, 1994; Dodge, 1980; Huesmann, 1988). In support of this idea is evidence showing that aggressive youth and adults are significantly more likely than nonaggressive peers to attribute hostile intent to others (Dodge, Price, Bachorowski, & Newman, 1990; Sancilio, Plumert, & Hartup, 1989).

The bias that is measured in the Facial Expression Recognition Task (FERT) is qualitatively different from the bias measured in the Attentional Dot-Probe. The FERT investigates subtle biases in the perception and interpretation of ambiguous facial expressions. The Attentional Dot-Probe, on the other hand, provides a direct measure of visual attention (Mogg et al., 2000), which is inferred from differences in reaction times towards probes that replace emotional faces compared to probes that replace neutral faces. Despite its methodological limitations (as discussed in Chapter Three), it is possible that the Attentional Dot-Probe was more sensitive than the FERT in detecting biases in anger processing in HTA participants receiving placebo vs. fluoxetine. Indeed, it is conceivable that attentional biases to anger emerge more prominently when a competition between stimuli is directly compared. This argument has been raised previously in

anxiety research, given that threat-related biases are indeed more frequently seen in Attentional Dot-Probe paradigms (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007).

Surprisingly, however, a lack of group differences was found in the Continuous Flash Suppression (CFS) paradigm, which is considered a particularly robust measure of early attentional biases (Kim & Blake, 2005). Both groups showed equivalent reaction times when detecting emotional (happy, fearful and anger) and neutral faces. This suggests that the attentional bias towards anger seen in the Attentional Dot-Probe may not be associated with an automatic bias to detect this emotion. However, this interpretation is limited by the fact that this paradigm failed to replicate the main effect of emotion that was seen in Chapter Three (specifically, faster detection of fear vs. happiness faces by healthy volunteers). Given that this effect was seen in both studies with healthy volunteers reported in Chapter Three (and in an additional study from a separate research group; Yang, Zald, & Blake, 2007), these results may instead reflect an abnormal early processing of neutral and emotional stimuli by HTA participants. It is possible that HTA males do not discriminate valence during the early processing of happy, fearful and angry faces, conceivably due to a lack of sensitivity or impaired early visual emotion discrimination in the context of CFS. However, because of the absence of a group scoring low in trait anger the interpretation of this finding is limited. Another possibility is that this study was underpowered to detect any emotion effects in the modulation of attention, although it should be noted that Yang and colleagues (2007) previously replicated a main effect of emotion in a sample of only 14 participants.

There was no effect of fluoxetine on the categorisation and recall of self-referential positive and negative words. It is interesting to note that a single dose of the SSRI citalopram also failed to alter processing in this task (Browning et al., 2007), despite other classes of antidepressants being shown to facilitate the encoding and memory for positive words after a single dose (e.g., Arnone, et al., 2009; Harmer et al., 2008). It is possible that acute administration of SSRIs does not modulate emotional memory processes. However, the fact that this task was not included in the study from Chapter Two further constrains any interpretation of the potential differences between fluoxetine and other drugs on emotional memory, and we are therefore unable to distinguish between the population and drug characteristics as potential reasons for the lack of effects on the memory task.

Contrary to initial predictions, fluoxetine failed to modulate the cardiovascular reactivity

experienced during an anger recall paradigm. The groups showed comparable heart rate variability (HRV) across conditions. Fluoxetine-treated participants showed a decrease in nervousness, dizziness and heart palpitations ratings compared to the placebo group; however, these differences did not emerge as significantly different across the timepoints of testing. A visual inspection of the data indicated that such differences appeared in the baseline and recovery conditions during the anger recall paradigm. Given the imperfect group matching achieved in this study, it is possible that these differences represent baseline disparities rather than a direct effect of the drug. However, the finding that the fluoxetine group felt less nervous in the anticipation of and the recovery from a stressor is somewhat consistent with the anxiolytic effect seen in Chapter Two. Of note, in that chapter, the anxiolytic-like finding was seen in the Emotion-Potentiated Startle Paradigm rather than emerging as a group difference in subjective levels of anxiety. This raises the intriguing (yet speculative) possibility that the influence of fluoxetine under the presence of an acute stressor produces subtle changes that are more visibly experienced at a subjective level in susceptible HTA males.

In this study, no group differences were seen in autonomic reactivity (as measured using HRV) during the anger recall paradigm. This could reflect a real lack of effects of acute fluoxetine in this measure, or a lack of power to detect differences due to the inherent inter-subjects variability associated with such a modest sample size. The initial hypothesis was that fluoxetine would decrease autonomic reactivity during the recollection of an anger-provoking situation, an effect that would be consistent with the potential anxiolytic-like properties of this medication. Interestingly, a pre-clinical study of Tiradentes and colleagues (2014) reported that a single dose of fluoxetine reduced renal sympathetic nerve activity and heart rate in anesthetised rats, which is in line with this hypothesis. However, studies in humans have focused mostly on the effects of long-term use with antidepressants on HRV and the results reported are inconsistent (see Ramage, 2001). A meta-analysis by Kemp and colleagues (2010) suggests that continuous antidepressant use in depressed samples (with the exception of tricyclic drugs) has minimal influence on heart rate variability (HRV), but there is evidence showing that SSRI administration decreases respiratory sinus arrhythmia, a measure of HRV reflecting cardiac vagal control (Licht, de Geus, van Dyck, & Penninx, 2010; for a discussion on this topic see Kemp, Quintana, & Malhi, 2011). Future studies should include larger samples in order to clarify the effects of acute and chronic fluoxetine

administration on autonomic reactivity during the experience of anger.

The effect of fluoxetine on happiness processing

In line with the finding seen in Chapter Two, fluoxetine was shown in this study to enhance the processing of happy faces. An increased processing of happiness is an effect that is seen with a wide range of antidepressant drugs. A single dose of the selective noradrenergic reuptake inhibitor (NRI) reboxetine, the selective serotonin reuptake inhibitor (SSRI) citalopram and the serotonin-norepinephrine reuptake inhibitor (SNRI) duloxetine have all been shown to increase the recognition of happy facial expressions (e.g., Harmer, Bhagwagar et al. 2003; Harmer et al. 2008). As postulated by the model of antidepressant drug action (e.g., Harmer, Hill et al., 2003), an increase in positive emotional processing counteracts the presence of negative biases and may represent a critical mechanism in the action of different antidepressant drugs. Such findings are consistent with a transdiagnostic formulation whereby different treatments target common cognitive processes that characterise a wide range of psychological disorders (see Abasi, Fata, Sadeghi, Banihashemi, & Mohammadee, 2013). Depressed, anxious and angry individuals are all thought to demonstrate a negative bias in their perception of external and internal stimuli, but towards different cues. Depressed patients are biased to perceive sad-related information (Gotlib & Joormann, 2010), anxious individuals are hypervigilant to threat (Mathews & MacLeod, 2005), and angry/aggressive people show a bias towards detecting hostile information (Owen, 2011). The increased processing of positive information produced by the administration of antidepressants produces a shift in attention away from negative cues, therefore helping reinstall a balance in the way that negative and positive information is processed (Harmer & Cowen, 2013). This shift in processing could be beneficial for individuals with anger or aggressive traits, as it may help them develop a more tolerant (and less hostile) outlook and ultimately reduce their propensity to act with anger or aggressively.

Of special relevance to this argument is a study by Penton-Voak and colleagues (2013), which suggests that an increased recognition of happiness over anger is associated with lower levels of self-reported anger and aggression. The authors conducted an experiment where they manipulated the processing of emotional facial information, such that participants were trained to perceive happiness over anger in ambiguously morphed faces. Notably, this change in perception

resulted in a decrease of self-report levels of anger and aggression in healthy adults and adolescents at high risk of delinquency. The lower levels of aggression persisted as long as after two weeks upon completion of the attentional training.

As part of this discussion, it is important to note the trend towards increased recognition of surprise in the group receiving fluoxetine. In a study by Harmer and colleagues (2009), a single dose of reboxetine was shown to normalise the pattern of reduced recognition of happy and surprised facial expressions seen in a sample of depressed patients, although the effect on surprise failed to reach statistical significance. An abnormal processing of surprised faces has also been reported in subjects with delinquent behaviours (McCown, Johnson, & Austin, 1986). Interestingly, surprise is considered a highly ambiguous emotion, and its interpretation appears to be influenced by the context in which the face is assessed (Kim et al., 2004). In a more negative context, surprise is interpreted more negatively, whereas in a positive context this emotion is perceived more positively. It is therefore conceivable that the effects seen on surprise and happiness could reflect the same perceptual pattern of increased positive bias, and that, together, these changes play an important role in the treatment of depression and anger disorders. Due to the limitations of the current study, these interpretations are speculative and remain to be tested in further samples.

Limitations

As mentioned before, a number of methodological limitations constrain the interpretation of the results: unfortunately, due to difficulties in the recruitment of HTA participants, the sample size was smaller than originally planned; further, participants were not completely matched for baseline characteristics and these pre-treatment differences were not included as covariates due to the exploratory nature of the analyses.

Finally, the study did not include a group of participants scoring low in trait anger, which limits the interpretation of any potential emotional biases seen in the placebo group. There was some suggestion that the placebo group showed an increased bias towards anger in the Attentional Dot-Probe Paradigm, but in order for this hypothesis of increased vigilance to anger to be clearly tested it would be useful to include a control group.

5.5. Conclusion

The current study demonstrates that a single dose of fluoxetine increases the processing of happy faces and may additionally modulate the processing of faces depicting surprise. Further, initial evidence for a decreased attentional vigilance towards anger was found. These findings are in line with studies showing that different classes of antidepressants act to increase the processing of positive vs. negative information (Harmer & Cowen, 2013). A production of a positive bias could help individuals high in trait anger modify their approach during social interactions, whereas the reduction of an attentional vigilance towards anger may help reduce the interpretation of others' behaviours as hostile. These effects are largely consistent with the previous studies in this thesis and could represent a key mechanism through which fluoxetine exerts its clinical effects.

Chapter 6:

General discussion

6.1. Summary of findings

Despite the relatively high prevalence of paediatric depression and the controversies associated with the use of pharmacological drug treatments in this population, the neuropsychological effects of antidepressants in children and adolescents remain poorly understood. This thesis uses a combination of neuroimaging and behavioural techniques in order to characterise the effects of acute fluoxetine on emotional processing. The aim is to achieve a greater understanding of the mechanisms underlying this medication in depressed adolescents, in light of differences seen in their clinical presentation and response to antidepressant drugs. It is hoped that this knowledge will assist the development of more effective treatments for adolescent MDD.

In the first experiment (Chapter Two), the effects of a single dose of fluoxetine (vs. placebo) were investigated in a group of young adult volunteers (aged 18 to 21). It was found that fluoxetine decreased the processing of anger and sadness in a facial recognition task. There was also a trend for a slower detection of angry faces and for an increased recognition of happiness in the same paradigm. Beyond this, fluoxetine was shown to eliminate the emotion-potentiated startle effect seen in the placebo group, therefore showing an anxiolytic-like mechanism. The lack of differences between groups in the subjective measures of mood and anxiety suggests that fluoxetine has early and direct effects on emotional processing, which are not mediated by secondary mood or anxiety changes.

In this same chapter (Chapter Two), the paradigms used to assess attentional vigilance towards threatening and positive cues (i.e., the Attentional Dot-Probe and the Rapid Visual Series Presentation), failed, however, to reveal any significant group differences. The lack of effects of fluoxetine on threat processing could be interpreted as additional evidence that this medication does not induce an anxiogenic-like profile in young healthy people. However, in the absence of a basic fear vigilance effect in the placebo group (particularly in the Attentional Dot-Probe), such an

interpretation of the data becomes problematic. Given this methodological limitation, two separate studies with healthy volunteers were conducted (Chapter Three), in order to validate a paradigm that is more robust and potentially more sensitive to early automatic biases towards threat – Continuous Flash Suppression (CFS). As predicted, threat-related (fearful) faces were faster to be detected than positive (happy) faces, and this effect was enhanced by anxiety symptoms. Indeed, higher levels of anxiety were associated with a faster detection of fear vs. happiness. A second study was subsequently conducted to examine the sensitivity of CFS in detecting anxiolytic effects after 7 days treatment with the anti-anxiety drug diazepam. The lack of effects in the group receiving the active treatment (vs. placebo) suggests that the early and automatic detection of facial stimuli, as measured using CFS, is not modulated by short-term administration of diazepam. Due to its sensitivity in detecting automatic biases towards threat, this paradigm was nonetheless included in a subsequent study investigating the effects of fluoxetine in high trait anger males (Chapter Five). It was not possible to include this task in the study with depressed adolescents (Chapter Four) due to testing time constraints, but it would be interesting for future studies to use CFS to investigate the effects of fluoxetine in this population.

In Chapter Four, a neuroimaging study was conducted with depressed adolescents. The aim was to fully characterise the early mechanisms underlying fluoxetine administration in a group of adolescents who had been recently prescribed fluoxetine for the treatment of depression. As predicted, a single dose of fluoxetine was shown to decrease amygdala activity towards angry (vs. happy) faces, an effect that was opposite to that seen in the comparison between depressed patients on placebo vs. healthy participants. A trend for an increased activation in this region towards fear was also apparent, and, again, this effect was opposite to that seen in the placebo group vs. healthy controls. Interestingly, increased activity in the amygdala to fear was associated with decreased symptoms of anxiety/agitation and depression over the first 7-10 days of treatment. Whole-brain responses in temporal, occipital and frontal regions were highly consistent with the pattern of findings seen in the amygdala. To my knowledge, this is the first demonstration of such an immediate effect of fluoxetine on neural activity in depressed adolescents.

Following the finding that a single dose of fluoxetine decreased the processing of anger (seen in Chapters Two and Four), a final exploratory study was conducted with the aim of testing whether fluoxetine would decrease emotional processing and reactivity towards anger-related

stimuli in individuals high in trait anger. This study found an increased recognition of happy faces and a trend for an increased recognition of surprise following a single dose of fluoxetine, which is in line with the enhanced processing of positive information reported in Chapters Two and Four. These findings should nonetheless be interpreted with caution given the baseline differences between groups and the small sample size. Additionally, there was a very preliminary suggestion that fluoxetine decreased the processing of angry faces in the Attentional Dot-Probe, but this particular effect was statistically weak and therefore needs further replication.

Taken as a whole, the studies in this thesis provide behavioural and neuroimaging support for the notion that fluoxetine exerts immediate effects on emotional processes that could be relevant for the treatment of adolescent depression. Three lines of findings emerged. First, a reduction in anger was prominent and seen across studies, and may therefore represent a critical mechanism underlying fluoxetine administration. This effect was mediated by the amygdala, an area known to be involved in adolescent depression (Kerestes et al., 2014) and antidepressant drug effects (Harmer & Cowen, 2013). Second, the reduction of startle reactivity was suggestive of an anxiolytic effect of fluoxetine. This mechanism represents a key difference relative to the SSRI citalopram in which acute administration is associated with an anxiogenic action. Such effect may therefore reflect an important mechanism underlying the beneficial profile of fluoxetine, which may also help alleviate the comorbid anxiety symptoms frequently seen in depressed adolescents (Axelson & Birmaher, 2001). Third, the enhanced processing of positive vs. negative cues may represent a broader mechanism of action underlying fluoxetine, relevant for the remediation of the negative biases that characterise this disorder (Beek & Dubas, 2008; Schepman et al., 2012).

In the following sections, these findings and their implications for the treatment of adolescent depression will be discussed in more detail. Some methodological aspects will also be considered, as well as directions for future research.

6.2. The neuropsychological model of antidepressant drug action

The neuropsychological model of antidepressant drug action provided the basis for the experimental studies conducted in this thesis. According to this model, antidepressants act early in treatment to reverse the negative biases that are characteristic of depression and anxiety, even before changes in subjective mood become apparent. These effects can be detected by using

sensitive emotional processing tasks and fMRI measures (Harmer, Cowen, et al., 2011).

In this thesis, fluoxetine was found to decrease the processing of negative biases (in particular anger but also sadness), whilst increasing the recognition of happiness. These findings were seen independently of overall effects on mood and across different studies and populations, therefore corroborating the suggestion that a key mechanism underlying antidepressant treatment is to act directly to reverse the negative biases that play a role in the development and maintenance of depressive disorders.

6.2.1. Early behavioural effects of fluoxetine and potential mechanism of action

The influence of fluoxetine on anger processing may represent a critical effect of this specific antidepressant, which is less typically seen with other types of antidepressant drugs. Acute administration of a wide range of antidepressants, such as citalopram (Harmer, Bhagwagar et al., 2003; Browning et al., 2007), reboxetine (Harmer, Hill, et al., 2003), duloxetine (Harmer et al., 2008) and mirtazapine (Arnone et al., 2009) have not been shown to affect the processing of angry faces. With longer (7-day) administration, there is evidence that both citalopram and reboxetine reduce the recognition of angry faces (Harmer et al. 2004), though this effect was not seen with 7 days of agomelatine treatment (Harmer, de Bodinat, et al., 2011).

The extent to which flouxetine's influence on anger could represent a more prominent effect of this drug is nonetheless a preliminary hypothesis, given that the effects of this medication were not directly compared with other drugs. A second, related, question pertains to whether the effects on anger could be specific to younger populations. The exploratory findings reported in Chapter Five of a reduced attention towards anger in older males tentatively argues against this possibility, but due to the absence of effects on anger recognition in that study this hypothesis should be interpreted cautiously.

Beyond the effects on anger, fluoxetine was found to modulate the recognition of sadness and happiness. The decreased recognition of sad faces seen in Chapter Two is consistent with a study by Harmer, de Bodinat, and colleagues (2011), in which 7-day treatment with the 5-HT_{2C} antagonist agomelatine reduced the accuracy to detect sad facial expressions in the same facial recognition task. Similarly, the increased detection of happy faces (seen in Chapters Two and Five)

parallels several studies involving acute doses of different antidepressants. For instance, a single dose of the selective noradrenergic reuptake inhibitor (NRI) *reboxetine*, the selective serotonin reuptake inhibitor (SSRI) *citalopram* and the serotonin-norepinephrine reuptake inhibitor (SNRI) *duloxetine* have all been shown to increase the accuracy to detect happy faces (Harmer, Hill et al., 2003; Harmer, Bhagwagar et al., 2003; Harmer et al. 2008). The production of a bias away from negative information and towards positive cues may therefore represent a critical mechanism underlying a wide range of antidepressant drugs.

It is possible that the effects seen with fluoxetine result from a 5-HT_{2C} antagonistic action of the drug not seen with other SSRIs such as citalopram. In support of this idea, drugs that act via 5-HT_{2C} antagonism have been shown to decrease emotional reactivity in startle responses, as seen in Chapter Two (e.g., mirtazapine; Arnone et al., 2009; agomelatine: Harmer, de Bodinat, et al., 2011). Additionally, and in line with the decreased accuracy to detect sadness (also seen in Chapter Two), sub-chronic (7-day) treatment with the 5-HT_{2C} antagonist agomelatine decreased the recognition of sad facial expressions (Harmer, de Bodinat, et al., 2011). Finally, there is some suggestion that antagonism at 5-HT_{2C} receptors reduces aggression in pre-clinical models, which is consistent with the reduction in anger processing by fluoxetine (Dekeyne et al., 2008). The anxiolytic-like effect of fluoxetine, as well as the effects on anger and sadness, could therefore represent a common neuropsychological mechanism of antidepressant drugs that also act as 5-HT_{2C} antagonists. However, it remains critical to conduct studies directly comparing fluoxetine and other types of antidepressants (such as citalopram and mirtazapine) within the same experiment, in order to clearly dissociate the effects of these drugs on emotional processing and the likely contribution of their pharmacological properties to these effects.

6.2.2. Using early drug effects to predict initial responses to treatment

Due to its sensitivity in detecting beneficial effects of antidepressant drugs, even following a single dose and in the absence of subjective changes in mood or anxiety, the potential for the *neuropsychological model of antidepressant drug action* to predict subsequent clinical responses to treatment has been explored in recent studies (Godlewska et al., submitted; Tranter et al., 2009). In this vein, one of the aims of the study reported in Chapter Four was therefore to correlate the

acute effects of fluoxetine on amygdala activity with initial therapeutic outcomes. Due to the small sample size, these findings should nonetheless be treated as preliminary.

There was a negative correlation between amygdala responses to fearful faces and initial anxiety and agitation symptoms, with higher activity in this region in response to fear being associated with lower agitation/anxiety symptoms over the first 5 and 10 days of treatment (at a near-significant and significant levels, respectively). These findings are interesting as they suggest that acute changes in the amygdala in response to fear could help predict the experience of reduced anxiety/agitation symptoms early in treatment.

To my knowledge, this is the first study that correlated acute effects of antidepressant medication with initial therapeutic responses. These findings clearly need further replication, but the possibility that such immediate changes in neural processing could give an indication of how patients are going to respond to the medication is encouraging. A similar approach has been recently implemented in a study with adult patients (Godlewska et al., submitted), but here, neural responses were measured after 7 days of treatment rather than acutely. The sample was divided into non-responders and responders (defined as a reduction in depressive symptoms of 50% or more from baseline), an approach that is particularly useful in characterising the neural mechanisms that are associated with successful vs. unsuccessful pharmacological treatment. Early changes in amygdala activation, seen after 7 days, predicted later clinical responses at 6 weeks in those patients who benefited from treatment (i.e., in the “responders”). This strategy in data analysis was not implemented in the fMRI study presented in this thesis (Chapter Four) due to the modest sample size, but it offers great potential for future studies in the field of adolescent depression.

6.2.3. Lack of anxiogenic effects of fluoxetine

One of the initial study hypotheses presented in Chapter Two was that fluoxetine would increase the processing of threatening information. This prediction resulted from two lines of thinking. Firstly, acute administration of the antidepressant citalopram (which belongs to the same class of antidepressants as fluoxetine) has been show to produce anxiogenic-like effects on emotional processing tasks (e.g., Browning et al., 2007; Grillon, Levenson, & Pine, 2007). This increase in threat processing is thought to reflect the worsening of anxiety seen in some patients at

the initiation of SSRI treatment (Harmer et al., 2009). Secondly, antidepressants have been suggested to induce more adverse effects when administered in youth (Jureidini et al., 2004). The findings reported in Chapter Two nevertheless failed to support this hypothesis, with fluoxetine even showing the opposite pattern consistent with an anxiolytic-like mechanism.

An important methodological issue emerged from these findings. The Attentional Dot-Probe Task, which was used to assess attentional vigilance to threat, failed to replicate the fear vigilance effect expected in the placebo group. In the absence of such main effect of the task, any interpretation of the results becomes difficult. This led to the investigation of an additional paradigm that is more robust and potentially more sensitive to the detection of early automatic biases towards threat - Continuous Flash Suppression (CFS). The findings presented in Chapter Three support the validity of CFS in detecting automatic biases towards threatening vs. positive information. This paradigm was subsequently used in Chapter Five in order to more fully characterise the effects of fluoxetine on automatic processing.

A lack of anxiogenic effects underlying fluoxetine emerged in two additional studies: the fMRI study with clinically depressed adolescents (Chapter Four) and in the exploratory study with males high in trait anger (Chapter Five). In the first, fluoxetine was shown to decrease the neural processing of angry faces, and, despite increasing activation to fear, this latter effect was associated with decreased anxiety responses in the first days of treatment, and was also opposite to the hypoactivation pattern seen in the placebo group vs. healthy controls. In the second, there was some preliminary suggestion that fluoxetine decreased feelings of nervousness, heart palpitations and dizziness in males high in trait anger, but as emphasized previously, these effects are limited by methodological limitations (i.e. modest sample size and imperfect group matching).

