The role of the 5-HT_{2C} receptor in emotional processing in healthy adults

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Abstract

Serotonin (5-HT) has long been implicated in the pathophysiology of depression and anxiety, and the therapeutic effect of treatments. Several drugs useful in treatment produce either acute or neuroadaptive changes in 5-HT_{2C} receptor activity, and there has been growing interest in how alterations in the 5-HT_{2C} receptor might be important in mediating antidepressant and anxiolytic activity.

The neuropsychological hypothesis of drug action implies that the clinical effects of medications active in anxiety and depression are best understood through the effects of these agents on the processing of emotional information. Thus far, however, there has been no systematic attempt to identify the role of the 5-HT_{2C} receptor in drug-induced changes in emotional processing in humans. The current research therefore investigated the effects of drug treatments with 5-HT_{2C} blocking properties on neural and behavioural responses to emotional information in healthy volunteers. An fMRI study demonstrated that a single dose of mirtazapine, an antidepressant with action at the 5-HT_{2C} receptor, reduces activation in regions important in emotional processing, such as the amygdala and the fusiform gyrus, to threat-relevant stimuli. A series of behavioural studies utilized drugs acting, at least in part, as 5-HT_{2C} antagonists and agonists to show that these drugs are able to alter emotional processing, particularly emotional memory. A seven-day administration of mirtazapine was shown to increase the recall of positive versus negative personality characteristics. A single dose of agomelatine, also an antidepressant with putative action at the 5-HT_{2C} receptor, did not increase slow wave sleep, suggesting, the drug had no effect of 5-HT_{2C} blockade in the brain. In Chapter 4, agomelatine and mCPP, a 5-HT_{2C} agonist, also shown to had no significant effect on emotional processing measures, but there was a statistical trend for agomelatine to increase memory for positive stimuli, and for mCPP to increase memory for negative stimuli. These findings suggest that antidepressants may work by altering the bias in emotional processing. Overall, the results

of this exploration of the role of the 5-HT_{2C} receptor in emotional processing have contributed to the understanding of antidepressant treatment, and raise new possibilities for the continuation of study in this field.

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List of Abbreviations

Ag/Ag Cl	silver/silver chloride
BNST	bed nucleus of the stria terminalis
SWS	slow wave sleep
5-HT	serotonin
5-HT_{2C}	serotonin 2C
mPFC	medial prefrontal cortex
GABA	gamma-aminobutyric acid
GAD	generalized anxiety disorder
mCPP	meta-Chlorophenylpiperazine
ETB	Emotional Test Battery
PFC	prefrontal cortex
BOLD	blood oxygen level dependence
fMRI	functional magnetic resonance imaging
NA	noradrenaline
SSRI	selective serotonin reuptake inhibitor
NRI	noradrenaline reuptake inhibitor
SNRI	serotonin and noradrenaline reuptake inhibitor
ACTH	adrenocorticotropic hormone
BDI	Beck Depression Inventory
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
ECT	electro-convulsive therapy
IV	intravenous
MAOI	monoamine oxidase inhibitor
REM	rapid eye movement
NREM	non rapid eye movement
EEG	electroencephalography
EMG	electromyography
PSG	polysomnography
VAS	visual analogue scale
STAI	State-Trait Anxiety Inventory
EPQ	Eysenck Personality Questionnaire
BFS	Befindlichkeits Scale
OCMR	Oxford Centre for Clinical Magnetic Resonance Research
MNI	Montreal Neurological Institute
SD	standard deviation
SEM	standard error of the mean
PCMCIA	personal computer memory card international association
DTI	diffusion tensor imaging
VLPFC	ventro-lateral prefrontal cortex
DA	dopamine
NART	National Adult Reading Test

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Declaration

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Chapter 3

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Chapter 6

Sharpley AL, Rawlings NB, Brain S, McTavish SF, Cowen PJ (2010) Does agomelatine block 5-HT2(C) receptors in humans? Psychopharmacology (Berl) DOI 10.1007/s00213-010-1993-6.

A portion of the sleep study data (Chapter 6) collection and processing was conducted by Dr. Ann Sharpley, and the cortisol analysis (Chapter 7) was conducted by Mike Franklin.

Chapter 1

Research background

1.1 General Introduction

This thesis investigates the effects of a number of psychotropic compounds, which all have purported action at the serotonin 2C (5-HT_{2C}) receptor, on the processing of emotional information. Serotonin (5-HT) has long been implicated in the pathophysiology of depression and anxiety, and the therapeutic effect of treatments. Several drugs useful in treatment produce either acute or neuroadaptive changes in 5-HT_{2C} receptor activity, and there has been growing interest in how alterations in the 5-HT_{2C} receptor might be important in mediating antidepressant and anxiolytic activity. It is hoped that a fuller understanding of the relevant mechanisms will facilitate the development of more effective treatments for anxiety and depression.

1.2 Major Depression

1.2.1 Definition and Symptoms

Major depressive disorder (MDD; also known as unipolar depression, or depression for the purpose of this thesis) is a psychiatric disorder characterized by a number of symptoms, encompassing behavioural, emotional and cognitive domains. The central elements of MDD are depressed mood, negative thinking, lack of enjoyment (anhedonia), reduced energy, and slowness. These main features of the disorder lead to decreased social and occupational functioning in individuals suffering from depression (Gelder et al. 2006). This brief overview will

focus primarily on MDD, although the disorder is frequently comorbid with anxiety (Gorman 1996) and commonalities of symptoms and treatment lead to these disorders being considered together in some contexts (Ressler and Mayberg 2007; Elliott et al. 2010). There are also differences to note between the disorders as well. For example, it is common for anxiety symptoms precede the onset of depressive symptoms and also outlast them (Wittchen et al. 2003). Another important difference between anxiety and depression can be found in affective processing, particularly in attention processing and the negative bias seen in these disorders. Based on the results from numerous studies, it has been proposed that the process leading to attention to threat cues in anxiety begins at an early, pre-conconscious, processing stage. This early sensitivity to threat cues may be absent or inhibited in depression, and is replaced by slower, strategically directed attention to mood-congruent information (Mathews and MacLeod 2005). Another difference in affective processing between the disorders can be found in memory. There is much stronger evidence for a negative bias in memory in depression than there is for a bias in anxiety, which has mixed results (Mathews and MacLeod 2005). It has been suggested that the mixed results are due to the fact that anxiety groups have differences in encoding rather than differences in recall (Mathews and MacLeod 2005).

While there are meaningful divisions to be made between emotional disorders, there are several reasons for considering these disorders together, as mentioned above. Anxiety symptoms overlap highly with those of depression (Ressler and Mayberg 2007), and common features include worry and apprehension, muscle

tension, psychological arousal such as irritability, autonomic overactivity, and sleep disturbances (Gelder et al. 2006). There are also common pharmacological treatment for the disorders, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin and noradenaline reuptake inhibitors (SNRIs), and non-pharmacological treatment such as cognitive behavioural therapy is also used for both disorders (Ressler and Mayberg et al. 2007). Drugs used for the treatment of both disorders, such as SSRIs, have also been shown to alter emotional processing (Harmer et al 2004). While there is overlap between drug treatment in the disorders and their effect on affective processing. it should be noted that drugs used to treat anxiety rather than depression can have a different pattern or results from drugs used to treat depression and not anxiety. Diazepam, a benzodiazepine used for anxiety treatment, alters processing in the masked attentional dot probe task and the emotion-potentiated startle task (Murphy et al. 2008). These tasks have been suggested to measure the anxiolytic action of drugs on emotional processing due to the fact that they involve more automatic processing rather than more strategic processing required in tasks such as the facial expression recognition and emotional memory task (Murphy et al. 2008). In contrast, reboxetine, a noradrenergic antidepressant that is not typically used in the treatment of anxiety, showed the opposite pattern to diazepam on these tasks with effects on the recognition of facial expressions and emotional memory but no effect on the emotion potentiation of the startle response in the presence of negative pictures (Harmer et al. 2004). There are also similarities in the neurobiology of these disorders, as both have been shown to involve dysregulation in the prefrontal cortex, subgenual cingulate cortex, and the hippocampus and amygdala

(Ressler and Mayberg 2007). The amygdala has been shown to be overactive in both disorders (Drevets 2000), and will be further discussed in Section 1.2.5 and Chapter 3. Overall, given the commonalities between depression and anxiety, the remaining sections will focus primarily on depression.

The main features of depression, mentioned above, require further characterization. Depressed mood may be diurnal, meaning it is worse in the morning and improves as the day goes on. Negative thoughts can be categorized into several different themes such as worthlessness, pessimism, and guilt. Anhedonia is characteristic, and there is no enjoyment in activities an individual would normally wish to pursue. Additionally, individuals may withdraw from social encounters. A reduction in energy is common and most tasks can become an effort. Also present may be anxiety, irritability, a poor memory, psychomotor retardation, or conversely, agitation. Another set of symptoms, often referred to as biological symptoms, is also important in depression. These symptoms may involve sleep disturbance, loss of appetite, and loss of weight (Gelder et al. 2006).

Depression is diagnosed on the basis of a number of criteria described in the Diagnostic and Statistical Manual (DSM-IV) (American Psychiatric Association, 2000) and the International Statistical Classification of Diseases and Related Health Problems (ICD-10) (World Health Organization, 1994). Diagnosis of depression requires at least five out of nine symptoms to be present during the same two-week period, and must represent a change from previous functioning. Also, at least one of the symptoms must either be depressed mood or loss of

interest or pleasure in activities. The other symptoms include those mentioned above: weight loss or gain, sleep disturbances, psychomotor agitation or retardation, loss of energy, feelings of worthlessness or excessive or inappropriate guilt, diminished ability to think or concentrate, and recurrent thoughts of death or suicide ideation, attempt, or plan (Gelder et al. 2006).

1.2.2 Epidemiology and Health Burden

There are a number of important statistics often used to highlight the prevalence and suffering associated with depression, as well as the need for research into causes and treatment of the disorder. For example, the prevalence of depression in a 12-month period within an industrialized country is between two and five percent, and lifetime rates are likely between 10 and 20 percent (Alonso et al. 2004). These estimates make depression one of the most widespread psychiatric disorders (Gotlib and Joormann 2010), and the World Health Organization Global Burden of Disease Study has ranked depression as the most burdensome disease in the world when considering total disability-adjusted years among people in the middle years of life (Gotlib and Joormann 2010; Murray and Lopez 1996). In the United States, the recent annual cost of lost productivity due to depression was estimated to be over $36 billion (Kessler et al. 2006). One reason for the substantial health burden of depression is that in about 80% of patients, it runs a recurrent course and in some of the cases, it becomes chronic and unremitting. Depression is associated with an increased mortality, due largely to the risk of suicide, which is elevated to 12 times that of the general population (Gelder et al. 2006).

1.2.3 Aetiology

The aetiology of depression is complex, and is most likely due to a number of factors in combination, such as genetics, early life experiences, personality and stressful life events. The relative contributions of genetics and environmental factors have been assessed through twin and adoption studies. Based on meta-analyses, the heritability of depression is around 40% (Kendler and Prescott 1999; Sullivan et al. 2000). These genetic findings indicate that environmental factors are also important variables in the aetiology of depression. Childhood adversity, particularly sexual abuse, increases the risk for depression later in life (Bifulco et al. 1991). Neuroticism has also been associated with the development of depression (Kendler et al. 2006). Additionally, episodes of depression are often triggered by stressful life events, such as a death in the family or a divorce (Mundt et al. 2000). Thus, it appears that depression may result from both genetics and environmental factors (Kendler et al. 1995).

While depression is diagnosed based on the symptoms described above, depressive conditions are also thought to be characterized by neuropsychological and neurochemical changes, but the understanding of these changes is incomplete. The neuropsychology and neurochemistry of depression are considered in the following sections.

1.2.4 Neuropsychology of Depression

Traditional psychological views of depression have focused on the importance of negative biases of information processing in this mood disorder. These negative

biases are hypothesized to play a role in the pathophysiology and maintenance of depression (Beck et al. 1979). Beck's (1967) cognitive model of depression proposed that individuals with depression have a negative schema, or recurrent patterns of depressed thinking, and that this schema causes individuals to preferentially process information in the environment, which is congruent with negative thinking. Also part of Beck's theory is a cognitive triad, which may underlie depression. This triad of negative thoughts consists of pessimistic views about the self, world, and future. These negative thoughts are maintained by cognitive distortions such as overgeneralization and focusing on one detail of a situation and ignoring more important features, known as selective abstraction (Beck et al. 1979).

The negative bias suggested by Beck contributes to the cognitive and neuropsychological impairments that are characteristic of depression. Increased elaboration of negative information, difficulties disengaging from negative material, and deficits in cognitive control when processing negative information are all reported in depression (Gotlib and Joormann 2010). In addition to these cognitive biases in processing of emotional material, there are also suggestions of a more general or global deficit in cognitive functioning, which would affect both emotional and non-emotional processing. These more general changes in depression such as difficulties with memory and concentration are reported by patients (Burt et al. 1995), although results supporting this proposition are not always clear, and the nature of cognitive deficits in depression remains to be determined (Gotlib and Joormann 2010). It may be that there is not a general

deficit in cognitive processing in depression but that depressed individuals have difficulties inhibiting the processing of irrelevant information during cognitive tasks (Gotlib and Joormann et al. 2010). A number of factors may contribute to the lack of clarity in the literature, and influence findings, such as type of task used (explicit versus implicit, duration of stimuli etc.), medication state, age, and severity of depression (Elliott 1998).

Evidence for abnormalities in affective cognition are more clear, with studies showing negative biases in facial processing and interpretation as well as attention and memory in individuals with depression (Mathews and MacLeod 2005; Elliott et al. 2010). Abnormalities in identifying facial expressions of emotion have been found in depression, with depressed individuals exhibiting negative biases in identifying emotions. Patients with depression interpret neutral or ambiguous facial expressions as negative and happy faces as neutral (Gur et al. 1992; Surguladze et al. 2004). It has also been shown that individuals with depression have greater sensitivity to sad faces and reduced sensitivity to happy faces, as well as a greater response bias to identifying faces as sad, as measured by facial stimuli with varying emotional intensity (Joormann and Gotlib 2006; Harmer et al. 2009). Though these findings suggest an overall increase of negative bias in facial processing in depression, the directionality of the results are sometimes inconsistent. For example, some studies report a reduced sensitivity to happy or positive faces, while others report an increased sensitivity to sad or negative faces. The interpretation of ambiguous situations as negative is also important in depression (Beck 1967). For example, studies have also used self-report measures

to assess interpretation selection, and show that depressed individuals interpret ambiguous situations as negative (Krantz and Hammen 1979). The facial processing tasks described above could also be considered a probe of this bias, as participants interpret the emotional content of the face before pressing a button to identify the emotion of the face. Other tasks have used ambiguous homophones or sentences to assess interpretation bias in depression (Lawson and MacLeod 1999; Mogg et al. 2006), although results are not as clear as with self-report measures (Mathews and MacLeod 2005).

Memory abnormalities are also associated with depression, and there is evidence particularly for a tendency to remember negative material in explicit memory paradigms (Matt et al. 1992; Harmer et al. 2009). A number of studies have demonstrated this bias in memory for negative information. After being presented with lists of emotional words, depressed patients selectively remember more negative words (Bradley et al. 1995; Ruiz-Caballero and Gonzalez 1994; Denny and Hunt 1992). One interpretation of this negative bias is that it may be the absence of a normal positive bias seen in healthy individuals (Ellwart et al. 2003; Elliott et al. 2010). It should be noted, however, that some studies have not been able to demonstrate a negative bias in depression at all (Danion et al. 1995; Banos et al. 2001). A negative bias in attention has also been associated with depression (Gotlib et al. 2004). This negative bias has been observed using several different paradigms measuring attention, such as the emotional Stroop task and the dot-probe task. This attentional bias in depression, however, is evident only at longer exposure durations, perhaps suggesting a strategic direction of attention to

negative emotional information (Mathews and MacLeod 2005). It is also possible that the bias observed could be a response bias where depressed individuals deploy attention in a similar manner to non-depressed individuals, but display an altered response strategy to negative material (Mogg et al. 2006). Under the emotional Stroop task, individuals with depression showed slower reaction times to negative words, indicating increased interference to those words (Gotlib and McCann 1984; Segal et al. 1995; Broomfield et al. 2007; Williams et al. 1996). In the dot-probe task, depressed patients showed an attentional bias to socially threatening words (Mathews et al. 1996). Dot-probe tasks utilizing emotional faces also reveal a negative bias in depression. Gotlib et al. (2004) demonstrated depressed participants had a selective bias towards attending sad faces. Again, some studies have not demonstrated a negative attentional bias (Hill and Knowles 1991; Neshat-Doost et al. 2000), but it is possible these null results are due to the nature of the task and the use of subclinical populations (Rinck and Becker 2005; Elliott et al. 2010)

1.2.5 Structural and Functional Brain Differences in Depression

Depression is associated with both structural and functional brain abnormalities in a number of areas, which can be linked to some of the neuropsychological deficits mentioned above (Drevets et al. 2008). Particularly, structural abnormalities have been found in patients with depression in regions important for the processing of emotion, such as the prefrontal cortex (PFC), hippocampus, subgenual cortex, and amygdala. Reduced grey matter volume of the orbital, ventrolateral, and frontal polar/dorsal anterolateral PFC has been found in depression (Coffey et al. 1993;

Goodwin et al. 1997; Bremner et al. 2002; Drevets et al. 2004; Drevets and Price 2005), as has a decreased volume in the subgenual anterior cingulate cortex (Botteron et al. 2002; Drevets 1997). There are discrepant results regarding volume differences in the hippocampus and amygdala in depression. Some studies report reductions in hippocampal volume (Bremner et al. 2000; Sheline et al. 1999), but others report no change (Ashtari et al. 1999). In the amygdala, a decrease in volume has been reported to be associated with depression (Sheline et al. 1998), but also no difference between depressed individuals and healthy individuals has been reported (Pantel et al. 1997). Future studies with more homogeneous clinical populations and studies controlling for differences in medication status may help to clear up these inconsistent results. For example, besides the medication status, these studies have not accounted for personality differences in individuals such as introversion or extroversion, nor for differences in trait anxiety levels.

In addition to grey matter volume abnormalities in depression, several white matter differences have also been described. White matter is decreased in the corpus collosum of adults with depression, both in the genu and the splenium (Drevets et al. 2008). Studies using diffusion tensor imaging (DTI) to measure white matter in the brain have also identified regions of white matter abnormalities. For example, DTI studies have found changes in the frontal gyrus (Li et al. 2007; Ma et al. 2007), parietal lobe and occipito-temporal gyrus (Ma et al. 2007), and left striatum (Kieseppä et al. 2010) in depression.

There have been many studies utilizing functional brain imaging in depressed individuals. A few key regions found to have altered activity in depression are discussed below, and many are also the regions with structural abnormalities found in depression, described above. First, the prefrontal cortex (including the orbitofrontal, dorsolateral and dorsomedial cortices) has been implicated in depression. The prefrontal cortex plays a role in modulating emotional behaviour (Phillips et al. 2003a). The ventrolateral prefrontal cortex (VLPFC) has increased metabolism and blood flow in depression, and these measures are inversely correlated to ratings of depression (Drevets 1992). The VLPFC and posterior orbital cortex play a role in modulating behavioural and cognitive responses associated with defensive, fear and reward-directed behaviour as reinforcement contingencies change (Drevets 2000). It has been hypothesized that the increase in VLPFC activity during depression may reflect the effort to decrease aversive thought and emotion. From that concept, it is possible then, that changes in VLPFC activity may be related to abnormal emotional processing and perseverative responses to stressors seen in depression.

The dorsomedial and dorsolateral PFC are also associated with depression, but with a decrease in metabolism and blood flow rather than an increase (Baxter et al. 1989; Buchsbaum et al. 1997; Bench et al. 1993; Soares and Mann 1997). The dorsomedial PFC appears to play a role in the regulation of autonomic responses and arousal associated with emotional states and behaviour (Phillips et al. 2003a). Studies have shown decreased activation during a sad mood induction in healthy volunteers (Mayberg et al. 1999), and an inverse relationship between activation

and measures of autonomic function and anxiety during a novel cognitive task (Simpson et al 2001).

The dorsolateral PFC is implicated in working memory (Goldman-Rakic 1987) and studies have demonstrated activation during demanding cognitive tasks requiring attention, however, during some emotional states, activation decreases in this region (Mayberg et al. 1999). It has been suggested that the decrease in activity during depression in the dorsolateral PFC reflects a suppression of activity during negative processing, and that this suppression may relate to impairments of attention and memory that may be seen in depression (Drevets and Raichle 1998).

Abnormalities in the anterior cingulate are also implicated in depression, and are thought to reflect impaired attentional processes and abnormal emotional processing although findings across studies are inconsistent. The dorsal anterior cingulate has been associated with reduced blood flow in depression, but the pregenual anterior cingulate cortex has been shown to have increased blood flow and metabolism in most studies (Drevets 2000).

In individuals with depression, elevated blood flow within the amygdala has also been reported (Drevets et al. 1992). This increase is positively correlated to the severity of depression (Drevets et al. 1992; Abercrombie et al. 1998). The amygdala has been implicated in the modulation of vigilance and attention to emotionally salient information (Davis and Whalen 2001) as well as in the production of emotional states (Phillips et al. 2003b). Functional imaging studies

have shown an increase in amygdala activation to masked and unmasked emotional facial expressions in patients with depression (Drevets 2003), and an increase in left amygdala activation to fearful faces in depressed individuals (Sheline et al. 2001). The increase in amygdala activity is reduced following eight weeks of serotonergic antidepressant treatment, and although this change in depressed individuals following antidepressant treatment has not been compared directly to changes in healthy individuals, it is likely the responses would be the same (Sheline et al. 2001; Fu et al. 2004). It has been suggested that the increased activity of the amygdala might play a role in the hypervigilant monitoring of negative information found in depression and anxiety (Whalen et al. 2002; Harmer et al. 2006a). These imaging studies examine the responses to negative emotion, but some use different examples of negative emotion, including fear and sadness. There are recognizable differences between fearful and sad faces, and there is some evidence for specific brain regions being involved in the processing of these individual emotions (Phan et al. 2002). A recent meta-analysis reported that fearful facial expressions are the most consistent probes of amygdala function and are therefore included in the studies in this thesis (Phan et al. 2002). In general, however, depression is considered to be associated with a negative bias, and it is useful to be ale to consider studies together which include different types of negative emotion. It can also be useful to include a range of emotions to capture a full profile of emotion processing, to try and determine if there are deficits in processing positive emotions or an access of processing negative emotions. Examples used in this thesis include the gender processing task (using fear and

happy faces) and the facial expression recognition task, which will be explained in further detail in Chapters 2 and 3.

Overall, the structural changes, mainly volume reductions, associated with depression are found in regions implicated in emotion processing and regulation. The functional abnormalities implicated in depression also suggest impairments in emotional processing (Phillips et al. 2003b). In combination, it is possible that these changes result in an increased tendency to identify stimuli as emotional and experience them in a negative context (Phillips et al. 2003b).

1.3 The 5-HT System and Depression

1.3.1 Monoamine Hypothesis

Neurochemical approaches to depression have focused on the monoamine neurotransmitters as these pathways have been found to be important in mediating antidepressant drug action (Anderson and Reid 2004). Three monoamine transmitters are usually implicated: 5-HT, noradrenaline (NA), and dopamine (DA). Each of the neurotransmitters has been implicated in mood disorders, but the focus here will be on 5-HT. The original monoamine hypothesis of depression suggested that a lowered availability or activity of monoamines may be the cause of depression. It is now thought, however, that the relationship between monoamines and mood disorders is much more complicated than a direct depletion of neurotransmitters causing depression. For example, depletion of noradrenaline and 5-HT in healthy individuals does not lead them to become depressed (Ruhe et al. 2007). Nevertheless, the monoamine theory does provide a

useful framework for the discussion on the involvement of 5-HT in disorders affecting emotional processing such as depression and anxiety, especially as much of the search for antidepressants has been driven by this hypothesis.