Despite the need for future research to establish the anxiolytic profile of fluoxetine, these findings, taken as a whole, suggest that this SSRI, when administered acutely, does not produce anxiogenic effects on emotional processing. Whilst still preliminary, this opens a new line of research focusing on the potential anxiolytic-like action of fluoxetine, which, if further replicated, could help mitigate some of the concerns associated with administering this medication to young people.

6.3. Implications for the treatment of adolescent depression

The ultimate goal of the current thesis was to improve the current knowledge about how antidepressants exert their action in young people. There are still widespread concerns that antidepressants can induce adverse effects in children and adolescents, but current neuropsychological research examining the mechanisms of drug action in young ages is limited. In the sub-sections below, the implications of the current findings for the treatment of adolescent depression are discussed.

6.3.1. Fluoxetine and anger/irritability

The effect of fluoxetine on the processing of anger was a notable finding seen in this thesis. In the first experimental study with healthy young adults (Chapter Two), fluoxetine was shown to decrease both the accuracy of correct identification of angry faces, as well as the percentage of emotions incorrectly labelled as angry. A trend for a slower detection of this emotion was also seen. Similarly, in the fMRI study with depressed adolescents (Chapter Four), a single dose of fluoxetine reduced the pattern of functional amygdala hyperactivity in response to anger (vs. happiness), whilst decreasing the activity of an extended network of temporal, frontal and occipital regions involved in the emotional processing of this same emotion. Finally, in the study with males high in trait anger (Chapter Five), there was some very preliminary suggestion that fluoxetine decreased attentional vigilance towards angry faces. Together, these findings may highlight an important mechanism through which fluoxetine acts to alleviate symptoms in adolescent MDD.

Anger is a hallmark feature of irritability, one of the core symptoms of childhood and adolescent depression (American Psychiatric Association, & DSM-5 Task Force, 2013). Although a formal definition is lacking within the psychiatric field, irritability is often defined as the propensity to react with grouchiness, anger or temper tantrums (Stringaris, 2011; Stringaris & Goodman, 2009) in response to external or internal frustrations (Donovan et al. 2003; Leibenluft, 2011). There is growing evidence for the implications of irritability as a developmental symptom relevant for both the aetiology and the clinical presentation of depression. It has been suggested that irritability not only predicts depression in early and late adulthood, but also shares common genetic underpinnings with this disorder (Dougherty et al., 2013; Stringaris & Goodman 2009). For

instance, in a study by Stringaris, Zavos, et al. (2012), it was found that irritability showed a phenotypic relationship with depression, one that was even stronger than the link with delinquency. This finding highlights the need to examine irritability outside the traditional scope of delinquency and disruptive behaviours exclusively (Stringaris, Zavos, et al., 2012). Recent studies also emphasise the emerging role of irritability within adult depression (Fava et al., 2010; Judd et al., 2013) and in other disorders like premenstrual dysphoric disorder (Ko et al., 2013).

Fluoxetine is known to be effective in several conditions that are characterised by irritability, including depression with anger attacks (e.g., Fava et al*.,* 1991), intermittent explosive disorder (Coccaro & Kavoussi, 1997) and premenstrual dysphoric disorder (Steinberg et al., 2012). In line with this, experimental manipulations that reduce serotonin levels via acute tryptophan depletion (ATD) have the opposite effect of increasing aggression-related responses, particularly in individuals high in irritability (e.g., Bjork et al., 2000; Bond et al., 2001; Cleare & Bond, 1995; for a review see Young, 2013). The mechanisms that underlie the potential efficacy of fluoxetine in treating irritability are unclear, but, as hypothesised in the current thesis, it is possible that this medication acts to decrease a bias to perceive anger, which could ultimately help reduce behavioural symptoms of irritability.

The presence of facial processing biases in depressed youth has been little investigated, although there is evidence suggesting that depression in adolescence is associated with an increased perception of anger (van Beek et al. 2006). In adults, aggressive traits have also been associated with an increased bias to perceive this emotion (Hall, 2006). Despite being preliminary, these findings could suggest a link between the perception of anger and symptoms of irritability, aggression and depression. Of note, irritability was present in a significant proportion of depressed adolescents in the sample included in Chapter Four (53.85% presented with threshold levels and 38.46% with sub-threshold). These values are consistent with previous findings from clinical samples (Ryan et al. 1987; Strober et al., 1981), although the ranges can vary widely.

However, two issues pose significant challenges to the hypothesis that a reduction in anger processing is a critical mechanism mediating the alleviation of irritability symptoms. First, there was no significant correlation between activity in the amygdala and subsequent changes in irritability symptoms (as measured using single items from the CDRS-R and VAS) (Chapter Four). Despite irritability being significantly reduced after six weeks of treatment with fluoxetine, it is unclear if the

immediate effect seen on anger processing contributed to this clinical change. The absence of a significant correlation should nonetheless be interpreted in light of a modest sample size in the fluoxetine group (N=12) and limited measures of irritability. Second, despite some preliminary evidence that fluoxetine reduced attentional vigilance to anger in high trait anger males, this effect was statistically weak and was only seen in one paradigm (Chapter Five).

Future studies are clearly needed, including larger samples, and a combination of neuroimaging techniques with improved measures of anger and irritability (Stringaris, Goodman, et al., 2012). Recently, the mechanisms underlying irritability have been more frequently explored in the context of severe mood dysregulation (SMD), a disorder characterised by frequent temper outbursts and persistent irritability. A study by Thomas and colleagues (2013) found that patients with SMD demonstrated amygdala hyperactivity in comparison to healthy controls in response to angry, fearful and neutral faces, but deactivation in response to fear in the posterior cingulate cortex, precuneus and posterior insula. An additional study by Thomas and colleagues (2014) found that youth with severe mood dysregulation (vs. healthy controls) exhibited a pattern of increased reactivity to angry faces in the posterior cingulate, middle occipital gyrus, and superior temporal gyrus, but not in the amygdala. Together, these findings suggest that more pervasive problems of irritability may be associated with a pattern of increased neural activity towards anger, in addition to dysfunctional activation in other regions during the processing of neutral and fearful faces. The effect on anger may nonetheless represent a common pathophysiological substrate underpinning adolescent depression and SMD, especially given the developmental and phenotypic association between SMD or (chronic) irritability and depressive disorders (Stringaris et al., 2009; Stringaris, Zavos, et al., 2012). This hypothesis is still preliminary, given the lack of studies examining the neural correlates of irritability in adolescent depression.

6.3.2. Beyond anger – the anxiolytic and antidepressant effects of fluoxetine

The anxiolytic-like effect underlying fluoxetine administration (seen most notably in Chapter Two) suggests a mechanism of action potentially relevant for the treatment of depression in adolescence, given the high comorbidity with anxiety disorders (Axelson & Birmaher, 2001). Even when an anxiety disorder is not formally diagnosed, depression in this age group is often associated with feelings of anxiety.

Anxiety and depression have some similarities in their biological and cognitive profiles, and many authors even suggest that there is a common domain of negative affectivity underlying these disorders (Axelson & Birmaher, 2001). Indeed, both depressed and anxious youth show a negative bias in information processing (Wilson & Rapee, 2005), and patterns of dysfunction in the amygdala have been reported in both populations (Beesdo et al., 2009). It is therefore not surprising that SSRIs, including fluoxetine, are frequently used in the treatment of paediatric anxiety disorders (Clark et al., 2005; Walkup et al., 2008).

Together with the influence on anger, the effects of fluoxetine on sadness (Chapter Two) and happiness (Chapters Two, Four and Five) highlight the antidepressant effects of this medication. As mentioned previously, there is evidence suggesting that adolescents with depression are biased to perceive facial displays of anger (van Beek et al., 2006) and sadness (Schepman et al., 2012), whilst showing low sensitivity for detecting happiness (van Beek et al., 2006). Fluoxetine may therefore act to reverse these biases in information processing, a mechanism that could contribute to gradual mood improvements over time. As the depressed adolescent starts to interpret different situations under a more positive outlook, he or she may become more receptive towards rekindling or establishing new social connections and his or her mood may gradually improve as a consequence (see Harmer et al., 2009). Reinstalling positive social interactions is particularly important for a depressed adolescent, given the critical role played by friendships during this specific developmental age (Steinberg & Morris, 2001).

6.3.3. Fluoxetine effects on neural circuitry relevant to adolescent depression

In Chapter Four, not only was fluoxetine found to modulate the activity of the amygdala (a critical site involved in adolescent depression), but it also influenced an extended network of temporal, frontal and occipital regions. Whilst these findings clearly need further replication, they offer a distinctive insight into the very immediate effects of this medication at a systems level.

Amygdala as a critical site mediating the acute effects of fluoxetine

One of the predictions raised in this thesis was that fluoxetine would have immediate effects on the amygdala. This region is implicated in adolescent depression (Beesdo et al., 2009;

Yang and colleagues, 2010), and has been previously considered a critical site mediating both the acute and long-term effects of antidepressants (e.g., Godlewska et al., 2012; Murphy et al., 2009; Sheline et al., 2001). The finding that a single dose of fluoxetine decreased the processing of angry faces (vs. happy) therefore corroborates one of the thesis' main hypotheses, whilst extending the results from the only previous study that investigated the neural effects of fluoxetine in adolescents (Tao et al., 2012).

In line with the finding seen here, Tao and colleagues (2012) found that 8 weeks treatment with fluoxetine normalised the abnormal pattern of amygdala reactivity seen at baseline. However, in this study, the changes in neural activity could not be dissociated from the improvement in mood seen with chronic treatment with antidepressants. The present thesis therefore assessed the effects of fluoxetine prior to symptomatic improvement, and confirmed the hypothesis that the changes in amygdala are seen independently of subjective changes in mood or anxiety. Such effects may therefore represent a key mechanism of action through which fluoxetine acts to alleviate symptoms. Consistent with this notion is the tentative evidence that early changes in amygdala activity (in response to fear) are predictive of initial therapeutic responses. Despite being very preliminary, this finding highlights the potential of using a similar approach in future studies in order to identify biomarkers of treatment response, a much-needed requirement in the field of Child and Adolescence Psychiatry.

Opposite effects seen with fear and anger when compared to happy faces

A unique finding seen in this thesis is the opposite pattern of neural activation in response to fear vs. anger. Due to the paucity of studies looking at the influence of a single dose of fluoxetine on emotional processing (both in adults and adolescents), these findings are difficult to interpret. A number of speculative explanations can nonetheless be considered.

As mentioned previously, this differential pattern of activation could result from the contrasting meanings conveyed by anger vs. fear, which may become especially salient during adolescent depression. Fearful faces convey the message that there is the possibility of danger, but with an ambiguous source; whereas angry faces represent themselves the source of threat, therefore communicating the possibility of direct aggression towards the observer (Whalen, 1998). In line with this, it has been found that despite angry faces being rated as equally unpleasant,

threatening, and anxiety-provoking as fearful faces by adults, they are perceived as being significantly more likely to cause harm (Strauss et al., 2005). It is therefore tempting to speculate that these differential meanings could be especially salient to young people, and in particular to depressed adolescents.

Indeed, adolescence is characterised by increased social awareness and fears of peer rejection and of negative judgments more broadly (Harper, Dickson, & Welsh, 2006; Kloep, 1999). When depression develops during this age period, such fears are intensified (Platt et al., 2013). The trend for an excessive activation in the amygdala in response to anger could therefore represent an increased sensitivity to rejection. Interestingly, a similar pattern of excessive reactivity in the amygdala has been seen during the anticipation and experience of peer rejection in clinically anxious adolescents (Guyer et al., 2008; Lau et al., 2012). Facial displays of anger, *per se*, are not a sign of peer rejection, but they may evoke similar reactions during a developmental period that is particularly sensitive to the possibility of social harm.

It may also be useful to interpret these opposite findings in light of the approach-withdrawal model adopted within emotion research (Moons, Eisenberger, & Taylor, 2010). Anger is usually associated with appraisals of certainty, as well as being considered a motivation to approach and become aggressive (Carver & Harmon-Jones, 2009; Harmon-Jones & Sigelman, 2001; Lerner & Keltner, 2001; Mackie, Devos, & Smith, 2000). Fear, on the other hand, is associated with appraisals of uncertainty and potential avoidance or withdrawal (Carver & Harmon-Jones, 2009; Lerner & Keltner, 2001; Mackie et al., 2000). The increased activation towards fear following fluoxetine administration could therefore reflect decreased avoidance towards an emotion that represents a "more benign" threatening cue. In line with this is evidence suggesting that an accurate perception of fear predicts pro-social behaviour in adults (Marsh et al., 2007), therefore highlighting the ambiguous nature of this emotion and the fact that it may not always be associated with threat *per se* or with negative consequences for the individual. Anger, on the other hand, represents a more immediate harmful sign, for which a pattern of avoidance may be more normative in certain contexts. Interestingly, the need for adopting a more flexible and contextually-driven approach in the interpretation of emotions has been recently highlighted by developmental psychopathology theorists (Franklin, Jamieson, Glenn, & Nock, 2014). Of relevance to the findings seen here, is the argument that a pattern of "approach motivation" may not always be adaptive in

response to anger, especially during certain developmental stages where the pervasive experience of irritability and anger can lead to the development and maintenance of psychological disorders (Franklin, Jamieson, Glenn, & Nock, 2014).

In order to clarify the meanings conveyed by these two emotions, it would have been useful to ask participants to rate the faces in a number of dimensions. Examples include valence (positive, neutral, negative) and hostility, as well as proneness to induce fear/anxiety, rejection and harm. Similar rating procedures have been used in previous studies with adolescents. Interestingly, in a study by McClure and colleagues (2007), adolescents gave the highest fear ratings for angry faces (compared to fearful and neutral), but this effect was similar in both anxious adolescents and healthy controls. In a study by Beesdo et al. (2009), both angry and fearful faces received the highest fear and hostility ratings[6] across groups (i.e., in depressed, anxious and healthy controls). A visual inspection of the values made available by the authors showed higher hostility and fear ratings for angry faces (compared to fearful and happy). Thus, the implementation of similar ratings in the current thesis could help elucidate the cognitive mechanisms that are behind the opposite pattern of neural activity seen towards anger vs. fear (Chapter Four).

A number of additional measures could have also helped characterise the full cognitive and affective experiences triggered by these emotions (see Di Simplicio et al., 2012, 2014; Jonassen, Chelnokova, Harmer, Leknes, & Landrø, 2014; Yang, Simmons, et al., 2007;). Such measures include eye-tracking and indices of skin conductance and electrocardiography (ECG). For instance, by indexing eye gaze, it would be possible to quantify any changes in the ocular exploration of the stimuli and therefore understand how antidepressants may affect visual perceptual mechanisms. Interestingly, previous work by Di Simplicio and colleagues (2014) implemented this approach in a study with highly neurotic participants and found that 7-day treatment with citalopram increased ocular exploration of facial stimuli. Skin conductance and ECG recordings provide, in turn, a measure of arousal and autonomic reactivity. Together, these methods would help clarify, for instance, if the pattern of neural deactivation seen here towards fear (in the placebo group vs. healthy controls) results from a blunted affective response despite adequate perceptual and autonomic processing; or if the pattern of increased reactivity towards anger is associated with increased arousal and facial ocular exploration.

[6] Participants were asked: “How hostile is this face?” and “How afraid are you of this face?”

Finally, differences in accuracy during the recognition of fear vs. anger could have also affected the results. Fear is a particularly difficult emotion for young populations to identify (Wade, Lawrence, Mandy, & Skuse, 2006), showing a gradual improvement with age. Sensitivity to anger, on the other hand, increases sharply from adolescence to adulthood (Thomas et al., 2007). Interestingly, the facial recognition task administered after the functional scan showed that anger was indeed recognised more accurately than fear in the adolescent sample as a whole (see Chapter Four). These differences could have therefore influenced the results.

Amygdala and prefrontal responses

The pattern of decreased activity in the amygdala and prefrontal cortex (both in the DLPFC and VLPFC) during the processing of fearful faces in the placebo group could be seen as inconsistent with traditional theories of depression, which highlight a pattern of fronto-limbic imbalance (Mayberg, 2003; Price & Drevets, 2012). It is generally thought that decreased activation in the amygdala in response to emotionally salient stimuli results from insufficient (i.e. decreased) top-down recruitment from prefrontal regions. However, findings in the adolescent patient literature have been especially mixed. For instance, Tao and colleagues (2012) found a pattern of increased frontal activity along with enhanced amygdala responses to fear in a sample of depressed unmedicated adolescents vs. healthy controls. In contrast, Chantiluke and colleagues (2012) found reduced activation in the dorsolateral prefrontal cortex, also in depressed medication-free adolescents, during a task involving motivated attention. The degree of co-activation between subcortical and prefrontal regions in clinical samples is similarly unclear. Whilst adult studies typically find reduced connectivity between these regions in patients - supporting the traditional hypothesis that hyperactivity in the amygdala results from insufficient top-down regulation - the direction of this relationship is especially complex in youth (Lau, 2013). For instance, a preliminary study from Perlman and colleagues (2012) found reduced connectivity between the amygdala, the medial prefrontal cortex (mPFC) and insula in a sample of unmedicated depressed adolescents, which is consistent with the adult literature. In contrast, McClure and colleagues (2007) found a positive correlation between the amygdala, anterior cingulate and ventroprefrontal cortex in clinically anxious adolescents. Whilst these discrepancies may be the result of using different tasks and populations, they highlight the complexities surrounding prefrontal cortex development and

how it may influence (or be influenced by) psychopathology.

In light of these inconsistencies, dual-systems models of brain function may be inadequate to understand the complex interactions between limbic and higher-level cognitive systems occurring during early-onset depression and adolescence more generally (Pfeifer & Allen, 2012). Indeed, traditional theories postulate that the PFC is still underdeveloped during adolescence, therefore showing poor regulatory control over the excessive reactivity seen in subcortical limbic structures. This pattern is thought to reflect the increased emotionality and poor impulse inhibition that characterises adolescence (Romer, 2010; Steinberg & Morris, 2001; Willoughby, Good, Adachi, Hamza, & Tavernier, 2013). However, as noted by Pfeifer and Allen (2012), it is unclear how 'immaturity' translates into functional activation, given that there is evidence showing both an increased and reduced prefrontal activation with age in tasks probing cognitive control (e.g., Bunge & Wright, 2007; Eshel, Nelson, Blair, Pine, & Ernst, 2007 vs. McRae et al., 2012; Pitskel, Bolling, Kaiser, Crowley, & Pelphrey, 2011). Non-linear changes have also been reported (e.g., Scherf, Sweeney, & Luna, 2006; Somerville, Hare, & Casey, 2011). With this complexity in mind, any findings obtained in clinical samples should be interpreted with further caution.

The pattern of responses seen in subcortical and prefrontal regions in Chapter Four is intriguing and adds to the intricate scenario described above. In the placebo group (vs. healthy controls), fearful faces were associated with a pattern of deactivation in the amygdala and in both the dorsolateral (DLPFC) and ventrolateral (VLPFC) prefrontal cortices. The amygdala and VLPFC are linked anatomically (Ongür & Price, 2000) and have both been implicated in threat processing (Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003; Phillips, Drevets, Rauch, & Lane, 2003). Interestingly, a study by Guyer and colleagues (2008) found stronger positive amygdala-VLPFC connectivity in clinically anxious patients (vs. healthy controls) during the anticipation of feedback from peers previously rated as undesirable. This pattern was seen along with increased amygdala reactivity in the patient group. As noted by the authors, this pattern could reflect a positive VLPFC-amygdala coupling that arises within the amygdala, therefore suggesting a process of strong bottom-up signalling. This region might signal the need for the VLPFC to adjust its activity accordingly - in this case, to deal with the potentially threatening aspect of anticipating virtual feedback from peers perceived as undesirable. Despite the findings from the current thesis being in the opposite direction (i.e., decreased activation in both amygdala and prefrontal regions), it is

possible that they represent a similar pattern of co-activation, also driven by bottom-up signalling. However, in the absence of connectivity analyses, this hypothesis is merely speculative.

It is notable that a single dose of fluoxetine was found to produce immediate effects on a large network of temporal, frontal and occipital regions involved in affective processing. Harmer (2013) has previously suggested that acute and sub-chronic administration of antidepressants act to decrease the activation of the amygdala and other limbic structures, therefore operating through a bottom-up route, rather than modulating the activity of strategic prefrontal processes directly. However, recent evidence suggests that antidepressants may have an earlier and wider effect on both subcortical and prefrontal regions than previously thought. For instance, a single dose of the antidepressant mirtazapine has been shown to increase activity in both limbic and prefrontal regions to happy faces, and to reduce activity in the same regions to fear (Rawlings et al., 2010). These effects were seen in the amygdala–hippocampal region, as well as in a fronto-striatal cluster (including the ventral striatum, caudate, orbitofrontal cortex and frontal pole). While the degree of co-activation between these regions was not analysed, this finding, together with the results reported in the current thesis, raises the intriguing possibility that acute administration of certain antidepressants can produce similar (and potentially even synchronised) effects on both limbic and prefrontal regions. Speculatively, this effect could be mediated by a strong bottom-up response originated in the amygdala, which signals the need for the prefrontal cortex to adjust activation accordingly.

6.3.4. Contribution of these findings to the SSRI debate

One of the topics that motivated the research outlined in this thesis is the question repeatedly raised in the literature of whether antidepressants are sufficiently safe to be used in young people. The fact that this debate continues today shows how little we still know about the effects of antidepressants in young people. Although the answer to this question needs a combination of efforts from researchers from different backgrounds - as well the implementation of larger randomised clinical trials (which is beyond the scope of this thesis) -, the current findings may have implications to this debate that are worth considering.

Fluoxetine was shown in this study to decrease the processing of anger, and to have a broader effect on the processing of positive vs. negative information. An anxiolytic-like effect was

also apparent. This anxiolytic effect stands in contrast with studies conducted previously with acute citalopram administration, in which a single dose of this SSRI was found to increase the processing of threat-related information (e.g., Browning et al., 2007). These differences are pertinent in light of evidence that fluoxetine has a more favourable benefit-to-risk profile in young people when compared to citalopram and other antidepressants (Whittington et al., 2004).