The monoamine hypothesis developed following incidental findings in 1950 of an increase in depressed symptoms in patients treated with reserpine for hypertension. It was found that reserpine depletes 5-HT, NA and DA in the brain by inhibiting the uptake of the transmitter into synaptic vesicles in the presynaptic cell, where it is instead degraded by monoamine oxidase. A more serendipitous finding also contributed to the idea that depletion of monoamines can cause depression. A drug developed to treat tuberculosis in the 1950s, a monoamine oxidase inhibitor (MAOI) called isoniazid, also elevated the mood of depressed patients with tuberculosis. MAOIs increase the concentration of 5-HT and NA by reducing their breakdown by monoamine oxidase. Following these discoveries, other types of drugs acting on 5-HT and NA systems were found to be effective antidepressants. Tricyclic antidepressants extend the action of neurotransmitters in the synaptic cleft by blocking the reuptake of 5-HT and noradrenaline by presynaptic neurons. Another set of antidepressants, SSRIs also affect 5-HT neurotransmission by blocking the reuptake of 5-HT into the presynaptic cell.

While the effectiveness of SSRIs provides some evidence that the enhancement of 5-HT neurotransmission brings about therapeutic response in some antidepressants, other observations in individuals with depression have also supported this idea. Patients with depression and mood disorders have some

abnormalities in measures of 5-HT neurochemistry. For example, individuals with untreated depression have decreased plasma tryptophan, the precursor to 5-HT. In addition, there is evidence that 5-HT neuroendocrine responses are also impaired in depressed patients (Anderson et al. 1992; Golden et al. 1992; Bhagwagar et al. 2002). A decrease in $5HT_{1A}$ receptor binding in cortical and subcortical regions as measured by PET has also been found (Drevets et al. 1999; Drevets et al. 2000; Sargent et al. 2000). However, not all studies are in agreement (Parsey et al. 2006). Another issue is that some of the 5-HT abnormalities persist in patients following clinical recovery and withdrawal of antidepressant treatment (Bhagwagar et al. 2004).

The psychological effect of altering 5-HT transmission has also been studied. Depletion of tryptophan, by administering a mixture of amino acids that lacks tryptophan, leads to a temporary clinical relapse in patients with a history of mood disorder (Smith et al. 1997; Ruhe et al. 2007). In people at risk for depression through a positive family history of depression, tryptophan depletion produces a mild subjective lowering of mood but not overt clinical symptomatology (Ruhe et al. 2007). These findings suggest that impaired serotonin function may play a role in mediating vulnerability to mood disorder but is neither sufficient nor necessary to cause depression. However, it does seem clear that drugs whose acute action is confined to potentiation of brain 5-HT function are helpful in the treatment of depression and anxiety (Anderson and Reid 2004; Baldwin et al. 2005). Understanding the mode of action of these agents in neuropsychological terms

may also shed light on how low 5-HT function may predispose to clinical depression.

1.3.2 The 5-HT System

5-HT is a biogenic amine, derived from the amino acid tryptophan. The enzyme tryptophan hydroxylase converts the amino acid tryptophan to 5-hydroxytryptophan, which is then converted to 5-HT by the enzyme amino acid decarboxylase. 5-HT is known to modulate a wide range of behavioural processes such as attention, perception, sleep, memory, aggression, sexuality, appetite, and mood (Glennon and Lucki 1988; Barnes and Sharp 1999; Ursin 2002; Blundell 1984; Millan 2003). The cell bodies of 5-HT neurons are located in the raphe nuclei in the brainstem, and project widely throughout the brain, to regions including the amygdala, hippocampus, nucleus accumbens, striatum, frontal cortex, thalamus, and cingulate, as well as the spinal cord and cerebellum (Azmitia and Segal 1978; Gray 1987; Deakin and Graeff 1991; Wilson et al. 1993; Eison and Eison 1994; Handley 1995; Baumgarten and Grozdanovic 1997; McQuade and Sharp 1997; Vertes et al. 1999; Kirby et al. 2003). Post-synaptic receptors for 5-HT are classified into at least seven categories, or families (Barnes and Sharp 1999). All except the ligand-gated ion channel $5HT_3$ receptor are G protein-coupled receptors that activate a second messenger cascade. These seven families contain 14 distinct mammalian 5-HT receptor subtypes (Hoyer et al. 1994) (Table 1).

5-HT system	Receptors	Action on post-synaptic neuron	Molecular Action
5-HT_1	1A, 1B, 1D, 1E, 1F	Inhibitory	G-protein coupled decreases cellular levels of cAMP
5-HT_2	2A, 2B, 2C	Excitatory	G-protein coupled increases cellular levels of IP3 and DAG
5-HT_3		Excitatory	Ligand-gated Na+ and K+ cation channel depolarises cell membrane
5-HT_4		Excitatory	G-protein coupled increases cellular levels of cAMP
5-HT_5	5A, 5B	Inhibitory	G-protein coupled decreases cellular levels of cAMP
5-HT_6		Excitatory	G-protein coupled increases cellular levels of cAMP
5-HT_7		Excitatory	G-protein coupled increases cellular levels of cAMP

Table 1.
5-HT receptor type and physiology. cAMP: cyclic adenosine monophosphate; IP3: inositol triphosphate; DAG: diacylglycerol.

The 5-HT_2 receptor family is split into three subtypes whose second messenger signaling involves inositol cycling. 5-HT_{2A} and 5-HT_{2C} receptors have a high sequence homology and many psychotropic drugs have a substantial affinity for both receptor subtypes, though more selective ligands are available experimentally. In particular, a number of selective 5-HT_{2C} agonists and antagonists have been developed for various therapeutic purposes including the management of depression, anxiety, schizophrenia and obesity. This demonstrates the importance of 5-HT_{2C} receptors on fundamental processes such as anxiety and feeding as well as the interactions of 5-HT_{2C} receptors with other

neurotransmitters for example, dopamine (Millan 2005; Dekeyne et al. 2008). Hypotheses concerning the neurochemical basis of the antidepressant actions of 5-HT_{2C} receptor blockade include the interruption of post-synaptic 5-HT feedback loops and increased release of dopamine and NA in the PFC (Sharp et al. 2007; Millan et al. 2000; Stahl 2007).

1.3.3 The 5-HT_{2C} Receptor

The 5-HT_{2C} receptor is part of the 5-HT_2 receptor family along with the 5-HT_{2A} and 5-HT_{2B} receptors, and is a G protein-coupled receptor. The molecular structure of the receptors and distribution of receptors in the brain have been well-characterised (Barnes and Sharp 1999). 5-HT_{2C} receptors are distributed extensively throughout the brain, but the greatest density is found in the choroid plexus (Pazos et al. 1987). The frontal cortex, hippocampus, amygdala, cingulate, nucleus accumbens, subthalamic nucleus, suprachiasmatic nucleus, and the grey matter of the spinal cord are also enriched with 5-HT_{2C} receptors. Notably, many of these structures are implicated in mood and cognitive changes often found in depression (Millan et al. 2003; Sharma et al. 1997; Lopez-Gimenez et al. 2002). The 5-HT_{2C} receptor has been shown to exert an inhibitory influence on frontocortical dopaminergic and adrenergic pathways (Millan 2003; Di Giovanni et al. 1999; Gobert et al. 2000).

Interestingly, 5-HT_{2C} receptors downregulate in the brain in response to chronic exposure to both agonists and antagonists. This could, in part, relate to apparent inverse agonist properties of some 5-HT_{2C} receptor antagonists (Barker et al. 1994;

Labrecque et al. 1995). Thus, some antagonists appear to have constituent 'negative' activity at the 5-HT_{2C} receptor and do not merely prevent the action of the endogenous neurotransmitter. Thus an inverse agonist (in contrast to a 'classical' or neutral antagonist), will produce pharmacological effects no matter what the endogenous 'tone' of the system.

5-HT_{2C} receptor function

In this section, relevant studies relating to the function of the 5-HT_{2C} receptor are reviewed. Generally, activation of central 5-HT_{2C} receptors is associated with the following behavioural responses: hypolocomotion, hypophagia, anxiety, and hyperthermia (Koek et al. 1992). The association between 5-HT_{2C} receptors and mood and anxiety disorders are the focus of the studies discussed here. These findings are based on studies in both animals and humans of the consequences of administration of 5-HT_{2C} agonists and antagonists. One important compound to note is meta-chlorophenylpiperazine (mCPP), a metabolite of the antidepressants trazodone and nefazodone, which has been widely used in the study of 5-HT_{2C} receptors. mCPP is a 5-HT agonist whose pharmacological effects have been extensively studied in both humans and animals. According to ligand-binding studies, mCPP binds to a variety of 5-HT receptors but its among its highest affinities is that for the 5-HT_{2C} receptor (Hoyer 1998) (Table 2). It also binds as an agonist to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, and 5_HT_3 receptors as well as α_2-adrenoceptors (Hoyer 1988; Meneses 2002). It is a partial agonist for 5-HT_{2C} receptors and may also be one for 5-HT_{1B} receptors (Middlemiss and Hutson 1990; Fiorella et al. 1995). 5-HT_{1B} receptors are absent in the human brain, so mCPP is

thought to exert agonist actions mainly through 5-HT_{2C} receptor stimulation (Anderson et al. 2002). mCPP is often used as a neuroendocrine probe in humans and elevates plasma levels of prolactin, cortisol and adrenocorticotropic hormone (ACTH) as well as increasing temperature and decreasing food intake (Kahn and Wetzler 1991). However, the lack of specificity of mCPP for the 5-HT_{2C} receptor does complicate interpretation of its effects.

5-HT Receptor	Affinity (pK_i)
5-HT_{1A}	6.5
5-HT_{1B}	6.6
5-HT_{1D}	5.1
5-HT_{2A}	6.7
5-HT_{2B}	7.6
5-HT_{2C}	7.7
5-HT_3	7.0
5-HT_7	6.6

Table 2.
mCPP 5-HT receptor affinities (from Hoyer 1988; Meneses 2002).

5-HT$_{2C}$ receptor modulation and antidepressant action

Animal studies have taken several different approaches when investigating the antidepressant action of 5-HT$_{2C}$ receptor modulation. Some studies have focused on pharmacological manipulation while others have examined the functional status of 5-HT$_{2C}$ receptors while using paradigms modeling depression or depression treatment. For example, it has been shown that long-term tryptophan depletion in rats leads to the upregulation of 5-HT$_{2C}$ receptors (Franklin et al. 1999). Mitchell et al. (2003) examined the effect of repeated electroconvulsive shock (ECS) treatment in rats on 5-HT$_{2C}$ receptor-mediated hypolocomotor induced by mCPP. ECS treatment is used as an effective treatment for major depression in humans, and has been shown to induce effects similar to chronic antidepressant treatment in animal models of depression (Mitchell et al. 2003). Following repeated ECS, the 5-HT$_{2C}$ receptor-mediated hypolocomotion induced by acute mCPP challenge was abolished, which suggests ECS treatment may be associated with adaptive changes in 5-HT$_{2C}$ function.

Another paradigm used in animal studies of 5-HT$_{2C}$ receptor function is the forced swim test. This test involves placing a rat or mouse in a cylinder filled with water and forcing them to swim for a set amount of time. A second trial is performed 24 hours later, and the time spent immobile is recorded. Antidepressant action decreases immobility time. While, like most animal models, the forced swim test does not have great face validity as a model of depression, it is often used as a screen to detect potential antidepressant drug activity (Nestler and Hyman 2010).

The forced swim test has also been used to demonstrate antidepressant effects of 5-HT_{2C} receptor antagonists (Millan 2005).

Human studies concerning 5-HT_{2C} receptors and depression are less clear. Studies involving endocrine models, such as mCPP challenge studies, do not show consistent results. mCPP has been shown to increase physical symptoms in individuals with depression compared to healthy controls, but no other behavioural differences were found (Kahn and Wetzler 1991). Additionally, cortisol and prolactin responses of depressed patients did not differ from healthy controls (Kahn and Wetzler 1991). Adding to the limited findings, it has been difficult to study the brain 5-HT_{2C} receptor in humans in vivo, as specific imaging ligands for this receptor have apparently proved difficult to develop.

Some post-mortem studies do hint that 5-HT_{2C} receptors could be involved in depression. For example, Gurevich et al. (2002) found altered 5-HT_{2C} pre-mRNA editing site preferences in the PFC in depressed suicide victims. This study also examined changes in editing site preferences in mice chronically treated with fluoxetine. The changes in the mice were opposite to those found in the suicide victims with a history of depression. This finding suggests suicide victims with a history of depression may have an overall decrease in 5-HT_{2C} receptor activity in the PFC, although the inclusion of subjects on antidepressant treatment prior to suicide makes interpretation difficult (Gurevich et al. 2002).

Adding support to the involvement of 5-HT_{2C} receptors in depression is the number of antidepressants that appear to modify 5-HT_{2C} receptor function. One interesting point regarding SSRIs and other antidepressants, which has been made repeatedly over the last couple of decades, is the fact that the well-known delayed onset of therapeutic efficacy of SSRIs can be matched with their progressive down-regulation of 5-HT_{2C} receptors (Deakin 1998; Kennett et al. 1994; Cowen 1998). It is also worth noting that several clinically effective antidepressants directly antagonize 5-HT_{2C} receptors. For example, the tricyclic antidepressants amitriptyline and clomipramine, as well as trazodone and nefazodone, mirtazapine and agomelatine, are all reported to have antagonistic actions at 5-HT_{2C} receptors. Some of these antidepressants will be used for pharmacological manipulation in the experimental chapters of this thesis, along with mCPP, and require more detailed characterisation.

The antidepressant mirtazapine acts as an antagonist at the 5-HT_{2C} receptor, among other receptor sites. Mirtazapine is a new-generation antidepressant, sometimes referred to as a noradrenaline and selective serotonin antidepressant (NaSSA). Mirtazapine's unique and complex pharmacological actions include the blockade of monoamine receptors such as noradrenaline α_2-adrenoceptors and 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors (de Boer 1995; de Boer 1996; Davis and Wilde 1996) (Table 3). Mirtazapine's action as an α_2-adrenoceptor antagonist is thought to increase both norepinephrine and serotonin (5-HT) neurotransmission, although it is unclear if an increase in 5-HT neurotransmission occurs in humans during real world clinical treatment (Whale et al. 2000). α_2 antagonism blocks

pre-synaptic α_2 autoreceptors on noradrenergic neurons, which generally inhibit the neuron. This antagonism of the autoreceptors causes the noradrenergic neurons to become disinhibited and increase NA neurotransmission (Stahl 2000). α_2 antagonism may increase 5-HT neurotransmission by blocking pre-synaptic α_2-heteroceptors, which generally inhibit 5-HT neurons. The 5-HT neurons are then disinhibited and increase 5-HT neurotransmission as NA can no longer block 5-HT release. Another potential method for the increase of 5-HT release via α_2 antagonism is through the innervation of 5-HT neurons by NA neurons. The NA input enhances 5-HT release via the post-synaptic α_1 receptors on 5-HT neurons. When NA is disinhibited via α_2 antagonism, increased NA neurotransmission causes α_1 receptor stimulation, which causes more 5-HT neurotransmission (Stahl 2000). The 5-HT_{2C} receptors exert tonic, inhibitory control on fronto-cortical DA and adrenergic pathways via GABAergic interneurons, and the activity of these pathways are compromised in depression (Gobert et al. 2000; Millan et al. 2000). Blockade of the 5-HT2C receptors, then, increases levels of DA and NA in the frontal cortex (Millan et al. 2003). The α_2-adrenoceptor antagonism and $5\text{-HT}_{2A/2C}$ receptor antagonism of mirtazapine are thought to be critical in its clinical antidepressant action (Invernizzi and Garattini 2004; Dekeyne and Millan 2009). Mirtazapine's action as an antagonist at other receptors, for example histamine H_1 receptors, is believed to underlie some of its characteristic side effects such as sedation and weight gain. It has also been suggested that 5-HT_3 receptor blockade may prevent mirtazapine from causing nausea during clinical use, and and perhaps

prevent serotonin-induced sexual side effects (Anttila and Leinonen 2001; Davis and Wilde 1996).

Agomelatine is a recently licensed antidepressant with a novel mechanism of action. It acts as a potent agonist at melatonin receptors (MT_1 and MT_2) and a less potent antagonist at 5-HT_{2C} receptors (Millan et al. 2003) (Table 4). Melatonin, a hormone generated by the pineal gland, regulates the suprachiasmatic nucleus (SCN), which controls circadian and biological rhythms in mammals. Melatonin and 5-HT_{2C} receptors are enriched in the SCN and help modulate SCN function. The anxiolytic properties of agomelatine have been linked to 5-HT_{2C} blockade, as has its antidepressant profile, rather than the agonism of the melatonin receptors (Millan et al. 2003). This profile is thought to result from the blockade of 5-HT_{2C} receptors which increases extracellular levels of DA and NA in the frontal cortex, but not the nucleus accumbens and striatum (Millan et al. 2003). Agomelatine has also been shown to resychronize altered circadian rhythms in humans with depression and in animal models (Leproult et al. 2005; Fuchs et al. 2006) which may be relevant to its mode of action because depression has been associated with disturbed circadian rhythms, and this process likely involves the MT_1 and MT_2 receptor agonism (Lam 2008). Agomelatine has a favourable side-effect profile relative to more standard agents, with neutral effects on sexual function, bodyweight, and an absence of discontinuation symptoms (Zupancic and Guilleminault 2006).

Receptor	Affinity (pK_i or pA_2)
α_2-adrenergic autoreceptor	7.7
α_2-adrenergic heteroreceptor	8.0
Postsynaptic α_2-adrenoceptor	7.3
Presynaptic α_2-adrenoceptor	6.8
α_1-adrenoceptor	6.5
5-HT_{1A}	5.3
5-HT_{1B}	4.9
5-HT_{1D}	5.3
5-HT_{2A}	8.2
5-HT_{2B}	6.7
5-HT_{2C}	7.9
5-HT_3	8.1
D_1	5.8
D_2	5.6
H_1	9.3

Table 3.
Mirtazapine affinity for neurotransmitter receptors (from Anttila and Leinonen 2001; De Boer 1996; Kasper et al. 1997).

Receptor	Affinity (pK_i)
MT_1	10.0
MT_2	9.9
5-HT_{2A}	5.3
5-HT_{2B}	6.6
5-HT_{2C}	6.2

Table 4.
Agomelatine receptor affinity at cloned human receptor subtypes (Millan et al. 2003).

5-HT_{2C} receptor blockade studies examining anxiolytic action

Closely related to studies examining the antidepressant action of 5-HT_{2C} blockade are those examining the anxiolytic action of 5-HT_{2C} receptor antagonism. Both animal and human studies have found evidence of anxiolytic properties and action of 5-HT_{2C} receptor blockade. For example, male 5-HT_{2C} knockout mice exhibit an anxiolytic phenotype, as assessed by exposure to novelty-related anxiogenic stimuli such as the open field test, elevated maze, and novel object test (Heisler et al. 2007). Numerous behavioural studies have also shown that antagonism of 5-HT_{2C} receptors in rodents has anxiolytic effects (Griebel et al. 1997; Millan et al. 2005; Dekeyne et al. 2008). Both the 5-HT_{2C} /5-HT_{2B} antagonist SB-206553 and the 5-HT_{2C} inverse agonist SB-243213 have displayed anxiolytic effects in the rat by increasing scores in a social interaction test and increasing punished responding in the Geller-Seifter conflict paradigm (Kennett et al. 1996; Wood et al. 2001), an established predictor of anxiolytic clinical efficacy (Pollard and Howard 1979).

Another 5-HT_{2C} antagonist, FR260010, also demonstrated anxiolytic activity in four rodent models of anxiety. Following administration, rats increased time in the open arms in an elevated plus-maze and increased the number of interactions in the social interaction test. Similarly, mice increased time in the light zone of the testing chamber in a light/dark test and increased the duration of head dips in a hole-board test (Harada et al. 2006). Ritanserin, a 5-$HT_{2A/2C}$ antagonist, also decreases anxiety measures after chronic administration (Gatch et al. 2000; Graeff et al. 1998). In direct contrast to these results, 5-HT_{2C} agonists, such as mCPP, produce anxiogenic effects in rodents, increasing anxiety-like behaviours in models of anxiety including the social interaction test (Kennett et al. 1989), the elevated T-maze (Mora et al. 1997), and the elevated plus maze (Kshama et al. 1990; Fone et al. 1996).

Studies in humans also implicate 5-HT_{2C} receptors in mediating anxiety. The 5-$HT_{2A/2C}$ antagonist ritanserin produces mild anxiolytic effects in acute doses in healthy volunteers (Graeff et al. 1996), although some studies in individuals with panic disorder have found no changes in biochemical measures or psychometric assessments following ritanserin administration (Den Boer and Westenberg 1990). Conversely, administration of mCPP, which activates 5-HT_{2C} receptors, has been shown to increase measures of anxiety in healthy humans (Mueller et al. 1985; Charney et al. 1987; Murphy et al. 1989). This increase in anxiety after mCPP administration can be attenuated by 5-HT_{2C} antagonists such as ritanserin (Graeff et al. 1996; Bagdy et al. 2001).

While the evidence for specific abnormalities in 5-HT_{2C} receptors in depression is limited and incomplete, it is clear that many drugs with important psychotropic properties have pharmacological actions at the 5-HT_{2C} receptors (Dekeyne et al. 2008). The neuropsychological hypothesis of drug action implies that the clinical effects of medications active in anxiety and depression are best understood through the effects of these agents on the processing of emotional information. Thus far, however, there has been no systematic attempt to identify the role of the 5-HT_{2C} receptor in drug-induced changes in emotional processing in humans. The aim of the current thesis was to initiate an investigation of this question.

1.4 Cognitive Neuropsychological Model of Antidepressant Action

One approach which has the potential to evaluate the role of 5-HT_{2C} receptors in human emotion is the proposed cognitive neuropsychological model of antidepressant drug action. This global treatment model takes into account both the cognitive biases and psychological mechanisms implicated in depression and the neurochemical mechanisms of antidepressants discussed above. The model has been built up from a body of research investigating the early effects of antidepressants on emotional processing, and provides the basis for the research presented in this thesis.

The cognitive neuropsychological model suggests that all antidepressant treatment, regardless of a specific pharmacological mechanism, works by correcting negative bias in cognitive processing (Harmer et al. 2009a). As

mentioned previously, there is a negative cognitive bias found in depression, and this bias can be measured with tasks examining different elements of emotional processing. Emotional processing is a type of cognitive processing of emotionally salient information that includes perception, interpretation, attention and memory. There are multiple tasks used to measure emotional processing, but those utilized here have been shown to be sensitive to antidepressant-induced changes. Specifically, the recognition of facial expressions of emotion is designed to measure biases in perception and interpretation; the categorization of self-referent information measures interpretation and encoding and is a platform for emotional recognition and recall, which measures biases in memory; the dot-probe task measures biases of attention. The emotion-potentiated startle task is also sensitive to antidepressant-induced changes, and measures automatic processing of fear. It has been shown that changes in emotional processing following antidepressant administration can be detected in the absence of changes in mood, and that these processing changes can be found early in antidepressant treatment, often after a single dose, in both healthy subjects and depressed patients (Harmer et al. 2009a).

The hypothesis suggests that after the initiation of antidepressant treatment, there are relatively rapid changes in emotional processing with decreases in negative biases and increases in the salience of positive information; however, these changes in bias may not reach subjective awareness immediately. Thus no change in subjective mood is perceived. Over time, as the change in negative bias following antidepressant administration allows for the processing of more positive

information and as the individual interacts with their environment in this new emotional state, a change in mood develops. The delay to antidepressant treatment efficacy is well known but is yet to be fully accounted for. It generally takes several weeks before the clinical effect of antidepressants is fully apparent and obvious improvement in mood occurs. The model suggests this delay in antidepressant action is due to the time it takes for individuals to relearn new associations and process information without a negative bias rather than by delayed neurochemical effects.

The latter notion, that antidepressant action is a consequence of neuroadaptive changes triggered by the acute pharmacological effect of the drug (which usually occurs within hours) has been predominant (Frazer and Benmansour 2002). However, careful examination of clinical ratings scales using meta-analytic techniques does indeed suggest that clinically perceived improvement in mood can be seen with conventional antidepressants within the first week of treatment of depressed patients, when analyzing the difference between the SSRI and placebo groups (Taylor 2007). Moreover, the rate of improvement is as great at this time as it is subsequently, indicating that the time course suggests a continuous linear improvement in mood from the start of treatment rather than a discontinuous resolution as neuroadaptive changes manifest themselves. However, this theory does not, of course, mean that adaptive changes may not be important in the effects of antidepressants on neuropsychological processes. For example, the biphasic effect of SSRIs on anxiety (often increased initially but diminished later) hints strongly at such an action (Kent et al. 1998). Additionally, the idea that

clinical antidepressant action may involve the "relearning" of new emotional associations ties intriguingly into the effects of antidepressants to increase synaptic plasticity and neurogenesis (Malberg et al. 2000; Manji et al. 2003; Harmer et al. 2009a; Pringle et al. 2010). There are also some effects of drugs that are not readily explained with the cognitive neuropsychological model. For example, some drugs have a very rapid effect on mood, which appears to conflict with the time-course of the change in cognitive biases. Ketamine hydrochloride administered to individuals with depression has been shown to produce antidepressant effects and alter mood rapidly, within two hours after infusion (Zarate et al. 2006). It may be that concurrent changes are occurring, both in the underlying cognitive schema and in neuroadaptive effects (Harmer et al. 2009a), but it is necessary to further clarify the exact mechanisms underlying the neuropsychological hypothesis of antidepressant action.

Studies supporting this neuropsychological hypothesis of antidepressant action were initially focused on traditional antidepressants such as the reuptake inhibitors, NRIs and SSRIs. The NRI reboxetine has been shown to increase the processing of positive emotional information, both in behavioural and fMRI studies. Behaviourally, a single dose of reboxetine increases recognition of happy facial expressions and speeds response to categorize positive words relative to negative words compared to a single dose of placebo (Harmer et al. 2003b). Further, a seven-day course of reboxetine reduced the identification of negative facial expressions of fear and anger and increased the relative recall of positive

emotional words compared to placebo (Harmer et al. 2004). These effects occurred without any significant changes in mood and anxiety.

The SSRI citalopram has a rather different pattern of effect on emotional processing. A single dose of citalopram increased recognition of happy facial expressions (Harmer et al. 2003a) and increased attention to positive social stimuli compared to placebo (Browning et al. 2007). However, fitting with clinical descriptions of an increase in anxiety and agitation in some subgroups of patients early in SSRI treatment, a single dose of citalopram has also been shown to increase fearful face recognition (Harmer et al. 2003a; Browning et al. 2007) and increase the emotion-potentiated startle response compared to placebo (Browning et al. 2007; Grillon et al. 2007). Repeated doses of citalopram decreased the identification of negative facial expressions and increased the recall of positive emotional words similar to reboxetine, but also abolished the increased startle response following negative images, and this change in facial recognition and startle response suggests a delayed onset anxiolytic effect of citalopram(Harmer et al. 2004).

fMRI studies generally support the behavioural findings of the effect of antidepressants on emotional processing, and suggest that the effects seen on brain regions involved in emotional processing are important in the therapeutic actions of antidepressants (Harmer et al. 2009a). Thus, seven-days treatment with reboxetine reduced amygdala activity to fearful faces and increased fusiform activity to happy relative to neutral facial expressions (Norbury et al. 2007). A

neural effect of reboxetine has also been found in emotional categorization and memory tasks. Repeated administration of reboxetine increased activation in fronto-parietal regions during categorization of positive words, including the left precuneus and right inferior frontal gyrus. This increase in activation during positive words suggests an increase in salience to positive stimuli (Norbury et al. 2008). During the recognition of positive words compared to negative, reboxetine was associated with decreased activation in fronto-parietal regions such as the left precuneus, anterior cingulate, and medial frontal gyrus (Nobury et al. 2008). Similarly, a single dose of reboxetine decreased activation in a fronto-parietal network during the correct recognition of positive words relative to matched distractors (Miskowiak et al. 2007). This decrease in activation to positive words during recognition suggests a reduced cognitive load or effort to recognize the positive words following reboxetine, as it is thought this decrease in activation reflects a decreased recruitment of neural resources (Miskowiak et al. 2007). This fits with the suggestion that the neural correlates of increased effort is demonstrated as increased activity in brain regions which are involved in retrieval (Rugg and Wilding 2000).

The effect of a single dose of citalopram on the neural processing of emotion is less clear, as some studies demonstrate a decrease in amygdala activity to fear and aversive stimuli (Del-Ben et al. 2005; Anderson et al. 2007; Murphy et al. 2009) while others report an increase in amygdala activity to fear stimuli (Bigos et al. 2008). Repeated citalopram administration (7-10 days) decreases activity in the amygdala to fearful faces (Harmer et al. 2006a) and increases amygdala activity to

happy faces (Norbury et al. 2009b) in healthy volunteers. Additionally, reduced responses were also found in the hippocampus and medial PFC to fearful faces (Harmer et al. 2006a). Similarly, reductions in amygdala activation in healthy volunteers were reported following the condition in which participants received repeated treatment with the SSRI escitalopram compared to the condition where the same participants received placebo treatment (Arce et al. 2008).

Neuroimaging studies have also investigated the effects of antidepressants in individuals with depression. These studies have reported that the increased amygdala activation to negative facial expressions found in depression is reduced after eight-weeks of serotonergic antidepressant administration (Sheline et al. 2001; Fu et al. 2004). Generally, however, it can be difficult to determine the reason for differences in brain activation from these studies as mood changes and symptom state changes occur over the study, and the effects of repeated testing are not quantified with a placebo condition (Harmer et al. 2006a).

In order to extend the model to general antidepressant action, these results need to be shown with antidepressants that act via different pharmacological mechanisms. Antidepressants and other agents acting on the 5-HT_{2C} receptors are interesting candidates to be studied in this manner. This approach allows for further testing of the cognitive neuropsychological model and potential clarification of the role of 5-HT_{2C} receptors in emotional processing, though this is somewhat limited by the lack of pharmacological selectivity of the drugs concerned. Initial behavioural studies with the antidepressants mirtazapine and

agomelatine, both of which are 5-HT_{2C} receptor antagonists, have already been carried out. These studies support the previous findings of antidepressant drug action in showing that antidepressants can shift emotional biases in a positive direction without any change in subjective mood. It is important to note that thus far these studies have been carried out principally in healthy volunteers. Using healthy volunteers is advantageous as it allows the behavioural effects of drugs to be explored unconfounded by changes in clinical state or remission of symptoms. These healthy volunteers, however, tend to be young and high functioning, which is not representative of the range of the depressed population. Additionally, individuals who have been identified as vulnerable to depression or who are in remission from depression have been shown to have altered responses to emotional processing (Mannie et al. 2007; Bhagwagar and Cowen 2008), suggesting a trait difference from healthy individuals. It is clear that studies are needed to determine if similar effects following antidepressant treatment are seen in depressed patients. It is promising, then, that a preliminary study of reboxetine suggests similar results are seen in a depressed population (Harmer et al. 2009b). Implications for examining these effects in healthy volunteers rather than depressed individuals are considered in the final chapter.

1.5 Aims and Objectives

Despite interest in 5-HT_{2C} receptors as a target for treatment of depression and anxiety, experimental support for antidepressant properties in selective 5-HT_{2C} antagonists remains limited (Dekeyne et al. 2008). Given these gaps in the field of antidepressant research, this thesis attempts to explore the role of the 5-HT_{2C}

receptor in emotional processing in healthy adults, which has been hypothesized to be a key mediator of antidepressant drug action. It is important to further characterize the role of this receptor because it may lead to the development of more effective and better tolerated agents for the treatment of depression and anxiety disorders. It is possible to probe 5-HT_{2C} receptors in humans using several different drugs. The approach used here was to administer antidepressants and other drugs acting as antagonists and agonists at 5-HT_{2C} receptors to healthy volunteers, and following administration, examine the effect on emotional processing and other measures, particularly slow wave sleep which is an established measure of 5-HT_{2C} receptor blockade (Sharpley et al. 1994; Sharpley and Cowen 1995).

This introductory chapter has covered the background research relating to the field of inquiry. In the following chapter, the general methods used in subsequent experimental chapters are explained. The first experimental chapter, Chapter 3, examines the effect of a single dose of the antidepressant mirtazapine, a 5-HT_{2C} receptor antagonist, on the neural processing of emotional faces in healthy volunteers. It was hypothesized that mirtazapine would decrease the amygdalae and fusiform gyrus responses to fearful faces in healthy volunteers. In Chapter 4 the effect of mirtazapine on neural processing during self-referent emotional word categorization and subsequent memory is investigated. It was hypothesized that mirtazapine would decrease activation in fronto-parietal regions to negative compared to positive self-referenct words during encoding, and that mirtazapine would increase the recall of positive words following the scan. Chapter 5 focuses

on the behavioural effects of a seven-day administration of mirtazapine on emotional processing, while Chapter 6 examines the ability of a new antidepressant, agomelatine, to block 5-HT_{2C} receptors as measured by increases in slow wave sleep. It was hypothesized that the repeated administration of mirtazapine would decrease both the recognition of fearful faces and the emotion-potentiated startle effect and increase recall memory of positive personality characteristics. For Chapter 6, it was hypothesized that agomelatine would increased SWS in healthy human volunteers when administered as a single dose just prior to sleep onset. In Chapter 7, a single dose of agomelatine was again used to investigate changes in emotional processing along with mCPP, which acts as an agonist at the 5-HT_{2C} receptor. It was hypothesized that mCPP would increase the emotion-potentiated startle response and increase fearful face recognition while agomelatine would decrease recognition of fearful faces and increase recall of positive words relative to placebo. The overall findings are further discussed in Chapter 8, and possible lines of further research, based on the implications of these studies are considered.

Chapter 2

General methods

2.1 Participant Selection and Screening

The Oxfordshire Research Ethics Committee approved all studies, and all participants provided written informed consent prior to enrollment. For each study, participants were recruited using the following methods: free or paid advertisements in local websites and mailouts, emails to students sent by their individual colleges, or flyers posted in libraries and colleges.

All potential participants underwent an approximately hour-long screening session in which eligibility for participation was determined. This assessment included an interview using the Structured Clinical Interview for DSM-IV (First et al. 1997). From this interview, participants were determined to be free of current or past axis-1 disorder (including anxiety disorders, depression, eating disorder, psychosis and substance abuse). Additionally, all participants were required to have a brief physical exam with a medical doctor to ensure physical fitness and eligibility for the study. The doctor also reviewed participants' medical history. General inclusion and exclusion criteria were applied for all participants. Inclusion criteria include: Participants must be 18-55 years of age, have a body mass index in the range of 19-30, be fluent in English, be willing and able to give informed consent for participation, be physically fit as assessed by a medical doctor, and females must only be tested outside of their pre-menstrual week. This requirement for female participants is an attempt to control for potential effects on

behavioural testing due to phase of menstrual cycle. Previous drug studies have found differing behavioural and hormonal effects according to the menstrual cycle (Cowen et al. 1995). Exclusion criteria include: Participants must not be taking psychotropic medication, be free of past or current Axis 1 psychiatric disorder on DSM-IV, have any medical contra-indication, be currently pregnant or breastfeeding, or have a current or past history of drug or alcohol dependency.

Each study also had specific inclusion and exclusion criteria. The imaging study required all participants to be right-handed with no contraindications for fMRI examination, such as pacemakers or metal implants. The polysomnogram study required participants to be free of any sleep disorders. The two studies using agomelatine excluded participants if they were taking oestrogen treatment or were on oestrogen-based contraception, as oestrogens may increase agomelatine blood levels. All participants in these studies also had a blood draw to examine levels of liver enzymes (bilirubin 3-17 umol/L, alanine transaminase 10-45 IU/L, alkaline phosphatase 95-280 IU/L, albumin 35-50 G/L). A large safety concern with agomelatine has been an increase in blood levels of liver enzymes during treatment (European Medicines Agency 2008; Servier Laboratories Ltd. 2009). These increases in liver enzymes occurred during repeated agomelatine treatment so it is not believed that a single dose of agomelatine would pose a significant risk, however, as recommended by the manufacturer, liver enzymes were checked to ensure normal levels prior to study inclusion (European Medicines Agency 2008; Servier Laboratories Ltd. 2009; Howland 2009). For the same reason, participants could not have a history of hepatitis or be taking any medication known to inhibit

CYP 1A2 liver enzymes. Participants with a history of migraines were excluded from the study with a mCPP treatment group, as mCPP has been shown to induce migraine attacks (Leone et al. 2000).

2.2 Randomisation

All studies were of randomised double-blind design. An experienced researcher within the group, not otherwise involved in the studies, drew up a randomisation schedule and organized the capsules in correct order. In the imaging study, sleep study and seven-day study, capsules were administered or dispensed by the experimenter as all capsules were matched to maintain blinding. In order to avoid possible experimenter bias in the behavioural mCPP and agomelatine single dose study, capsules were administered by a researcher not involved in the study, as the mCPP and matching placebo capsules were not matched in colour to the agomelatine and placebo capsules. In anecdotal questioning following the study, individuals often reported they were unaware of which group (drug versus placebo) they were in. It is possible that if participants were aware of which group they were randomized to, they could unconsciously, or consciously, alter their responses and behaviour accordingly and thus create a confound. Although subjective evidence suggests participants here were not aware of their randomization, future studies may wish to quantitatively assess this possibility and take it into account during analysis.

2.3 Questionnaires and Rating Scales

The questionnaires and rating scales administered in all studies were used to both establish baseline levels of personality and anxiety as well as detect changes in mood, anxiety, and physical side effects during the experimental sessions. This is important in studies examining emotional processing in volunteers, as groups should be matched on baseline levels to avoid confounds. Additionally, it is important to monitor any changes in mood and anxiety throughout the study.

The sleep study did not require these levels of monitoring of mood and anxiety as the main measure of interest was the slow wave sleep outcome. This study, therefore, used the side-effects questionnaire and quality of sleep measure after each night of the experimental session. All of the following questionnaires have been used extensively in psychopharmacological testing paradigms (Serretti et al. 2010).

In all other studies, the Beck Depression Inventory (BDI) (Beck et al. 1961) was used as an indicator of mood at baseline. This inventory was developed to measure the severity of depression rather than be a diagnostic tool, but has been used in many previous psychopharmacological studies as a measure of mood (Serretti et al. 2010). The BDI is a self-rated scale composed of 21 categories of attitudes and statements. Each category consists of four to five self-evaluative statements. Numerical values between zero and three are assigned to each statement in order to denote the measure of severity with three being the most severe. Scores of 11 or less suggest no depression is present, scores between 11

and 18 suggest mild depression, 19 to 29 suggests moderate depression, and 30 and above is severe depression (Beck et al. 1961).

The Eysenck Personality Questionnaire (EPQ) (Eysenck et al. 1985) was used to determine dimensions of personality at baseline. The EPQ consists of 90 yes/no statements which fall into one of four scales measured in the questionnaire: extroversion/introversion, neuroticism/stability, psychoticism/socialization and lie.

The State and Trait Anxiety Inventory (STAI) (Spielberger et al. 1970) was used to determine state (in the present moment) and trait anxiety levels at baseline. The STAI consists of two 20-item scales with four possible responses to each question, ranging from one to four (not at all - very much). The focus areas of the questions include worry, tension, apprehension, and nervousness.

All studies also used the state anxiety inventory, visual analogue scales (VAS) (Bond and Lader 1974) and Befindlichkeits scale of mood and energy (BFS) (von Zerssen et al. 1974) in repeated fashion to determine baseline levels and to monitor changes throughout the study. The state anxiety measure is described above. Visual analogue scales have been used extensively in neuro-endocrine and psychopharmacological testing paradigms and are used to report physiological and behavioural responses to the particular probe in question (Silverstone et al. 1994; Cowen et al. 1995). For example, physiological measures include nausea, drowsiness, and dizziness while psychological measures include mood states such

as happiness and sadness. The rating scales consist of 10 cm lines with participants instructed to draw a horizontal line crossing the 10 cm line at the point that represents how they presently feel.

The BFS is a self-rating mood scale consisting of 56 pairs of adjectives that characterize opposite states of mood. Participants indicate which of the word pairs most corresponds with their present state. Positive words are given a score of zero, negative words two, and neither/nor choice is scored as one. Additionally, scores can be divided into mood and energy sub-scales.

The final study also used the Positive and Negative Affect Schedule (PANAS) (Watson et al. 1988) to measure positive and negative affect at hourly intervals. 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 – extremely).

2.4 Functional Magnetic Resonance Imaging

The first two study chapters (Chapters 3 and 4) discuss the experiment in which participants were given either a single dose of mirtazapine or placebo and underwent a functional magnetic resonance imaging (fMRI) scan. fMRI is a non-invasive technique for measuring brain activity, and is frequently used in psychiatry and psychology research. fMRI detects changes in blood oxygenation and flow that occur in response to neural activity. It is important to note that fMRI does not create a direct image of neural activity; rather it creates an image of

physiological activity correlated to neural activity. The blood-oxygenation-level-dependent (BOLD) contrast is the main principle behind fMRI studies. When a brain area is more active, it consumes more oxygen and to meet this increased demand, blood flow increases to the active area. Haemoglobin, which delivers oxygen to neurons, is diamagnetic when oxygenated and paramagnetic when deoxygenated, and it is this change in magneticism which is reflected in the MR signal depending on the amount of oxygenation. This change in oxygenation varies with the levels of neural activity so can be used to detect brain activity, and this is what is know as BOLD imaging.

Participants are given a scanner safety briefing and metal check before entering the scanner. Participants are briefed on the scan protocol (types and lengths of scans, tasks, emergency procedures) and are instructed to remain as still as possible throughout the scan. Participants are then aligned in the scanner and a test of screen visibility and button box function is conducted. The initial scan is a structural scan lasting 5 minutes 15 seconds. This scan is high-resolution and has much greater tissue contrast compared to the functional images, as it measures differences between tissues. Structural scans are used in fMRI to facilitate coregistration of the functional data to standard space. First the functional and structural scans are co-registered, or mapped to each other. Then, the scans are normalized to a standard space, which allows for the combination of data across individuals. After the structural scan, the functional scans, described below, were run. Functional scans measure changes in the blood oxygenation of the brain over time, and from these changes, inferences about underlying neuronal activity can be

made. Participants perform tasks in the scanner, and the scanner records signal throughout the brain while the task is performed. In brain areas that respond to the stimulus presented in the task the signal increases or decreases as the stimulus is presented or not. The activity in a voxel is defined as how closely the time course of the signal from that voxel matches the expected time-course. Voxels whose signal corresponds tightly are given a high activation score, voxels showing no correlation are given a low score and voxels showing the opposite are given a negative score. These scores are translated into activation maps.

2.4.1 fMRI Data Acquisition

All images were acquired using a 1.5 T Siemens Sonata scanner located at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR). Functional imaging consisted of 24 T2*-weighted echo-planar image (EPI) slices (TR=2000 ms, TE=45 ms, matrix 64x64, slice thickness=5 mm), 3.0x3.0x5.0 mm voxels. A Turbo FLASH sequence (TR=12 ms, TE=5.65 ms), voxel size= 1 mm^3 was also acquired to facilitate later coregistration of the fMRI data into standard space.

2.4.2 fMRI Task Designs

Gender discrimination task

An fMRI block design with presentations of fearful and happy facial expressions was used to assess the effect of mirtazapine on the neural response to emotional faces (faces taken from the NimStim Face Stimulus Set). Fearful and happy faces have been used in previous paradigms within the lab to investigate the effect of antidepressants on emotional processing (Harmer et al. 2006; Norbury et al. 2007;

Norbury et al. 2009b). Sad facial expressions are often used as stimuli in studies of depression, but were not included here since we aimed to explore the effects of drug treatment on amygdala function and fearful faces have been shown to be the emotional expression that activates the amygdala most strongly (Phan et al. 2002). Neutral faces are not included in this study as these expressions can be considered ambiguous and participants may not interpret them consistently (Cooney et al. 2006). For these imaging tasks, the fixation cross comprises the baseline condition to contrast with other conditions, so a neutral face condition is not necessary. Participants were instructed to make gender discrimination responses via appropriate button presses on an MRI-compatible button-box. Each trial consisted of a single emotional face presented for 100ms followed by a fixation cross lasting 2900ms. Four blocks of each valence (fear and happy) were presented for 30 seconds interspersed with 30-second fixation cross blocks, resulting in a scan duration of 8 minutes 36 seconds. Stimuli were presented on a computer using E-Prime (version 1.0; Psychology Software Tools Inc., Pittsburgh, PA, USA) and projected onto an opaque screen at the foot of the scanner bore, which participants viewed using angled mirrors. Both accuracy and reaction time were recorded by E-Prime. This task can be considered a covert emotion processing task, as opposed to an overt or explicit emotion processing task, as participants do not directly evaluate the emotional expression of the facial stimuli. For example, a similar task of explicit emotional processing would be a facial expression matching task, in which participants match the emotional expression of the top face (fear, happy) to either the left or right face on the bottom of the screen. This particular gender discrimination task was chosen as it has been demonstrated that neural responses

to emotional stimuli in limbic regions during implicit tasks (such as gender discrimination) are greater and more reliable than performance during explicit emotional labeling tasks (Critchley et al. 2000, Lange et al. 2003, Morris et al. 1996, Phillips et al. 1997).

Visual checkerboard task

A visual checkerboard task was used to control for a possible confounding effect of global drug-related modulation of the BOLD signal. This task is a passive viewing condition used to determine the effect of mirtazapine on the BOLD signal in the primary visual cortex. A flashing checkerboard (frequency 8 Hz) was presented in blocks of 15 seconds alternating with 15 seconds of a fixation cross for a total of 20 cycles. Participants were instructed to watch the screen throughout the task.

Categorisation task

An fMRI event-related design was used to assess the effect of mirtazapine on the neural response to the categorisation of self-referent emotional words. In the word categorisation task, participants viewed single words on the screen and were told to decide whether or not they would like to be described with that word. Participants made responses via appropriate button presses on an MRI-compatible button-box. The scan duration for this task was 10 minutes and 54 seconds. Each trial consisted of a single emotional word presented for 1000 ms followed by a fixation cross with a duration between 1500 ms-7500 ms (variable). A total of 110 (45 positive, 45 negative, 20 control (left or right)) words were presented. Personality-trait adjectives were constructed from Anderson's list of personality

trait words (Anderson 1968). Half of the words were unambiguously positive and half were negative. Positive and negative words were matched on length, written frequency (Francis and Kucera 1982), and meaningfulness (Anderson 1968). Each list contained 20 control words (10 presentations each of left and right) included as a baseline control condition. The words left and right were selected in order to control for the sensorimotor aspects of the tasks without requiring subjective assessment of the positive or negative aspects of the word. Stimuli were presented on a computer using E-Prime (version 1.0; Psychology Software Tools Inc., Pittsburgh, PA, USA) and projected onto an opaque screen at the foot of the scanner bore, which participants viewed using angled mirrors. Both accuracy and reaction time were recorded by E-Prime.

2.4.3 fMRI Data Analyses

Functional MRI data were pre-processed and analyzed using freely available software tools within FMRIB's Software Library (FSL,version 5.92; Smith et al. 2004). Pre-processing involves a number of steps designed to reduce unwanted variability in the data and to improve the validity of statistical analyses (Huettel et al. 2004). Pre-processing included motion correction (Jenkinson et al. 2002), as it is impossible for participants to remain motionless in the scanner throughout 5-10 minute tasks. Even slight head motion causes some of the images to be in the wrong location. Motion correction algorithms adjust the time series so that the brain is in the same position in each image of the scan. Independent component analysis was used to identify and remove excessive motion (Beckmann and Smith 2004), and volumes with a large movement spike were excluded from analysis.