As a whole, these findings could be interpreted as tentative evidence against the notion that antidepressant use, in this case with fluoxetine, produces (exclusively) adverse effects during the first stages of treatment in youth. The findings cannot, however, be used to directly scrutinise the clinical risks and benefits of fluoxetine. Despite the study in Chapter Four having included measures of suicidal ideation, it was not designed (nor powered) to assess the influence of fluoxetine on the intensity and frequency of these symptoms over time. Participants reported high levels of suicidal ideation at baseline (as measured using the SIQ-JR), which were significantly reduced after 6 weeks of treatment. There was also a trend for a reduction in suicidal thinking (as measured using the CDRS-R) after one week of treatment, but this finding failed to reach statistical significance. Acutely, fluoxetine did not seem to influence the experience of suicidal thoughts when compared to placebo (as measured using a VAS item). Interestingly, however, the participants receiving fluoxetine showed values of suicidal ideation that were statistically comparable to healthy controls. Despite supporting the notion that a single dose of fluoxetine does not seem to increase suicidal thoughts, this measure is limited in its assessment of acute changes in ideation. Future work is needed in order to explore whether fluoxetine could be associated with a protective effect against suicidal thinking. For instance, it could be speculated that the anxiolytic effects of this medication might help reduce the initial propensity to experience agitation/anxiety symptoms, often considered as precursors of suicidal thoughts and behaviour (see Goodman, Murphy, & Storch, 2007). To my knowledge, the only study that investigated suicidal thoughts in depth and during a long course of fluoxetine treatment in adolescents was the TADS study (March et al., 2004). The authors found that fluoxetine combined with CBT was associated with a greater decrease in the severity of suicidal ideation (also measured using the SIQ-JR) compared to fluoxetine and CBT alone; but fluoxetine alone was not significantly different from CBT alone or placebo. Adding fluoxetine to CBT may therefore have a protective effect but it is unknown what exactly is driving

this synergy. Only after additional research is conducted, especially large clinical trials, will we have a more comprehensive picture of the effects underlying fluoxetine treatment in youth.

6.4. Methodological considerations

There are a number of methodological considerations that need to be considered in relation to the studies presented in this thesis, which are described in the sub-sections below.

6.4.1. Definition of adolescence

Adolescence is often considered to start with puberty, but these terms are not synonymous. Adolescence is a gradual and complex period of various transitions, from being a "child" to becoming a "teenager" and then an "adult". It is therefore marked by a sequence of events that cannot be easily defined by a discrete age range (Spear, 2000). Due to this uncertainty, different researchers have defined adolescence differently, from 12 to 18 years (Spear, 2000) or from 10 to as late as 25 (Baumrind, 1987). In this thesis, due to practical and ethical reasons, two separate age ranges were used. In the healthy volunteer study described in Chapter Two, the age range between 18 and 21 was chosen as it falls between the late adolescence and young adulthood period, and avoids the ethical constraints of administering antidepressants to healthy younger teenagers. In the study with depressed adolescents described in Chapter Four, only participants aged 13 to 18 were included. The upper age limit of 18 aligns with the criterion used by the CAMHS services when providing care to adolescents (after 18, adolescents are seen by their family doctor). This division poses important constraints in the interpretation of the data. Of note, however, the similarities in the findings obtained across studies may suggest that these results are applicable to both adolescents and young adults as a whole.

6.4.2. The validity of the paradigms for a young population

The tasks used in this thesis were carefully selected in order to capture a wide range of antidepressant effects - both positive and negative. However, a key question is whether they were adequate for implementation in adolescents. Notably, the high performance of the adolescent sample in Chapter Four (accuracy of over 95%) when completing the emotional faces task inside the scanner corroborates the validity of using this paradigm across different age groups. However,

in the facial recognition task (administered outside the scanner), the accuracy levels for detecting fear were inferior to those seen in adult studies using the same paradigm (adolescent sample: 37% vs. adults: 50%-55%). These differences are nonetheless consistent with the developmental literature and seem to represent a real age difference in emotional recognition abilities rather than an artefact of this paradigm (Wade et al., 2006). Future studies should extend the findings seen here, if possible using facial stimuli from both adults and adolescents. The inclusion of more ecologically valid paradigms (e.g., “Cyberball”, “Chatroom”, etc.) is also of high interest (see Lau et al., 2012), as they may reveal increased sensitivity to certain developmental processes especially salient during adolescent depression, such as peer rejection (e.g., Platt et al., 2013).

6.4.3. Using behavioural tasks, self-report measures and BOLD fMRI to detect antidepressant drug effects

In this thesis, the effects of antidepressants were investigated using a wide battery of emotional processing tasks, fMRI and self-report measures. The combination of these methods represents a powerful approach to detect subtle changes in emotional processing after acute antidepressant administration (Harmer & Cowen, 2013).

As mentioned earlier, the behavioural tasks used in this thesis were carefully selected in order to detect both positive and negative effects of antidepressants. Each task measures different levels of information processing but they may also present methodological limitations that need to be considered in the interpretation of the results. A new paradigm has been validated in this thesis - Continuous Flash Suppression (CFS) -, in an attempt of overcoming some of the methodological constraints associated with the Attentional Dot-Probe. However, whilst the Attentional Dot-Probe failed to show the expected fear vigilance effect in Chapter Two, a modified version of this paradigm may have shown increased sensitivity to detect attentional biases in males high in trait anger. Similarly, although CFS proved useful in detecting early automatic biases towards threat in Chapter Three, it failed to be modulated by diazepam. This highlights the need to continuously refine the methodological features associated with these paradigms in order to clearly understand any variations in results across studies and populations [for useful reviews see Browning, Holmes, & Harmer (2010); Harmer, Goodwin, et al. (2009); Kim & Blake (2005)].

An important question that emerges from this thesis, however, is whether the choice of

paradigms was the most adequate to detect antidepressant effects in a young population. The neuropsychological model of antidepressant drug action supports the inclusion of these tasks, as they are sensitive to both the positive and adverse effects of antidepressants. It would nonetheless be useful, in future experiments, to implement additional paradigms (such as negative mood induction) as they may reveal additional biases and drug effects that were not detected in both the healthy volunteer and clinical studies presented here. The inclusion of healthy participants has the advantage of directly measuring the effects of antidepressants without the confounding effect of mood. However, given the high plasticity that characterises adolescence and the relevance of stress-diathesis models for understanding affective biases (Ingram & Luxton, 2005), it may be useful to investigate the effects of antidepressants after inducing negative mood or stress/anxiety. Indeed, the use of an anger recall paradigm proved useful in characterising the effects of fluoxetine in the last experimental study (Chapter Five). Finally - and again in light of the high plasticity that marks adolescence -, it would be useful for future studies to explore if attentional training paradigms could boost the effects of antidepressants in correcting the negative biases that characterise paediatric depression (see Lau 2013).

The self-report measures used in chapter Four were also carefully chosen in order to assess a wide range of symptoms, including depression, anxiety, irritability and suicidal ideation. This was important not only to determine that the acute effects of the drug on emotional processing were independent of changes in mood or anxiety, but also to detect changes in symptoms over the course of the fluoxetine treatment and to correlate them with acute changes in neural activity. As predicted, acute fluoxetine did not influence subjective reporting on any of the scales, despite producing subtle changes in behavioural and neuroimaging measures. Interestingly, whilst acute amygdala activity to fear correlated with symptoms of anxiety/agitation over the first 10 days of treatment, amygdala activity to anger failed to show a significant correlation with irritability. However, it should be noted that the measures used to assess irritability were limited, as only single items from the CDRS-R and VAS were used. Future studies should include more sensitive measures of irritability (see Stringaris, Goodman, et al., 2012) in order to explore the link between amygdala reactivity to anger and subsequent clinical changes in this domain.

Finally, despite the immense potential of applying fMRI in psychiatric research (Murphy, 2010), a few limitations associated with this technique are worth noting. First, it should be noted

that BOLD fMRI measures the physiological changes that are associated with neural activity, rather than “neural activity” itself (Iannetti & Wise, 2007). This limitation raises the possibility that the fMRI signal detected using this technique reflects global physiological changes rather than a specific modulation of the drug on neural activity (Murphy & Mackay, 2011). One approach frequently used to overcome this problem is the implementation of a control task, which measures BOLD responses in a region of “no interest” – i.e., region that is not expected to be influenced by the drug (such as examining the activity in the occipital cortex in response to a visual stimulation paradigm; Murphy & Mackay, 2011). If this area fails to be modulated by the drug agent, one could be more certain that the medication is having a specific effect on localised brain activity. Additionally, an alternative approach that can be used to control for confounding drug effects on vascular reactivity is to implement arterial spin labelling. This perfusion technique provides an absolute measure of cerebral blood flow (CBF), which can be used to compare CBF during a baseline condition vs. a task of direct relevance. This separation helps dissociate global drug effects on baseline activity from those that are mediated by task-induced activation (Wang, Chen, Fernandez-Seara, & Detre, 2011). It would be advantageous to include both techniques in future fMRI studies investigating the effects of fluoxetine in youth.

6.4.4. Between-groups design

All of the studies in this thesis used a between-subjects design, which has a number of drawbacks. As seen in Chapter Two and Five specifically, the groups showed some baseline differences, despite participants being allocated to each of the groups in a randomised and double-blind design. This problem was especially prominent in the study with high trait anger males. An alternative approach would have been to use a within-subjects crossover design, where the same participants are administered the same experimental condition but at different timepoints (to allow for the necessary washout period between interventions). This approach has the benefit of removing inter-subject differences, which may arise from imperfect group matching or from uncontrolled variables that are unknown to the experimenter. However, a within-subjects design has a number of disadvantages in the context of this type of study. Using the same participants would involve a repeated administration of the same emotional processing tasks, therefore leading to unavoidable practice or habituation effects. Indeed, in our laboratory, it has been found that the

effects of antidepressants are stronger on the first testing session (Harmer, unpublished observations). Habituation is also a particular problem in fMRI studies, given that the amygdala, for instance, is known to habituate rapidly to repeated presentation of emotional stimuli (e.g. Breiter et al., 1996; Wright et al., 2001), although it is unclear if these effects persist across testing sessions (e.g. Carver & Harmon-Jones, 2009). For these reasons, the studies reported in this thesis adopted a between-group design, in which a careful matching on a range of variables was attempted.

6.4.5. Sample sizes and power calculations

The studies included in this thesis made use of power calculations in order to determine desired sample sizes. However, recruitment in the two final experiments was more difficult than anticipated and therefore these studies could have been underpowered. It has nonetheless been argued that small sample sizes usually contribute to type II ("false negatives") rather than type I ("false positives") errors, which can conceal the presence of real effects. Given that both these studies (Chapters Four and Five) revealed positive findings, type II errors may be less of a concern (McClure et al. 2007). Future experiments with larger samples are needed to replicate and clarify these results.

6.5. Future studies

This thesis has hopefully advanced our current knowledge about the early effects of antidepressants in young people, but the need for future research is clear.

First, it is crucial for future studies to include larger and more homogenous clinical samples when investigating the effects of both acute and chronic treatment with antidepressants. A larger sample would be especially useful to explore the potential of using fMRI to predict clinical responses to antidepressant medication prospectively.

Future studies should also include a combination of multi-modal measures (ecologically valid paradigms, fMRI, ECG, eye-tracking, genetic analysis, etc.) as well as different approaches to data analysis (e.g., connectivity analysis, resting state), in order to more fully understand the mechanisms underlying antidepressant use in young people. For instance, implementing connectivity analyses would be important to understand how distributed subcortical and prefrontal networks are coupled together during the processing of specific emotions.

In this thesis, the pubertal status of the adolescent participants was not considered. This is an important limitation of this and other studies in the field, which should be addressed in future work, especially in light of emerging evidence showing that puberty influences cognitive development (Blakemore, Burnett, & Dahl, 2010).

Further research should also explore how the experience of depression across different adolescent age ranges may have an impact on brain functioning. Most studies combine data from different ages, which are then averaged in order to compare brain activity between groups as a whole. Given the profound brain changes occurring throughout adolescence (Blakemore et al., 2010), a better stratification of age would be helpful.

Finally, forthcoming work should explore the factors that may contribute to the adverse effects experienced upon initiation of antidepressant treatment. The clinical study in Chapter Four lacked power to identify those patients who may have developed more adverse effects after initiating the treatment. In clinical trials, only a small percentage of adolescents seem to develop harmful reactions when administered antidepressants, at rates that vary between 2% to 8% for hostility and behavioural activation, and 3% to 12% for suicide-related events (Cheung, Dewa, & Levitt, 2005). Additional studies are therefore critical in order to identify both genetic and psychological markers that can reliably predict which patients are most susceptible to encountering side effects after initiating antidepressant treatment. For instance, a polymorphism in the serotonin-transporter gene has been associated with a greater risk to experience adverse SSRI-induced effects in both adult (Perlis et al., 2003) and adolescent populations (Kronenberg et al., 2007). It would be useful to investigate how this polymorphism influences brain activity in response to fluoxetine treatment in youth.

6.6. Conclusions

In conclusion, the series of studies conducted as part of this thesis have provided behavioural and neuroimaging support for the notion that fluoxetine exerts direct and immediate effects on emotional processing. It was hypothesised that the behavioural and neural effects on anger could represent a mechanism particularly relevant to the treatment of adolescent depression, given the prominence of irritability in the clinical presentation of this disorder. The broader effect on positive vs. negative processing may also have important implications for reversing the negative

biases that seem to characterise adolescent MDD. Finally, the potential anxiolytic-like property of fluoxetine seen acutely is of particular interest, as it may reflect an important mechanism underlying the beneficial profile of fluoxetine that is not seen with acute administration of other antidepressant drugs. This effect could also help reduce the anxiety symptoms that are often seen in adolescent MDD.

At a neural level, the amygdala emerged as a critical site underpinning the acute effects of fluoxetine administration. This finding is important, as it elucidates a neural mechanism through which fluoxetine may act to alleviate symptoms. Indeed, there was some suggestion that early effects on the amygdala were associated with reduced anxiety symptoms during the first ten days of treatment. Whilst still very preliminary, these findings are encouraging and offer a useful approach to investigate antidepressant effects in youth. A combination of efforts is needed in order to bridge the gap between clinical trials and neuropsychological research, and assist the development of more effective pharmacological and psychological treatments for such a devastating, yet still poorly understood, disease. Adolescence represents a time of increased vulnerability for the experience of a wide range of psychological disorders, but it is also increasingly being recognised as a period of immense plasticity and of unique opportunities to promote health and resilience.

Appendices

Appendix 1

Structured clinical interview for DSM-IV Axis-II

The semi-structured SCID-I interview (First et al. 1997) was administered to ascertain Axis I diagnoses. The SCID-I is divided into six modules: mood episodes; psychotic symptoms; psychotic disorders; mood disorders; substance use disorders; and anxiety, adjustment, and other disorders.

National Adult Reading Test (NART)

The National Adult Reading Test (NART; Nelson, 1991) is a measure of verbal intelligence widely used in studies with adult healthy volunteers. This test includes a list of 50 words printed in order of increasing difficulty. The participant is required to read aloud these words and the experimenter records the number of mistakes. The full verbal IQ is then calculated.

Eysenck-Personality Scales (EPQ)

The Eysenck Personality Questionnaire (EPQ; Eysenck et al. 1985) was used to determine dimensions of personality at baseline. The EPQ consists of 90 “yes” and “no” statements and is composed by four scales: extroversion/introversion, neuroticism/stability, psychoticism/socialization and lie.

State-Trait anxiety Inventory (STAI)

The State and Trait Anxiety Inventory (STAI; Spielberger et al. 1970) determines state and trait anxiety levels. The STAI consists of two 20-item scales with four possible responses to each question, ranging from one to four (“not at all”/”almost never” – “very much”/”almost always”). Possible scores range from 20 to 80, with higher scores indicating higher levels of anxiety.

Beck Depression Inventory (BDI)

The BDI (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) is a 21 item self-report instrument designed to assess the severity of depressive symptoms occurring during the past week. The questions reflect the criteria used to diagnose Major Depressive Disorder and each item is rated on a 4-point scale (0–3). Total scores range from 0 to 63, with higher scores reflecting higher levels of depression.

The Positive and Negative Affective Schedule (PANAS)

Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) measures positive and negative affect. Ten descriptors for the positive affect scale and ten descriptors for the negative affect scale are given. Participants indicate to what extent they feel on each descriptor using a rating from one to five ("very slightly or not at all" to "extremely").

Visual Analogue Scales (VAS)

The Visual Analogue Scales (VAS) questionnaire consists of 15 items that assess mood. The scales consist of 15 horizontal lines, 10 cm in length, with antonymous adjectives (e.g. alert vs. drowsy) on either pole. The scales have been validated experimentally (Bond & Lader, 1974), and each item has been attributed to 1 of 3 factors: Alertness, Calmness and Contentedness.

Antidepressant Side-Effects Checklist (ASEC)

The Antidepressant Side-Effect Checklist (ASEC; Uher et al., 2009) is a self-report instrument that measures 21 adverse reactions to antidepressants (e.g., dry mouth, drowsiness, blurred vision, headache, etc.). This questionnaire is yet to be validated, but it has been derived from a systematic review by Uher and colleagues (2009). For each item, the participant rates the severity of the specified symptom on a four-point scale (0=absent; 1=mild; 2=moderate; 3=severe) and specifies whether a symptom (if present) was likely to be a side-effect of the antidepressant drug ("yes" or "no"). An adapted version of this questionnaire was used in this study, in which some items not adequate to acute administration were removed (e.g., insomnia, problems with sexual function).

Jitteriness Rating Questionnaire (JRQ)

The Jitteriness Rating Questionnaire (JRQ; Sinclair et al., 2009) is a self-report instrument that measures 21 adverse reactions to antidepressants (e.g., dry mouth, drowsiness, blurred vision, headache, etc.). This is a non-validated questionnaire that has been derived from a systematic review by Sinclair and colleagues (2009). For each item, the participant rates the severity of the specified symptom on a six-point scale (0 for “none of the time” to 5 “all of the time”). The maximum score is 120.

Appendix 2

Kiddie-Sads-Present and Lifetime Version (K-SADS-PL)

The K-SADS-PL (Kaufman et al., 2000) is a semi-structured psychiatric interview designed to assess the presence of current and past episodes of psychological disorders in children and adolescents using DSM-IV criteria. K-SADS-PL includes an initial screen interview (82 symptoms related to 20 diagnostic areas) and five diagnostic supplements: (1) affective disorders (major depression, dysthymia, mania/hypomania); (2) psychotic disorders; (3) anxiety disorders (social phobia, agoraphobia, specific phobia, obsessive-compulsive disorder, separation anxiety disorder, generalized anxiety disorder, panic disorder, posttraumatic stress disorder); (4) disruptive behavioural disorders (attention deficit hyperactivity disorder/ADHD, conduct disorder, oppositional defiant disorder); and (5) substance abuse, tic disorders, eating disorders, and elimination disorders (enuresis, encopresis).

Socio-demographic questionnaire

A socio-demographic questionnaire was administered to all parents in order to determine their education level and average annual income.

Child Behaviour Checklist (CBCL)

The CBCL (Achenbach & Rescorla, 2001) is a parent-report questionnaire that measures competency in general activities, social interactions and school (part I), as well as the presence of various behavioural and emotional problems (part II) in children and adolescent aged between 6 and 18 years-old. Only the second part of the questionnaire (i.e., part II) was administered in this study, which includes 113 items measuring the presence of behavioural or emotional problems during the past 6 months. The parent was asked to rate “how true” a given behaviour was for their child. It yields eight behavioural subscales: withdrawn, somatic complaints, anxious/depressed, social problems, thought problems, attention problems, delinquent behaviour, and aggressive behaviour. These scales are then quantified in three behavioural composite scores: *Internalizing Problems*, *Externalizing Problems* and *Total Problems*. Higher scores are indicative of more problems. These three composite scores and the anxious/depressed scale are reported here.

Wechsler Abbreviated Scale of Intelligence (WASI)

The WASI (Wechsler, 1999) is a standardised test designed to measure general intellectual ability in individuals aged 6 to 89 years. The two-subtest version was administered in this study, which includes the sub-tests *Vocabulary* (in which the subject is required to orally define words presented both orally and visually) and *Matrix Reasoning* (in which incomplete grid patterns are presented and the participant needs to select the correct response from five possible choices). Together they provide an estimate of Full Scale Intelligence Quotient (FSIQ).

Children Depression Rating Scale (CDRS-R)

The CDRS-R (Poznanski & Mokros, 1996) is a 17-item semi-structured interview that assesses the presence and severity of depressive symptoms in young people. It was designed for 6- to 12-years children, but it has been successfully used with adolescents, showing good reliability and validity in this age group (Mayes, Bernstein, Haley, Kennard, & Emslie, 2010). Total scores range from 17 to 113, with a score of 40 or higher being indicative of clinical depression.

Children Depression Inventory (CDI)

The CDI (Kovacs, 1992) is a 27 self-report questionnaire used to quantify depressive symptomatology in children/adolescents aged between 7 and 17 years-old. Each item includes three descriptions of graded severity and the adolescent is asked to indicate which statement describes him best. Responses are scored on a scale 0–2, providing a total range of 0-54. Higher scores indicate increased severity. A research version of 26-items was used in this study (consistent with Smucker, Craighead, Craighead, & Green, 1986).

Suicidal Ideation Questionnaire- Junior (SIQ-JR)

The SIQ-JR (Reynolds, 1987) is a 15-item self-report questionnaire used to measure the frequency of suicidal ideation over a 1-month period in adolescents aged between 12 and 18 years old. Items are scored on a 7-point scale, ranging from 0 (“I never had this thought”) to 6 (“almost every day”). Total scores range from 0 to 90. A clinical cut-off of 31 is frequently used as an indication of high suicide risk.

State-Trait Anxiety Inventory for Children (STAI-C)

The STAI-C (Spielberger, 1973) consists of two 20-item questionnaires that measure state and trait anxiety (A-State and A-Trait questionnaires, specifically) in children between the ages of 8 and 14. However, it has been validated for use in adolescents (Kirisci & Clark, 1996). The A-State scale quantifies anxiety that is specific to the situation, whereas the A-trait scale measures the general predisposition of the child to feel anxious. Responses are given on a three-point scale, ranging from “hardly ever” to “often true”. Total scores range from 0-40.

Visual Analogue Scale

This study used a simplified version of a Visual Analogue Scale, consisting of 6 items in total: “tired”, “happy”, “sad”, “nervous/tense”, “irritable” and “thinking about death”. The rating scales consisted of 10 cm lines and participants were instructed to draw a horizontal line crossing the line at the point that represented how they currently felt. Similar visual analogue scales have been used successfully in paediatric samples (e.g., Chapman & Kirby-Turner, 2002)

Antidepressant side-effects checklist

An Antidepressant side-effects checklist was created in order to measure the occurrence of potential side effects. Participants were given a list of potential side-effects and were asked to write down which ones (if any) they were currently experiencing (testing session) and/or which ones were more prominent in the past 7 days (measured at follow-up 1) or 6 weeks (follow-up 2). The list of side-effects was adapted from the Antidepressant Side-Effect Checklist used in Chapter Two (ASEC; Uher et al., 2009).

Children's Global Assessment Scale (CGAS)

The Children's Global Assessment Scale (CGAS; Shaffer et al., 1983) is a clinician-rated instrument used to quantify the general functioning of children and adolescents under 18. This measure provides a single global rating only, ranging from 0 to 100. Higher scores represent better functioning.

Clinical Global Impression Scale (CGI)

The Clinical Global Impression Scale (CGI; Guy, 1976) is an observer-rated scale that measures illness severity (CGI-S), global improvement or change (CGI-I) and therapeutic response/efficacy (CGI-E). Consistent with previous studies (e.g., Forkmann et al., 2011), only the illness severity and global improvement items are reported, both of which use a 7-point scale to assess the severity of the illness and therapeutic improvement, respectively.

Appendix 3

Structured clinical interview for DSM-IV Axis-II

The semi-structured SCID-II interview (First, 1997) was administered to ascertain Axis II (Personality Disorder) diagnosis. This interview includes 12 categories: Avoidant, Dependent, Obsessive-Compulsive, Passive-Aggressive, Depressive, Paranoid, Schizotypal, Schizoid, Histrionic, Narcissistic, Borderline and Anti-social. This interview was administered in a stepped procedure. Participants were first asked to fill in the SCID-II questionnaire (in online format), which assesses the presence of traits for all twelve categories. These questions required simple “yes” or “no” answers. Once completed by the participant, the questionnaire was immediately scored during the screening session. Only those sections that fulfilled the minimum criteria required to make a diagnosis were then covered in more detail using the SCID-II semi-structured interview.

ADHD Module from the MINI International Neuropsychiatric Interview (M.I.N.I.), v 0.5

The ADHD Module from the MINI was used to screen for the presence of past and/or current ADHD. The M.I.N.I. 5.0 (Sheehan et al., 1998) is a brief, structured diagnostic interview schedule used to address different disorders according to the DSM-IV and ICD-10 criteria. The ADHD module covers both inattentive and hyperactive/impulsive symptoms observed during childhood and adulthood.

State-Trait Anger Expression Inventory (STAXI)

The STAXI scale (Spielberg, 1988) is a self-report measure that measures anger symptoms. Responses are made on a 4-point scale ranging from 1 (almost never) to 4 (almost always). It is composed by 6 scales: State Anger (intensity of current angry feelings), Trait Anger (disposition to experience anger), Anger-in (frequency in which angry feelings are suppressed), Anger-out (frequency in which angry feelings are expressed), Anger Control (frequency with which the individual tries to control the expression of anger) and Anger Expression (frequency with which anger is expressed, regardless of the direction of the expression). Higher scores indicate greater presence of the symptoms.

Barrat Impulsiveness Scale (BIS-11)

BIS-11 (Patton et al., 1995) is a 30-item test that measures impulsivity. Items are scored to yield six first-order factors (attention, motor, self-control, cognitive complexity, perseverance, and cognitive instability impulsiveness), three second-order factors (attentional, motor, and non-planning impulsiveness) and a total score (taken as a global measure of impulsivity). Only the second-order factors and the total score will be reported in this thesis.

Buss Perry Aggression Questionnaires (BPAQ)

The BPAQ (Buss & Perry, 1992) is a 29-item questionnaire that measures aggression, being composed by four scales: physical aggression, verbal aggression, anger and hostility. Responses to items on a 5-point scale range from 1 (extremely uncharacteristic of me) to 5 (extremely characteristic of me). Higher scores are indicative of greater aggression.

References

Abasi, I., Fata, L., Sadeghi, M., Banihashemi, S., & Mohammadee, A. (n.d.). A Comparison of Transdiagnostic Components in Generalized Anxiety Disorder, Unipolar Mood Disorder and Nonclinical Population. Depression, 8(22), 23.

Abela, J. R. Z., & Hankin, B. L. (2008). Cognitive Vulnerability to Depression in Children and Adolescents: a Developmental Psychopathology Perspective. In *Handbook of Depression in Children and Adolescents*. New York: Guilford Press.

Achenbach, T. M., & Rescorla, L. A. (2001). *The manual for the ASEBA school-age forms & profiles. Burlington*. Burlington, VT: University of Vermont, Research Center for Children, Youth, and Families.

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Berger, A. C., & Cohen, R. J. (1981). *Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science (New York, N.Y.), 213(4504), 220–222.*

American Psychiatric Association, American Psychiatric Association, & DSM-5 Task Force. (2013). *Diagnostic and statistical manual of mental disorders: DSM-5.* Arlington, Va.: American Psychiatric Association.

Andersen, S. L., & Navalta, C. P. (2004). Altering the course of neurodevelopment: a framework for understanding the enduring effects of psychotropic drugs. *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience*, *22*(5-6), 423–440. doi:10.1016/j.ijdevneu.2004.06.002

Andersen, S. L., & Teicher, M. H. (2008). Stress, sensitive periods and maturational events in adolescent depression. *Trends in Neurosciences*, *31*(4), 183–191. doi:10.1016/j.tins.2008.01.004

Anderson, A. K., & Phelps, E. A. (2001). Lesions of the human amygdala impair enhanced perception of emotionally salient events. *Nature*, *411*(6835), 305–309. doi:10.1038/35077083

Anderson, N. H. (1968). Likableness ratings of 555 personality-trait words. *Journal of Personality and Social Psychology*, *9*(3), 272–279.

Andersson, J.L.R., Jenkinson, M., & Smith, S. (2007). Non-linear registration, aka Spatial normalisation. FMRIB technical report TR07JA2 from www.fmrib.ox.ac.uk/analysis/techrep.

Anderson, J. C., Linden, W., & Habra, M. E. (2005). The importance of examining blood pressure reactivity and recovery in anger provocation research. International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology, 57(3), 159–163. http://doi.org/10.1016/j.ijpsycho.2004.12.011

Ansorge, M. S., Zhou, M., Lira, A., Hen, R., & Gingrich, J. A. (2004). Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science (New York, N.Y.)*, 306(5697), 879–881. doi:10.1126/science.1101678

Appel, M. L., Berger, R. D., Saul, J. P., Smith, J. M., & Cohen, R. J. (1989). Beat to beat variability in cardiovascular variables: noise or music? Journal of the American College of Cardiology, 14(5), 1139–1148.

Arnone, D., Horder, J., Cowen, P. J., & Harmer, C. J. (2009). Early effects of mirtazapine on emotional processing. *Psychopharmacology*, *203*(4), 685–691. doi:10.1007/s00213-008-1410-6

Axelson, D. A., & Birmaher, B. (2001). Relation between anxiety and depressive disorders in childhood and adolescence. *Depression and Anxiety*, *14*(2), 67–78.

Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J., & van IJzendoorn, M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychological Bulletin*, *133*(1), 1–24. doi:10.1037/0033-2909.133.1.1

Bateson, A. N. (2002). Basic pharmacologic mechanisms involved in benzodiazepine tolerance and withdrawal. *Current Pharmaceutical Design*, *8*(1), 5–21.

Baumrind, D. (1987). A developmental perspective on adolescent risk taking in contemporary America. *New Directions for Child and Adolescent Development*, *1987*(37), 93–125. doi:10.1002/cd.23219873706

Bechara, A., Damasio, H., & Damasio, A. R. (2000). Emotion, Decision Making and the Orbitofrontal Cortex. *Cerebral Cortex*, *10*(3), 295–307. doi:10.1093/cercor/10.3.295

Beck, A. T. (1976). *Cognitive therapy and the emotional disorders*. International Universities Press.

Beck, A. T., & Clark, D. A. (1997). An information processing model of anxiety: automatic and strategic processes. *Behaviour Research and Therapy*, *35*(1), 49–58.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Archives of General Psychiatry*, *4*(6), 561.

Beckers, T., Wagemans, J., Boucart, M., & Giersch, A. (2001). Different effects of lorazepam and diazepam on perceptual integration. *Vision Research*, *41*(17), 2297–2303.

Beek, Y. van, & Dubas, J. S. (2008). Decoding Basic and Non-basic Facial Expressions and Depressive Symptoms in Late Childhood and Adolescence. *Journal of Nonverbal Behavior*, *32*(1), 53–64. doi:10.1007/s10919-007-0041-7

Beesdo, K., Lau, J. Y. F., Guyer, A. E., McClure-Tone, E. B., Monk, C. S., Nelson, E. E., … Pine, D. S. (2009). Common and distinct amygdala-function perturbations in depressed vs anxious adolescents. *Archives of General Psychiatry*, *66*(3), 275–285. doi:10.1001/archgenpsychiatry.2008.545

Belli, S. R., Rogers, R. D., & Lau, J. Y. F. (2012). Adult and adolescent social reciprocity: experimental data from the Trust Game. *Journal of Adolescence*, *35*(5), 1341–1349. doi:10.1016/j.adolescence.2012.05.004

Berkowitz, L. (1999). Anger. In *Handbook of cognition and emotion* (pp. 411–428). New York: Wiley.

Berzsenyi, P., Galateo, E., & Valzelli, L. (1983). Fluoxetine activity on muricidal aggression induced in rats by p-chlorophenylalanine. *Aggressive Behavior*, *9*(4), 333–338. doi:10.1002/1098-2337(1983)9:4<333::AID-AB2480090408>3.0.CO;2-R

Bhansali, P., Dunning, J., Singer, S. E., David, L., & Schmauss, C. (2007). Early life stress alters adult serotonin 2C receptor pre-mRNA editing and expression of the alpha subunit of the heterotrimeric G-protein G q. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*(6), 1467–1473. doi:10.1523/JNEUROSCI.4632-06.2007

Bigos, K. L., Pollock, B. G., Aizenstein, H. J., Fisher, P. M., Bies, R. R., & Hariri, A. R. (2008). Acute 5-HT reuptake blockade potentiates human amygdala reactivity. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *33*(13), 3221–3225. doi:10.1038/npp.2008.52

Birmaher, B., Brent, D., AACAP Work Group on Quality Issues, Bernet, W., Bukstein, O., Walter, H., … Medicus, J. (2007). Practice parameter for the assessment and treatment of children and adolescents with depressive disorders. *Journal of the American Academy of Child and Adolescent Psychiatry*, *46*(11), 1503–1526. doi:10.1097/chi.0b013e318145ae1c

Birmaher, B., Brent, D. A., & Benson, R. S. (1998). Summary of the practice parameters for the assessment and treatment of children and adolescents with depressive disorders. American Academy of Child and Adolescent Psychiatry. *Journal of the American Academy of Child and Adolescent Psychiatry*, *37*(11), 1234–1238.

Birmaher, B., Ryan, N. D., Williamson, D. E., Brent, D. A., Kaufman, J., Dahl, R. E., … Nelson, B. (1996). Childhood and adolescent depression: a review of the past 10 years. Part I. *Journal of the American Academy of Child and Adolescent Psychiatry*, *35*(11), 1427–1439. doi:10.1097/00004583-199611000-00011

Birmaher, B., Williamson, D. E., Dahl, R. E., Axelson, D. A., Kaufman, J., Dorn, L. D., & Ryan, N. D. (2004). Clinical presentation and course of depression in youth: does onset in childhood differ from onset in adolescence? *Journal of the American Academy of Child and Adolescent Psychiatry*, *43*(1), 63–70. doi:10.1097/00004583-200401000-00015

Bishop, S. J., Dalgleish, T., & Yule, W. (2004). Memory for emotional stories in high and low depressed children. *Memory (Hove, England)*, *12*(2), 214–230. doi:10.1080/09658210244000667

Bjork, J. M., Dougherty, D. M., Moeller, F. G., & Swann, A. C. (2000). Differential behavioral effects of plasma tryptophan depletion and loading in aggressive and nonaggressive men. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *22*(4), 357–369. doi:10.1016/S0893-133X(99)00136-0

Blair, R. J., & Curran, H. V. (1999). Selective impairment in the recognition of anger induced by diazepam. *Psychopharmacology*, *147*(3), 335–338.

Blakemore, S.-J., Burnett, S., & Dahl, R. E. (2010). The Role of Puberty in the Developing Adolescent Brain. *Human Brain Mapping*, *31*(6), 926–933. doi:10.1002/hbm.21052

Boes, A. D., McCormick, L. M., Coryell, W. H., & Nopoulos, P. (2008). Rostral anterior cingulate cortex volume correlates with depressed mood in normal healthy children. *Biological Psychiatry*, *63*(4), 391–397. doi:10.1016/j.biopsych.2007.07.018

Bond, A. J., Wingrove, J., & Critchlow, D. G. (2001). Tryptophan depletion increases aggression in women during the premenstrual phase. *Psychopharmacology*, *156*(4), 477–480.

Bond, A., & Lader, M. (1974). The use of analogue scales in rating subjective feelings. *British Journal of Medical Psychology*, *47*(3), 211–218. doi:10.1111/j.2044-8341.1974.tb02285.x

Borsini, F., Podhorna, J., & Marazziti, D. (2002). Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology*, *163*(2), 121–141. doi:10.1007/s00213-002-1155-6

Botteron, K. N., Raichle, M. E., Drevets, W. C., Heath, A. C., & Todd, R. D. (2002). Volumetric reduction in left subgenual prefrontal cortex in early onset depression. *Biological Psychiatry*, *51*(4), 342–344.

Bourke, C., Douglas, K., & Porter, R. (2010). Processing of facial emotion expression in major depression: a review. The Australian and New Zealand Journal of Psychiatry, 44(8), 681–696. http://doi.org/10.3109/00048674.2010.496359

Bradley, B. P., Mogg, K., Falla, S. J., & Hamilton, L. R. (1998). Attentional bias for threatening facial expressions in anxiety: Manipulation of stimulus duration. *Cognition & Emotion*, *12*(6), 737–753.

Bradley, M. M., & Lang, P. J. (1999). *Affective norms for English words (ANEW): Instruction manual and affective ratings*. Citeseer. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.306.3881&rep=rep1&type=pdf

Breiter, H. C., Etcoff, N. L., Whalen, P. J., Kennedy, W. A., Rauch, S. L., Buckner, R. L., … Rosen, B. R. (1996). Response and habituation of the human amygdala during visual processing of facial expression. *Neuron*, *17*(5), 875–887.

Bremner, J. D., Vythilingam, M., Vermetten, E., Nazeer, A., Adil, J., Khan, S., … Charney, D. S. (2002). Reduced volume of orbitofrontal cortex in major depression. *Biological Psychiatry*, *51*(4), 273–279. doi:10.1016/S0006-3223(01)01336-1

Bridge, J. A., & Kelleher, K. J. (2011). The 2004 FDA warning on antidepressant suicidality risk changed outpatient treatment patterns for young people with new-onset depression. *Evidence Based Mental Health*, *14*(2), 42–42. doi:10.1136/ebmh.14.2.42

Broadbent, D., & Broadbent, M. (1988). Anxiety and Attentional Bias: State and Trait. *Cognition & Emotion*, *2*(3), 165–183. doi:10.1080/02699938808410922.

Brocco, M., Dekeyne, A., Gobert, A., De Nanteuil, G., & Millan, M. J. (2000). Actions of the neurokinin1 antagonists, GR205, 171 and CP99, 994, in behavioral and neurochemical models of potential antidepressant activity. Int J Neuropsychopharmacol, 3, S376.

Brøsen, K. (2004). Some aspects of genetic polymorphism in the biotransformation of antidepressants. *Thérapie*, *59*(1), 5–12.

Browning, M., Holmes, E. A., Charles, M., Cowen, P. J., & Harmer, C. J. (2012). Using Attentional Bias Modification as a Cognitive Vaccine Against Depression. *Biological Psychiatry*, *72*(7), 572–579. doi:10.1016/j.biopsych.2012.04.014

Browning, M., Holmes, E. A., & Harmer, C. J. (2010). The modification of attentional bias to emotional information: A review of the techniques, mechanisms, and relevance to emotional disorders. *Cognitive, Affective & Behavioral Neuroscience*, *10*(1), 8–20. doi:10.3758/CABN.10.1.8

Browning, M., Reid, C., Cowen, P. J., Goodwin, G. M., & Harmer, C. J. (2007). A single dose of citalopram increases fear recognition in healthy subjects. *Journal of Psychopharmacology (Oxford, England)*, *21*(7), 684–690. doi:10.1177/0269881106074062

Buffett-Jerrott, S. E., & Stewart, S. H. (2002). Cognitive and sedative effects of benzodiazepine use. Current Pharmaceutical Design, 8(1), 45–58.

Bunge, S. A., & Wright, S. B. (2007). Neurodevelopmental changes in working memory and cognitive control. Current Opinion in Neurobiology, 17(2), 243–250. http://doi.org/10.1016/j.conb.2007.02.005

Burg, M. M., Soufer, A., Lampert, R., Collins, D., & Soufer, R. (2011). Autonomic Contribution to Endothelin-1 Increase during Laboratory Anger-Recall Stress in Patients with Coronary Artery Disease. Molecular Medicine, 17(5-6), 495–501. http://doi.org/10.2119/molmed.2010.00083

Burghardt, N. S., Sullivan, G. M., McEwen, B. S., Gorman, J. M., & LeDoux, J. E. (2004). The selective serotonin reuptake inhibitor citalopram increases fear after acute treatment but reduces fear with chronic treatment: a comparison with tianeptine. *Biological Psychiatry*, *55*(12), 1171–1178. doi:10.1016/j.biopsych.2004.02.029

Busch, S. H., Frank, R. G., Martin, A., & Barry, C. L. (2011). Characterizing declines in pediatric antidepressant use after new risk disclosures. Medical Care Research and Review: MCRR, 68(1), 96–111. http://doi.org/10.1177/1077558710374197

Buss, A. H., & Perry, M. (1992). The Aggression Questionnaire. *Journal of Personality and Social Psychology*, *63*(3), 452–459. doi:10.1037/0022-3514.63.3.452

Cadesky, E. B., Mota, V. L., & Schachar, R. J. (2000). Beyond words: how do children with ADHD and/or conduct problems process nonverbal information about affect? *Journal of the American Academy of Child and Adolescent Psychiatry*, *39*(9), 1160–1167. doi:10.1097/00004583-200009000-00016

Caetano, S. C., Fonseca, M., Hatch, J. P., Olvera, R. L., Nicoletti, M., Hunter, K., … Soares, J. C. (2007). Medial temporal lobe abnormalities in pediatric unipolar depression. *Neuroscience Letters*, *427*(3), 142–147. doi:10.1016/j.neulet.2007.06.014

Carver, C. S., & Harmon-Jones, E. (2009). Anger is an approach-related affect: evidence and implications. *Psychological Bulletin*, *135*(2), 183–204. doi:10.1037/a0013965

Casey, B., Jones, R. M., & Somerville, L. H. (2011). Braking and Accelerating of the Adolescent Brain. *Journal of Research on Adolescence : The Official Journal of the Society for Research on Adolescence*, *21*(1), 21–33. doi:10.1111/j.1532-7795.2010.00712.x

Cash, S. J., & Bridge, J. A. (2009). Epidemiology of Youth Suicide and Suicidal Behavior. *Current Opinion in Pediatrics*, *21*(5), 613–619. doi:10.1097/MOP.0b013e32833063e1

Chantiluke, K., Barrett, N., Giampietro, V., Brammer, M., Simmons, A., Murphy, D. G., & Rubia, K. (2014). Inverse Effect of Fluoxetine on Medial Prefrontal Cortex Activation During Reward Reversal in ADHD and Autism. *Cerebral Cortex (New York, N.Y.: 1991)*. doi:10.1093/cercor/bht365

Chantiluke, K., Halari, R., Simic, M., Pariante, C. M., Papadopoulos, A., Giampietro, V., & Rubia, K. (2012). Fronto-striato-cerebellar dysregulation in adolescents with depression during motivated attention. *Biological Psychiatry*, *71*(1), 59–67. doi:10.1016/j.biopsych.2011.09.005

Chapman, H. R., & Kirby-Turner, N. (2002). Visual/verbal analogue scales: Examples of brief assessment methods to aid management of child and adult patients in clinical practice. *British Dental Journal*, *193*(8), 447–450. doi:10.1038/sj.bdj.4801593

Chen, H.-H., Rosenberg, D. R., MacMaster, F. P., Easter, P. C., Caetano, S. C., Nicoletti, M., … Soares, J. C. (2008). Orbitofrontal cortex volumes in medication naïve children with major depressive disorder: a magnetic resonance imaging study. *Journal of Child and Adolescent Psychopharmacology*, *18*(6), 551–556. doi:10.1089/cap.2008.053

Chetty, S., Friedman, A. R., Taravosh-Lahn, K., Kirby, E. D., Mirescu, C., Guo, F., … Kaufer, D. (2014). Stress and glucocorticoids promote oligodendrogenesis in the adult hippocampus. Molecular Psychiatry, 19(12), 1275–1283. http://doi.org/10.1038/mp.2013.190

Cheung, A. H., Dewa, C. S., & Levitt, A. J. (2005). Clinical review of mania, hostility and suicide-related events in children and adolescents treated with antidepressants. *Paediatrics & Child Health*, *10*(8), 457–463.

Cisler, J. M., & Koster, E. H. W. (2010). Mechanisms of Attentional Biases towards Threat in the Anxiety Disorders: An Integrative Review. *Clinical Psychology Review*, *30*(2), 203. doi:10.1016/j.cpr.2009.11.003

Clark, D. A., & Beck, A. T. (2010). Cognitive theory and therapy of anxiety and depression: Convergence with neurobiological findings. *Trends in Cognitive Sciences*, *14*(9), 418–424. doi:10.1016/j.tics.2010.06.007

Clark, D. B., Birmaher, B., Axelson, D., Monk, K., Kalas, C., Ehmann, M., … Brent, D. (2005). Fluoxetine for the treatment of childhood anxiety disorders: open-label, long-term extension to a controlled trial. *Journal of the American Academy of Child and Adolescent Psychiatry*, *44*(12), 1263–1270. doi:10.1097/01.chi.0000183464.41777.c1

Cleare, A. J., & Bond, A. J. (1995). The effect of tryptophan depletion and enhancement on subjective and behavioural aggression in normal male subjects. *Psychopharmacology*, 118(1), 72–81.

Coccaro, E. F., & Kavoussi, R. J. (1997). Fluoxetine and impulsive aggressive behavior in personality-disordered subjects. *Archives of General Psychiatry*, *54*(12), 1081–1088.

Coccaro, E. F., Lee, R. J., & Kavoussi, R. J. (2009). A double-blind, randomized, placebo-controlled trial of fluoxetine in patients with intermittent explosive disorder. *The Journal of Clinical Psychiatry*, *70*(5), 653–662. doi:10.4088/JCP.08m04150

Coccaro, E. F., McCloskey, M. S., Fitzgerald, D. A., & Phan, K. L. (2007). Amygdala and orbitofrontal reactivity to social threat in individuals with impulsive aggression. *Biological Psychiatry*, *62*(2), 168–178. doi:10.1016/j.biopsych.2006.08.024

Cohen, D. J., Eckhardt, C. I., & Schagat, K. D. (1998). Attention allocation and habituation to anger-related stimuli during a visual search task. *Aggressive Behavior*, *24*(6), 399–409. doi:10.1002/(SICI)1098-2337(1998)24:6<399::AID-AB1>3.0.CO;2-I

Coltheart, M. (1981). The MRC psycholinguistic database. *The Quarterly Journal of Experimental Psychology Section A*, *33*(4), 497–505. doi:10.1080/14640748108400805

Cowen, P. J. (2008). Serotonin and depression: pathophysiological mechanism or marketing myth? *Trends in Pharmacological Sciences*, *29*(9), 433–436. doi:10.1016/j.tips.2008.05.004

Crawford, J. R., & Henry, J. D. (2004). The Positive and Negative Affect Schedule (PANAS): Construct validity, measurement properties and normative data in a large non-clinical sample. *British Journal of Clinical Psychology*, *43*(3), 245–265. doi:10.1348/0144665031752934

Crick, N. R., & Dodge, K. A. (1994). A review and reformulation of social information-processing mechanisms in children's social adjustment. *Psychological Bulletin*, *115*(1), 74–101. doi:10.1037/0033-2909.115.1.74

Critchley, H., Daly, E., Phillips, M., Brammer, M., Bullmore, E., Williams, S., … Murphy, D. (2000). Explicit and implicit neural mechanisms for processing of social information from facial expressions: a functional magnetic resonance imaging study. *Human Brain Mapping*, *9*(2), 93–105.