Fieldmap-based EPI unwarping (Jenkinson 2003; Jenkinson et al. 2004) was used to correct for distortion often caused by inhomogeneities in the static or excitation fields. A field map, an image of the intensity of the static magnetic field, can be incorporated into the processing steps to correct for geometric distortions. The whole head images were segmented into brain and non-brain tissue and the non-brain tissue removed so analysis is only performed on actual brain tissue (Smith 2002). Grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor was used to scale each volume by the same amount so that higher-level analyses are valid. Highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=60.0 s) improves the quality of data by removing low frequency artefacts such as scanner drift. Normalisation of data is required to allow for combination of data across individuals, and when normalization is done to a common space, data from multiple studies can be compared. Here, data were normalised to the Montreal Neurological Institute (MNI) template (Jenkinson and Smith 2001; Jenkinson et al. 2002), created from 152 brain images. Data was smoothed with a Gaussian kernel (5mm Full-Width-Half-Maximum) to reduce noise.

Lower level analysis

For the gender discrimination task, analyses of data from individual subjects were computed using the general linear model with local autocorrelation correction (Woolrich et al. 2001). Two explanatory variables were modeled: 'fear' and 'happy' faces. Temporal derivatives were included in the model as covariates of no interest to increase statistical sensitivity. Variables were modeled by convolving

each block with a haemodynamic response function, using a variant of a gamma function (i.e. a normalization of the probability density function of the gamma function) with a standard deviation of 3 seconds and a mean lag of 6 seconds.

An event-related design was used for the self-referent categorisation task. Events were defined as occurring at the presentation of word stimuli and were designated as positive, negative or control. Events were modeled separately by convolving the onset of each correctly categorized word using a haemodynamic response function, using a variant of a gamma function (i.e. a normalization of the probability density function of the gamma function) with a standard deviation of 3 seconds and a mean lag of 6 seconds.

Group level analysis

Generally, tasks were analysed at the group-level using mixed-effects analysis (Woolrich et al. 2004). This analysis allows for inferences to be made beyond the participants involved in the study to the population from which the participants were recruited. Mixed-effects assumes that the experimental effect is different across subjects, so statistical tests are performed on each subject and then at a second level of analysis the distribution of the individual subjects' statistics is tested for significance. For the whole brain analysis, significant activations were identified using cluster-based thresholding of statistical images. As the name suggests, in this analysis the entire brain and all voxels within it are considered when conducting statistical tests on activation. Cluster-based thresholding is an approach used to correct for multiple comparisons, a serious problem in fMRI

analysis. If fMRI data is analysed on a voxel-by-voxel basis, many thousands of statistical tests would be carried out, and Type I errors increase as the number of statistical tests increases. With this cluster-based method, a certain number of contiguous voxels must be active in order to be labeled as significant rather than single voxels.

Significant interactions from whole brain analyses were further explored by extracting percent BOLD signal change, which were analysed using appropriate post hoc *t*-tests to determine directionality of the effect. Further task specific analyses of regions of interest (ROIs) were generated and also examined by extracting percent BOLD signal change, which were analysed using appropriate post hoc *t*-tests to determine directionality of the effect. ROI analysis is a more targeted approach to analysis and constrains the search for active voxels to a particular area of the brain thought to be involved in the process performed during the scan. These ROIs may be anatomical, chosen based on anatomical criteria, or functional, chosen based on functional criteria such as the output of a whole-brain analysis. The specifics of these higher-level analyses varied depending on task, and are presented in more detail below.

Gender discrimination task

For the gender discrimination task, higher-level analysis was carried out using mixed-effects (Woolrich et al. 2004). For the whole brain analysis, 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 et al. 1994). Fear faces were contrasted with happy faces between groups. Significant interactions from whole brain analyses were further explored by extracting percent BOLD signal change, which were analysed using appropriate post hoc *t*-tests to determine directionality of the effect.

ROI analysis

ROIs were created in a priori regions (amygdalae and fusiform face area) by using functional masks created from significant clusters of activation to fear faces across both groups within structural anatomical masks of specific regions. BOLD signal change extracted from ROIs were analysed using repeated measures ANOVA (between-participants variable=group; within-participants variable=valence). Significant interactions were investigated further with appropriate post hoc *t*-tests to determine directionality of the effect.

Categorization task

For the categorization task, higher-level analysis was carried out using mixed-effects (Woolrich et al. 2004). For the whole brain analysis, 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 et al. 1994). Positive words were contrasted with negative words between groups.

ROI analysis

ROI of the right medial PFC (a priori region of interest) was used from a previous study examining the effect of antidepressants on self-referent word processing (Norbury et al. 2008). BOLD signal change extracted from ROIs were analysed using repeated measures ANOVA (between-participants variable = group; within-participants variable = valence). Significant interactions were investigated further with appropriate post hoc *t*-tests to determine directionality of the effect.

Visual stimulation task

For the visual checkerboard task, a region of occipital cortex activated by the task (compared to fixation cross baseline) was identified and the percentage BOLD signal extracted and compared between mirtazapine and placebo groups with a one-way ANOVA (between-participants variable = group).

2.5 Polysomnography

Polysomnography was developed in the 1970s to examine physiologic changes that occurred in relation to different states of sleep and wakefulness (Holland et al. 1974). Electroencephalography (EEG), electromyography (EMG) and eye movement recordings were combined along with cardiac and respiratory sensors. Now, standard polysomnography can consist of simultaneous recordings of multiple physiological variables during sleep such as EEG, recorded from central and occipital locations on the scalp (C3 or C4 and O1 or O2); electrooculography (EOG), recorded from electrodes placed in the vicinity of the right and left outer canthus; and chin EMG, recorded from electrodes placed over the chin muscles.

Electrocardiography (ECG), leg EMG, and nasal and oral airflow and respiration are also often measured (Lee-Chiong et al. 2002).

2.5.1 Sleep Architecture

There is a pattern of sequential stages and cycles to sleep, known as sleep architecture, which polysomnography details. Stage 1 sleep is light and is the transition between wakefulness and other sleep stages, thus only lasts a few minutes. It consists of low-voltage slow theta (4-7 Hz) and delta (2-3 Hz) band activity. There may be slow eye movements and EMG levels decrease relative to wake. Stage 2 sleep consists of high-voltage vertex waves with K-complexes and sleep spindles. A K-complex is a sharp negative wave followed by slower positive component, lasting at least 0.5 seconds. Sleep spindles last at least 0.5 seconds and are in the 7 to 14 Hz range. Stage 2 occurs 5 to 7 minutes after stage 1 sleep. Stage 3 follows 15 to 30 minutes after stage 2, and consists of high-voltage waves in the delta range, but sleep spindles may still be present. Stage 3 can be defined as greater than 20% but less than 50% of the epoch being occupied by slow activity. Stage 4 has high-voltage slow waves also, but it must occur at more than 50% of the epoch. Stages 3 and 4 make up slow wave sleep (SWS), and lasts about 30 to 45 minutes. After these stages, there is a reversal to stage 2 and then the onset of the first rapid eye movement (REM) episode follows. REM sleep is characterized by low-voltage intermixed cerebral electrical activity associated with reduced muscle tone in submental muscles and rapid eye movements. This pattern makes up one cycle of sleep. There are four to five 90 minute cycles of sleep in a night,

and as time progresses, REM sleep increases in duration and slow-wave decreases in duration.

2.5.2 Application

Polsomnogram (PSG) was used here to investigate the effect of agomelatine on SWS. EEG electrodes were applied in accordance with the International 10/20 system of electrode placement (Jasper 1958), with four elecroencephalogram channels (C3/A2, C4/A1, O1/A2, O2/A1) used. The measurements for electrode placement were made in relation to anatomical landmarks: the nasion, inion, and left and right pre-auricular points. Self-adhesive electrodes were used for EOG and submentalis (chin) EMG. Following measurements, the skin surface where electrodes were to be placed were cleaned and abraded. This cleaning is done in order to lower the impedence levels, or resistance to the electrical impulses to be recorded. Then EEG electrodes were filled with gel and attached to the scalp with glue. Impedance readings were taken for each electrode and electrodes were adjusted if found to be out of range. After all electrodes were attached and impedances checked, the electrodes were sealed with glue. Operational and clean channel signals were verified by participants moving eyes, closing eyes and grinding teeth. Following application of the electrodes and testing of signal, participants returned home and were instructed to turn on the recording as they went to bed. Participants removed the electrodes at home the following morning, and all equipment was returned to the lab the day after participation.

2.5.3 Processing

Each study night, PSGs were recorded as each participant slept at home, using the Embla A10 digital data recording system (Medcare, Broomfield, CO, USA). Participants went to bed and got up at their usual time, and this schedule was kept constant for all study nights and the night directly before the study night. PSGs were staged in 30 second epochs using the Embla diagnostic software, Somnologica Studio. The data from the Embla is downloaded to the program from PCMCIA disks (PC card) after the ambulatory study. This software provides measures for all aspects of sleep architecture according to standard criteria in accordance with Rechtschaffen and Kales criteria, developed for the scoring on healthy adult PSG (Rechtschaffen and Kales 1968). These criteria consist of the stages of sleep architecture discussed above: Stage wake, Stage 1 sleep, Stage 2 sleep, Stages 3 and 4 sleep (SWS), Stage REM sleep, Stage movement time. The parameters measured in the PSG allow for a range of sleep variables to be assessed. These include total sleep period (defined as time between sleep onset and final awakening), total sleep time (defined as total time spent asleep during the total sleep period, not including awakenings), sleep efficiency (calculated as total sleep time/total sleep period x 100), sleep cycles of non-REM (NREM) and REM sleep segments, and sleep stages during NREM sleep. Sleep onset was defined as the beginning of the first 2 minutes that were not scored as wake or movement. The number of awakenings was defined as the total number of awakenings occurring after sleep onset. Additional sleep continuity and sleep stage variables are also presented. In addition, the polysomnograms were scored by an

experienced sleep physiologist, blind to treatment status, using the same criteria (Rechtschaffen and Kales 1968).

2.6 Psychological Tests

The Emotional Test Battery (ETB) was developed to investigate the effects of antidepressant and other drugs on emotion processing. The battery consists of six different tasks including facial expression recognition, emotional memory and emotion-potentiated startle responses and has been shown to be sensitive to SSRI, SNRI, and NRI treatment (Harmer et al. 2004). One limitation of the ETB that should be noted, however, is the issue of the number of analyses performed with this testing battery. There are a number of measures that can be calculated for each test, and these are not corrected for multiple comparisons, although within each analysis, corrections for multiple comparisons are applied.

2.6.1 Facial Expression Recognition

The facial recognition task featured six basic emotions (anger, disgust, fear, happiness, sadness, and surprise) taken from the Pictures of Facial Affect series (Ekman and Friesen 1976). These had been morphed between each prototype and neutral using techniques described by Young et al. (1997), which involved taking a variable percentage of the shape and texture differences between the two standard images 0% (neutral) and 100% (full emotion) in 10% steps. Four examples of each emotion at each intensity were presented (total of ten individuals). Each face was also given in a neutral expression, giving a total of 250 stimuli presentations.

The face stimuli were presented in random order on a computer screen for 500 ms each and replaced by a blank screen. Participants indicated which expression they thought the face depicted by pressing a labeled key on the keyboard. Participants were asked to respond as quickly and as accurately as possible. Accuracy (number of correct out of 40, out of 10 for neutral) and response time was recorded for each emotion.

The facial expression recognition test examines the interpretation of key social signals and can be considered a probe of interpretation bias. The differing intensity levels allows for increasing difficulty, ambiguity and sensitivity to drug effects (Harmer et al. 2010b). Accuracy and reaction time for each emotion are the main outcome measures of this task. Results from previous studies utilizing this task suggest this task is most sensitive to altered perception of happy and fearful facial following antidepressant administration (Harmer et al. 2003a; Harmer et al. 2004; Arnone et al. 2009).

2.6.2 Emotional Categorisation and Memory

Sixty personality characteristics selected to be disagreeable (e.g. domineering, hostile) or agreeable (e.g. cheerful, generous), taken from Anderson (1968), were presented on a computer screen for 500 ms. These words were matched for length and ratings of frequency and meaningfulness (Anderson 1968; Francis and Kucera 1982). Each trial began with a fixation cross lasting 500 ms followed by the word

and ending with a post-trial interval of three seconds. Participants were asked to categorize 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. Classifications and response times for correct identification were recorded.

Immediately following the emotional categorization task, participants were asked to recall as many of the personality characteristics as possible in a surprise free recall test where as many words as participants could remember were written within a two minute time limit. Recognition memory was then assessed by asking participants to indicate whether they thought the word presented on the screen was one of the personality characteristics from the categorization task or not ('familiar' or 'novel'). The task comprised of 60 target words plus 60 matched distractors (30 positive, 30 negative) (Anderson 1968; Francis and Kucera 1982). Each trial consisted of a 500 ms fixation cross, followed by the word presentation for 500 ms, and a post-trial interval of three seconds.

The emotional categorization task assesses speed to respond to positive and negative self-referent personality descriptors and can be considered a probe for interpretation and encoding. Accuracy and reaction time for each valence type are the main outcome measures of this task, but differences in reaction time are more often found (Harmer et al. 2010b). The task was initially developed to test for later memory recall, but subsequent studies revealed that reaction time was altered by

antidepressant administration. Results from previous studies utilizing this task suggest antidepressant treatment facilitates reaction times for positive stimuli (Harmer et al. 2004; Harmer et al. 2003b). The emotional memory task assesses the incidental encoding of emotional stimuli from the categorization task, and can be considered a probe of memory bias. Accuracy in the recall of emotional words is the main outcome measure of this task. Results from previous studies utilizing this task suggest antidepressant treatment facilitates recall of positive versus negative stimuli (Harmer et al. 2004; Harmer et al. 2003b; Arnone et al. 2009).

2.6.3 Recognition Task

A recognition task, based on the computerized memory task described above, was used in the fMRI study to assess recognition for words presented in the categorization task performed in the scanner. This task was conducted on a laptop after the scan was completed. Words were presented on the screen for 500 ms with a fixation cross, presented for 2000 ms, between each word. Participants were instructed to indicate whether they thought the word presented on the screen was one of the personality characteristics used in the scanner categorization task or not ('old' or 'new'). The task comprised of 90 target words plus 90 matched distractors (45 positive, 45 negative) (Anderson 1968; Francis and Kucera 1982).

The recognition task assesses accuracy to respond to positive and negative self-referent personality descriptors and can be considered a probe for interpretation

and encoding. Accuracy for each valence type is the main outcome measure for this task. The recognition memory task is similar to the recall memory task, but is a different test of underlying emotional memory, and includes a retrieval cue whereas the recall test does not.

2.6.4 Attentional Dot-Probe

Two types of emotional words were used in this task: 60 social threatening negative words and 60 positive words. Each emotional word was paired with a neutral word that began with the same letter and was matched for length. There were, therefore, 60 socially threatening-neutral pairs and 60 positive-neutral pairs, plus 60 neutral-neutral pairs. Each trial started with a fixation cross for 500 ms, followed by a word pair where one of the words appeared above and the other below the central fixation position. The emotional words appeared in the top and bottom location with equal frequency. In the unmasked condition, the word pair was presented for 500 ms, and then a probe of either one or two stars appeared in the location of one of the preceding words. Participants were asked to indicate as quickly and as accurately as possible the number of stars present on the screen by pressing a labeled key on a keyboard, and their response terminated the probe display. The probe appeared at the top and bottom of the screen with equal frequency: in the same position as the emotional word for half of the trials and in the same position as the neutral word on the other half. The sequence of events was the same in the masked condition, except the duration of the word pair was 14 ms, and the display of the word pair was immediately followed by a mask, which

was displayed for 186 ms. The mask was constructed from digits, letters and other non-letter symbols (for example, @B%2#) and was matched for word position and length. There were 180 trials in total (masked: 30 positive-neutral, 30 negative-neutral, 30 neutral-neutral; unmasked: 30 positive-neutral, 30 negative-neutral, 30 neutral-neutral), and masked and unmasked trials were presented in a random order.

Median reaction time and accuracy scores were recorded, and attentional vigilance scores were calculated for each participant by subtracting the mean accuracy or reaction time from trials when probes appeared in the opposite position to the emotional word (incongruent trials) from trials when probes appeared in the same position as the emotional word (congruent trials).

The dot-probe task assesses attention to positive versus negative stimuli, and can be considered a probe of attention bias. Accuracy and reaction time for each valence type are the main outcome measures of this task, but differences in reaction time are more often found as accuracy is often at ceiling (Harmer et al. 2010b). Results from previous studies utilizing this task suggest antidepressant treatment either increases attentional vigilence to positive stimuli or decreases attentional vigilance to negative stimuli (Browning et al. 2007; Murphy et al. 2009).

2.6.5 Emotion-Potentiated Startle Response

The involuntary reaction to a sudden and unexpected stimulus is known as the startle response. This response can be found in both animals and humans and includes the forward thrusting of the head, contraction of muscles in limbs, and blinking. Human studies have focused on the eyeblink component, as it is the first and fastest element of the startle response (Lang et al. 1990). The eyeblink response alone can also occur when stimuli are not strong enough to initiate the whole startle reflex (Lang et al. 1990). Fear conditioning studies have shown that animals that have been conditioned to be fearful have an enhanced response to startle probes when presented during conditioned stimulus at extinction (Brown et al. 1951). Administration of diazepam, an anxiolytic, attenuates the increased response in conditioned fear in rats (Berg and Davis 1984). These general findings have also been demonstrated in human studies (Ross 1961; Spence and Runquist 1958).

This concept has been extended to an emotion potentiated startle paradigm in which the muscle activity of the orbicularis oculi is measured with EMG electrodes following presentation of an acoustic startle probe while participants view positive, negative, or neutral photographs. Startle responses are enhanced (or potentiated) when subjects anticipate unpleasant stimuli compared to when subjects are resting. (Lang et al. 1990). The emotion-potentiated startle is a non-invasive technique that can be used to study fear and anxiety in humans. The neural circuitry underlying the startle effect has been suggested to involve the amygdala. More specifically, rodent studies have found that the projections from

the central nucleus of the amygdala and the bed nucleus of the stria terminalis (BNST), a rostral extension of the central nucleus, to the nucleus reticularis pontis caudalis are involved in the fear-potentiated startle effect (Davis and Whalen 2001). Some studies suggest the central amygdala is involved in processing fear while the BNST is involved in long-lasting fear, more like anxiety (Davis et al. 1997). Additionally, direct projections to the trigeminal and facial motor nuclei mediate the eyeblink potentiation. There are parallel pathways in humans, and studies with patients with lesions and amygdala damage indicate these pathways may also be involved in the human startle reflex (Funayama et al. 2001).

The EMG recording site was first cleaned to remove makeup, skin oil, and dead skin cells to try and reduce impedance between the skin and electrode gel and maximize the flow of current from the skin surface to the conductive surface of the electrodes. Ag/AgCl electrodes (In Vivo Metric, Healdsburg, CA, USA) are filled with a high-conductivity electrode gel (Spectra 360, Parker Laboratories Inc., Fairfield, NJ, USA) and attached with a double-sided adhesive collar. Electrode placement/configuration is made up of one electrode below the lower eyelid in line with the pupil in forward gaze and a second electrode placed approximately 1-2 cm lateral to the first. A single ground electrode (isolated ground, not earth ground) attached at the electrically inactive site (mastoid, forehead, temple). Recordings were on the right side of the face due to room setup, and it has been shown that with binaural stimulation, laterality effects are not significant (Bradley et al. 1996).

EMG signal was checked for noise artifacts that may obscure the blink reflex. The noise artifacts could be due to physical sources such as mobile phones and power lines (50-60 Hz interference), or to biosignals from other facial muscles. Spontaneous and voluntary blinks and eye movements were observed, and if noise levels were too high to detect the blinks, the electrodes and gel application were adjusted or replaced as needed. Participants were instructed to sit quietly and watch the screen for the duration of the session.

Picture stimuli from the International Affective Picture System, designed to elicit positive, negative, or neutral emotions were used (Lang et al. 1998). Stimuli were presented for 13 seconds (inter-trial interval of between 11-15 seconds, mean 13 seconds) on a 43 cm computer screen approximately 1 m away from the participant. Pictures were presented in three blocks each containing seven pictures of each category in a fixed random order with the constraint that no two of the same type (neutral, positive, or negative) were presented successively. 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 50 ms, 95 dB bursts of white noise with a nearly instantaneous rise time and are 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).

EMG signals were sampled at a rate of 1,000 samples per second, filtered (30-300 Hz), then smoothed with a filter window of 5 ms and rectified. Eye-blink reflex magnitudes (in millivolts) were calculated by subtracting the amount of integrated EMG at reflex onset from the peak amplitude maximum amount of integrated EMG between 20 and 120 ms following probe onset. Trials with no perceptible eye-blink reflex were assigned a magnitude of zero and included in the analysis. Eye-blink reflexes with excessive noise during a 20 ms prestartle baseline period were excluded. Participants' data were not included in they if they displayed fewer than 25% satisfactory blink responses.

2.7 Statistical Analyses for Psychological Tests and Emotion-Potentiated Startle Task

Tasks were analyzed using repeated-measures ANOVA with group (drug group(s), placebo) as the between-subjects factor. The within-subjects factors were facial expression (facial expression recognition, fMRI faces task), picture valence (affective startle) and word valence (emotion categorisation and memory). Statistically significant interactions and main effects of treatment group were followed up by comparing each drug vs. placebo in a separate ANOVA with group and valence as factors. Significant interactions and group main effects in this

analysis were completed with simple main effect analyses (one-way ANOVA), comparing each drug with placebo for each emotion. Analyses specific to individual chapters and further detail are provided in each chapter.

2.8 Sample sizes

The number of participants included in each study varied depending on the number of groups being examined and on the types of testing method used. Ultimately, numbers also varied across studies due to participant exclusions, but generally, groups of at least 15 participants each were recruited. For all the studies, participant numbers were based on previous studies conducted in the lab rather than on power calculations. One previous imaging studies examining the effect of antidepressants on neural processing of emotion performed in the lab had a total of 24 participants (12 in the citalopram group and 12 in the placebo group), and found a significant whole-brain result as well as results from an ROI analysis (Harmer et al. 2006), and was the basis of the number of participants per group in the imaging studies. A behavioural study performed in the lab with three groups (two drug groups and a placebo group) found moderate effect sizes with 16 participants per group, including results in the ETB tasks of facial emotion recognition and emotional memory (both effect sizes of r=0.40) (Harmer et al. 2010a). This study examined the same drug as one of the studies conducted as part of this thesis.

Chapter 3

The effect of mirtazapine on the neural processing of facial expressions

3.1 Introduction

This study explored the effects of mirtazapine, an antidepressant with 5-HT_{2c} antagonist properties, compared to placebo using a covert emotional facial processing task. As mentioned in the Introduction, part of the negative bias associated with depression and anxiety can be observed with emotional facial processing tasks where individuals with depression selectively attend to sad faces or other negative faces compared to happy faces. Several behavioural studies have already shown that more established antidepressants affect emotion processing, specifically facial processing. For example, single doses of the SSRI citalopram and the serotonin and SNRI duloxetine increased recognition of happy facial expressions in healthy volunteers (Harmer et al. 2003a; Murphy et al. 2009; Harmer et al. 2008). A similar effect was seen with the selective noradrenaline re-uptake inhibitor, reboxetine, in both healthy volunteers and depressed patients (Harmer et al. 2003b; Harmer et al. 2009b). The effect of these monoamine re-uptake inhibitors on the recognition of fearful faces was less consistent. While citalopram increased the detection of fearful faces (Harmer et al. 2003a; Browning et al. 2007), neither duloxetine nor reboxetine produced a significant change (Harmer et al. 2003b; Harmer et al. 2008). It is possible that the effect of citalopram on fear processing could be related to the propensity of SSRIs to increase anxiety early in treatment in some patients (Kent et al. 1998).