Crowe, M., Ward, N., Dunnachie, B., & Roberts, M. (2006). Characteristics of adolescent depression. *International Journal of Mental Health Nursing*, *15*(1), 10–18. doi:10.1111/j.1447-0349.2006.00399.x

Dalgleish, T., Taghavi, R., Neshat-Doost, H., Moradi, A., Canterbury, R., & Yule, W. (2003). Patterns of processing bias for emotional information across clinical disorders: a comparison of attention, memory, and prospective cognition in children and adolescents with depression, generalized anxiety, and posttraumatic stress disorder. *Journal of Clinical Child and Adolescent Psychology: The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*, *32*(1), 10–21. doi:10.1207/S15374424JCCP3201_02

Datla, K. P., Mitra, S. K., & Bhattacharya, S. K. (1991). Serotonergic modulation of footshock induced aggression in paired rats. *Indian Journal of Experimental Biology*, *29*(7), 631–635.

David, D. J., Samuels, B. A., Rainer, Q., Wang, J.-W., Marsteller, D., Mendez, I., … Hen, R. (2009). Behavioral effects of fluoxetine in an animal model of anxiety/depression are mediated by

both neurogenisis-dependent and independent mechanisms. *Neuron*, *62*(4), 479–493. doi:10.1016/j.neuron.2009.04.017

Davis, M., Falls, W. A., Campeau, S., & Kim, M. (1993). Fear-potentiated startle: a neural and pharmacological analysis. *Behavioural Brain Research*, *58*(1-2), 175–198.

Deffenbacher, J. L., Oetting, E. R., Lynch, R. S., & Morris, C. D. (1996). The expression of anger and its consequences. *Behaviour Research and Therapy*, *34*(7), 575–590. doi:10.1016/0005-7967(96)00018-6

Dekeyne, A., Mannoury la Cour, C., Gobert, A., Brocco, M., Lejeune, F., Serres, F., … Millan, M. J. (2008). S32006, a novel 5-HT2C receptor antagonist displaying broad-based antidepressant and anxiolytic properties in rodent models. *Psychopharmacology*, *199*(4), 549–568. doi:10.1007/s00213-008-1177-9

De Martino, B., Strange, B. A., & Dolan, R. J. (2008). Noradrenergic neuromodulation of human attention for emotional and neutral stimuli. *Psychopharmacology*, *197*(1), 127–136. doi:10.1007/s00213-007-1015-5

Di Simplicio, M., Costoloni, G., Western, D., Hanson, B., Taggart, P., & Harmer, C. J. (2012). Decreased heart rate variability during emotion regulation in subjects at risk for psychopathology. *Psychological Medicine*, *42*(8), 1775–1783. doi:10.1017/S0033291711002479

Di Simplicio, M., Doallo, S., Costoloni, G., Rohenkohl, G., Nobre, A. C., & Harmer, C. J. (2014). “Can you look me in the face?” Short-term SSRI Administration Reverts Avoidant Ocular Face Exploration in Subjects at Risk for Psychopathology. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*. doi:10.1038/npp.2014.159

Di Simplicio, M., Norbury, R., & Harmer, C. J. (2012). Short-term antidepressant administration reduces negative self-referential processing in the medial prefrontal cortex in subjects at risk for depression. *Molecular Psychiatry*, *17*(5), 503–510. doi:10.1038/mp.2011.16

Di Simplicio, M., Norbury, R., Reinecke, A., & Harmer, C. J. (2014). Paradoxical effects of short-term antidepressant treatment in fMRI emotional processing models in volunteers with high neuroticism. *Psychological Medicine*, *44*(2), 241–252. doi:10.1017/S0033291713000731

Dodge, K. A. (1980). Social cognition and children's aggressive behavior. *Child Development*, *51*(1), 162–170.

Dodge, K. A., Price, J. M., Bachorowski, J. A., & Newman, J. P. (1990). Hostile attributional biases in severely aggressive adolescents. *Journal of Abnormal Psychology*, *99*(4), 385–392.

Donovan, S. J., Nunes, E. V., Stewart, J. W., Ross, D., Quitkin, F. M., Jensen, P. S., & Klein, D. F. (2003). "Outer-directed irritability": a distinct mood syndrome in explosive youth with a disruptive behavior disorder? *The Journal of Clinical Psychiatry*, *64*(6), 698–701.

Doty, T. J., Japee, S., Ingvar, M., & Ungerleider, L. G. (2013). Fearful face detection sensitivity in healthy adults correlates with anxiety-related traits. *Emotion*, *13*(2), 183–188. doi:10.1037/a0031373

Dougherty, L. R., Smith, V. C., Bufferd, S. J., Stringaris, A., Leibenluft, E., Carlson, G. A., & Klein, D. N. (2013). Preschool Irritability: Longitudinal Associations With Psychiatric Disorders at Age 6 and Parental Psychopathology. *Journal of the American Academy of Child and Adolescent Psychiatry*, *52*(12), 1304–1313. doi:10.1016/j.jaac.2013.09.007

Drevets, W. C. (2007). Orbitofrontal Cortex Function and Structure in Depression. *Annals of the New York Academy of Sciences*, *1121*(1), 499–527. doi:10.1196/annals.1401.029

Dulawa, S. C., Holick, K. A., Gundersen, B., & Hen, R. (2004). Effects of Chronic Fluoxetine in Animal Models of Anxiety and Depression. *Neuropsychopharmacology*, *29*(7), 1321–1330. doi:10.1038/sj.npp.1300433

Eckhardt, C. I., & Cohen, D. J. (1997). Attention to anger-relevant and irrelevant stimuli following naturalistic insult. *Personality and Individual Differences*, *23*(4), 619–629. doi:10.1016/S0191-8869(97)00074-3

Eizenman, M., Yu, L. H., Grupp, L., Eizenman, E., Ellenbogen, M., Gemar, M., & Levitan, R. D. (2003). A naturalistic visual scanning approach to assess selective attention in major depressive disorder. *Psychiatry Research*, 118(2), 117–128.

Ekman, P., & Friesen, W. V. (1976). *Pictures of Facial Affect*. Consulting Psychologists Press.

Emslie, G. J., Heiligenstein, J. H., Wagner, K. D., Hoog, S. L., Ernest, D. E., Brown, E., … Jacobson, J. G. (2002). Fluoxetine for acute treatment of depression in children and adolescents: a placebo-controlled, randomized clinical trial. *Journal of the American*

Academy of Child and Adolescent Psychiatry, *41*(10), 1205–1215. doi:10.1097/00004583-200210000-00010

Emslie, G. J., Rush, A. J., Weinberg, W. A., Kowatch, R. A., Hughes, C. W., Carmody, T., & Rintelmann, J. (1997). A double-blind, randomized, placebo-controlled trial of fluoxetine in children and adolescents with depression. *Archives of General Psychiatry*, *54*(11), 1031–1037.

Ernst, M., Pine, D. S., & Hardin, M. (2006). Triadic model of the neurobiology of motivated behavior in adolescence. *Psychological Medicine*, *36*(3), 299–312. doi:10.1017/S0033291705005891

Eshel, N., Nelson, E. E., Blair, J., Pine, D. S., & Ernst, M. (2007). Neural substrates of choice selection in adults and adolescents: development of the ventrolateral prefrontal and anterior cingulate cortices. *Neuropsychologia*, *45*(6), 1270–1279. doi:10.1016/j.neuropsychologia.2006.10.004

Etkin, A., & Cuthbert, B. (2014). Beyond the DSM: development of a transdiagnostic psychiatric neuroscience course. *Academic Psychiatry: The Journal of the American Association of Directors of Psychiatric Residency Training and the Association for Academic Psychiatry*, *38*(2), 145–150. doi:10.1007/s40596-013-0032-4

Eysenck, HJ, & Eysenck BJ. (1975). *Eysenck Personality Inventory* (Hodderand Stoughton.). London.

Fairburn, C. G., Cooper, Z., & Shafran, R. (2003). Cognitive behaviour therapy for eating disorders: a “transdiagnostic” theory and treatment. *Behaviour Research and Therapy*, *41*(5), 509–528.

Fava, M., Anderson, K., & Rosenbaum, J. F. (1990). “Anger attacks”: possible variants of panic and major depressive disorders. *The American Journal of Psychiatry*, 147(7), 867–870.

Fava, M., Hwang, I., Rush, A. J., Sampson, N., Walters, E. E., & Kessler, R. C. (2010). The importance of irritability as a symptom of major depressive disorder: results from the National Comorbidity Survey Replication. *Molecular Psychiatry*, *15*(8), 856–867. doi:10.1038/mp.2009.20

Fava, M., Rosenbaum, J. F., McCarthy, M., Pava, J., Steingard, R., & Bless, E. (1991). Anger attacks in depressed outpatients and their response to fluoxetine. *Psychopharmacology Bulletin*, *27*(3), 275–279.

Field, D. J., & Brady, N. (1997). Visual sensitivity, blur and the sources of variability in the amplitude spectra of natural scenes. *Vision Research*, *37*(23), 3367–3383.

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1997). *Structured Clinical Interview for DSM–IV Axis I Disorders (SCID–I)*. American Psychiatric Press: Washington, DC.

First, M. B. (1997). *User's Guide for the Structured Clinical Interview for DSM-IV Axis II Personality Disorders: SCID-II*. American Psychiatric Pub.

Forkmann, T., Scherer, A., Boecker, M., Pawelzik, M., Jostes, R., & Gauggel, S. (2011). The clinical global impression scale and the influence of patient or staff perspective on outcome. *BMC Psychiatry*, *11*(1), 83. doi:10.1186/1471-244X-11-83

Fox, E. (2002). Processing emotional facial expressions: the role of anxiety and awareness. *Cognitive, Affective & Behavioral Neuroscience*, 2(1), 52–63.

Francis, W., & Kučera, H. (1982). *Frequency analysis of English usage: Lexicon and grammar.* Boston: Houghton Mifflin.

Franklin, J. C., Jamieson, J. P., Glenn, C. R., & Nock, M. K. (2014). How Developmental Psychopathology Theory and Research Can Inform the Research Domain Criteria (RDoC) Project. *Journal of Clinical Child and Adolescent Psychology: The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*. doi:10.1080/15374416.2013.873981

Friston, K. J., Worsley, K. J., Frackowiak, R. S. J., Mazziotta, J. C., & Evans, A. C. (1994). Assessing the significance of focal activations using their spatial extent. *Human Brain Mapping*, 1(3), 210–220. http://doi.org/10.1002/hbm.460010306

Fu, C. H. Y., Williams, S. C. R., Cleare, A. J., Brammer, M. J., Walsh, N. D., Kim, J., … Bullmore, E. T. (2004). Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. *Archives of General Psychiatry*, *61*(9), 877–889. doi:10.1001/archpsyc.61.9.877

Fuster, J. M. (2002). Frontal lobe and cognitive development. *Journal of Neurocytology*, *31*(3-5), 373–385.

Gaffrey, M. S., Luby, J. L., Belden, A. C., Hirshberg, J. S., Volsch, J., & Barch, D. M. (2011). Association between depression severity and amygdala reactivity during sad face viewing in depressed preschoolers: an fMRI study. *Journal of Affective Disorders*, *129*(1-3), 364–370. doi:10.1016/j.jad.2010.08.031

Garland, E. J., Kutcher, S., & Virani, A. (2009). 2008 Position Paper on Using SSRIs in Children and Adolescents. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *18*(2), 160–165.

Gaspar, P., Cases, O., & Maroteaux, L. (2003). The developmental role of serotonin: news from mouse molecular genetics. *Nature Reviews. Neuroscience*, *4*(12), 1002–1012. doi:10.1038/nrn1256

Gee, D. G., Humphreys, K. L., Flannery, J., Goff, B., Telzer, E. H., Shapiro, M., … Tottenham, N. (2013). A developmental shift from positive to negative connectivity in human amygdala-prefrontal circuitry. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(10), 4584–4593. doi:10.1523/JNEUROSCI.3446-12.2013

Geisler, W. S. (2008). Visual Perception and the Statistical Properties of Natural Scenes. *Annual Review of Psychology*, *59*(1), 167–192. doi:10.1146/annurev.psych.58.110405.085632

Gibbons, R. D., Brown, C. H., Hur, K., Marcus, S. M., Bhaumik, D. K., Erkens, J. A., … Mann, J. J. (2007). Early evidence on the effects of regulators' suicidality warnings on SSRI prescriptions and suicide in children and adolescents. *The American Journal of Psychiatry*, *164*(9), 1356–1363. doi:10.1176/appi.ajp.2007.07030454

Gibbons, R. D., Hur, K., Bhaumik, D. K., & Mann, J. J. (2006). The relationship between antidepressant prescription rates and rate of early adolescent suicide. *The American Journal of Psychiatry*, *163*(11), 1898–1904. doi:10.1176/appi.ajp.163.11.1898

Gibbons, R. D., Hur, K., Brown, C. H., Davis, J. M., & Mann, J. J. (2012). Benefits from antidepressants: synthesis of 6-week patient-level outcomes from double-blind placebo-controlled randomized trials of fluoxetine and venlafaxine. *Archives of General Psychiatry*, *69*(6), 572–579. doi:10.1001/archgenpsychiatry.2011.2044

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., … Rapoport, J. L. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience*, *2*(10), 861–863. doi:10.1038/13158

Giedd, J. N., Keshavan, M., & Paus, T. (2008). Why do many psychiatric disorders emerge during adolescence? *Nature Reviews. Neuroscience*, *9*(12), 947–957. doi:10.1038/nrn2513

Giedd, J. N., & Rapoport, J. L. (2010). Structural MRI of Pediatric Brain Development: What Have We Learned and Where Are We Going? *Neuron*, *67*(5), 728–734. doi:10.1016/j.neuron.2010.08.040

Giersch, A., Boucart, M., Speeg-Schatz, C., Muller-Kauffmann, F., & Danion, J. M. (1996). Lorazepam impairs perceptual integration of visual forms: a central effect. *Psychopharmacology*, *126*(3), 260–270.

Giersch, A., & Herzog, M. H. (2004). Lorazepam strongly prolongs visual information processing. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *29*(7), 1386–1394. doi:10.1038/sj.npp.1300429

Gläscher, J., & Adolphs, R. (2003). Processing of the arousal of subliminal and supraliminal emotional stimuli by the human amygdala. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *23*(32), 10274–10282.

Glaser, K. (1967). Masked depression in children and adolescents. *American Journal of Psychotherapy*, *21*(3), 565–574.

Goddings, A.-L., Burnett Heyes, S., Bird, G., Viner, R. M., & Blakemore, S.-J. (2012). The relationship between puberty and social emotion processing. *Developmental Science*, *15*(6), 801–811. doi:10.1111/j.1467-7687.2012.01174.x

Godlewska, B. R., Norbury, R., Selvaraj, S., Cowen, P. J., & Harmer, C. J. (2012). Short-term SSRI treatment normalises amygdala hyperactivity in depressed patients. *Psychological Medicine*, *42*(12), 2609–2617. doi:10.1017/S0033291712000591

Godlewska, B. R., Browning, B., Norbury, R., Cowen, P. J., Harmer, C. J. (submitted for publication). Early changes in neural response to emotional stimuli predict clinical response to SSRI treatment in depression.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., … Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through

early adulthood. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(21), 8174–8179. doi:10.1073/pnas.0402680101

Goodman, W. K., Murphy, T. K., & Storch, E. A. (2007). Risk of adverse behavioral effects with pediatric use of antidepressants. *Psychopharmacology*, *191*(1), 87–96. doi:10.1007/s00213-006-0642-6

Goodyer, I., Dubicka, B., Wilkinson, P., Kelvin, R., Roberts, C., Byford, S., … Harrington, R. (2007). Selective serotonin reuptake inhibitors (SSRIs) and routine specialist care with and without cognitive behaviour therapy in adolescents with major depression: randomised controlled trial. *BMJ*, *335*(7611), 142. doi:10.1136/bmj.39224.494340.55

Gotlib, I. H., & Joormann, J. (2010). Cognition and Depression: Current Status and Future Directions. *Annual Review of Clinical Psychology*, *6*, 285–312. doi:10.1146/annurev.clinpsy.121208.131305

Gotlib, I. H., McLachlan, A. L., & Katz, A. N. (1988). Biases in Visual Attention in Depressed and Nondepressed Individuals. *Cognition & Emotion*, *2*(3), 185–200. doi:10.1080/02699938808410923

Gray, K. L. H., Adams, W. J., & Garner, M. (2009). The influence of anxiety on the initial selection of emotional faces presented in binocular rivalry. *Cognition*, *113*(1), 105–110. doi:10.1016/j.cognition.2009.06.009

Gray, K. L. H., Adams, W. J., Hedger, N., Newton, K. E., & Garner, M. (2013). Faces and Awareness: Low-Level, Not Emotional Factors Determine Perceptual Dominance. *Emotion (Washington, D.C.)*. doi:10.1037/a0031403

Grillon, C., Levenson, J., & Pine, D. S. (2007). A single dose of the selective serotonin reuptake inhibitor citalopram exacerbates anxiety in humans: a fear-potentiated startle study. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *32*(1), 225–231. doi:10.1038/sj.npp.1301204

Gur, R. C., Erwin, R. J., Gur, R. E., Zwil, A. S., Heimberg, C., & Kraemer, H. C. (1992). Facial emotion discrimination: II. Behavioral findings in depression. *Psychiatry Research*, 42(3), 241–251.

Guyer, A. E., Lau, J. Y. F., McClure-Tone, E. B., Parrish, J., Shiffrin, N. D., Reynolds, R. C., … Nelson, E. E. (2008). Amygdala and ventrolateral prefrontal cortex function during

anticipated peer evaluation in pediatric social anxiety. *Archives of General Psychiatry*, *65*(11), 1303–1312. doi:10.1001/archpsyc.65.11.1303

Guyer, A. E., Monk, C. S., McClure-Tone, E. B., Nelson, E. E., Roberson-Nay, R., Adler, A. D., … Ernst, M. (2008). A developmental examination of amygdala response to facial expressions. *Journal of Cognitive Neuroscience*, *20*(9), 1565–1582. doi:10.1162/jocn.2008.20114

Guy, W., & National Institute of Mental Health (U. S.). Psychopharmacology Research Branch. Division of Extramural Research Programs. (1976). *ECDEU assessment manual for psychopharmacology*. Rockville, Md. : U.S. Dept. of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs. Retrieved from http://archive.org/details/ecdeuassessmentm1933guyw

Hair, J. F., Anderson, R. E., Tatham, R. L., & Black, W. C. (1995). *Multivariate Data Analysis with Readings*. Prentice Hall.

Halari, R., Simic, M., Pariante, C. M., Papadopoulos, A., Cleare, A., Brammer, M., … Rubia, K. (2009). Reduced activation in lateral prefrontal cortex and anterior cingulate during attention and cognitive control functions in medication-naïve adolescents with depression compared to controls. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *50*(3), 307–316. doi:10.1111/j.1469-7610.2008.01972.x

Hall, C. W. (2006). Self-Reported Aggression and the Perception of Anger in Facial Expression Photos. *Journal of Psychology*, *140*(3), 255–267.

Hall, L. M. J., Klimes-Dougan, B., H. Hunt, R., M. Thomas, K., Houri, A., Noack, E., … R. Cullen, K. (2014). An fMRI study of emotional face processing in adolescent major depression. *Journal of Affective Disorders*, *168*, 44–50. doi:10.1016/j.jad.2014.06.037

Hamilton, J. P., Etkin, A., Furman, D. J., Lemus, M. G., Johnson, R. F., & Gotlib, I. H. (2012). Functional Neuroimaging of Major Depressive Disorder: A Meta-Analysis and New Integration of Baseline Activation and Neural Response Data. *American Journal of Psychiatry*, *169*(7), 693–703. doi:10.1176/appi.ajp.2012.11071105

Hammad, T. A., Laughren, T., & Racoosin, J. (2006). Suicidality in pediatric patients treated with antidepressant drugs. *Archives of General Psychiatry*, *63*(3), 332–339. doi:10.1001/archpsyc.63.3.332

Handley, S. L., & McBlane, J. W. (1992). Opposite effects of fluoxetine in 2 animal-models of anxiety. In British Journal of Pharmacology (Vol. 107, pp. P446–P446). Wiley-Blackwell Commerce Place, 350 main street, Malden 02148, MA USA.

Hankin, B. L., Abramson, L. Y., Moffitt, T. E., Silva, P. A., McGee, R., & Angell, K. E. (1998). Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study. *Journal of Abnormal Psychology*, *107*(1), 128–140.

Hankin, B. L., Gibb, B. E., Abela, J. R. Z., & Flory, K. (2010). Selective attention to affective stimuli and clinical depression among youths: role of anxiety and specificity of emotion. *Journal of Abnormal Psychology*, *119*(3), 491–501. doi:10.1037/a0019609

Hansen, H. H., Sánchez, C., & Meier, E. (1997). Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immobility in adult rats: a putative animal model of depression? *The Journal of Pharmacology and Experimental Therapeutics, 283*(3), 1333–1341.

Hare, T. A., Tottenham, N., Galvan, A., Voss, H. U., Glover, G. H., & Casey, B. J. (2008). Biological substrates of emotional reactivity and regulation in adolescence during an emotional go-nogo task. *Biological Psychiatry*, *63*(10), 927–934. doi:10.1016/j.biopsych.2008.03.015

Hariri, A. R., Bookheimer, S. Y., & Mazziotta, J. C. (2000). Modulating emotional responses: effects of a neocortical network on the limbic system. *Neuroreport*, *11*(1), 43–48.

Hariri, A. R., Mattay, V. S., Tessitore, A., Fera, F., & Weinberger, D. R. (2003). Neocortical modulation of the amygdala response to fearful stimuli. *Biological Psychiatry*, *53*(6), 494–501.

Harmer, C. J. (2013). Emotional processing and antidepressant action. *Current Topics in Behavioral Neurosciences*, *14*, 209–222. doi:10.1007/7854_2012_210

Harmer, C. J., Bhagwagar, Z., Perrett, D. I., Völlm, B. A., Cowen, P. J., & Goodwin, G. M. (2003). Acute SSRI administration affects the processing of social cues in healthy volunteers.

Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, *28*(1), 148–152. doi:10.1038/sj.npp.1300004

Harmer, C. J., & Cowen, P. J. (2013). “It”s the way that you look at it’--a cognitive neuropsychological account of SSRI action in depression. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *368*(1615), 20120407. doi:10.1098/rstb.2012.0407

Harmer, C. J., Cowen, P. J., & Goodwin, G. M. (2011). Efficacy markers in depression. *Journal of Psychopharmacology (Oxford, England)*, *25*(9), 1148–1158. doi:10.1177/0269881110367722

Harmer, C. J., de Bodinat, C., Dawson, G. R., Dourish, C. T., Waldenmaier, L., Adams, S., … Goodwin, G. M. (2011). Agomelatine facilitates positive versus negative affective processing in healthy volunteer models. *Journal of Psychopharmacology (Oxford, England)*, *25*(9), 1159–1167. doi:10.1177/0269881110376689

Harmer, C. J., Goodwin, G. M., & Cowen, P. J. (2009). Why do antidepressants take so long to work? A cognitive neuropsychological model of antidepressant drug action. *The British Journal of Psychiatry: The Journal of Mental Science*, *195*(2), 102–108. doi:10.1192/bjp.bp.108.051193

Harmer, C. J., Heinzen, J., O’Sullivan, U., Ayres, R. A., & Cowen, P. J. (2008). Dissociable effects of acute antidepressant drug administration on subjective and emotional processing measures in healthy volunteers. *Psychopharmacology*, *199*(4), 495–502. doi:10.1007/s00213-007-1058-7

Harmer, C. J., Hill, S. A., Taylor, M. J., Cowen, P. J., & Goodwin, G. M. (2003). Toward a neuropsychological theory of antidepressant drug action: increase in positive emotional bias after potentiation of norepinephrine activity. *The American Journal of Psychiatry*, *160*(5), 990–992.

Harmer, C. J., Mackay, C. E., Reid, C. B., Cowen, P. J., & Goodwin, G. M. (2006). Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. *Biological Psychiatry*, *59*(9), 816–820. doi:10.1016/j.biopsych.2005.10.015

Harmer, C. J., O’Sullivan, U., Favaron, E., Massey-Chase, R., Ayres, R., Reinecke, A., … Cowen, P. J. (2009). Effect of acute antidepressant administration on negative affective bias in

depressed patients. *The American Journal of Psychiatry*, *166*(10), 1178–1184. doi:10.1176/appi.ajp.2009.09020149

Harmer, C. J., Shelley, N. C., Cowen, P. J., & Goodwin, G. M. (2004). Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *The American Journal of Psychiatry*, *161*(7), 1256–1263.

Harmon-Jones, E., & Sigelman, J. (2001). State anger and prefrontal brain activity: evidence that insult-related relative left-prefrontal activation is associated with experienced anger and aggression. *Journal of Personality and Social Psychology*, *80*(5), 797–803.

Harper, M. S., Dickson, J. W., & Welsh, D. P. (2006). Self-Silencing and Rejection Sensitivity in Adolescent Romantic Relationships. *Journal of Youth and Adolescence*, *35*(3), 435–443. doi:10.1007/s10964-006-9048-3

Hazell, P., O'Connell, D., Heathcote, D., Robertson, J., & Henry, D. (1995). Efficacy of tricyclic drugs in treating child and adolescent depression: a meta-analysis. *BMJ*, *310*(6984), 897–901. doi:10.1136/bmj.310.6984.897

Hensher, D. A., Rose, J. M., & Greene, W. H. (2005). *Applied Choice Analysis: A Primer*. Cambridge University Press.

Hetrick, S. E., McKenzie, J. E., Cox, G. R., Simmons, M. B., & Merry, S. N. (2012). Newer generation antidepressants for depressive disorders in children and adolescents. *The Cochrane Database of Systematic Reviews*, *11*, CD004851. doi:10.1002/14651858.CD004851.pub3

Hoffman, B. J., Mezey, E., & Brownstein, M. J. (1991). Cloning of a serotonin transporter affected by antidepressants. *Science*, *254*(5031), 579–580. doi:10.1126/science.1948036

Homberg, J. R., Schubert, D., & Gaspar, P. (2010). New perspectives on the neurodevelopmental effects of SSRIs. *Trends in Pharmacological Sciences*, *31*(2), 60–65. doi:10.1016/j.tips.2009.11.003

Honk, J. V., Tuiten, A., de Haan, E., vann de Hout, M., & Stam, H. (2001). Attentional biases for angry faces: Relationships to trait anger and anxiety. *Cognition & Emotion*, *15*(3), 279–297. doi:10.1080/02699930126112

Huang, G.-J., Bannerman, D., & Flint, J. (2008). Chronic fluoxetine treatment alters behavior, but not adult hippocampal neurogenesis, in BALB/cJ mice. *Molecular Psychiatry*, *13*(2), 119–121. doi:10.1038/sj.mp.4002104

Huesmann, L. R. (1988). An information processing model for the development of aggression. *Aggressive Behaviour, 14, 13-24.*

Hyde, J. S., Mezulis, A. H., & Abramson, L. Y. (2008). The ABCs of depression: integrating affective, biological, and cognitive models to explain the emergence of the gender difference in depression. *Psychological Review*, *115*(2), 291–313. doi:10.1037/0033-295X.115.2.291

Iannetti, G. D., & Wise, R. G. (2007). BOLD functional MRI in disease and pharmacological studies: room for improvement? *Magnetic Resonance Imaging*, *25*(6), 978–988. doi:10.1016/j.mri.2007.03.018

Immordino-Yang, M. H., & Singh, V. (2013). Hippocampal contributions to the processing of social emotions. *Human Brain Mapping*, *34*(4), 945–955. doi:10.1002/hbm.21485

Ingram, R. E. & Luxton, D. (2005). Vulnerability-stress models. In B. L. Hankin & J. R. Z. Abela(Eds.). Development of psychopathology: A vulnerability-stress perspective. New York: Sage.

Iñiguez, S. D., Warren, B. L., & Bolaños-Guzmán, C. A. (2010). Short- and long-term functional consequences of fluoxetine exposure during adolescence in male rats. *Biological Psychiatry*, *67*(11), 1057–1066. doi:10.1016/j.biopsych.2009.12.033

Jenkinson, M., Bannister, P., Brady, M., & Smith, S. (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage*, *17*(2), 825–841.

Jiang, Y., Costello, P., Fang, F., Huang, M., & He, S. (2006). A gender- and sexual orientation-dependent spatial attentional effect of invisible images. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(45), 17048–17052. doi:10.1073/pnas.0605678103

Jiang, Y., Costello, P., & He, S. (2007). Processing of invisible stimuli: advantage of upright faces and recognizable words in overcoming interocular suppression. *Psychological Science*, *18*(4), 349–355. doi:10.1111/j.1467-9280.2007.01902.x

Jiang, Y., & He, S. (2006). Cortical responses to invisible faces: dissociating subsystems for facial-information processing. *Current Biology: CB*, *16*(20), 2023–2029. doi:10.1016/j.cub.2006.08.084

Jonassen, R., Chelnokova, O., Harmer, C., Leknes, S., & Landrø, N. I. (2014). A single dose of antidepressant alters eye-gaze patterns across face stimuli in healthy women. *Psychopharmacology*. doi:10.1007/s00213-014-3729-5

Joormann, J., Talbot, L., & Gotlib, I. H. (2007). Biased processing of emotional information in girls at risk for depression. *Journal of Abnormal Psychology*, *116*(1), 135–143. doi:10.1037/0021-843X.116.1.135

Judd, L. L., Schettler, P. J., Coryell, W., Akiskal, H. S., & Fiedorowicz, J. G. (2013). Overt irritability/anger in unipolar major depressive episodes: past and current characteristics and implications for long-term course. *JAMA Psychiatry*, *70*(11), 1171–1180. doi:10.1001/jamapsychiatry.2013.1957

Jureidini, J. N., Doecke, C. J., Mansfield, P. R., Haby, M. M., Menkes, D. B., & Tonkin, A. L. (2004). Efficacy and safety of antidepressants for children and adolescents. *BMJ (Clinical Research Ed.)*, *328*(7444), 879–883. doi:10.1136/bmj.328.7444.879

Kaufman, J., Birmaher, B., Brent, D. A., Ryan, N. D., & Rao, U. (2000). K-SADS-PL. *Journal of the American Academy of Child and Adolescent Psychiatry*, *39*(10), 1208. doi:10.1097/00004583-200010000-00002

Kelvin, R. G., Goodyer, I. M., Teasdale, J. D., & Brechin, D. (1999). Latent negative self-schema and high emotionality in well adolescents at risk for psychopathology. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, 40(6), 959–968.

Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., & Gatt, J. M. (2010). Impact of Depression and Antidepressant Treatment on Heart Rate Variability: A Review and Meta-Analysis. *Biological Psychiatry*, 67(11), 1067–1074. http://doi.org/10.1016/j.biopsych.2009.12.012

Kemp, A. H., Quintana, D. S., & Malhi, G. S. (2011). Effects of serotonin reuptake inhibitors on heart rate variability: methodological issues, medical comorbidity, and clinical relevance. *Biological Psychiatry*, 69(8), e25–26; author reply e27–28. http://doi.org/10.1016/j.biopsych.2010.10.035

Kennett, G. A., Wood, M. D., Bright, F., Trail, B., Riley, G., Holland, V., … Blackburn, T. P. (1997). SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. *Neuropharmacology*, *36*(4-5), 609–620.

Kent, J. M., Coplan, J. D., & Gorman, J. M. (1998). Clinical utility of the selective serotonin reuptake inhibitors in the spectrum of anxiety. *Biological Psychiatry*, *44*(9), 812–824.

Kerestes, R., Davey, C. G., Stephanou, K., Whittle, S., & Harrison, B. J. (2014). Functional brain imaging studies of youth depression: A systematic review. *NeuroImage: Clinical*, *4*, 209–231. doi:10.1016/j.nicl.2013.11.009

Kim, C.-Y., & Blake, R. (2005). Psychophysical magic: rendering the visible “invisible.” *Trends in Cognitive Sciences*, *9*(8), 381–388. doi:10.1016/j.tics.2005.06.012

Kim, H., Somerville, L. H., Johnstone, T., Polis, S., Alexander, A. L., Shin, L. M., & Whalen, P. J. (2004). Contextual modulation of amygdala responsivity to surprised faces. Journal of Cognitive Neuroscience, 16(10), 1730–1745. http://doi.org/10.1162/0898929042947865

Kim, P., Thomas, L. A., Rosen, B. H., Moscicki, A. M., Brotman, M. A., Zarate, C. A., … Leibenluft, E. (2012). Differing amygdala responses to facial expressions in children and adults with bipolar disorder. *The American Journal of Psychiatry*, *169*(6), 642–649. doi:10.1176/appi.ajp.2012.11081245

King, R. A., Riddle, M. A., Chappell, P. B., Hardin, M. T., Anderson, G. M., Lombroso, P., & Scahill, L. (1991). Emergence of Self-Destructive Phenomena in Children and Adolescents during Fluoxetine Treatment. *Journal of the American Academy of Child and Adolescent Psychiatry*, *30*(2), 179–186. doi:10.1097/00004583-199103000-00003

Kirisci, L., & Clark, D. B. (1996). Reliability and Validity of the State-Trait Anxiety Inventory for Children in an Adolescent Sample: Confirmatory Factor Analysis and Item Response Theory. Retrieved from http://eric.ed.gov/?id=ED400304

Kloep, M. (1999). Love is all you need? Focusing on adolescents’ life concerns from an ecological point of view. *Journal of Adolescence*, *22*(1), 49–63. doi:10.1006/jado.1998.0200

Ko, C.-H., Long, C.-Y., Chen, S.-Y., Chen, I.-J., Huang, T.-H., & Yen, J.-Y. (2013). Depression, irritability, and anxiety in women with premenstrual dysphoric disorder. *International Journal of Psychiatry in Medicine*, *46*(1), 39–55.

Kostowski, W., Valzelli, L., Kozak, W., & Bernasconi, S. (1984). Activity of desipramine, fluoxetine and nomifensine on spontaneous and p-CPA-induced muricidal aggression. *Pharmacological Research Communications*, *16*(3), 265–271.

Kovacs, M. (1992). *Children's Depression Inventory.* North Tonawanda, NY: Multi-Health Systems, Inc.

Kroeze, S., & van den Hout, M. A. (2000). Selective attention for cardiac information in panic patients. *Behaviour Research and Therapy*, *38*(1), 63–72. doi:10.1016/S0005-7967(99)00023-6

Kronenberg, S., Apter, A., Brent, D., Schirman, S., Melhem, N., Pick, N., … Weizman, A. (2007). Serotonin transporter polymorphism (5-HTTLPR) and citalopram effectiveness and side effects in children with depression and/or anxiety disorders. *Journal of Child and Adolescent Psychopharmacology*, *17*(6), 741–750. doi:10.1089/cap.2006.0144

Kučera, H., & Francis, W. N. (1967). *Computational Analysis of Present-Day American English*. Brown University Press.

Kurt, M., Arik, A. C., & Celik, S. (2000). The effects of sertraline and fluoxetine on anxiety in the elevated plus-maze test in mice. *Journal of Basic and Clinical Physiology and Pharmacology*, *11*(2), 173–180.

Lacerda, A. L. T., Keshavan, M. S., Hardan, A. Y., Yorbik, O., Brambilla, P., Sassi, R. B., … Soares, J. C. (2004). Anatomic evaluation of the orbitofrontal cortex in major depressive disorder. *Biological Psychiatry*, *55*(4), 353–358. doi:10.1016/j.biopsych.2003.08.021

Lakdawalla, Z., Hankin, B. L., & Mermelstein, R. (2007). Cognitive theories of depression in children and adolescents: a conceptual and quantitative review. *Clinical Child and Family Psychology Review*, *10*(1), 1–24. doi:10.1007/s10567-006-0013-1

Lange, K., Williams, L. M., Young, A. W., Bullmore, E. T., Brammer, M. J., Williams, S. C. R., … Phillips, M. L. (2003). Task instructions modulate neural responses to fearful facial expressions. *Biological Psychiatry*, *53*(3), 226–232.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1990). Emotion, attention, and the startle reflex. *Psychological Review*, *97*(3), 377–395.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2005). *International Affective Picture System (IAPS): Affective Ratings of Pictures and Instruction Manual*. NIMH, Center for the Study of Emotion & Attention.

Larkin, K. T., Martin, R. R., & McClain, S. E. (2002). Cynical hostility and the accuracy of decoding facial expressions of emotions. *Journal of Behavioral Medicine*, *25*(3), 285–292.

Laugesen, N., Dugas, M. J., & Bukowski, W. M. (2003). Understanding Adolescent Worry: The Application of a Cognitive Model. *Journal of Abnormal Child Psychology*, *31*(1), 55–64. doi:10.1023/A:1021721332181

Lau, J. Y., Britton, J. C., Nelson, E. E., Angold, A., Ernst, M., Goldwin, M., … Pine, D. S. (2011). Distinct neural signatures of threat learning in adolescents and adults. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(11), 4500–4505. doi:10.1073/pnas.1005494108

Lau, J. Y. F. (2013). Developmental aspects of mood disorders. *Current Topics in Behavioral Neurosciences*, *14*, 15–27. doi:10.1007/7854_2012_214

Lau, J. Y. F., & Eley, T. C. (2006). Changes in genetic and environmental influences on depressive symptoms across adolescence and young adulthood. *The British Journal of Psychiatry: The Journal of Mental Science*, *189*, 422–427. doi:10.1192/bjp.bp.105.018721

Lau, J. Y. F., Goldman, D., Buzas, B., Fromm, S. J., Guyer, A. E., Hodgkinson, C., … Ernst, M. (2009). Amygdala Function and 5-HTT Gene Variants in Adolescent Anxiety and Major Depressive Disorder. *Biological Psychiatry*, *65*(4), 349–355. doi:10.1016/j.biopsych.2008.08.037

Lau, J. Y. F., Guyer, A. E., Tone, E. B., Jenness, J., Parrish, J. M., Pine, D. S., & Nelson, E. E. (2012). Neural responses to peer rejection in anxious adolescents: Contributions from the amygdala-hippocampal complex. *International Journal of Behavioral Development*, *36*(1), 36–44. doi:10.1177/0165025411406854

Lefkowitz, M. M., & Burton, N. (1978). Childhood depression: a critique of the concept. *Psychological Bulletin*, *85*(4), 716–726.

Leibenluft, E. (2011). Severe mood dysregulation, irritability, and the diagnostic boundaries of bipolar disorder in youths. *The American Journal of Psychiatry*, *168*(2), 129–142. doi:10.1176/appi.ajp.2010.10050766

Leibenluft, E., Cohen, P., Gorrindo, T., Brook, J. S., & Pine, D. S. (2006). Chronic versus episodic irritability in youth: a community-based, longitudinal study of clinical and diagnostic associations. *Journal of Child and Adolescent Psychopharmacology*, *16*(4), 456–466. doi:10.1089/cap.2006.16.456

Leppänen, J. M., Milders, M., Bell, J. S., Terriere, E., & Hietanen, J. K. (2004). Depression biases the recognition of emotionally neutral faces. *Psychiatry Research,* 128(2), 123–133. http://doi.org/10.1016/j.psychres.2004.05.020

Lerner, J. S., & Keltner, D. (2001). Fear, anger, and risk. *Journal of Personality and Social Psychology*, *81*(1), 146–159. doi:10.1037/0022-3514.81.1.146

Licht, C. M. M., de Geus, E. J. C., van Dyck, R., & Penninx, B. W. J. H. (2010). Longitudinal evidence for unfavorable effects of antidepressants on heart rate variability. *Biological Psychiatry*, 68(9), 861–868. http://doi.org/10.1016/j.biopsych.2010.06.032

Lisboa, S. F. S., Oliveira, P. E., Costa, L. C., Venâncio, E. J., & Moreira, E. G. (2007). Behavioral evaluation of male and female mice pups exposed to fluoxetine during pregnancy and lactation. *Pharmacology, 80*(1), 49–56. doi:10.1159/000103097

Lewinsohn, P. M., Clarke, G. N., Seeley, J. R., & Rohde, P. (1994). Major depression in community adolescents: age at onset, episode duration, and time to recurrence. *Journal of the American Academy of Child and Adolescent Psychiatry*, *33*(6), 809–818. doi:10.1097/00004583-199407000-00006

Lewinsohn, P. M., Rohde, P., Klein, D. N., & Seeley, J. R. (1999). Natural course of adolescent major depressive disorder: I. Continuity into young adulthood. *Journal of the American Academy of Child and Adolescent Psychiatry*, *38*(1), 56–63. doi:10.1097/00004583-199901000-00020

Lewinsohn, P. M., Rohde, P., Seeley, J. R., & Fischer, S. A. (1993). Age-cohort changes in the lifetime occurrence of depression and other mental disorders. *Journal of Abnormal Psychology*, *102*(1), 110–120.

Libby, A. M., Brent, D. A., Morrato, E. H., Orton, H. D., Allen, R., & Valuck, R. J. (2007). Decline in treatment of pediatric depression after FDA advisory on risk of suicidality with SSRIs. *The American Journal of Psychiatry*, *164*(6), 884–891. doi:10.1176/appi.ajp.164.6.884

Licinio, J., & Wong, M.-L. (2005). Depression, antidepressants and suicidality: a critical appraisal. *Nature Reviews. Drug Discovery*, *4*(2), 165–171. doi:10.1038/nrd1634

Liljequist, R., Linnoila, M., & Mattila, M. J. (1978). Effect of diazepam and chlorpromazine on memory functions in man. *European Journal of Clinical Pharmacology*, *13*(5), 339–343.

Lopez, F. G., & Thurman, C. W. (1993). High-Trait and Low-Trait Angry College Students: A Comparison of Family Environments. *Journal of Counseling & Development*, *71*(5), 524–527. doi:10.1002/j.1556-6676.1993.tb02235.x

Louters, L. L. (2004). Don't overlook childhood depression. *JAAPA: Official Journal of the American Academy of Physician Assistants*, *17*(9), 18–24.

Lu, C. Y., Zhang, F., Lakoma, M. D., Madden, J. M., Rusinak, D., Penfold, R. B., … Soumerai, S. B. (2014). Changes in antidepressant use by young people and suicidal behavior after FDA warnings and media coverage: quasi-experimental study. *BMJ*, *348*(jun18 24), g3596–g3596. doi:10.1136/bmj.g3596

Mackie, D. M., Devos, T., & Smith, E. R. (2000). Intergroup emotions: explaining offensive action tendencies in an intergroup context. *Journal of Personality and Social Psychology*, *79*(4), 602–616.

MacLeod, C., Mathews, A., & Tata, P. (1986). Attentional bias in emotional disorders. *Journal of Abnormal Psychology*, *95*(1), 15–20.

MacMaster, F. P., Mirza, Y., Szeszko, P. R., Kmiecik, L. E., Easter, P. C., Taormina, S. P., … Rosenberg, D. R. (2008). Amygdala and hippocampal volumes in familial early onset major depressive disorder. *Biological Psychiatry*, *63*(4), 385–390. doi:10.1016/j.biopsych.2007.05.005

MacMillan, S., Szeszko, P. R., Moore, G. J., Madden, R., Lorch, E., Ivey, J., … Rosenberg, D. R. (2003). Increased amygdala: hippocampal volume ratios associated with severity of anxiety in pediatric major depression. *Journal of Child and Adolescent Psychopharmacology*, *13*(1), 65–73. doi:10.1089/104454603321666207

Mandrioli, R., Mercolini, L., & Raggi, M. (2008). Benzodiazepine Metabolism: An Analytical Perspective. *Current Drug Metabolism*, *9*(8), 827–844. doi:10.2174/138920008786049258

Manhães de Castro, R., Barreto Medeiros, J. M., Mendes da Silva, C., Ferreira, L. M., Guedes, R. C., Cabral Filho, J. E., & Costa, J. A. (2001). Reduction of intraspecific aggression in adult

rats by neonatal treatment with a selective serotonin reuptake inhibitor. *Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Médicas E Biológicas / Sociedade Brasileira De Biofísica ... [et Al.]*, 34(1), 121–124.

Mann, J. J., Emslie, G., Baldessarini, R. J., Beardslee, W., Fawcett, J. A., Goodwin, F. K., … Wagner, K. D. (2006). ACNP Task Force report on SSRIs and suicidal behavior in youth. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 31(3), 473–492. http://doi.org/10.1038/sj.npp.1300958

March, J., Silva, S., Petrycki, S., Curry, J., Wells, K., Fairbank, J., … Treatment for Adolescents With Depression Study (TADS) Team. (2004). Fluoxetine, cognitive-behavioral therapy, and their combination for adolescents with depression: Treatment for Adolescents With Depression Study (TADS) randomized controlled trial. *JAMA: The Journal of the American Medical Association*, 292(7), 807–820. doi:10.1001/jama.292.7.807

Marini, Z., & Case, R. (1994). The Development of Abstract Reasoning about the Physical and Social World. *Child Development*, *65*(1), 147. doi:10.2307/1131372

Marsh, A. A., Kozak, M. N., & Ambady, N. (2007). Accurate identification of fear facial expressions predicts prosocial behavior. *Emotion (Washington, D.C.)*, *7*(2), 239–251. doi:10.1037/1528-3542.7.2.239

Martinez, C., Rietbrock, S., Wise, L., Ashby, D., Chick, J., Moseley, J., … Gunnell, D. (2005). Antidepressant treatment and the risk of fatal and non-fatal self harm in first episode depression: nested case-control study. *BMJ (Clinical Research Ed.)*, *330*(7488), 389. doi:10.1136/bmj.330.7488.389

Martin, J. R., Ballard, T. M., & Higgins, G. A. (2002). Influence of the 5-HT2C receptor antagonist, SB-242084, in tests of anxiety. *Pharmacology, Biochemistry, and Behavior*, *71*(4), 615–625.

Mathews, A., & MacLeod, C. (2005). Cognitive Vulnerability to Emotional Disorders. *Annual Review of Clinical Psychology*, *1*(1), 167–195. doi:10.1146/annurev.clinpsy.1.102803.143916

Matsumoto, D., & Ekman, P. (1988). Japanese and Caucasian facial expressions of emotion (JACFEE)[Slides]. *San Francisco, CA: Intercultural and Emotion Research Laboratory, Department of Psychology, San Francisco State University*.

Matthews, G., & Wells, A. (2000). Attention, automaticity, and affective disorder. *Behavior Modification*, *24*(1), 69–93.

Ma, Y. (2014). Neuropsychological mechanism underlying antidepressant effect: a systematic meta-analysis. *Molecular Psychiatry*. doi:10.1038/mp.2014.24

Mayberg, H. S. (2003). Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. *British Medical Bulletin*, *65*, 193–207.