A recent behavioural study indicates that the antidepressant mirtazapine also alters emotional facial processing, raising the possibility that 5-HT_{2c} antagonism might contribute to this change in emotional processing (Arnone et al. 2009). As discussed in the introduction, mirtazapine is an antidepressant drug whose therapeutic effects are thought to depend on blockade of noradrenaline α_2-adrenoceptors, perhaps in concert with 5-HT_{2A} and 5-HT_{2C} receptor antagonism (Davis and Wilde 1996). Clinically mirtazapine has a sedating profile, which is probably due to H_1 histamine receptor blockade (Davis and Wilde 1996) and it does not increase anxiety on treatment initiation. Indeed, it was found in healthy volunteers that in contrast to the monoamine re-uptake inhibitors, a single dose of mirtazapine had no effect on recognition of happy facial expressions but instead decreased recognition of fear, suggesting an acute anxiolytic action (Arnone et al. 2009).

The present study attempted to extend these initial behavioural findings by using fMRI to assess the effects of a single dose of mirtazapine on the neural substrates implicated in the processing of emotional faces. In this facial processing task, participants were told to watch the faces and make button press responses to the gender of the face presented. This task was adapted from previous studies that have shown that the amygdala, a region that plays a key role in emotional appraisal, as well as other brain regions, are reliably activated in response to fearful faces (Surguladze et al. 2003; Whalen et al. 2001). Activity in the fusiform

gyrus, which has direct feedback connections with the amygdala, is also known to be increased when the face presented is fearful, in line with increased attention and visual processing of these salient stimuli (Vuillemier et al. 2005).

The primary objective of this project was to answer the following question: Does administration of a single dose of mirtazapine alter the neural processing of emotional faces in healthy volunteers? To answer this question, participants underwent an fMRI scan while performing a facial processing task where participants made gender discriminations with happy or fearful faces. Based on behavioural findings outlined above, it was predicted that mirtazapine would decrease the amygdalae and fusiform gyrus responses to fearful faces in healthy volunteers. This decision to limit our regions of interest to these two specific regions previously highlighted in our lab and others as reliably activated by fearful face stimuli was made to limit the search and number of tests performed in the analysis.

3.2 Methods

3.2.1 Participants

Thirty-seven healthy volunteers were recruited into the study. However, due to the presence of a structural abnormality (one participant), button-box and scanner malfunctions (four participants), and extreme sedation in the scanner (four participants), twenty-eight participants were included in the final imaging analysis. It should be noted that the four participants excluded due to sedation

were all in the mirtazapine group. All participants were right-handed with a mean age of 23.3 (range 18-31) and a mean predicted full-scale intelligence quotient of 119.6 (as indexed by the National Adult Reading Test (Nelson and Willison 1991)). All participants provided written informed consent prior to study enrolment, which was approved by the Oxfordshire Research Ethics Committee, and received £60 reimbursement for their time while participating. Participants were briefed on scanner safety and had no contraindications for fMRI examination. Participants were not permitted to drink caffeine or smoke for the duration of the experimental session. For detailed information about participant recruitment and screening, see Chapter 2.

3.2.2 Experimental Design

The study was a double-blind, randomized, placebo-controlled between-groups design. Subjects were randomly assigned to receive either a single dose of mirtazapine (n=12, 15mg) or placebo (n=16), given in identical capsules to maintain blinding. Participants completed the following baseline measurements: the State and Trait Anxiety Inventory (Spielberger et al. 1970), the BDI (Beck et al. 1961), the EPQ (Eysenck and Eysenck 1975), the BFS (von Zerssen et al. 1974), and VAS (Bond and Lader 1974). Following completion of baseline questionnaires, mirtazapine or placebo was administered. Participants then spent 1.5 hours working or reading followed by mid-point questionnaires and mood scales (State Anxiety Inventory and VAS). fMRI scanning began approximately 2 hours after administration of mirtazapine/placebo, coinciding with the peak plasma concentration of mirtazapine in adults (Timmer et al. 2000). After the fMRI scan,

participants completed the facial expression recognition test and completed the State Anxiety Inventory and VAS as endpoint measurements of subjective mood and experience.

3.2.3 Behavioural Data Analyses

Baseline questionnaire scores were analyzed using independent-samples *t*-test. Questionnaires and scales measuring mood changes throughout the experiment, imaging task response accuracy and response latency, and facial expression recognition were analyzed using a repeated-measures ANOVA model (SPSS version 14.0 for Windows). Outliers in the facial expression recognition test were defined as data lying at more than three times the participants' interquartile range above their third or below their first quartile, and excluded from that analysis.

3.2.4 fMRI Task Design, Acquisition, and Analysis

All imaging data were collected using a 1.5 Siemens Sonata scanner at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR). For details, please see Chapter 2.4.

3.3 Results

3.3.1 Baseline Characteristics

There were no significant differences between the two groups in terms of age, verbal IQ (measured with the National Adult Reading Test), and BDI (all $p > 0.05$). There was, however, a significant difference between the groups on scores of Trait anxiety ($t(26) = -2.24$, $p = 0.03$) and the psychoticism score on the EPQ ($t(26) = -2.12$,

p=0.04). These scores were included as covariates in further image analysis, and all interactions remained significant in the analysis of variance of BOLD responses. For clarity, analyses without the inclusion of covariates are presented here. Baseline mood ratings on the VAS and the BFS did not differ between the two groups (all p>0.05) (Table 1).

3.3.2 Mood and Anxiety Scales

There were no significant effects of group or time on self-reported anxiety ratings assessed before treatment, 1.5 hours post-treatment (pre-testing), or 3 hours post-treatment (post-testing) measured by the State Anxiety Inventory, nor was there an interaction between time and group (all p>0.1). There was a main effect of time on the VAS measures of dizziness, hunger, and alertness (all p<0.02), with both groups getting more dizzy and hungry, and less alert over time. There were no group differences or interactions found (all p>0.10). There were no group or time differences on the BFS (p>0.3), but there was an interaction between time and group $F(2,52)=3.77$, p=0.03. This interaction was investigated further with post-hoc t-tests and no group differences were found (all p>0.1) (Table 2).

	Mirtazapine Mean ± SD	Placebo Mean ± SD	Significance
Age (years)	23.75 (2.86)	22.88 (3.96)	0.52
NART	119.66 (3.90)	118.89 (4.83)	0.65
BDI	2.96 (2.68)	4.47 (3.35)	0.21
Trait Anxiety	31.42 (5.71)	38.19 (9.22)	0.03
State Anxiety	28.25 (5.19)	32.19 (7.89)	0.15
EPQ-N	7.17 (3.10)	7.94 (4.74)	0.63
EPQ-P	2.00 (1.71)	3.69 (2.33)	0.04
EPQ-L	7.92 (7.92)	6.19 (2.71)	0.12
EPQ-E	15.58 (3.23)	14.44 (5.11)	0.50
BFS	17.15 (15.99)	27.75 (18.70)	0.13
VAS- nausea	6.11 (14.66)	6.16 (6.84)	0.99
VAS- dizziness	7.72 (11.62)	6.34 (9.05)	0.73
VAS- hunger	19.98 (17.17)	29.28 (27.55)	0.31
VAS- anxiety	8.77 (11.11)	11.91 (13.53)	0.52
VAS- sadness	12.09 (13.99)	13.65 (19.13)	0.81
VAS- happiness	65.66 (19.25)	57.25 (17.66)	0.24
VAS- alertness	67.86 (19.92)	57.64 (14.03)	0.12

Table 1.
Demographic characteristics and baseline ratings of mood. Means (± standard deviation).

	Time 1	Time 2	Time 3
State Anxiety			
Mirtazapine Placebo	28.25 (5.19) 32.19 (7.89)	29.92 (8.30) 31.41 (6.76)	29.00 (7.72) 33.91 (9.45)
BFS			
Mirtazapine Placebo	17.15 (15.99) 27.75 (18.70)	23.47 (17.24) 19.61 (16.95)	30.10 (21.25) 25.43 (18.64)
VAS-nausea			
Mirtazapine Placebo	6.11 (14.66) 6.16 (6.84)	8.21 (19.25) 7.18 (12.11)	8.92 (13.78) 10.81 (16.99)
VAS-dizziness			
Mirtazapine Placebo	7.72 (11.62) 6.34 (9.05)	14.06 (20.64) 9.13 (11.93)	26.72 (30.63) 19.59 (22.41)
VAS-hunger			
Mirtazapine Placebo	19.598 (17.17) 29.28 (27.55)	29.33 (20.27) 29.79 (20.50)	43.41 (20.00) 39.93 (21.64)
VAS-anxiety			
Mirtazapine Placebo	8.77 (11.11) 11.91 (13.53)	11.62 (18.20) 12.25 (13.95)	10.82 (19.01) 10.98 (19.73)
VAS-sadness			
Mirtazapine Placebo	12.09 (13.99) 13.65 (19.13)	13.02 (16.38) 6.15 (8.48)	9.91 (15.60) 10.57 (14.74)
VAS-happiness			
Mirtazapine Placebo	65.66 (19.25) 57.25 (17.66)	57.68 (21.59) 57.51 (16.21)	58.12 (15.47) 53.27 (23.13)
VAS-alertness			
Mirtazapine Placebo	67.86 (19.92) 57.64 (14.03)	51.85 (20.41) 50.89 (21.02)	36.65 (20.43) 43.69 (24.16)

Table 2.
Ratings of mood over time. Means (± standard deviation).

3.3.3 Behavioural Measures

There was no significant difference between the mirtazapine-treated group and the placebo-treated group in accuracy to identify faces as male or female and accuracy was at ceiling. Additionally, there was no significant effect of facial emotion on accuracy of gender classification, nor was there a significant interaction between the treatment group and facial emotion on accuracy (all $p>0.6$). All tests examining facial emotion included the same trials as the tests examining gender.

There was no significant difference between the mirtazapine-treated group and the placebo-treated group in median reaction time to classifying faces as male or female ($F(1,26)=2.29$, $p=0.14$). There was, however, a significant effect of facial emotion on speed to classify gender ($F(1, 26)=5.86$, $p=0.02$). Both groups were slower to classify fearful faces compared to happy faces. There was no significant interaction between the treatment group and facial emotion ($F(1, 26)=1.22$, $p=0.28$).

3.3.4 Functional Imaging

Main effect of Task

Presentations of fearful and happy facial expressions produced activation in a broad network of frontal, temporal, cingulate, parietal and occipital regions in both the mirtazapine and placebo groups (for peak cluster activation see Table 3). These regions included the middle, inferior and superior frontal gyri, anterior and posterior cingulate, middle temporal gyrus, amygdala, and hippocampus. These

findings result from two separate contrasts between fear versus baseline and happy versus baseline.

	Number of voxels	Brain regions	Maximum Z	Peak voxel (MNI)
Fear				X Y Z
Mirtazapine	35029	R temporal occipital fusiform cortex	5.42	36 -44 -24
	2601	L superior parietal lobe, supramarginal gyrus	3.67	-44 -44 46
Placebo	51293	L lateral occipital cortex, occipital fusiform gyrus	5.95	-40 -84 -22
Happy				
Mirtazapine	36910	R temporal occipital fusiform cortex	5.25	40 -48 -24
	1727	L lateral occipital cortex	3.83	-34 -60 54
Placebo	26776	R temporal occipital fusiform cortex	5.74	40 -56 -18
	798	L lateral occipital cortex, superior parietal lobe	4.36	-32 -60 48

Table 3.
Peak cluster activation in brain regions significantly activated during presentation of fearful and happy faces (main effect of task).

Group x task interactions

Whole brain analysis

A whole brain analysis (threshold $Z>2.0$, $p<0.05$, corrected) revealed decreased response under mirtazapine to fearful versus happy faces in two brain regions implicated in affective processing. Mirtazapine attenuated the neural response to fearful faces in the right temporal cortex (including amygdala and hippocampus)($Z=2.87$, $p<0.05$) and in a left fronto-striatal region ($Z=3.07$, $p<0.05$) (Table 4, Figures 1a & 3a). Post-hoc analysis of percent BOLD signal change for the group x valence interactions revealed increased responses in the right amygdala-hippocampal region in the placebo vs. mirtazapine group during presentation of fearful faces ($t(26)=-3.27$, $p=0.003$). Conversely, mirtazapine treated volunteers had increased responses to happy faces as compared to placebo ($t(26)=2.83$, $p=0.009$) (Figure 1b). This pattern was also apparent in the ROI specifically of the right amygdala (valence x laterality x group interaction $F(1,24)=4.45$, $p=.045$; group x valence interaction for right hemisphere $F(1,26)=5.91$, $p=0.02$); group x valence interaction for left hemisphere $F(1,26)=1.44$, $p=0.24$) (Figure 2). In the left fronto-striatal region, increased responses in the placebo group were also found during presentation of fearful faces, and were accompanied by relatively reduced responses in the mirtazapine group ($t(26)=2.64$, $p=0.014$). During the presentation of happy faces, the mirtazapine group had increased responses with an accompanying decreased response in the placebo group ($t(26)=-2.93$, $p=0.007$) (Figure 3b).

Region	Cluster size (voxels)	Coordinates	Z-score	*p*-value
Hippocampus – amygdala (right)	500	28, -18, -18	2.87	0.048
Caudate- orbitofrontal cortex (left)	587	-16, 24, 0	3.07	0.012

Table 4.
Regions showing significant activation in the placebo group compared to the mirtazapine group in response to fearful versus happy facial expressions. MNI coordinates (x,y,z) refer to the peak of activation within each cluster.

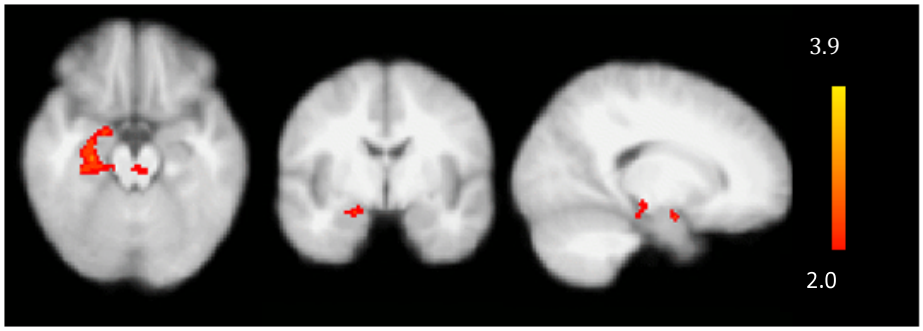


Figure 1.
(a) Axial, coronal, and sagittal images depicting significantly increased activation in placebos for the contrast fear v. happy facial expressions (images thresholded at Z=2.0, p=0.05, corrected) in the right temporal cortex, extending to hippocampus and amygdala (peak voxel: 28, -18, -18).



(b) BOLD percent signal change extracted from the significant cluster in the right temporal cortex to fear and happy faces. Error bars show standard error of the mean. $^{**}p<0.01$.



Figure 2.
(a) BOLD percent signal change extracted from right amygdala mask. Error bars show standard error of the mean. *p<0.05 for the interaction.



(b) BOLD percent signal change extracted from left amygdala mask. Error bars show standard error of the mean.

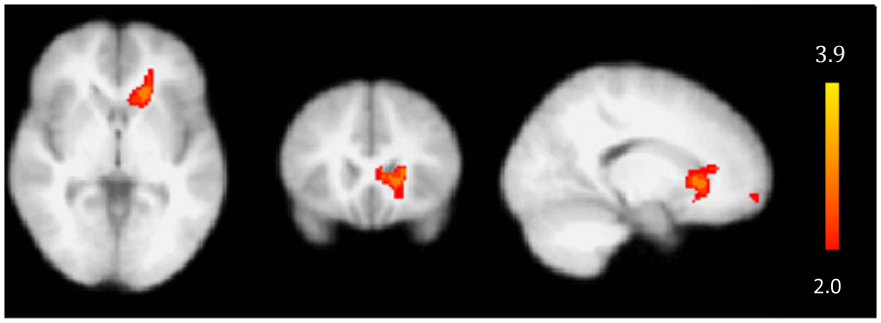


Figure 3.
(a) Axial, coronal, and sagittal images depicting significantly increased activation in placebos for the contrast fear v. happy facial expressions (images thresholded at Z=2.0, p=0.05, corrected) in left fronto-striatal region (peak voxel: -16, 24, 0).



(b) BOLD percent signal change extracted from the significant cluster in the left fronto-striatal region to fear and happy faces. Error bars show standard error of the mean. **p<0.01,*p<0.05.

Fusiform ROI

Repeated measures ANOVA of the percent signal change extracted from the mask of the fusiform (Figure 4) revealed a significant group x valence interaction ($F(1,26)=8.91$, $p=0.006$) which did not interact further with hemisphere ($F(1,26)=1.66$, $p=0.209$). A similar pattern of signal change was seen in both the left ($F(1,26)=10.10$, $p=0.004$) and right fusiform ($F(1,26)=5.35$, $p=0.029$), with the placebo group appearing to have an increased response to fear and a decreased response to happy faces, and the mirtazapine group having a decreased response to fear and an increased response to happy, although this pattern is stronger in the left fusiform (Figure 5).

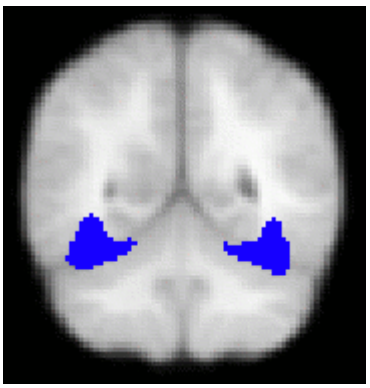


Figure 4. Left and right fusiform masks in coronal view, created across both mirtazapine and placebo groups from clusters of activation to fear faces.

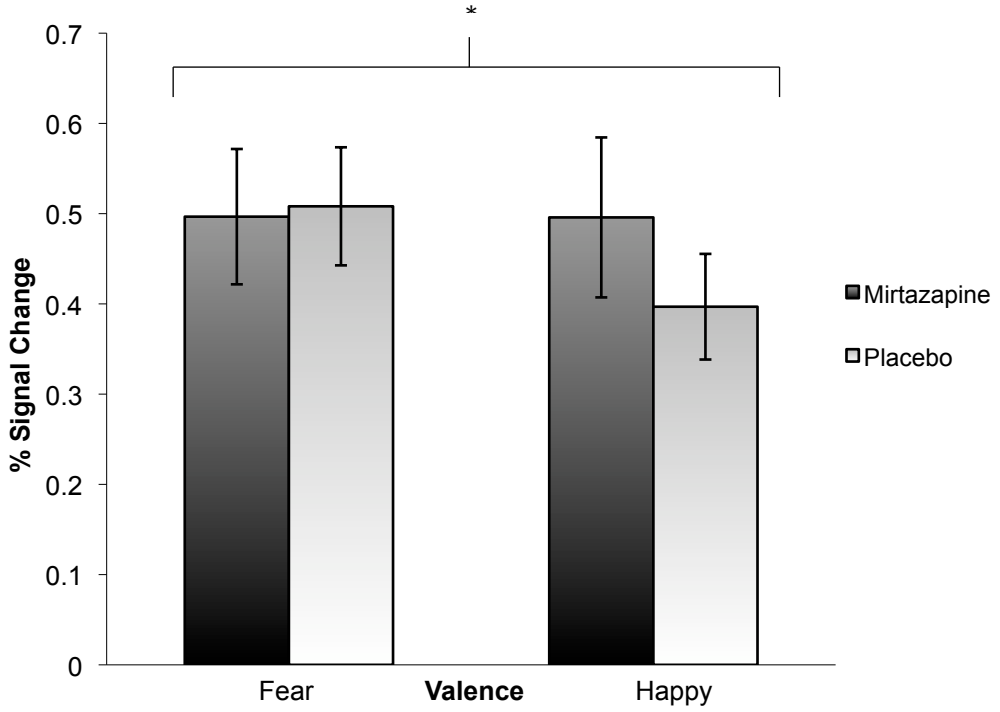
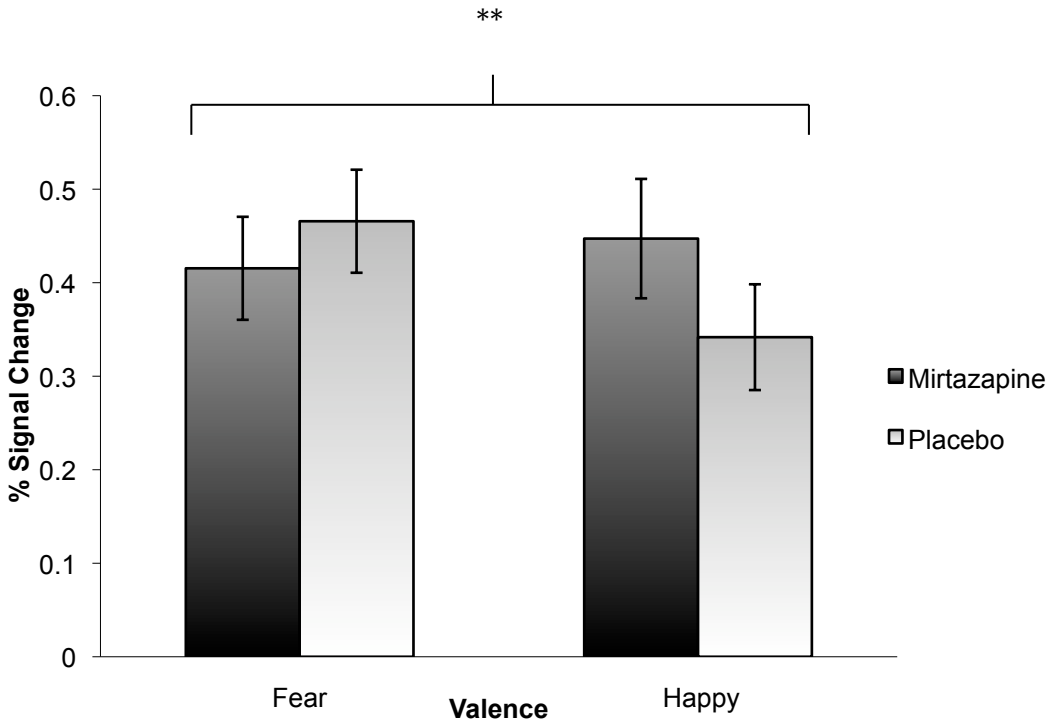


Figure 5.
(a) BOLD percent signal change extracted from right fusiform mask. Error bars show standard error of the mean. *p<0.05 for the interaction.



(b) BOLD percent signal change extracted from left fusiform mask. Error bars show standard error of the mean. **p<0.01 for the interaction.

Visual checkerboard control

In the checkerboard task, a whole-brain analysis of visual stimulation on vs. visual stimulation off between the two groups revealed no significant differences. There was also no significant difference between the mirtazapine group and placebo group on percentage BOLD signal change in this region of the occipital cortex during the visual stimulation ($t(26)=-0.98$, $p=0.34$) suggesting that the observed effects during face processing did not result from global haemodynamic changes.

3.3.5 Facial Expression Recognition

There was no main effect of treatment group or interaction between treatment group and facial expression for accuracy or reaction times in this task (all comparisons $p>0.4$) (Figure 6). There was a difference in the facial expression recognition of disgust over the different intensity levels presented in the task (group x intensity ($F(9,234)=2.30$, $p=0.038$)), with mirtazapine reducing the recognition of disgust at higher intensity levels (60%: ($F(1,27)=6.21$, $p=0.02$), 80%: ($F(1,27)=4.51$, $p=0.04$)).

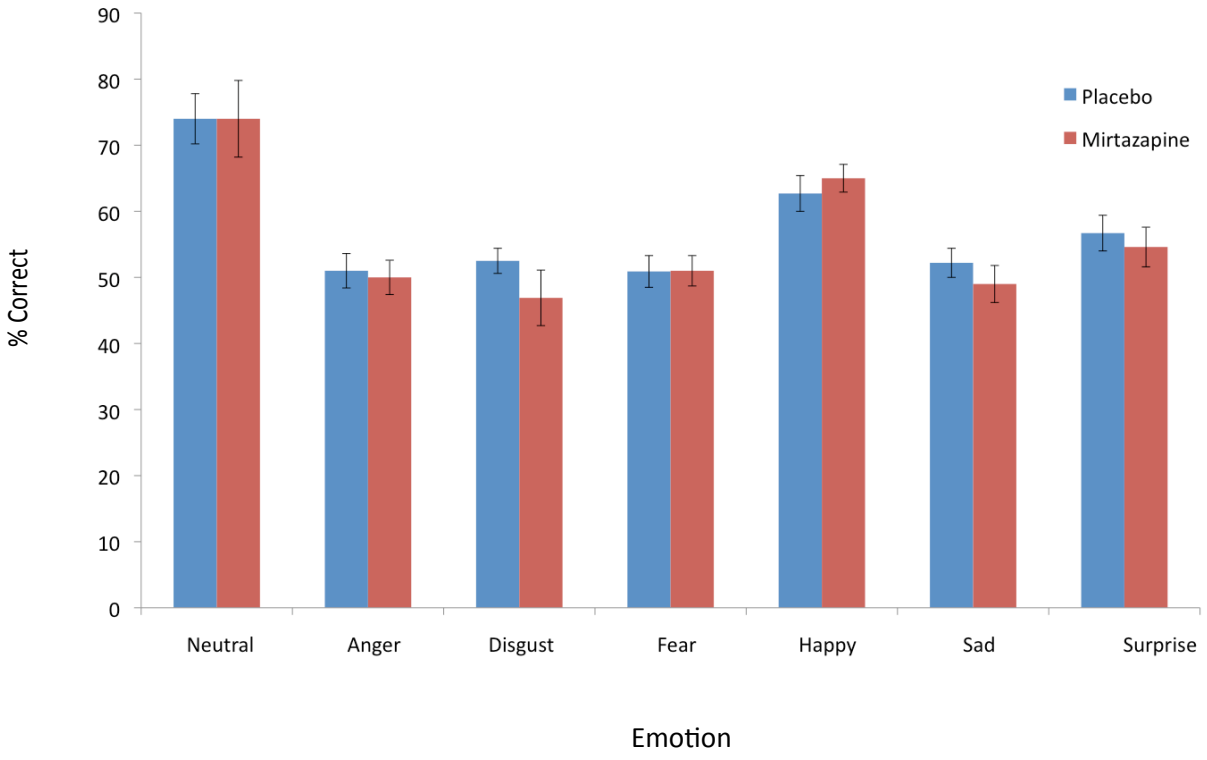


Figure 6.

The effect of mirtazapine and placebo on accuracy of facial expression recognition. Percent correct for all emotions summed over the different intensity levels used in the task. Values are mean % correct ±SEM.

3.4 Discussion

This study investigated the effect of a single dose of mirtazapine on the neural processing of emotional faces in healthy volunteers. The facial stimuli used in this study were happy and fearful, and were chosen for their reliable activation of the amygdala. It should be noted, however, that while fearful faces are often in fMRI studies of emotional processing, sad faces are the stimuli frequently used in studies of depression (Gur et al. 1992; Surguladze et al. 2004; Fu et al. 2004; Costafreda et al. 2009). In this study, mirtazapine significantly decreased BOLD response to fearful faces and increased response to happy faces in a network of

areas involved in emotional processing. These effects occurred in the absence of an improvement in mood or anxiety, which suggests an early, direct effect of mirtazapine on emotional processing. These changes may reflect an important means by which antidepressant treatment is able to resolve depression and anxiety disorders, where changes in emotional processing precede changes in mood and symptoms.

As noted in the introduction, acute administration of mirtazapine decreased fear processing in healthy adults in a facial recognition task (Arnone et al. 2009). In addition, mirtazapine increased recall of positive vs. negative self-referent words, and attenuated overall startle responses. The present study identifies potential neural substrates for these behavioural effects. Here, whole-brain analysis revealed a reduced response following mirtazapine within the amygdala-hippocampal region during presentation of fearful faces. The amygdala is implicated in emotional information processing and detection of threat-relevant cues such as fearful facial expressions (Morris et al. 1996), and the hippocampus is also involved in fear response (Surguladze et al. 2003; Williams et al. 2001). Previous studies have shown attenuation in the right amygdala-hippocampal region and specifically the amygdala during the presentation of aversive facial expressions in healthy volunteers after both treatment with acute citalopram and seven days of citalopram (Harmer et al. 2006a; Del-Ben et al. 2005; Murphy et al. 2009; though see Bigos et al. 2008). Additionally, the amygdala has been shown to be hyperactive to negative emotional stimuli in individuals with depression (Sheline et al. 2001; Fu et al. 2004; Siegle et al. 2007), and there is a growing body

of evidence that a key action of antidepressant drugs involves decreasing the hyperactivity of the amygdala (Fu et al. 2004; Sheline et al. 2001; Davidson et al. 2003). The current finding suggests that mirtazapine modulates amygdala-hippocampal activity to affective stimuli in healthy volunteers hours after a single dose.

Along with the amygdala-hippocampal region, activity in the left fronto-striatal region was also attenuated to fearful faces in participants receiving mirtazapine. This extensive region of activation includes the ventral striatum, caudate, orbital frontal cortex (OFC), and extends through the frontal pole. These regions have each been implicated in the processing of emotional information, and may have altered activation in acute depression and anxiety (Phillips et al. 2003b). For example, the OFC is a region implicated in the top-down processing of emotion, and it has been suggested to have a role in the automatic regulation of emotional behavior (Phillips et al. 2003a). Additionally, the OFC has connections to the amygdala (from the basolateral nucleus of amygdala) and may have a role in representing reward value of a stimulus (Philips et al. 2003a; Rolls 1999). Increased activation to negative emotional stimuli has also been shown in the caudate during acute depression (Fu et al. 2008) and after recovery from depression (Norbury et al. 2009a). However, the fronto-striatal region identified by the analysis is large and the activation relatively weak. Therefore, although these regions have been implicated in emotional processing and, in some studies, the effects of depression, the interpretation of the effects of mirtazapine identified here requires replication.

In addition to the decrease in activity to the fearful faces in these two regions, an increase in activity following mirtazapine to happy faces was found. Repeated treatment for one week with citalopram and reboxetine has been associated with an increase in amygdala responses to happy facial expressions in healthy volunteers (Norbury et al. 2007; Norbury et al. 2009b). This increase in amygdala activation to happy vs. fearful facial expressions by antidepressant treatment could reflect an increased salience of happy facial expressions. Conceivably, such changes may contribute to the improvement in social function seen after antidepressant treatment in depression (Tse and Bond 2003; Norbury et al. 2009b). Interestingly, we also found a similar increase in activation to happy faces in the fronto-striatal region, which may reflect a general increase in relevance or rewarding value of positive emotion processing following mirtazapine administration; however, as noted the changes found in this large area of activation should be received with caution.

Results from the ROI analysis of the fusiform are similar to those observed in the amygdala-hippocampal region, where mirtazapine decreased neural responses to fearful versus happy faces in healthy volunteers relative to placebo treatment. This interaction effect observed in the fusiform in our study is consistent with the hypothesis that antidepressant administration decreases the relative salience and/or attentional processing of threat versus approach cues (Vuillemier et al. 2005; Harmer et al. 2009a) and is similar to the effects seen with repeated reboxentine administration (Norbury et al. 2007).

As noted above, mirtazapine has complex pharmacological actions and attributing the effects observed on the neural responses to emotional stimuli to specific pharmacological actions is difficult. An anxiolytic action can be linked with 5-$HT_{2A/2C}$ receptor blockade, but H_1 receptor antagonism and 5-HT_3 receptor blockade may also be relevant, as both are implicated in anxiolytic effects (Lader and Scotto 1998; Olivier et al. 2000; Harmer et al. 2006b). The increased response to happy facial expressions seems more likely to be linked to antidepressant effects and here changes in noradrenergic neurotransmission, perhaps arising from blockade of presynaptic α_2-adrenoceptors (Davis and Wilde 1996), might be relevant. $5HT_{2C}$ receptor blockade might also produce antidepressant effects, perhaps through increased release of dopamine and noradrenaline in the frontal cortex (Millan et al. 2003).

Overall, the results of the current mirtazapine study and the previous behavioural mirtazapine study are similar to the profile of results found after repeated citalopram administration, and are consistent with the hypothesis that the anxiolytic actions of SSRIs may relate to downregulation of postsynaptic, possibly 5-HT_{2c}, sertotonin receptors (Deakin 1998). Acute SSRI treatment has been shown to increase behavioral measures of fear processing and anxiety (Browning et al. 2007; Harmer et al. 2003a). This effect is reversed after seven days of treatment with SSRIs, and thus fear processing is decreased (Harmer et al. 2004). Acute blockade of 5-HT_{2c} receptors is usually associated with both anxiolytic and antidepressant actions in animals (Kennett et al. 1996, 1997; Millan et al. 2005;

Dekeyne et al. 2008). As discussed in the introduction chapter, mirtazapine acts on the 5-HT_{2c} receptor immediately (acutely) rather than downregulating the receptor over time; it is therefore possible that mirtazapine has a similar effect on behavioural and neural measures to that of the repeated administration of citalopram. Our imaging data corroborate these behavioural results, and suggest that there is an immediate effect of mirtazapine on the amygdala-hippocampal response to fear.

3.4.1 Limitations

It is important to consider several limitations to the present study. First, despite the fact that the study was of a randomized double-blind design, there was a baseline difference between the placebo group and the mirtazapine group in trait anxiety and the EPQ measure of psychoticism. This initial discrepancy was resolved by including the scores as a covariate in subsequent imaging analysis. Second, mirtazapine has a sedating profile, and it could be argued that any between group differences could be due to changes in alertness. However, arguing against this point, the groups were matched in terms of alertness and sedation (as measured with VAS), and response latency in the scanner. Finally, when investigating the effects of drug manipulation on BOLD responses with fMRI, it is important to examine if the observed effects may be due to global effects of mirtazapine on baseline blood flow or neuronal coupling. Here, we included a control task to assess the BOLD response in a region of the brain not expected to be altered by the drug. There were no observed differences in the visual stimulation task between the mirtazapine and placebo groups. This finding

indicates that the effect of mirtazapine was specific for emotional facial processing rather than a global haemodyamic change.

3.5 Conclusions

In summary, the current study demonstrates that a single dose of mirtazapine modulates neural activity to affective stimuli, decreasing neural responses to fear and increasing responses to happy facial expressions. These results are consistent with the hypothesis that 5-HT_{2c} receptor antagonism may be important in decreased fear processing. Additionally, that such effects should be present after a single dose of medication is a striking demonstration of how initiation of antidepressant treatment is associated with rapid changes in emotional processing which are in the opposite direction to those seen in depression. In this respect, it may be of clinical relevance that a recent meta-analysis suggested that mirtazapine is among the most effective of currently available antidepressant drugs with a significantly earlier onset of action than SSRIs or SNRIs (Watanabe et al. 2008; Cipriani et al. 2009). Following from this result, the next chapter focuses on the effect of mirtazapine on self-referent emotional word categorisation and memory. Further chapters will examine the effect of other agents also acting on the 5-HT_{2c} receptor in order to learn more about the role of this receptor in altering emotion processing.

Chapter 4

The effect of mirtazapine on the neural processing of emotional word categorisation and subsequent memory

4.1 Introduction

The results shown in Chapter 3 demonstrated that a single dose of mirtazapine, an antidepressant with 5-HT_{2C} antagonist actions, has an effect on the neural processing of emotional faces. Emotional memory biases have been particularly associated with depression and with antidepressant drug treatment (Harmer et al. 2010b). Therefore, the current experiment explored the effect of a single dose of mirtazapine in healthy volunteers on self-referent emotional word categorisation and memory in order to further evaluate the role of 5-HT_{2C} receptors on the neural basis of emotional processing.

The self-referent word task employed here was designed to measure BOLD activity elicited by categorising positive and negative personality characteristics. The categorisation of these words can be considered an initial encoding task allowing for the recall and recognition tasks to test potential biases in emotional memory. In this task, participants view words on the screen, one at a time, while in an fMRI scanner, and are told to categorise the word as a word they would like or dislike to be described as, thus making the task self-referent. More specifically, participants are instructed to imagine they overhear someone describe them with the words on the screen, and must decide if they would like or dislike to be described with that

word. It has been shown previously that the encoding and processing of emotional self-referent words activates the medial prefrontal cortex (mPFC) and parietal and occipital cortices of the brain (Craik et al. 1999; Fossati et al. 2003; Moran et al. 2006; Vuilleumier 2005). This self-referent processing task was developed from these studies as well as studies examining the effect of antidepressants on the cognitive processing of emotional words (Harmer et al. 2003b; Miskowiak et al. 2007).

In addition to word categorisation, the experiment also involved word recognition and recall components performed outside of the scanner. In these sections, participants were asked to recall and write down as many of the personality characteristic words presented in the categorisation task as possible within a 5-minute limit, completed approximately 10 minutes after the categorisation task. Recognition memory was assessed following the free recall task by asking participants to respond with 'old' or 'new' to words presented on a laptop. Emotional word recall and recognition have been tested with similar paradigms in behavioural studies examining the effect of antidepressants on emotional memory (Harmer et al. 2003b; Harmer et al. 2004).

There have been multiple behavioural studies concerned with the effect of antidepressants on emotional word memory in healthy volunteers. Acute and repeated doses of reboxetine each enhanced memory for positive words (Harmer et al. 2003b; Harmer et al. 2004). Conversely, a single dose of citalopram had no

effect on memory (Browning et al. 2007). Repeated administration of citalopram, however, did enhance positive compared to negative word recall (Harmer et al. 2004). The difference between the two citalopram findings may be due to the length of drug exposure, and could, for example, be related to the time it takes for 5-HT post-synaptic receptor downregulation to start to occur. Given most studies do support the positive bias in emotional memory following antidepressant treatment, it might be expected that differences may be found in the initial encoding stages of the memory process between antidepressant and placebo treatment.

Studies on the processing of emotional words have found some behavioural differences in the speed to categorise self-referent personality characteristics following antidepressant treatment (Harmer et al. 2003b; Harmer et al. 2004). Acute and repeated doses of reboxetine decreased the speed to categorise positive versus negative self-referent personality characteristics. A single dose of citalopram, however, had no effect on speed of word categorisation (Browning et al. 2007). Repeated administration of citalopram also did not affect word categorisation (Harmer et al. 2004). These findings, although mixed, do also indicate that there may be neural differences underlying the behavioural differences seen in the reaction time to positive versus negative emotional stimuli during categorisation.

The effect of a single dose of mirtazapine on emotional word categorisation and memory has also been investigated. Similar to the citalopram studies, the

mirtazapine and placebo group did not differ in speed to categorise positive versus negative word categorisation, but the mirtazapine group did show a positive bias in word recall compared to the placebo group (Arnone et al. 2009). These complementary findings may be due to the underlying common effect of decreasing activity at 5-HT_{2C} receptors.

The primary objective of this section of the project was to answer the following question: Does administration of a single dose of mirtazapine change the neural processing of self-referent emotional information and subsequent memory in healthy volunteers? As recall has been shown to be positively biased following mirtazapine administration, it was hypothesized that the placebo-treated group compared to the mirtazapine-treated group would have greater activation in fronto-parietal regions to negative self-referent words than to positive self-referent words during the initial encoding of self-referent emotional information. Additionally, it was hypothesized that the mirtazapine group would have an increase in positive words recalled after the scan was complete.

4.2 Methods

4.2.1 Participants

The same thirty-seven healthy volunteers recruited for the study were used both in Chapter 3 and the current chapter. However, due to the presence of a structural abnormality (one participant), button-box/scanner malfunctions (four participants), extreme sedation in the scanner (four participants), adverse reaction

to medication (one participant), and poor behavioural response (one participant) twenty-six participants were included in the final imaging analysis. For further information on participants, please see Chapters 2.1 and 3.2.1.

4.2.2 Experimental Design

The study was a double-blind, randomized, placebo-controlled between-groups design. Subjects were randomly assigned to receive either a single dose of mirtazapine (n=12, 15 mg) or placebo (n=14), given in identical capsules to maintain blinding. Participants completed baseline questionnaires, and then were given the single dose of mirtazapine or placebo. Participants then spent one and a half hours working or reading followed by mid-point questionnaires and mood scales. fMRI scanning began approximately two hours after administration of mirtazapine/placebo. After the fMRI scan, participants completed endpoint measurements of subjective mood and experience. Following these questionnaires, participants completed a free recall test based on the words presented in the categorisation scanner task, and then a word recognition task on a laptop. For more details, please see Chapter 3.2.2.

4.2.3 fMRI Task Design, Acquisition, and Analysis

All imaging data were collected using a 1.5 Siemens Sonata scanner at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR). For details, please see Chapter 2.4.

4.2.4 Behavioural Tasks and Analyses

For information on the categorisation task, please see Chapter 2.4.2. After the fMRI scan and final questionnaires were completed participants, were assessed with the recall task. Participants were given pen and paper and asked to write down as many words from the categorisation task as they could remember in a five-minute period. Following the recall task, participants completed a recognition task (see Chapter 2.6.4 for details). Please see Chapter 2.7 for information on statistical analysis for the categorisation, recall, and recognition tasks.

4.3 Results

4.3.1 Baseline Characteristics

Baseline questionnaire results did not differ from results presented in Chapter 3.3.1.

4.3.2 Mood and Anxiety Scales

Mood and anxiety results did not differ from results presented in Chapter 3.3.2.

4.3.3 Behavioural Measures

Emotional word categorisation task

There was no significant difference between the mirtazapine-treated group and the placebo-treated group in accuracy to categorise words as positive or negative ($F(1,24)=3.83$, $p=0.06$), although there was a trend for the placebo group to categorise more words correctly (Table 1). There was no significant effect of word

valence on accuracy to categorise words ($F(1,24)=1.00$, $p=0.33$), nor was there an interaction between the treatment group and the word valence ($F(1,24)=1.00$, $p=0.33$). There was no significant difference between the mirtazapine-treated group and the placebo-treated group in median reaction time to categorise words ($F(1,24)=0.00$, $p=0.99$). There was, however, a significant effect of word valence on speed to categorise words ($F(1,24)=20.22$, $p<0.01$). Both groups were faster to categorise the positively-valenced words. There was no significant interaction between the treatment group and word valence on median reaction time ($F(1,24)=0.03$, $p=0.86$).

Recall

The total number of words recalled outside the scanner was not significantly different between the mirtazapine and placebo group, although there was a trend for the placebo group to recall more words ($F(1,25)=3.82$, $p=0.06$) (Table 2). The percentage of these words that were positive, however, was higher in the mirtazapine group ($F(1,25)=5.00$, $p=0.035$) (Figure 1).

Recognition

There was a main effect of treatment group on accuracy in the recognition of positive and negative personality characteristics outside the scanner ($F(1,23)=10.59$, $p=0.003$), with the placebo group correctly recognizing more words than the mirtazapine group (Table 1). There was, however, no interaction ($F(1,23)=0.26$, $p=0.62$). There was no main effect of treatment group and no interaction between word valence and treatment group on median reaction time in

the recognition of personality characteristics (main effect of group ($F(1,23)=0.08$, $p=0.78$; group x valence ($F(1,23)=0.13$, $p=0.72$)).

Word Type	Placebo (±SD)	Mirtazapine (±SD)
Categorisation reaction time (ms)		
Negative Positive	1165.54 (282.01) 947.68 (173.99)	1158.67 (323.84) 957.29 (230.49)
Categorisation accuracy (%)		
Negative Positive	94.60 (4.15) 94.60 (4.33)	84.26 (18.70) 87.04 (15.88)
Recognition reaction time (ms)		
Negative Positive	1155.50 (154.57) 1157.43 (141.08)	1125.81 (268.79) 1143 (236.36)
Recognition accuracy (%)		
Negative Positive	67.78 (15.80) 55.24 (16.19)	67.68 (14.58) 53.74 (20.58)

Table 1.
Response time (milliseconds), percentage total number of correct items (accuracy) in the categorisation and recognition tasks for participants following placebo (n=14) and mirtazapine (n=12) administration. Data are means ± SD.

Word Type	Placebo (± SD)	Mirtazapine (± SD)
Recall accuracy (% from total number of words written)	77.69 (4.18)	64.63 (4.81)

Table 2.
Recall accuracy of personality characteristics as a percent of total number of words written following placebo and mirtazapine administration. Data are means ± SD.

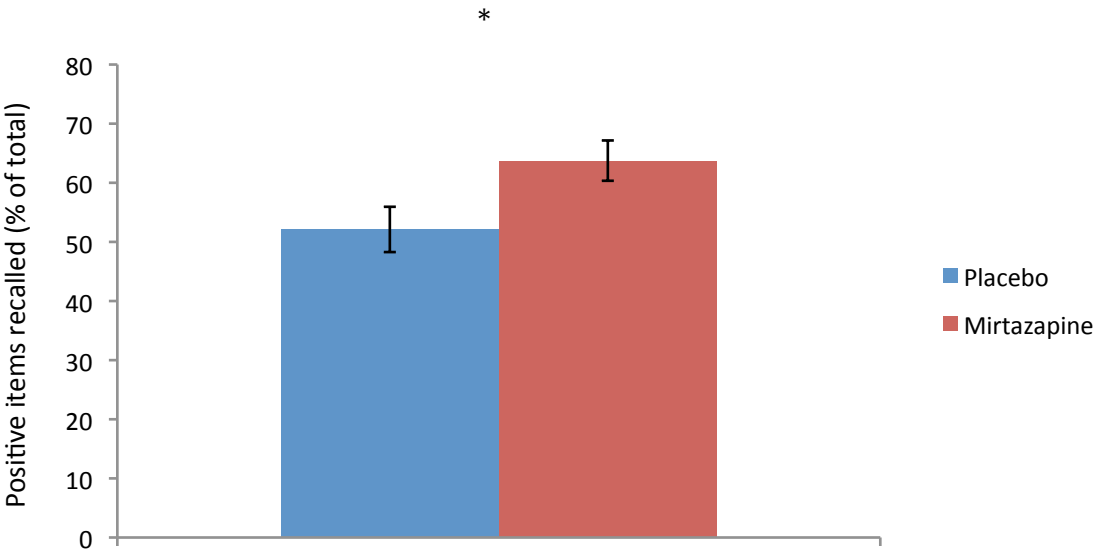


Figure 1.
Recall of positive personality characteristics as a percent of total correct words recalled in participants following placebo and mirtazapine administration. Data are means ± SEM. *p<0.05.

4.3.4 Functional Imaging

Whole brain analysis

Main effect of task

Self-referent categorisation of positive and negative personality characteristics activated a large network of left-lateralised inferior and medial frontal gyri and right-side occipito-temporal regions to both positive and negative personality characteristics (for cluster maxima see Table 3). Negative words activated the occipital pole, inferior frontal gyrus, and cingulate gyrus, while positive words activated the occipital pole and the middle frontal and inferior frontal gyri in

placebo-treated volunteers. These findings result from two separate contrasts, negative words versus baseline and positive words versus baseline.

Group x valence interactions

A whole-brain analysis revealed no interaction between treatment group and word valence. Additionally, there was no effect of mirtazapine on neural responses when considering positive and negative words separately. There was no significant group by valence interaction in the right mPFC. Also, extraction of mean percent signal change from the network of regions activated by self-referent categorisation of positive and negative words showed no effects of mirtazapine to the words (all $p>0.3$).

As exploratory analyses, two further whole-brain analyses were conducted. The first was designed to model the events at the time of participant response rather than the time of word presentation, but no differences were found between groups. The second whole-brain analysis examined the neural differences between words later correctly recognized by participants, but this also revealed no group differences.