Mayes, T. L., Bernstein, I. H., Haley, C. L., Kennard, B. D., & Emslie, G. J. (2010). Psychometric properties of the Children's Depression Rating Scale-Revised in adolescents. *Journal of Child and Adolescent Psychopharmacology*, *20*(6), 513–516. doi:10.1089/cap.2010.0063

McCutcheon, J. E., & Marinelli, M. (2009). Age matters. *The European Journal of Neuroscience,* 29(5), 997–1014. http://doi.org/10.1111/j.1460-9568.2009.06648.x

McClure, E. B., Monk, C. S., Nelson, E. E., Parrish, J. M., Adler, A., Blair, R. J. R., … Pine, D. S. (2007). Abnormal attention modulation of fear circuit function in pediatric generalized anxiety disorder. *Archives of General Psychiatry*, *64*(1), 97–106. doi:10.1001/archpsyc.64.1.97

McClure, E. B., Pope, K., Hoberman, A. J., Pine, D. S., & Leibenluft, E. (2003). Facial expression recognition in adolescents with mood and anxiety disorders. *The American Journal of Psychiatry*, *160*(6), 1172–1174.

McCown, W., Johnson, J., & Austin, S. (1986). Inability of delinquents to recognize facial affects. *Journal of Social Behavior & Personality*, 1(4), 489–496.

McRae, K., Gross, J. J., Weber, J., Robertson, E. R., Sokol-Hessner, P., Ray, R. D., … Ochsner, K. N. (2012). The development of emotion regulation: an fMRI study of cognitive reappraisal in children, adolescents and young adults. *Social Cognitive and Affective Neuroscience*, *7*(1), 11–22. doi:10.1093/scan/nsr093

Medicinal and Healthcare Products Regulatory Agency (MHRA, 2003). Safety review of antidepressants used by children completed (reference 2003/0505). [online], http://www.antidepressantsfacts.com/2003-10-12-05-05-MHRA-CSM-SSRIs.htm

Miller, M., Swanson, S. A., Azrael, D., Pate, V., & Stürmer, T. (2014). Antidepressant dose, age, and the risk of deliberate self-harm. *JAMA Internal Medicine*, *174*(6), 899–909. doi:10.1001/jamainternmed.2014.1053

Mogg, K., & Bradley, B. P. (1999). Orienting of Attention to Threatening Facial Expressions Presented under Conditions of Restricted Awareness. *Cognition & Emotion*, *13*(6), 713–740. doi:10.1080/026999399379050

Mogg, K., Bradley, B. P., de Bono, J., & Painter, M. (1997). Time course of attentional bias for threat information in non-clinical anxiety. *Behaviour Research and Therapy*, *35*(4), 297–303.

Mogg, K., Bradley, B. P., Dixon, C., Fisher, S., Twelftree, H., & McWilliams, A. (2000). Trait anxiety, defensiveness and selective processing of threat: an investigation using two measures of attentional bias. *Personality and Individual Differences*, *28*(6), 1063–1077. doi:10.1016/S0191-8869(99)00157-9

Mogg, K., Mathews, A., & Eysenck, M. (1992). Attentional bias to threat in clinical anxiety states. *Cognition & Emotion*, *6*(2), 149–159. doi:10.1080/02699939208411064

Möhler, H., Fritschy, J. M., & Rudolph, U. (2002). A New Benzodiazepine Pharmacology. *Journal of Pharmacology and Experimental Therapeutics*, *300*(1), 2–8. doi:10.1124/jpet.300.1.2

Monk, C. S., Klein, R. G., Telzer, E. H., Schroth, E. A., Mannuzza, S., Moulton, J. L., … Ernst, M. (2008). Amygdala and nucleus accumbens activation to emotional facial expressions in children and adolescents at risk for major depression. *The American Journal of Psychiatry*, *165*(1), 90–98. doi:10.1176/appi.ajp.2007.06111917

Monk, C. S., McClure, E. B., Nelson, E. E., Zarahn, E., Bilder, R. M., Leibenluft, E., … Pine, D. S. (2003). Adolescent immaturity in attention-related brain engagement to emotional facial expressions. *NeuroImage*, *20*(1), 420–428.

Moons, W. G., Eisenberger, N. I., & Taylor, S. E. (2010). Anger and fear responses to stress have different biological profiles. *Brain, Behavior, and Immunity*, *24*(2), 215–219. doi:10.1016/j.bbi.2009.08.009

Morris, J. S., Friston, K. J., Büchel, C., Frith, C. D., Young, A. W., Calder, A. J., & Dolan, R. J. (1998). A neuromodulatory role for the human amygdala in processing emotional facial expressions. *Brain: A Journal of Neurology*, *121 (Pt 1)*, 47–57.

Mudrik, L., Breska, A., Lamy, D., & Deouell, L. Y. (2011). Integration Without Awareness Expanding the Limits of Unconscious Processing. *Psychological Science*. doi:10.1177/0956797611408736

Munn, M. A., Alexopoulos, J., Nishino, T., Babb, C. M., Flake, L. A., Singer, T., … Botteron, K. N. (2007). Amygdala Volume Analysis in Female Twins with Major Depression. *Biological Psychiatry*, *62*(5), 415–422. doi:10.1016/j.biopsych.2006.11.031

Murphy, S. E., Downham, C., Cowen, P. J., & Harmer, C. J. (2008). Direct effects of diazepam on emotional processing in healthy volunteers. *Psychopharmacology*, *199*(4), 503–513. doi:10.1007/s00213-008-1082-2

Murphy, S. E., & Mackay, C. E. (2011). Using MRI to measure drug action: caveats and new directions. *Journal of Psychopharmacology (Oxford, England)*, *25*(9), 1168–1174. doi:10.1177/0269881110372547

Murphy, S. E., Norbury, R., O'Sullivan, U., Cowen, P. J., & Harmer, C. J. (2009). Effect of a single dose of citalopram on amygdala response to emotional faces. *The British Journal of Psychiatry: The Journal of Mental Science*, *194*(6), 535–540. doi:10.1192/bjp.bp.108.056093

Murphy, S. (2010). Using Functional Neuroimaging to Investigate the Mechanisms of Action of Selective Serotonin Reuptake Inhibitors (SSRIs). *Current Pharmaceutical Design*, *16*(18), 1990–1997. doi:10.2174/138161210791293051

Murray, M. L., Thompson, M., Santosh, P. J., & Wong, I. C. K. (2005). Effects of the Committee on Safety of Medicines advice on antidepressant prescribing to children and adolescents in the UK. *Drug Safety: An International Journal of Medical Toxicology and Drug Experience*, *28*(12), 1151–1157.

Murrin, L. C., Sanders, J. D., & Bylund, D. B. (2007). Comparison of the Maturation of the Adrenergic and Serotonergic Neurotransmitter Systems in the Brain: Implications for Differential Drug Effects on Juveniles and Adults. *Biochemical Pharmacology*, *73*(8), 1225–1236. doi:10.1016/j.bcp.2007.01.028

Nakamura, K., & Kurasawa, M. (2001). Anxiolytic effects of aniracetam in three different mouse models of anxiety and the underlying mechanism. *European Journal of Pharmacology*, *420*(1), 33–43. doi:10.1016/S0014-2999(01)01005-6

National Institute for Health and Clinical Excellence (2005) CG28 depression in children and young people: NICE guideline. London: National Institute for Health and Clinical Excellence.

Nelson, HE, W., J. (1991). The Revised National Adult Reading Test–Test manual. Windsor, UK: NFER-Nelson.

Nelson, E. E., Leibenluft, E., McClure, E. B., & Pine, D. S. (2005). The social re-orientation of adolescence: a neuroscience perspective on the process and its relation to psychopathology. *Psychological Medicine*, *35*(2), 163–174.

Nelson, E. E., McClure, E. B., Monk, C. S., Zarahn, E., Leibenluft, E., Pine, D. S., & Ernst, M. (2003). Developmental differences in neuronal engagement during implicit encoding of emotional faces: an event-related fMRI study. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *44*(7), 1015–1024.

Ni, Y. G., & Miledi, R. (1997). Blockage of 5HT2C serotonin receptors by fluoxetine (Prozac). *Proceedings of the National Academy of Sciences of the United States of America*, *94*(5), 2036–2040.

Norbury, R., Mackay, C. E., Cowen, P. J., Goodwin, G. M., & Harmer, C. J. (2007). Short-term antidepressant treatment and facial processing. Functional magnetic resonance imaging study. *The British Journal of Psychiatry: The Journal of Mental Science*, *190*, 531–532. doi:10.1192/bjp.bp.106.031393

Norton, P. J. (2012). A Randomized Clinical Trial of Transdiagnostic CBT for Anxiety Disorder by Comparison to Relaxation Training. *Behavior Therapy*, *43*(3), 506–517. doi:10.1016/j.beth.2010.08.011

Oh, J., Zupan, B., Gross, S., & Toth, M. (2009). Paradoxical anxiogenic response of juvenile mice to fluoxetine. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *34*(10), 2197–2207. doi:10.1038/npp.2009.47

Öhman, A. (1993). Fear and anxiety as emotional phenomena. In M. Lewis & J. Haviland (Ed.), *Handbook of emotions* (pp. 511–536). New York, NY, US: Guilford Press.

Öhman, A., & Mineka, S. (2001). Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. *Psychological Review*, *108*(3), 483–522. doi:10.1037/0033-295X.108.3.483

Olfson, M., Shaffer, D., Marcus, S. C., & Greenberg, T. (2003). Relationship between antidepressant medication treatment and suicide in adolescents. *Archives of General Psychiatry*, *60*(10), 978–982. doi:10.1001/archpsyc.60.9.978

Olivier, J. D. A., Akerud, H., Kaihola, H., Pawluski, J. L., Skalkidou, A., Hogberg, U., & Sundstrom-Poromaa, I. (2013). The effects of maternal depression and maternal selective serotonin reuptake inhibitor exposure on offspring. *Frontiers in Cellular Neuroscience, 7*. doi:10.3389/fncel.2013.00073

Olivier, J. D. A., Blom, T., Arentsen, T., & Homberg, J. R. (2011). The age-dependent effects of selective serotonin reuptake inhibitors in humans and rodents: A review. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *35*(6), 1400–1408. doi:10.1016/j.pnpbp.2010.09.013

Ongür, D., & Price, J. L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral Cortex (New York, N.Y.: 1991)*, *10*(3), 206–219.

Ouimet, A. J., Gawronski, B., & Dozois, D. J. A. (2009). Cognitive vulnerability to anxiety: A review and an integrative model. *Clinical Psychology Review*, 29(6), 459–470. http://doi.org/10.1016/j.cpr.2009.05.004

Owen, J. M. (2011). Transdiagnostic cognitive processes in high trait anger. *Clinical Psychology Review*, *31*(2), 193–202. doi:10.1016/j.cpr.2010.10.003

Pagliaro, L. A. (1999). *Psychologists' Psychotropic Drug Reference*. Psychology Press.

Pälvimäki, E.-P., Kuoppamäki, M., Syvälahti, E., & Hietala, J. (1999). Differential effects of fluoxetine and citalopram treatments on serotonin 5-HT(2C) receptor occupancy in rat brain. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *2*(2), 95–99. doi:10.1017/S1461145799001406

Pannekoek, J. N., van der Werff, S. J. A., van den Bulk, B. G., van Lang, N. D. J., Rombouts, S. A. R. B., van Buchem, M. A., … van der Wee, N. J. A. (2014). Reduced anterior cingulate gray matter volume in treatment-naïve clinically depressed adolescents. *NeuroImage: Clinical, 4*, 336–342. doi:10.1016/j.nicl.2014.01.007

Parati, G., Frattola, A., Di Rienzo, M., Castiglioni, P., Pedotti, A., & Mancia, G. (1995). Effects of aging on 24-h dynamic baroreceptor control of heart rate in ambulant subjects. *The American Journal of Physiology*, 268(4 Pt 2), H1606–1612.

Patton, J. H., Stanford, M. S., & Barratt, E. S. (1995). Factor structure of the Barratt impulsiveness scale. *Journal of Clinical Psychology*, *51*(6), 768–774.

Pavuluri, M. N., O'Connor, M. M., Harral, E., & Sweeney, J. A. (2007). Affective neural circuitry during facial emotion processing in pediatric bipolar disorder. *Biological Psychiatry*, *62*(2), 158–167. doi:10.1016/j.biopsych.2006.07.011

Penton-Voak, I. S., Thomas, J., Gage, S. H., McMurran, M., McDonald, S., & Munafò, M. R. (2013). Increasing Recognition of Happiness in Ambiguous Facial Expressions Reduces Anger and Aggressive Behavior. *Psychological Science*, 0956797612459657. http://doi.org/10.1177/0956797612459657

Perlis, R. H., Fava, M., Trivedi, M. H., Alpert, J., Luther, J. F., Wisniewski, S. R., & Rush, A. J. (2009). Irritability is associated with anxiety and greater severity, but not bipolar spectrum features, in major depressive disorder. *Acta Psychiatrica Scandinavica*, *119*(4), 282–289. doi:10.1111/j.1600-0447.2008.01298.x

Perlis, R. H., Mischoulon, D., Smoller, J. W., Wan, Y.-J. Y., Lamon-Fava, S., Lin, K.-M., … Fava, M. (2003). Serotonin transporter polymorphisms and adverse effects with fluoxetine treatment. *Biological Psychiatry*, *54*(9), 879–883.

Perlman, G., Simmons, A. N., Wu, J., Hahn, K. S., Tapert, S. F., Max, J. E., … Yang, T. T. (2012). Amygdala response and functional connectivity during emotion regulation: a study of 14 depressed adolescents. *Journal of Affective Disorders*, *139*(1), 75–84. doi:10.1016/j.jad.2012.01.044

Perreault, H. A. N., Semsar, K., & Godwin, J. (2003). Fluoxetine treatment decreases territorial aggression in a coral reef fish. *Physiology & Behavior*, *79*(4-5), 719–724.

Pessoa, L., Japee, S., & Ungerleider, L. G. (2005). Visual awareness and the detection of fearful faces. *Emotion (Washington, D.C.)*, *5*(2), 243–247. doi:10.1037/1528-3542.5.2.243

Pfeifer, J. H., & Allen, N. B. (2012). Arrested development? Reconsidering dual-systems models of brain function in adolescence and disorders. *Trends in Cognitive Sciences*, *16*(6), 322–329. doi:10.1016/j.tics.2012.04.011

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

Phillips, M. L. (2003). Understanding the neurobiology of emotion perception: implications for psychiatry. *The British Journal of Psychiatry*, *182*(3), 190–192. doi:10.1192/bjp.02.185

Phillips, M. L., Drevets, W. C., Rauch, S. L., & Lane, R. (2003). Neurobiology of emotion perception II: Implications for major psychiatric disorders. *Biological Psychiatry*, *54*(5), 515–528.

Pitskel, N. B., Bolling, D. Z., Kaiser, M. D., Crowley, M. J., & Pelphrey, K. A. (2011). How grossed out are you? The neural bases of emotion regulation from childhood to adolescence. *Developmental Cognitive Neuroscience*, *1*(3), 324–337. doi:10.1016/j.dcn.2011.03.004

Pizzagalli, D. A. (2011). Frontocingulate Dysfunction in Depression: Toward Biomarkers of Treatment Response. *Neuropsychopharmacology*, *36*(1), 183–206. doi:10.1038/npp.2010.166

Platt, B., Cohen Kadosh, K., & Lau, J. Y. F. (2013). The role of peer rejection in adolescent depression. *Depression and Anxiety*, *30*(9), 809–821. doi:10.1002/da.22120

Poldrack, R. A. (2007). Region of interest analysis for fMRI. *Social Cognitive and Affective Neuroscience*, 2(1), 67–70. http://doi.org/10.1093/scan/nsm006

Pompéia, S., Pradella-Hallinan, M., Manzano, G. M., & Bueno, O. F. A. (2008). Effects of lorazepam on visual perceptual abilities. *Human Psychopharmacology: Clinical and Experimental*, *23*(3), 183–192. doi:10.1002/hup.927

Poznanski, E., & Mokros, H. (1996). *Children's Depression Rating Scale Revisited (CDRS-R).* Los Angeles: Western Psychological Service.

Price, J. L., & Drevets, W. C. (2012). Neural circuits underlying the pathophysiology of mood disorders. *Trends in Cognitive Sciences*, *16*(1), 61–71. doi:10.1016/j.tics.2011.12.011

Pringle, A., Browning, M., Cowen, P. J., & Harmer, C. J. (2011). A cognitive neuropsychological model of antidepressant drug action. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *35*(7), 1586–1592. doi:10.1016/j.pnpbp.2010.07.022

Putman, P., Hermans, E., & van Honk, J. (2004). Emotional stroop performance for masked angry faces: it's BAS, not BIS. *Emotion (Washington, D.C.)*, *4*(3), 305–311. doi:10.1037/1528-3542.4.3.305

Ramage, A. G. (2001). Central cardiovascular regulation and 5-hydroxytryptamine receptors. *Brain Research Bulletin*, 56(5), 425–439. http://doi.org/10.1016/S0361-9230(01)00612-8

Ramirez, C. A., Rosén, L. A., Deffenbacher, J. L., Hurst, H., Nicoletta, C., Rosencranz, T., & Smith, K. (1997). Anger and anger expression in adults with high ADHD symptoms. *Journal of Attention Disorders*, *2*(2), 115–128. doi:10.1177/108705479700200205

Ratcliff, R. (1993). Methods for dealing with reaction time outliers. *Psychological Bulletin*, *114*(3), 510–532.

Rawlings, N. B., Norbury, R., Cowen, P. J., & Harmer, C. J. (2010). A single dose of mirtazapine modulates neural responses to emotional faces in healthy people. *Psychopharmacology*, *212*(4), 625–634. doi:10.1007/s00213-010-1983-8

Raymond, J. E., Shapiro, K. L., & Arnell, K. M. (1992). Temporary suppression of visual processing in an RSVP task: an attentional blink? *Journal of Experimental Psychology. Human Perception and Performance*, *18*(3), 849–860.

Reinecke, A., Waldenmaier, L., Cooper, M. J., & Harmer, C. J. (2013). Changes in automatic threat processing precede and predict clinical changes with exposure-based cognitive-behavior therapy for panic disorder. *Biological Psychiatry*, *73*(11), 1064–1070. doi:10.1016/j.biopsych.2013.02.005

Reynolds, W. M. (1987). *About my Life: S. I. Q. -JR Form H. S.* Odessa, FL: Psychological Assessment Resources.

Rich, B. A., Vinton, D. T., Roberson-Nay, R., Hommer, R. E., Berghorst, L. H., McClure, E. B., … Leibenluft, E. (2006). Limbic hyperactivation during processing of neutral facial expressions in children with bipolar disorder. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(23), 8900–8905. doi:10.1073/pnas.0603246103

Rie, H. E. (1966). Depression in childhood. A survey of some pertinent contributions. *Journal of the American Academy of Child Psychiatry*, *5*(4), 653–685.

Roberson-Nay, R., McClure, E. B., Monk, C. S., Nelson, E. E., Guyer, A. E., Fromm, S. J., … Pine, D. S. (2006). Increased amygdala activity during successful memory encoding in adolescent major depressive disorder: An FMRI study. *Biological Psychiatry*, *60*(9), 966–973. doi:10.1016/j.biopsych.2006.02.018

Rodrigues-Filho, R., & Takahashi, R. N. (1999). Antinociceptive effects induced by desipramine and fluoxetine are dissociated from their antidepressant or anxiolytic action in mice. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *2*(4), 263–269. doi:10.1017/S1461145799001637

Romer, D. (2010). Adolescent Risk Taking, Impulsivity, and Brain Development: Implications for Prevention. *Developmental Psychobiology*, *52*(3), 263–276. doi:10.1002/dev.20442

Rosenberg, H. C., & Chiu, T. H. (1985). Time course for development of benzodiazepine tolerance and physical dependence. *Neuroscience and Biobehavioral Reviews*, *9*(1), 123–131.

Rossi, A., Barraco, A., & Donda, P. (2004). Fluoxetine: a review on evidence based medicine. *Annals of General Hospital Psychiatry*, *3*, 2. doi:10.1186/1475-2832-3-2

Rosso, I. M., Cintron, C. M., Steingard, R. J., Renshaw, P. F., Young, A. D., & Yurgelun-Todd, D. A. (2005). Amygdala and hippocampus volumes in pediatric major depression. *Biological Psychiatry*, *57*(1), 21–26. doi:10.1016/j.biopsych.2004.10.027

Rovai, A. P., Baker, J. D., & Ponton, M. K. (2013). *Social Science Research Design and Statistics: A Practitioner's Guide to Research Methods and IBM SPSS*. Watertree Press LLC.

Ryan, N. D., Puig-Antich, J., Ambrosini, P., Rabinovich, H., Robinson, D., Nelson, B., … Twomey, J. (1987). The clinical picture of major depression in children and adolescents. *Archives of General Psychiatry*, 44(10), 854–861.

Salchner, P., & Singewald, N. (2006). 5-HT receptor subtypes involved in the anxiogenic-like action and associated Fos response of acute fluoxetine treatment in rats. *Psychopharmacology*, 185(3), 282–288. doi:10.1007/s00213-005-0247-5

Sancilio, M. F., Plumert, J. M., & Hartup, W. W. (1989). Friendship and aggressiveness as determinants of conflict outcomes in middle childhood. *Developmental Psychology*, *25*(5), 812.

Sato, W., Uono, S., Matsuura, N., & Toichi, M. (2009). Misrecognition of facial expressions in delinquents. *Child and Adolescent Psychiatry and Mental Health*, *3*, 27. doi:10.1186/1753-2000-3-27

Schepman, K., Taylor, E., Collishaw, S., & Fombonne, E. (2012). Face emotion processing in depressed children and adolescents with and without comorbid conduct disorder. *Journal of Abnormal Child Psychology*, *40*(4), 583–593. doi:10.1007/s10802-011-9587-2

Scherf, K. S., Sweeney, J. A., & Luna, B. (2006). Brain basis of developmental change in visuospatial working memory. *Journal of Cognitive Neuroscience*, *18*(7), 1045–1058. doi:10.1162/jocn.2006.18.7.1045

Schmukle, S. C. (2005). Unreliability of the dot probe task. *European Journal of Personality*, *19*(7), 595–605. doi:10.1002/per.554

Schreiber, R., Melon, C., & De Vry, J. (1998). The role of 5-HT receptor subtypes in the anxiolytic effects of selective serotonin reuptake inhibitors in the rat ultrasonic vocalization test. *Psychopharmacology*, *135*(4), 383–391.

Serafini, G., Pompili, M., Borgwardt, S., Houenou, J., Geoffroy, P. A., Jardri, R., … Amore, M. (2014). Brain changes in early-onset bipolar and unipolar depressive disorders: a systematic review in children and adolescents. *European Child & Adolescent Psychiatry*. doi:10.1007/s00787-014-0614-z

Shaffer, D., Gould, M. S., Brasic, J., Ambrosini, P., Fisher, P., Bird, H., & Aluwahlia, S. (1983). A children's global assessment scale (CGAS). *Archives of General Psychiatry*, *40*(11), 1228–1231.

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., … Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal of Clinical Psychiatry*, *59 Suppl 20*, 22–33;quiz 34–57.