Task and Region	Maximum Z	Peak voxel (MNI)
Main effect of positive words		
R occipital fusiform gyrus / occipital pole	5.08	28 -86 -12
L precentral gyrus / middle frontal gyrus / inferior frontal gyrus	3.65	-44 8 30
L juxtapositional cortex	3.29	-2 2 62
Main effect of negative words		
R occipital fusiform cortex / occipital pole	5.29	22 -90 -10
L frontal orbital cortex / inferior frontal gyrus	3.78	-34 30 -2
R postcentral gyrus / precentral gyrus	3.90	44 -18 60
L paracingulate gyrus / anterior cingulate gyrus	3.09	-6 16 44

Table 3.
Peak cluster activation in brain regions of significantly increased BOLD response during the emotional categorisation task in the placebo-treated volunteers (main effect of task). MNI coordinates (x, y, z) refer to peak activation within each cluster identified thresholded at Z=2.0 and $p<0.05$, corrected.

4.4 Discussion

Here, a single dose of mirtazapine had no effect on neural activity during self-referent categorisation of positive and negative personality characteristics in healthy volunteers. In the ensuing recall task completed outside the scanner, however, the mirtazapine group had an increased recall of positive vs. negative words. These behavioural results are consistent with previous effects of acute mirtazapine administration and repeated citalopram administration to healthy volunteers (Arnone et al. 2009; Harmer et al. 2003b).

There was a main effect of the categorisation task, demonstrated by activation of a large network of left-lateralised inferior and medial frontal gyri and right-side occipito-temporal regions to both positive and negative personality characteristics. It is thought that the processing of self-referent information activates the medial frontal region of the brain, one of the regions activated in the present task. Fossati et al. (2003) investigated the neural regions involved in self-referential processing with an emotional context, and found the dorsomedial PFC to be unique in self-referential emotion processing compared to other-referential processing regardless of the valence of the emotional words. The study by Fossati et al. (2003) complements an earlier study also suggesting the right dorsomedial PFC is activated during self-encoding of personality trait words (Craik et al. 1999). Additionally, another study has also confirmed the role of the mPFC in self-reflection regardless of valence (Moran et al. 2006). It has been suggested that this brain region represents states of an emotional 'self' and then processes emotional stimuli with a personally relevant perspective (Fossati et al. 2003).

The other brain regions activated in this current task are also consistent with previous studies of emotional processing. For example, the results of the current study fit with the hypothesis that the processing of emotional information involves the inferior frontal region of the brain. Kuchinke et al. (2005) found greater inferior frontal gyrus activation and orbitofrontal gyrus activation to emotional words compared to neutral words, and the inferior frontal gyrus has also been shown previously to be involved in processing emotional information (Dolcos et al. 2004). Occipito-temporal areas are thought to be involved in attentional response

to emotional compared to neutral stimuli (Miskowiak et al. 2007; Kuchinke et al. 2005; Moratti et al. 2004), again, consistent with the activation pattern found in the present study during self-referent word categorisation. Additionally, increased activation in the occipito-parietal regions in response to emotional versus neutral information has been hypothesized to represent increased attentional processing of relevant events (Vuilleumier 2005). Overall, it has been suggested that activation in these above regions may reflect attention to emotion and appraisal/evaluation of emotion (Phan et al. 2002), and the increased activation during self-referential processing supports this suggestion.

There was no effect of a single dose of mirtazapine on speed or accuracy of categorisation to self-referent positive and negative words, but some previous studies have suggested that administration of antidepressants do alter the speed of categorisation. For example, a single dose of reboxetine increased speed to categorise positive versus negative self-referent personality characteristics (Harmer et al. 2003b). Similarly, seven-day administration of reboxetine also facilitated speed of categorisation for positive compared to negative words (Harmer et al. 2004). A single dose of citalopram, however, did not alter speed to categorise words as positive or negative (Browning et al. 2007), and neither did a seven-day administration (Harmer et al. 2004). A single dose of mirtazapine was found to decrease reaction time to correctly categorise words regardless of valence (Arnone et al. 2009), so the present findings fit with the previous studies of mirtazapine and citalopram action on speed to categorise words. It should be noted, however, that the behavioural data obtained here was collected in the

scanning environment rather than a normal computerised behavioural testing environment, which may contribute to the current results, as the scanning environment may be sub-optimal for detection of behavioural effects of drugs (Miskowiak et al. 2007; Norbury et al. 2008).

This study did not show an effect of mirtazapine on neural activity during self-referent categorisation of positive and negative personality characterisitics. Previous fMRI studies have also examined how neural processing in emotional word categorisation and memory are affected by antidepressants in healthy volunteers. One study involved the categorisation and subsequent recognition of self-referent positive and negative personality characteristics following seven-days administration of reboxetine (Norbury et al. 2008). Norbury et al. (2008) found a difference between the reboxetine and placebo groups during categorisation of positive words compared to negative words in two brain regions, suggesting the encoding process is affected by antidepressant treatment. Activation in the left precuneus and right inferior frontal gyrus was increased in the reboxetine group for positive words compared to negative words. Increases in activation in these regions under reboxetine can be interpreted as an increase in salience of positive stimuli. During recognition, the reboxetine group had reduced activation to positive words compared to negative words in the left precuneus, anterior cingulate, and medial frontal gyrus, regions implicated in episodic retrieval and self-referent memory, and effortful attentional processes in the case of the anterior cingulate (Cabeza et al. 2000; Fossati et al. 2004; Posner and Dehaene 1994). This

reduction in activity in these regions during positive word recognition may reflect a decrease in retrieval effort for the positive words (Norbury et al. 2008).

In another neuroimaging study, an acute dose of reboxetine did not have an effect on the categorisation of positive or negative personality characteristics, but did affect later recognition of these words (Miskowiak et al. 2007). A single dose of reboxetine decreased the neural activation to positive words compared to matched distractor words in a fronto-parietal network including the medial frontal gyrus in healthy volunteers during word recognition. Again, this decrease in activation during recognition can be interpreted as a facilitated memory for positive target words, as it has been suggested that the neural correlates of increased effort is increased brain activity in the region involved in the particular retrieval task (Rugg and Wilding 2000).

The single dose reboxetine fMRI study did not find any drug effect on brain activation during categorisation, whereas the repeated dose study did. This discrepancy in neural activation during categorisation across these two reboxetine studies may be due to the length of administration (Miskowiak et al. 2007). Both the acute reboxetine study and the acute mirtazapine study have similar results in that there was no effect of treatment group on categorisation. As these studies were both single dose administration, it may be that a single dose of an antidepressant does not have an effect on the neural basis of self-referent word encoding. The dose size or length of administration of mirtazapine may each contribute to the lack of effect on self-referent word categorisation. The standard

starting dose of mirtazapine for treatment of depression is 15 mg, but often doses of 30 mg are used on treatment initiation as well, so the 15 mg used here may have been too low to produce changes in encoding. However, behaviourally both a single dose of reboxetine and a single dose of mirtazapine have effects on either the encoding stage or the recall/recognition stage. These differences seem to suggest that antidepressants have an effect on the encoding and memory process, although neural differences were not seen.

Alternatively, it may be that mirtazapine does not act on brain regions subserving the encoding process and instead has actions on the regions involved in recognition or recall memory. These regions include the lateral, prefrontal, premotor, parietal, and occipital regions (Fossati et al. 2004). The mPFC also reactivates during successful recognition of words previously encoded (Fossati et al. 2004). As mentioned above, the two previous studies of reboxetine and self-referent encoding and memory both demonstrated decreased medial frontal and parietal response during recognition of positive self-referent words.

To date, no fMRI studies of self-referent emotional word processing following citalopram administration have been reported. In order to clarify the role of the serotonergic system in self-referent emotional word encoding and memory, studies involving both acute and repeated doses of citalopram should be conducted.

A single dose of mirtazapine increased recall of positive vs. negative words compared to placebo in this study. Arnone et al. (2009) found a difference between the mirtazapine and placebo group in the recall of positive versus negative emotional words, similar to the present findings. This replication suggests mirtazapine affects self-referent emotional memory. Additionally, acute and repeated doses of reboxetine and repeated doses of citalopram (Harmer et al. 2003b; Harmer et al. 2004) increase recall of positive trait words, as does seven-day administration of the agomelatine (Harmer et al. 2010a), an antidepressant with antagonistic actions at 5-HT_{2C} receptors. The memory effects described here are represented as an increase in recall of positive versus negative emotional words rather than a change in recognition of emotional words. This difference may be due to the fact that the recognition task is less challenging than the recall task, and participants perform much better on it than the recall task. The recall task, then, is not at ceiling, which allows for the discrimination of differences between groups. Taken together, these results suggest memory effects may be particularly relevant to antidepressant action (Harmer et al. 2009a).

4.4.1 Limitations

There are several limitations to this study including those discussed in the previous chapter, such as baseline group differences, the sedative properties of mirtazapine, and the possible effect of mirtazapine on baseline blood flow (see Chapter 3.4). Additionally, the categorisation task was the last task performed in the scanner, and it is possible that participants may have lost interest or engagement with the task, an effect which may have been more pronounced for the

drug group. There was a significant difference between groups in recognition accuracy, with the placebo group correctly recognizing more words than the mirtazapine group. However, as mentioned in Chapter 3.4, there were no differences between groups in terms of alertness and sedation (as measured with VAS).

4.5 Conclusions

Mirtazapine increased the recall of positive versus negative words following a categorisation task. This increase in recall of positive trait words has also been found in another single dose mirtazapine study as well as in a repeated citalopram study. These results generally provide support for the hypothesis that antidepressants bias emotional processing in a positive manner following administration. More specifically, it also supports the idea that mirtazapine biases memory in a positive fashion, potentially through 5-HT_{2C} receptors. However, mirtazapine produced no effect on neural processing during categorisation of self-referent words, suggesting that such effects may not occur during the encoding process. The next chapter further examines mirtazapine and the contribution of the 5-HT_{2C} receptor to emotional processing by investigating the impact of a seven-day administration of mirtazapine on a battery of emotional processing tasks, including facial processing and self-referent word categorisation and memory.

Chapter 5

The effect of repeated mirtazapine and hydroxyzine on emotional processing

5.1 Introduction

This study aims to extend the work of the single dose of mirtazapine fMRI study (Chapters 3 and 4) and a previous mirtazapine single dose behavioural study by examining the effect of a seven-day dose of mirtazapine on emotional processing. The distinction between acute and repeated doses of different antidepressants may be important, as the effect of acute and repeated (seven-day) administration of SSRIs leads to different patterns of influence on emotional bias. For example, a single dose of citalopram increases the processing of fear (Browning et al. 2007; Harmer et al. 2003a), but this effect is reversed after repeated administration (Harmer et al. 2004). Animal studies have also shown acute and chronic citalopram treatment differentially affects fear processing in rats (Burghardt et al. 2004). Acute treatment with citalopram facilitated acquisition of auditory fear conditioning while chronic treatment decreased acquisition. The reversal of increased threat processing seen with the SSRI citalopram is consistent with clinical observations that subgroups of patients treated with SSRIs report increased anxiety and agitation before anxiolytic actions are seen (Harmer 2008; Kent et al. 1998). It has been hypothesized that the therapeutic effects of SSRIs in clinical anxiety and depression may be related to the down regulation of 5-$HT_{2A/2C}$receptors over time (Deakin 1998).

Mirtazapine has direct antagonistic actions on 5-HT_{2C} receptors, and these actions are thought to contribute to its antidepressant and anxiolytic effects. A previous single dose study has shown mirtazapine decreases behavioural recognition of fearful faces and startle reactivity in the emotion potentiated startle paradigm, and increases recall of positive versus negative information compared to placebo in healthy volunteers (Arnone et al. 2009). Additionally, the fMRI studies reported in Chapters 3 and 4 demonstrate that a single dose of mirtazapine has action on the processing of emotional faces and emotional word recall. These behavioural results are similar to the profile seen with repeated SSRI administration and are therefore consistent with the hypothesis that the anxiolytic actions of SSRIs may relate to downregulation of postsynaptic, possibly 5-HT_{2C}, serotonin receptors. Conceivably, a single dose of mirtazapine, if it antagonised 5-HT_{2C} receptors sufficiently, might reproduce the effect of repeated SSRIs to desensitise 5-HT_{2C} receptors.

Additionally, fMRI studies have shown that the antidepressant reboxetine, which acts as a noradrenaline reuptake inhibitor, has differing effects on the categorisation of self-referent positive or negative words depending on the length of administration. A single dose of reboxetine had no effect (Miskowiak et al. 2007), while seven days of administration increased activation in frontal and parietal regions during classification of self-referent positive personality characteristics (Norbury et al. 2008). Together, these results indicate that the

repeated administration of some antidepressants have differing or greater effects on some types of emotional processing, such as categorisation, than a single dose or acute administration.

Mirtazapine is also a potent histamine H_1 receptor antagonist, and therefore, another important element to this study is the inclusion of a more selective antihistamine, hydroxyzine, in addition to the placebo group. Hydroxyzine, developed in 1956, is a first generation anti-histamine mainly prescribed for pruritis (itches), allergies, and other conditions such as hyperalgesia. Hydroxyzine is a piperazine derivation, and its main action is the blockade of H_1 receptors, and thus has a side-effect profile with some similarities to mirtazapine, causing, for example, sedation and a decrease in alertness (Lader and Scotto 1998; Llorca et al. 2002). This treatment group may help parse out any antidepressant effects of mirtazapine from those caused by sedation and by blockade of the H_1 receptors. Additionally, hydroxyzine is also licensed as an anxiolytic drug and is thought to be effective in generalized anxiety disorder (GAD) (Hidalgo et al. 2007). The current chapter will assess if the effects seen acutely with mirtazapine are maintained with repeated administration. As mentioned above, the effects of acute compared to repeated doses of SSRIs have been shown to differ, and it is possible this difference is due to specific actions of SSRIs rather than a general antidepressant effect. The issue of H_1 blockade as a possible confound to the results of the previous single dose mirtazapine study is also addressed.

For this study, participants took either 15 mg of mirtazapine, 50 mg of hydroxyzine, or placebo for seven days and then returned to the laboratory for behavioural testing, which included tasks such as the facial expression recognition task, dot-probe, emotional categorization and recognition/recall tasks, and emotion-potentiated startle task. It was hypothesized that mirtazapine would change recognition and bias of emotional stimuli compared to hydroxyzine and placebo. More specifically, the repeated administration of mirtazapine would decrease both the recognition of fearful faces and the emotion-potentiated startle response, and increase recall memory to positive personality characteristics.

5.2 Methods

5.2.1 Participants

Fifty-four participants were recruited into the study. All participants provided written informed consent prior to study enrolment, and received £125 reimbursement for participation. The study was approved by the Oxfordshire Research Ethics Committee. The mean age of participants at the time of participation was 22.8 years (range 18-39 years). Participants had a mean predicted full-scale intelligence quotient of 118.7 (as indexed by the National Adult Reading Test (Nelson and Willison 1991). For more information on recruitment and participants, please see chapter 2.1.

5.2.2 Experimental Design

The study was a double-blind, randomized, placebo-controlled between-groups design. Subjects were randomly assigned to receive either one-week administration of mirtazapine (n=18, 15 mg per day), hydroxyzine (n= 18, 50 mg per day), or placebo (n=18), given in identical capsules to maintain blinding. Participants completed the following baseline measurements in the laboratory the day before taking their first dose: the State and Trait Anxiety Inventory (Spielberger et al. 1970), BDI (Beck et al. 1961), EPQ (Eysenck and Eysenck 1975), BFS (von Zerssen et al. 1974), PANAS (Watson et al. 1988), and VAS (Bond and Lader 1974).

Following completion of baseline questionnaires, participants were instructed when to take the capsules and had the option to enrol in a SMS service reminder of when to take the capsules each day. Participants were then given the blinded capsules and daily questionnaires to take home for the following eight days. Hydroxyzine has a shorter half-life than mirtazapine (around 20 hours (Paton and Webster 1985; Simons and Simons 1994) compared to 20-40 hours for mirtazapine (Timmer et al. 2000), and it is usually prescribed in a thrice-daily dosing regime (Lader and Scotto 1998; Darcis et al. 1995). Therefore, each group received three daily capsules to maintain blinding. The mirtazapine group had a morning and afternoon dose of placebo, and an evening dose of 15 mg of mirtazapine. The hydroxyzine group had 12.5 mg of hydroxyzine in the morning

and afternoon, and a 25 mg dose in the evening. The placebo group had placebo for each daily dose.

Each morning before taking the first dose, participants completed the State Anxiety Inventory, VAS, PANAS, and a side-effects questionnaire. Participants would then take three doses throughout the day, at approximately 9 am, 4 pm, and 11 pm. On the eighth day, participants completed the last daily questionnaire pack and took their final morning dose before returning to the lab for their testing session, which lasted approximately two hours. The testing session began with a final set of questionnaires (BDI, BFS, State, PANAS, VAS, and side-effects questionnaire) followed by a set of computerized tasks (facial expression recognition, emotional categorization and memory, attentional dot-probe, and emotion-potentiated startle).

5.2.3 Psychological Tests

Please see Chapter 2.6 for more information on the ETB.

5.2.4 Statistical Analyses

Demographic characteristics, baseline questionnaire scores, and measures of subjective state were analyzed using one-way ANOVA with group (mirtazapine, hydroxyzine, placebo) as the factor. For more information on general statistical analyses for ETB tasks, please see Chapter 2.7.

Signal detection analysis was performed for the facial recognition task. Target sensitivity (d') and response bias (ß) are calculated by the formulae: $d' = 0.5 + [(y - x)(1 + y - x)]/[4y(1 - x)]$, $0 < d' < 1$, where a higher value is associated with increased accuracy. $ß = [y(1 - y) - x(1 - x)]/[y(1 - y) + x(1 - x)]$, $-1 < ß < 1$, where a higher value is associated a conservative response style (few false alarms). Here, x = the probability of a false alarm [number of false alarms / (number of false alarms + number of correct rejections)] and y = the probability of a hit [number of hits / number of targets]. Note that number of targets = (hits+misses) (Green and Swets 1966; Grier 1971).

Extreme outlying data (data lying at more than ± 2.5 times the standard deviation) were removed from psychological tasks. Outliers were defined by factoring by group in each measure (i.e. accuracy, reaction time, misclassifications). For the emotion-potentiated startle task, five of the 54 participants were not included in the analysis because of electrode interference or because they displayed fewer than 25% satisfactory blink responses in the paradigm (two in the mirtazapine group and three in the placebo group). Where assumptions of equality of variances were broken an ε factor was calculated and used to correct the degrees of freedom using the Greenhouse-Geisser procedure (Howell, 2002). For clarity, uncorrected degrees of freedom are reported.

5.3 Results

5.3.1 Baseline Characteristics

The three groups were comparable at baseline for age, gender, verbal IQ, BDI score, measures of anxiety, and measures of personality (Table 1).

5.3.2 Mood and Anxiety Scales

To allow change in mood to be quantified, baseline ratings were subtracted from scores at the end of treatment for each measure. All subjective state measures were unaffected by treatment group (Table 2). Ratings of dizziness, however, were close to significance ($F(2,53)=2.71$, $p=0.08$), which was driven by a trend of increased ratings of dizziness following mirtazapine administration ($F(1,35)=3.76$, $p=0.06$) but not hydroxyzine ($F(1,35)=0.41$, $p=0.53$) relative to placebo.

	Hydroxyzine (n=18)	Mirtazapine (n=18)	Placebo (n=18)	Significance p-value
Age	23.78 (5.41)	22.72 (3.95)	21.89 (3.44)	0.43
Gender	9M:9F	9M:9F	9M:9F	N/A
Verbal IQ	117.98 (4.17)	118.74 (4.24)	119.35 (3.42)	0.62
BDI	2.86 (2.55)	2.39 (3.50)	1.24 (1.74)	0.20
Trait Anxiety	34.67 (8.41)	32.71 (10.53)	30.53 (5.73)	0.34
State Anxiety	30.56 (8.69)	28.71 (7.88)	31.11 (9.30)	0.70
EPQ-N	6.81 (3.76)	5.44 (5.18)	4.12 (3.04)	0.18
EPQ-P	4.38 (3.16)	2.78 (2.13)	3.82 (2.86)	0.23
EPQ-L	7.38 (3.63)	8.33 (4.07)	7.12 (2.34)	0.55
EPQ-E	17.65 (2.61)	16.11 (4.55)	17.18 (3.63)	0.50
BFS-T	15.33 (9.18)	13.61 (18.01)	10.28 (10.36)	0.51
BFS-E	3.61 (4.54)	2.39 (3.01)	2.22 (2.88)	0.45
BFS-M	11.72 (7.87)	11.28 (15.59)	8.06 (8.13)	0.56

Table 1.

Demographic, mood, and personality measures pre-treatment. Means (± standard deviations). BDI: Beck Depression Inventory; EPQ: Eysenck Personality Questionnaire (N= neuroticism, P= psychoticism, L= lie, E= extraversion); BFS: the Befindlichkeits scale of mood and energy (T= total, E= energy, M= mood).

	Hydroxyzine (n=18)	Mirtazapine (n=18)	Placebo (n=18)	Significance p-value
BDI	0.53 (2.90)	0.06 (4.13)	0.81 (2.97)	0.80
State Anxiety	4.78 (5.81)	2.22 (7.63)	0.22 (7.85)	0.17
BFS-T	8.22 (21.89)	1.61 (21.87)	4.94 (16.67)	0.62
BFS-E	4.56 (7.30)	1.94 (5.31)	2.00 (5.39)	0.35
BFS-M	3.89 (16.29)	-0.39 (17.62)	2.94 (13.41)	0.70
PANAS-P	-4.83 (7.07)	-1.50 (7.18)	-3.33 (7.90)	0.41
PANAS-N	0.78 (19.35)	-0.39 (2.66)	-1.17 (2.15)	0.18
VAS-nausea	1.00 (19.35)	3.44 (21.42)	-0.72 (3.82)	0.76
VAS-dizziness	-2.44 (22.01)	10.89 (21.24)	0.94 (4.76)	0.08
VAS-hunger	4.89 (24.17)	7.44 (25.05)	-3.94 (10.72)	0.24
VAS-anxiety	-4.33 (15.80)	6.11 (27.96)	0.83 (7.33)	0.27
VAS-happy	-12.61 (26.87)	-15.78 (22.41)	-3.00 (8.17)	0.17
VAS-sad	4.22 (22.09)	4.56 (24.76)	2.50 (8.31)	0.95
VAS-alert	-4.89 (24.76)	-7.17 (25.05)	-2.00 (9.75)	0.76

Table 2.

Subjective state changes over time. Values are ratings at the end of treatment minus baseline ratings. Means (± standard deviations). BFS: the Befindlichkeits scale of mood and energy (T= total, E= energy, M= mood); PANAS: Positive and Negative Affective Schedules (P= positive, N= negative); VAS: Visual Analogue Scale.

5.3.3 ETB

Facial expression recognition

Reaction time

There was no main effect of treatment group or interaction between treatment group and facial expression for reaction time in the facial expression recognition task, although the interaction came close to significance (main effect of group: $F(2,45)=1.83$, $p=0.17$; group x expression: $F(12,270)=1.82$, $p=0.07$). To further

investigate this trend, mirtazapine vs. placebo and hydroxyzine vs. placebo were compared in separate ANOVAs with group and valence as factors. There were no significant differences in reaction time between the mirtazapine and placebo groups, nor between the hydroxyzine and placebo groups (all $p>0.26$).

Accuracy

There was a main effect of treatment group for accuracy of facial expression recognition, but no interaction in this task (main effect of group: $F(2,47)=4.18$, $p=0.02$; group x expression: $F(12,282)=0.56$, $p=.082$). This was driven by a difference between the mirtazapine group and placebo group (main effect of group: $F(1,33)=5.90$, $p=0.02$), and no difference between hydroxyzine and placebo (main effect of group: $F(1,30)=0.02$, $p=0.88$). Thus, volunteers receiving mirtazapine were generally worse at recognising emotions, though this was only significant for fearful facial expressions in exploratory post-hoc simple main effect analyses ($t(33)=2.01$, $p=0.05$) (Figure 2). However, it is likely that similar but non-significant reductions in performance are seen across the different emotions in the facial expression recognition task.