Sheline, Y. I., Barch, D. M., Donnelly, J. M., Ollinger, J. M., Snyder, A. Z., & Mintun, M. A. (2001). Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biological Psychiatry*, *50*(9), 651–658.

Sheline, Y. I., Gado, M. H., & Price, J. L. (1998). Amygdala core nuclei volumes are decreased in recurrent major depression. *Neuroreport*, *9*(9), 2023–2028.

Sheline, Y. I., Sanghavi, M., Mintun, M. A., & Gado, M. H. (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major

depression. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *19*(12), 5034–5043.

Sinclair, L. I., Christmas, D. M., Hood, S. D., Potokar, J. P., Robertson, A., Isaac, A., … Davies, S. J. C. (2009). Antidepressant-induced jitteriness/anxiety syndrome: systematic review. *The British Journal of Psychiatry: The Journal of Mental Science*, *194*(6), 483–490. doi:10.1192/bjp.bp.107.048371

Singer, N., Eapen, M., Grillon, C., Ungerleider, L. G., & Hendler, T. (2012). Through the eyes of anxiety: Dissecting threat bias via emotional-binocular rivalry. *Emotion (Washington, D.C.)*, *12*(5), 960–969. doi:10.1037/a0027070

Smith, E. G. (2009). Association between antidepressant half-life and the risk of suicidal ideation or behavior among children and adolescents: confirmatory analysis and research implications. *Journal of Affective Disorders*, *114*(1-3), 143–148. doi:10.1016/j.jad.2008.06.018

Smith, P., & Waterman, M. (2004). Role of experience in processing bias for aggressive words in forensic and non-forensic populations. *Aggressive Behavior*, *30*(2), 105–122. doi:10.1002/ab.20001

Smith, S. M. (2002). Fast robust automated brain extraction. *Human Brain Mapping*, *17*(3), 143–155. doi:10.1002/hbm.10062

Smucker, M. R., Craighead, W. E., Craighead, L. W., & Green, B. J. (1986). Normative and reliability data for the Children's Depression Inventory. *Journal of Abnormal Child Psychology*, *14*(1), 25–39.

Somerville, L. H., Hare, T., & Casey, B. J. (2011). Frontostriatal maturation predicts cognitive control failure to appetitive cues in adolescents. *Journal of Cognitive Neuroscience*, *23*(9), 2123–2134. doi:10.1162/jocn.2010.21572

Sowell, E. R., Thompson, P. M., Leonard, C. M., Welcome, S. E., Kan, E., & Toga, A. W. (2004). Longitudinal mapping of cortical thickness and brain growth in normal children. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *24*(38), 8223–8231. doi:10.1523/JNEUROSCI.1798-04.2004

Sparks, J. A., & Duncan, B. L. (2013). Outside the Black Box: Re-assessing Pediatric Antidepressant Prescription. *Journal of the Canadian Academy of Child and Adolescent*

Psychiatry = Journal de l'Académie Canadienne de Psychiatrie de L'enfant et de L'adolescent, *22*(3), 240–246.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews*, *24*(4), 417–463.

Spielberger, C. (1996). *State–Trait Anger Expression Inventory: Professional Manual.* Odessa, FL: Psychological Assessment Resources.

Spielberger, C. D. (1973). *Manual for the State Trait Anxiety Inventory for Children*. Palo Alto, CA: Consulting Psychologists Press.

Spielberger, C. D., Gorsuch, R. L., & Edward, L. R. (1970). *STAI manual for the state-trait anxiety inventory ("Self-evaluation questionnaire")*. Consulting Psychologists Press.

Spielberger, C. D., Krasner, S. S., & Solomon, E. P. (1988). The Experience, Expression, and Control of Anger. In M. P. Janisse (Ed.), *Individual Differences, Stress, and Health Psychology* (pp. 89–108). Springer New York. Retrieved from http://link.springer.com/chapter/10.1007/978-1-4612-3824-9_5

Spielberger, C. D., Reheiser, E. C., & Sydeman, S. J. (1995). Measuring the Experience, Expression, and Control of Anger. *Issues in Comprehensive Pediatric Nursing*, *18*(3), 207–232. doi:10.3109/01460869509087271

Spielberg, J. M., Olino, T. M., Forbes, E. E., & Dahl, R. E. (2014). Exciting fear in adolescence: Does pubertal development alter threat processing? *Developmental Cognitive Neuroscience*, *8*, 86–95. doi:10.1016/j.dcn.2014.01.004

Spielberg, S. (1988). *State-Trait Anger Expression Inventory (STAXI), research edition.* Odessa, FL: Psychological Assessment Resources,.

Springer, U. S., Rosas, A., McGetrick, J., & Bowers, D. (2007). Differences in startle reactivity during the perception of angry and fearful faces. *Emotion (Washington, D.C.)*, *7*(3), 516–525. doi:10.1037/1528-3542.7.3.516

Steinberg, E. M., Cardoso, G. M. P., Martinez, P. E., Rubinow, D. R., & Schmidt, P. J. (2012). Rapid response to fluoxetine in women with premenstrual dysphoric disorder. *Depression and Anxiety*, *29*(6), 531–540. doi:10.1002/da.21959

Steinberg, L., & Morris, A. S. (2001). Adolescent Development. *Annual Review of Psychology*, *52*(1), 83–110. doi:10.1146/annurev.psych.52.1.83

Sterzer, P., Hilgenfeldt, T., Freudenberg, P., Bermpohl, F., & Adli, M. (2011). Access of emotional information to visual awareness in patients with major depressive disorder. *Psychological Medicine*, *41*(8), 1615–1624. doi:10.1017/S0033291710002540

Stevens, J. P. (2009). *Applied Multivariate Statistics for the Social Sciences, Fifth Edition* (5th ed.). Routledge Academic.

Strauss, M. M., Makris, N., Aharon, I., Vangel, M. G., Goodman, J., Kennedy, D. N., … Breiter, H. C. (2005). fMRI of sensitization to angry faces. *NeuroImage*, *26*(2), 389–413. doi:10.1016/j.neuroimage.2005.01.053

Stringaris, A. (2011). Irritability in children and adolescents: a challenge for DSM-5. *European Child & Adolescent Psychiatry*, *20*(2), 61–6. doi:10.1007/s00787-010-0150-4

Stringaris, A., Cohen, P., Pine, D. S., & Leibenluft, E. (2009). Adult outcomes of youth irritability: a 20-year prospective community-based study. *The American Journal of Psychiatry*, *166*(9), 1048–1054. doi:10.1176/appi.ajp.2009.08121849

Stringaris, A., & Goodman, R. (2009). Three dimensions of oppositionality in youth. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *50*(3), 216–223. doi:10.1111/j.1469-7610.2008.01989.x

Stringaris, A., Goodman, R., Ferdinando, S., Razdan, V., Muhrer, E., Leibenluft, E., & Brotman, M. A. (2012). The Affective Reactivity Index: a concise irritability scale for clinical and research settings. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *53*(11), 1109–1117. doi:10.1111/j.1469-7610.2012.02561.x

Stringaris, A., Maughan, B., Copeland, W. S., Costello, E. J., & Angold, A. (2013). Irritable mood as a symptom of depression in youth: prevalence, developmental, and clinical correlates in the Great Smoky Mountains Study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *52*(8), 831–840. doi:10.1016/j.jaac.2013.05.017

Stringaris, A., Zavos, H., Leibenluft, E., Maughan, B., & Eley, T. (2012). Adolescent irritability: phenotypic associations and genetic links with depressed mood. *The American Journal of Psychiatry*, *169*(1), 47–54. doi:10.1176/appi.ajp.2011.10101549

Strober, M., Green, J., & Carlson, G. (1981). Phenomenology and subtypes of major depressive disorder in adolescence. *Journal of Affective Disorders*, *3*(3), 281–290.

Stroud, L. R., Foster, E., Papandonatos, G. D., Handwerger, K., Granger, D. A., Kivlighan, K. T., & Niaura, R. (2009). Stress Response and the Adolescent Transition: Performance versus Peer Rejection Stressors. *Development and Psychopathology*, *21*(1), 47–68. doi:10.1017/S0954579409000042

Tafrate, R. C., Kassinove, H., & Dundin, L. (2002). Anger episodes in high- and low-trait-anger community adults. *Journal of Clinical Psychology*, *58*(12), 1573–1590. doi:10.1002/jclp.10076

Taggart, P., Boyett, M. R., Logantha, S. J. R. J., & Lambiase, P. D. (2011). Anger, Emotion, and Arrhythmias: from Brain to Heart. *Frontiers in Physiology*, *2*. doi:10.3389/fphys.2011.00067

Tamnes, C. K., Ostby, Y., Fjell, A. M., Westlye, L. T., Due-Tønnessen, P., & Walhovd, K. B. (2010). Brain maturation in adolescence and young adulthood: regional age-related changes in cortical thickness and white matter volume and microstructure. *Cerebral Cortex (New York, N.Y.: 1991)*, *20*(3), 534–548. doi:10.1093/cercor/bhp118

Tao, R., Calley, C. S., Hart, J., Mayes, T. L., Nakonezny, P. A., Lu, H., … Emslie, G. J. (2012). Brain activity in adolescent major depressive disorder before and after fluoxetine treatment. *The American Journal of Psychiatry*, *169*(4), 381–388.doi:10.1176/appi.ajp.2011.11040615

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (TFESCNASPE) (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation*, 93(5), 1043–1065.

Teicher, M. H., Glod, C., & Cole, J. O. (1990). Emergence of intense suicidal preoccupation during fluoxetine treatment. *The American Journal of Psychiatry*, *147*(2), 207–210.

Tiradentes, R. V., Pires, J. G. P., Silva, N. F., Ramage, A. G., Santuzzi, C. H., & Futuro Neto, H. A. (2014). Effects of acute administration of selective serotonin reuptake inhibitors on sympathetic nerve activity. *Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Médicas E Biológicas* / Sociedade Brasileira De Biofísica ... [et Al.], 47(7), 554–559.

Thomas, K. M., Drevets, W. C., Dahl, R. E., Ryan, N. D., Birmaher, B., Eccard, C. H., … Casey, B. J. (2001). Amygdala response to fearful faces in anxious and depressed children. *Archives of General Psychiatry*, *58*(11), 1057–1063.

Thomas, L. A., Brotman, M. A., Bones, B. L., Chen, G., Rosen, B. H., Pine, D. S., & Leibenluft, E. (2014). Neural circuitry of masked emotional face processing in youth with bipolar disorder, severe mood dysregulation, and healthy volunteers. *Developmental Cognitive Neuroscience*, *8*, 110–120. doi:10.1016/j.dcn.2013.09.007

Thomas, L. A., De Bellis, M. D., Graham, R., & LaBar, K. S. (2007). Development of emotional facial recognition in late childhood and adolescence. *Developmental Science*, *10*(5), 547–558. doi:10.1111/j.1467-7687.2007.00614.x

Thomas, L. A., Kim, P., Bones, B. L., Hinton, K. E., Milch, H. S., Reynolds, R. C., … Leibenluft, E. (2013). Elevated amygdala responses to emotional faces in youths with chronic irritability or bipolar disorder. *NeuroImage. Clinical*, *2*, 637–645. doi:10.1016/j.nicl.2013.04.007

Timbremont, B., & Braet, C. (2004). Cognitive vulnerability in remitted depressed children and adolescents. *Behaviour Research and Therapy*, *42*(4), 423–437. doi:10.1016/S0005-7967(03)00151-7

Tranter, R., Bell, D., Gutting, P., Harmer, C., Healy, D., & Anderson, I. M. (2009). The effect of serotonergic and noradrenergic antidepressants on face emotion processing in depressed patients. *Journal of Affective Disorders*, *118*(1-3), 87–93. doi:10.1016/j.jad.2009.01.028

Tsuchiya, N., & Koch, C. (2005). Continuous flash suppression reduces negative afterimages. *Nature Neuroscience*, *8*(8), 1096–1101. doi:10.1038/nn1500

Uher, R., Farmer, A., Henigsberg, N., Rietschel, M., Mors, O., Maier, W., … Aitchison, K. J. (2009). Adverse reactions to antidepressants. *The British Journal of Psychiatry*, *195*(3), 202–210. doi:10.1192/bjp.bp.108.061960

Van Beek, Y., van Dolderen, M. S. M., & Demon Dubas, J. J. S. (2006). Gender-specific development of nonverbal behaviours and mild depression in adolescence. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *47*(12), 1272–1283. doi:10.1111/j.1469-7610.2006.01663.x

Van den Bulk, B. G., Meens, P. H. F., van Lang, N. D. J., de Voogd, E. L., van der Wee, N. J. A., Rombouts, S. A. R. B., … Vermeiren, R. R. J. M. (2014). Amygdala activation during emotional face processing in adolescents with affective disorders: the role of underlying depression and anxiety symptoms. *Frontiers in Human Neuroscience*, *8*, 393. doi:10.3389/fnhum.2014.00393

Van Loon, A. M., Knapen, T., Scholte, H. S., St. John-Saaltink, E., Donner, T. H., & Lamme, V. A. F. (2013). GABA Shapes the Dynamics of Bistable Perception. *Current Biology*, *23*(9), 823–827. doi:10.1016/j.cub.2013.03.067

Von Zerssen, D., Strian, F., & Schwarz, D. (1974). Evaluation of depressive states, especially in longitudinal studies. *Modern Problems of Pharmacopsychiatry,* 7, 189.

Vorhees, C. V., Acuff-Smith, K. D., Schilling, M. A., Fisher, J. E., Moran, M. S., & Buelke-Sam, J. (1994). A developmental neurotoxicity evaluation of the effects of prenatal exposure to fluoxetine in rats. *Fundamental and Applied Toxicology: Official Journal of the Society of Toxicology*, *23*(2), 194–205.

Wade, A. M., Lawrence, K., Mandy, W., & Skuse, D. (2006). Charting the development of emotion recognition from 6 years of age. *Journal of Applied Statistics*, *33*(3), 297–315. doi:10.1080/02664760500445756

Wagemans, J., Notebaert, W., & Boucart, M. (1998). Lorazepam but not diazepam impairs identification of pictures on the basis of specific contour fragments. *Psychopharmacology*, *138*(3-4), 326–333.

Wagner, G. C., Fisher, H., Pole, N., Borve, T., & Johnson, S. K. (1993). Effects of monoaminergic agonists on alcohol-induced increases in mouse aggression. *Journal of Studies on Alcohol.* Supplement, 11, 185–191.

Wagner, K. D., Ambrosini, P., Rynn, M., Wohlberg, C., Yang, R., Greenbaum, M. S., … Sertraline Pediatric Depression Study Group. (2003). Efficacy of sertraline in the treatment of children and adolescents with major depressive disorder: two randomized controlled trials. *JAMA,* 290(8), 1033–1041. http://doi.org/10.1001/jama.290.8.1033

Wagner, K. D., Robb, A. S., Findling, R. L., Jin, J., Gutierrez, M. M., & Heydorn, W. E. (2004). A Randomized, Placebo-Controlled Trial of Citalopram for the Treatment of Major Depression in Children and Adolescents. *American Journal of Psychiatry,* 161(6), 1079–1083. http://doi.org/10.1176/appi.ajp.161.6.1079

Walkup, J., & Labellarte, M. (2001). Complications of SSRI treatment. *Journal of Child and Adolescent Psychopharmacology*, 11(1), 1–4. doi:10.1089/104454601750143320

Walkup, J. T., Albano, A. M., Piacentini, J., Birmaher, B., Compton, S. N., Sherrill, J. T., … Kendall, P. C. (2008). Cognitive Behavioral Therapy, Sertraline, or a Combination in Childhood

Anxiety. *New England Journal of Medicine*, *359*(26), 2753–2766. doi:10.1056/NEJMoa0804633

Wang, D. J. J., Chen, Y., Fernandez-Seara, M. A., & Detre, J. A. (2011). Potentials and Challenges for Arterial Spin Labeling in Pharmacological Magnetic Resonance Imaging. *The Journal of Pharmacology and Experimental Therapeutics*, *337*(2), 359–366. doi:10.1124/jpet.110.172577

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology*, 54(6), 1063–1070.

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence*. New York, NY: The Psychological Corporation: Harcourt Brace & Company.

Weir, J. M., Zakama, A., & Rao, U. (2012). Developmental risk I: depression and the developing brain. *Child and Adolescent Psychiatric Clinics of North America*, *21*(2), 237–259, vii. doi:10.1016/j.chc.2012.01.004

Weissman, M. M., Wolk, S., Goldstein, R. B., Moreau, D., Adams, P., Greenwald, S., … Wickramaratne, P. (1999). Depressed adolescents grown up. *JAMA*, *281*(18), 1707–1713.

Wellman, C. L., Izquierdo, A., Garrett, J. E., Martin, K. P., Carroll, J., Millstein, R., … Holmes, A. (2007). Impaired stress-coping and fear extinction and abnormal corticolimbic morphology in serotonin transporter knock-out mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*(3), 684–691. doi:10.1523/JNEUROSCI.4595-06.2007

Whalen, P. J. (1998). Fear, vigilance, and ambiguity: Initial neuroimaging studies of the human amygdala. *Current Directions in Psychological Science*, *7*(6), 177–188.

Whittington, C. J., Kendall, T., Fonagy, P., Cottrell, D., Cotgrove, A., & Boddington, E. (2004). Selective serotonin reuptake inhibitors in childhood depression: systematic review of published versus unpublished data. *Lancet*, *363*(9418), 1341–1345. doi:10.1016/S0140-6736(04)16043-1

Whittle, S., Lichter, R., Dennison, M., Vijayakumar, N., Schwartz, O., Byrne, M. L., … Allen, N. B. (2014). Structural Brain Development and Depression Onset During Adolescence: A Prospective Longitudinal Study. *The American Journal of Psychiatry*. doi:10.1176/appi.ajp.2013.13070920

Why, Y. P., & Johnston, D. W. (2008). Cynicism, anger and cardiovascular reactivity during anger recall and human-computer interaction. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*, 68(3), 219–227.

Wijlaars, L. P. M. M., Nazareth, I., & Petersen, I. (2012). Trends in depression and antidepressant prescribing in children and adolescents: a cohort study in The Health Improvement Network (THIN). *PloS One*, *7*(3), e33181. doi:10.1371/journal.pone.0033181

Williams, J. M. G. (1997). *Cognitive psychology and emotional disorders*. John Wiley & Sons.

Willoughby, T., Good, M., Adachi, P. J. C., Hamza, C., & Tavernier, R. (2013). Examining the link between adolescent brain development and risk taking from a social-developmental perspective. *Brain and Cognition*, *83*(3), 315–323. doi:10.1016/j.bandc.2013.09.008

Wilson, J. K., & Rapee, R. M. (2005). The interpretation of negative social events in social phobia with versus without comorbid mood disorder. *Journal of Anxiety Disorders*, *19*(3), 245–274. doi:10.1016/j.janxdis.2004.03.003

Windfuhr, K., While, D., Hunt, I., Turnbull, P., Lowe, R., Burns, J., … National Confidential Inquiry into Suicide and Homicide by People with Mental Illness. (2008). Suicide in juveniles and adolescents in the United Kingdom. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *49*(11), 1155–1165. doi:10.1111/j.1469-7610.2008.01938.x

Wolitzky-Taylor, K. B., Ruggiero, K. J., McCart, M. R., Smith, D. W., Hanson, R. F., Resnick, H. S., … Kilpatrick, D. G. (2010). Has adolescent suicidality decreased in the United States? Data from two national samples of adolescents interviewed in 1995 and 2005. *Journal of Clinical Child and Adolescent Psychology: The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*, *39*(1), 64–76. doi:10.1080/15374410903401146

Woolrich, M. W., Behrens, T. E. J., Beckmann, C. F., Jenkinson, M., & Smith, S. M. (2004). Multilevel linear modelling for FMRI group analysis using Bayesian inference. *NeuroImage*, *21*(4), 1732–1747. doi:10.1016/j.neuroimage.2003.12.023

Woolrich, M. W., Ripley, B. D., Brady, M., & Smith, S. M. (2001). Temporal autocorrelation in univariate linear modeling of FMRI data. NeuroImage, 14(6), 1370–1386. http://doi.org/10.1006/nimg.2001.0931

Wright, C. I., Fischer, H., Whalen, P. J., McInerney, S. C., Shin, L. M., & Rauch, S. L. (2001). Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli. *Neuroreport*, *12*(2), 379–383.

Yang, E., Zald, D. H., & Blake, R. (2007). Fearful expressions gain preferential access to awareness during continuous flash suppression. *Emotion (Washington, D.C.)*, *7*(4), 882–886. doi:10.1037/1528-3542.7.4.882

Yang, E., Brascamp, J., Kang, M.-S., & Blake, R. (2014). On the use of continuous flash suppression for the study of visual processing outside of awareness. *Frontiers in Psychology*, 5, 724. http://doi.org/10.3389/fpsyg.2014.00724

Yang, T. T., Simmons, A. N., Matthews, S. C., Tapert, S. F., Bischoff-Grethe, A., Frank, G., … Paulus, M. P. (2007). Increased Amygdala Activation is Related to Heart Rate During Emotion Processing in Adolescent Subjects. *Neuroscience Letters*, *428*(2-3), 109–114. doi:10.1016/j.neulet.2007.09.039

Yang, T. T., Simmons, A. N., Matthews, S. C., Tapert, S. F., Frank, G. K., Bischoff-Grethe, A., … Paulus, M. P. (2009). Depressed adolescents demonstrate greater subgenual anterior cingulate activity. *Neuroreport*, *20*(4), 440–444. doi:10.1097/WNR.0b013e3283262e10

Yang, T. T., Simmons, A. N., Matthews, S. C., Tapert, S. F., Frank, G. K., Max, J. E., … Paulus, M. P. (2010). Adolescents with major depression demonstrate increased amygdala activation. *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*(1), 42–51.

Yoo, S. B., Kim, B.-T., Kim, J. Y., Ryu, V., Kang, D.-W., Lee, J.-H., & Jahng, J. W. (2013). Adolescence fluoxetine increases serotonergic activity in the raphe-hippocampus axis and improves depression-like behaviors in female rats that experienced neonatal maternal separation. *Psychoneuroendocrinology*, *38*(6), 777–788. doi:10.1016/j.psyneuen.2012.08.013

Young, A. W., Rowland, D., Calder, A. J., Etcoff, N. L., Seth, A., & Perrett, D. I. (1997). Facial expression megamix: tests of dimensional and category accounts of emotion recognition. *Cognition*, *63*(3), 271–313.

Young, S. N. (2013). The effect of raising and lowering tryptophan levels on human mood and social behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1615), 20110375. doi:10.1098/rstb.2011.0375

Zald, D. H. (2003). The human amygdala and the emotional evaluation of sensory stimuli. *Brain Research. Brain Research Reviews*, 41(1), 88–123.

Zangara, A., Blair, R., & Curran, V. H. (2002). A comparison of the effects of a β-adrenergic blocker and a benzodiazepine upon the recognition of human facial expressions. *Psychopharmacology*, *163*(1), 36–41. doi:10.1007/s00213-002-1120-4

Zito, J. M., Safer, D. J., DosReis, S., Gardner, J. F., Magder, L., Soeken, K., … Riddle, M. A. (2003). Psychotropic practice patterns for youth: a 10-year perspective. *Archives of Pediatrics & Adolescent Medicine*, *157*(1), 17–25.