Signal detection analysis revealed a main effect of treatment group on target sensitivity, but no interaction (main effect of group: ($F(2,43)=3.27$, $p=0.047$; group x expression: $F(12,258)=0.98$, $p=0.46$). This was driven by a difference between the mirtazapine group and placebo group (main effect of group: $F(1,30)=4.51$, $p=0.04$) and no difference between hydroxyzine and placebo (main effect of group:

$F(1,28)=0.13$, $p=0.72$), with the mirtazapine group being generally worse at discriminating between emotional faces. There was no significant effect of treatment group or interaction on the number of misclassifications of facial expressions (all $p>0.27$).

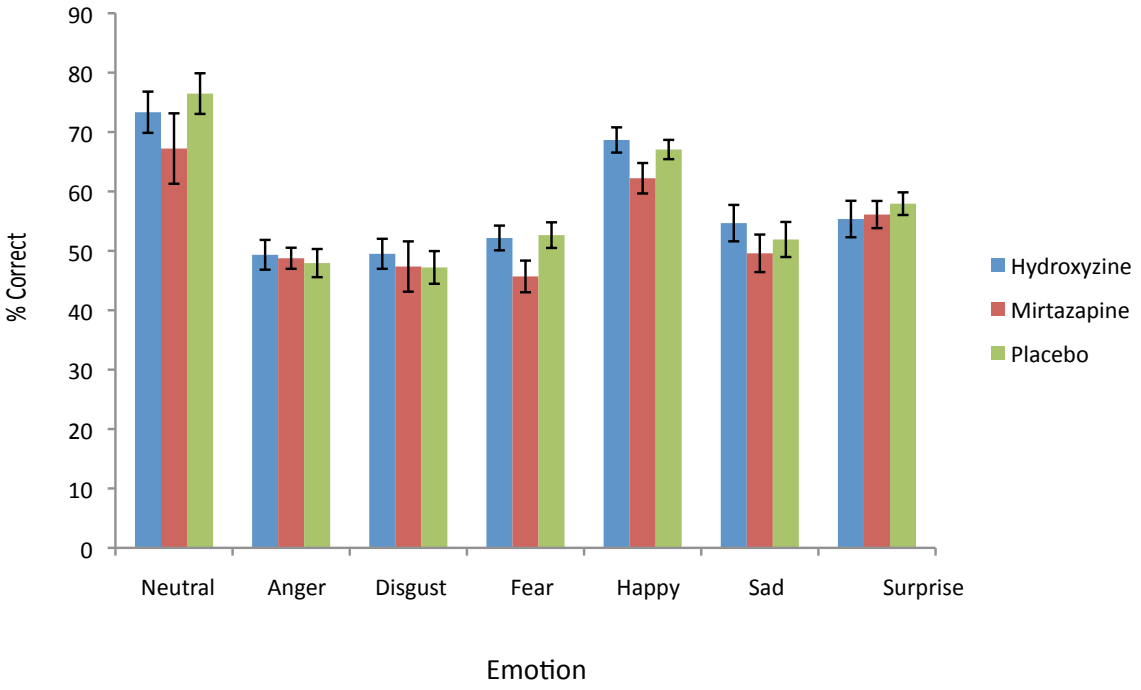


Figure 1.

The effect of mirtazapine, hydroxyzine, and placebo on accuracy of facial expression recognition. Percent correct for all emotions summed over the different intensity levels used in the task. Values are mean % correct ±SEM.

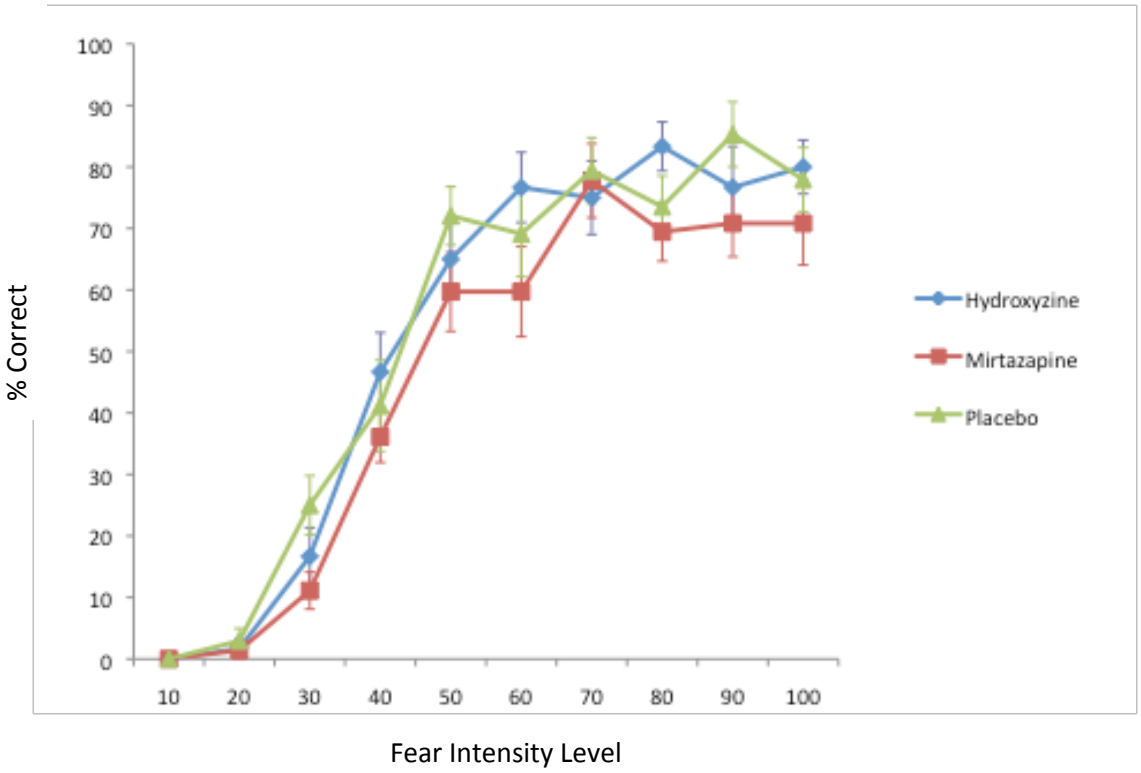


Figure 2.

Identification of fear across the different intensity levels. Values are mean % correct with ±SEM.

Emotional word categorisation

There was no main effect of treatment group and no interaction between word valence and treatment group on accuracy or reaction time in the categorisation of self-referent personality characteristics as positive or negative (Accuracy: main effect of group: $F(2,48)=2.37$, $p=0.10$; group x valence: $F(2,48)=2.13$, $p=0.13$; Reaction time: main effect of group: $F(2,50)=0.39$, $p=0.68$; group x valence: $F(2,50)=2.15$, $p=0.13$).

Emotional word recall

The total number of words correctly recalled did not differ significantly between the three groups ($F(2,53)=0.53$, $p=0.59$). However, the percentage of these words that were positive did differ between groups ($F(2,51)=3.66$, $p=0.03$), with the mirtazapine group having a greater difference score than the placebo group ($F(1,33)=6.97$, $p=0.01$) (Figure 3). The hydroxyzine and placebo groups did not differ ($F(1,34)=0.05$, $p=0.83$). Finally, there were no significant differences in the number of false alarms made in this task (main effect of group: $F(2,48)=1.87$, $p=0.17$; group x valence: $F(2,48)=.11$, $p=0.90$).

Emotional word recognition

Recognition of positive and negative personality characteristics was not significantly affected by treatment group nor was there an interaction between word valence and treatment group (main effect of group: $F(2,51)=0.27$, $p=0.77$; group × valence: $F(2,51)=0.86$, $p=0.43$). Reaction time was also not significantly affected by treatment group nor was there an interaction (main effect of group: $F(2,49)=0.13$, $p=0.88$; group x valence: $F(2,49)=0.95$, $p=0.39$). There was also no significant effect on false alarms (main effect of group: $F(2,49)=0.11$, $p=0.90$; group x valence: $F(2,49)=0.06$, $p=0.94$).

Dot probe

All groups had high accuracy (hydroxyzine mean: 95.52%, mirtazapine mean: 95.31%, placebo mean: 95.62%), which suggests a ceiling effect with the accuracy

measure. There were also no significant effects on the vigilance scores of reaction time (main effect of group: $F(2,48)=0.43$, $p=0.65$; group x valence x mask: $F(2,48)=0.06$, $p=0.95$). This was also true when only unmasked trials were considered (main effect of group: $F(2,48)=1.59$, $p=0.21$; valence x group: $F(2,48)=0.22$, $p=0.81$) (Table 3).

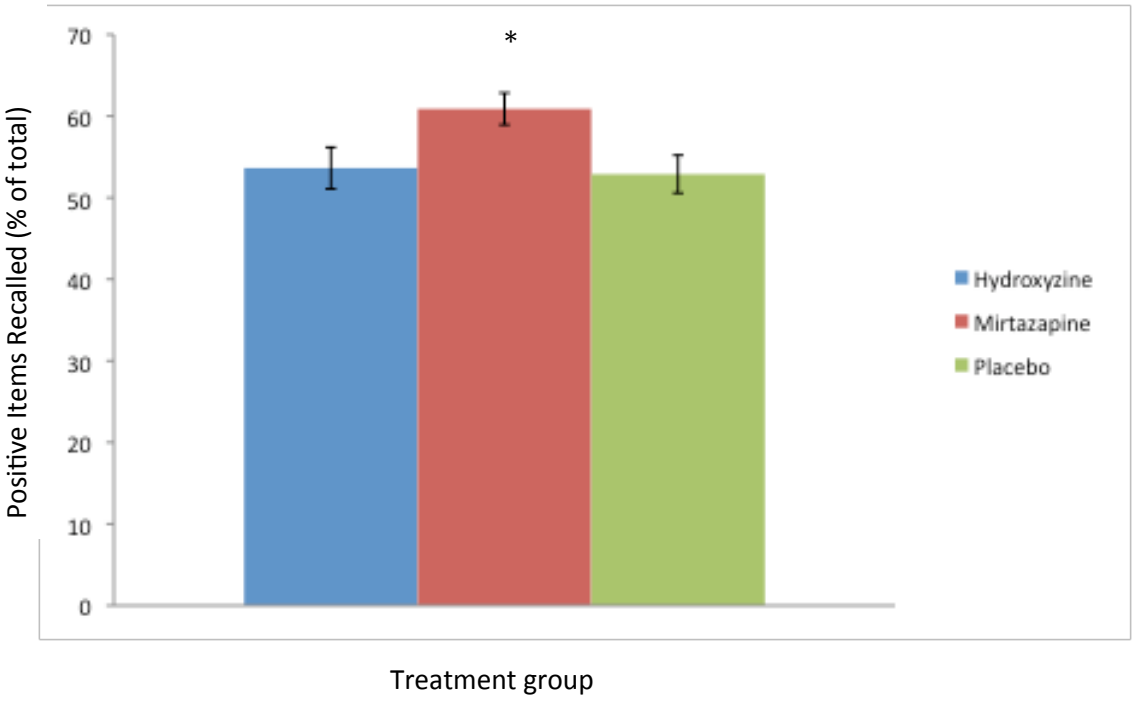


Figure 3.
Recall of positive personality characteristics as a percent of total correct words recalled in participants following hydroxyzine, mirtazapine, and placebo administration. Data are means means± SEM. *$p<0.05$ for the mirtazapine vs. placebo comparison.

		Hydroxyzine	Mirtazapine	Placebo
Masked condition				
Reaction time vigilance (ms)	Positive Negative	0.47 (36.93) 0.45 (50.00)	5.44 (39.68) 1.99 (26.25)	1.75 (31.38) -8.27 (45.34)
Unmasked condition				
Reaction time vigilance (ms)	Positive Negative	13.12 (44.41) 7.37 (47.34)	-6.49 (29.74) -6.60 (33.69)	9.64 (47.64) 3.31 (43.51)

Table 3.
Attentional bias as measured by the dot-probe task under masked and unmasked conditions. Values represent group means ± standard deviations.

Emotion-potentiated startle

There was a significant modulation of the relative startle amplitude (z-values) by the valence ($F(2,92)=14.11$, $p<0.01$), (Figure 4). There were trends for a main effect of treatment group ($F(2,46)=3.00$, $p=0.06$), and interaction between picture valence and treatment group ($F(4,92)=2.08$, $p=0.09$). The latter trend was driven by a reduction in the emotion-potentiated effect with hydroxyzine, as exploratory tests showed only the hydroxyzine group did not demonstrate a significant difference between the neutral and unpleasant condition (Hydroxyzine: $t(17)=-0.55$, $p=0.59$; Mirtazapine: $t(15)=-4.00$, $p<0.01$; Placebo: $t(14)=-4.13$, $p<0.01$). The mirtazapine group also showed a trend for a difference between the neutral and pleasant condition (Hydroxyzine: $t(17)=1.26$, $p=0.23$; Mirtazapine: $t(15)=-1.83$, $p=0.088$; Placebo: $t(14)=-0.76$, $p=0.46$). Generally, this same pattern of results is found when considering the raw amplitude of startle responses (valence: ($F(2,92)=9.65$, $p<.001$); valence x group: ($F(4,92)=1.90$, $p=0.12$); group: ($F(2,46)=0.12$, $p=0.88$)).

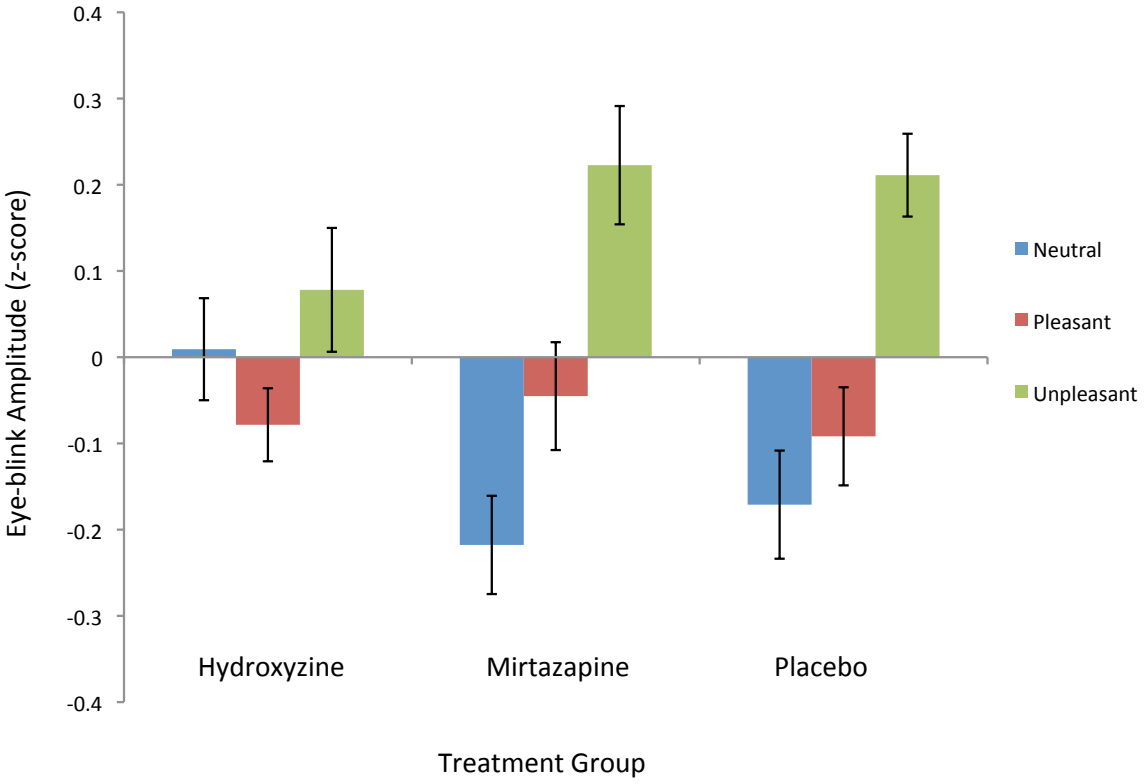


Figure 4.
Effect of hydroxyzine, mirtazapine, and placebo on emotion-potentiated startle responses. Values are relative startle response (z-transformed) to the white noise stimulus in the context of neutral, pleasant (positive), and unpleasant (negative) picture stimuli. Values are mean responses ±SEM in the three groups of volunteers.

5.4 Discussion

In this study, the effect of a seven-day administration of the antidepressant mirtazapine and the anti-histamine hydroxyzine was compared to placebo. Mirtazapine was found to increase the recall of positive versus negative personality characteristics compared to placebo and to decrease facial expression recognition performance, particularly for fearful faces, although this result was

found in exploratory post-hoc analysis only and should not be over-interpreted. Hydroxyzine had a tendency to reduce the emotion potentiated-startle response.

Arnone et al. (2009) found a similar increase in recall of positive personality characteristics following a single dose of mirtazapine compared to placebo. The study presented in Chapter 4 is also consistent with this result. There, a single dose of mirtazapine increased the relative recall of positive emotional words. Thus, this particular positive bias has been seen following mirtazapine administration, both as an acute dose and a repeated dose, which suggests in both dosing regimes mirtazapine alters this aspect of emotion processing in a positive manner. This effect seems to be consistent with antidepressant action, as acute and repeated administration of reboxetine and repeated citalopram administration also produce the same results (Harmer et al. 2003b; Harmer et al. 2004). As negative bias in emotional memory is believed to play an important role in the aetiology and maintenance of depression, these findings suggest that the modulation of emotional memory is particularly relevant to antidepressant action (Harmer et al. 2010b).

In addition to this increase in positive bias in emotional memory, Arnone et al. (2009) also found that a single dose of mirtazapine decreased recognition of fearful facial expressions. The results from the single dose of mirtazapine imaging study presented in Chapter 3 are consistent with those findings. In the scanner task, participants receiving a single dose of mirtazapine had a decrease in

amygdala activity during presentation of fearful faces compared to happy faces, which suggests that mirtazapine exercises an effect on the neural circuitry involved in fear processing. Consistent with this, volunteers receiving seven days of mirtazapine also showed reduced recognition of fearful facial expressions compared to placebo, although this only manifested as a trend in the omnibus analysis. It is possible that the effect was not maintained at a strong level over a week due to issues related to dosing, which are discussed below.

Along with fearful face recognition, the startle paradigm can be understood as a task assessing the processing of threatening stimuli. After a single dose of mirtazapine, the amplitude of blink responses was decreased across all conditions, and this decrease remained significant after the inclusion of alertness as a covariate (Arnone et al. 2009). Repeated citalopram administration and acute administration of anxiolytic drugs such as diazepam also decrease startle reactivity across conditions (Harmer et al. 2004; Murphy et al. 2008). In the present study, hydroxyzine, but not mirtazapine, decreased the emotion-potentiated startle effect.

Hydroxyzine was included in the current study as an additional control for the side-effect of drowsiness caused by antagonism at the H_1 receptor, as both mirtazapine and hydroxyzine have action there. It should be noted, however, that hydroxyzine is also used as a treatment for anxiety, particularly GAD, and has been shown to have a treatment effect size comparable to more standard treatments for

GAD (Hidalgo et al. 2007). Before being prescribed for GAD, hydroxyzine had been shown to relieve physical symptoms of psychosomatic and stress-related illnesses. Hydroxyzine has also been shown to decrease shock-motivated responses in rats and reduces plasma cortisol elevation and galvanic skin reflex (Matsuki and Zsigmond 1973; Pishkin et al. 1967). While the main action of hydroxyzine is the antagonism of the H_1 receptor, one study in rats has suggested that hydroxyzine also has an affinity for the 5-HT_2 receptor (Snowman and Snyder 1990), which may also mediate its anxiolytic actions.

The recommended dose for anxiety treatment in the British National Formulary (2010) is 50-100 mg of hydroxyzine, four times per day, which is much greater than the total 50 mg per day participants received in the present experiment. Early studies showed high doses of hydroxyzine (up to 400 mg/day) improved anxiety symptoms in 60-90% of cases (Barranco and Bridger 1977; Argyropoulos et al. 2000). However, more recent studies, including those used in the Hidalgo et al. meta-analysis (2007) found beneficial effects of hydroxyzine over placebo in GAD in doses of 50 mg daily (Ferrei and Hantouche 1998; Lader and Scotto 1998; Llorca et al. 2002). It was for this reason that the 50 mg daily dose was chosen for the present study, but perhaps a higher dose would have shown more robust effects on emotional processing. The tendency for hydroxyzine to reduce the emotion potentiated-startle response suggests it does have anxiolytic properties detectable in human laboratory models; perhaps using "real-world" doses similar

to those suggested by the British National Formulary would have enabled those properties to be more clearly identified.

The discrepancy between a single dose and repeated doses of mirtazapine in the emotion-potentiated startle task is important to consider, as is the small effect size of the decrease in fearful face processing following repeated mirtazapine administration. Some other serotonergic antidepressants such as the SSRI citalopram demonstrate different effects in healthy volunteers depending on the time course of treatment**.** Acute administration of citalopram increases fear processing, as measured by increased startle responses in the startle task and increased fearful facial expression recognition (Browning et al. 2007; Grillon et al. 2006; Harmer et al. 2003a). After repeated administration of citalopram, the initial effect of increased fear processing is reversed, and healthy volunteers show reduced perception of fearful, angry and disgusted facial expressions as well as reduced startle responses to threatening images in the startle task (Harmer et al. 2004). This difference corresponds to clinical descriptions in a subgroup of patients of increased anxiety early in SSRI treatment (Kent et al. 1998), and can be thought to reflect the downregulation of postsynaptic 5-$HT_{2A/2C}$ receptors following repeated SSRI administration (Deakin and Graeff 1991). As mirtazapine acts as an antagonist on these receptors immediately, rather than affecting them over time like SSRIs, it seems unlikely that there should be a change in emotion processing from a single dose to a seven-day administration unless some kind of

up-regulation of 5-$HT_{2A/2C}$ receptor sensitivity might take place. However, there is no clear evidence for this from animal studies (Rogóz et al. 2005).

It is also worth noting that although by seven days of treatment mirtazapine should be at steady state (Timmer et al. 1995), it is likely that the blood levels of mirtazapine would be somewhat lower about 12 hours after the final dose when the testing for the chronic regime was conducted, than during the acute study where levels would have been at a peak (Arnone et al. 2009). In a study of healthy volunteers, Timmer et al. (1996) found that in males after an acute single dose of mirtazapine (20 mg orally) mean peak values at about two hours are a little less than 30 mg/ml. In contrast, once steady state has been achieved, mean mirtazapine levels approximately 12 hours after an evening dose are about half this. Thus, if the effects of mirtazapine on emotional processing at seven days are still dependent to some extent on plasma levels of drug, it could in part explain why the effects of mirtazapine on emotional processing in the repeated treatment model seem less extensive than after acute dosing. Additionally, Timmer et al. (1996) found a gender effect, with women clearing mirtazapine more slowly. It may be that in this current study there would be a greater effect of mirtazapine in females, however, the statistical power is not great enough to test this prediction.

5.4.1 Limitations

There are several limitations to the current study, which may help explain the discrepancy between the acute and seven day studies of mirtazapine and emotion

processing in healthy volunteers. It has been shown that within the therapeutic dosage range of mirtazapine (15-45 mg per day), steady state is reached in approximately 5 days (Timmer at al. 1996, Croom et al. 2009). The recommended starting dose of mirtazapine is 15 mg daily, but doses of 30 mg and above are more often used in practice. In a recent naturalistic study in Sweden with 180 depressed patients, the mean dose of mirtazapine employed was 33.9 mg with the majority of patients receiving 30-45 mg daily (Walinder et al. 2006). The 15 mg daily dose used in this study may therefore not be a high enough dosage to have a notable effect on some aspects of emotion processing after seven days when testing was carried out 12 hours after the final dose. As noted above, it is also possible that the hydroxyzine dose was too low. A related concern is that participants may not have taken all of their doses as instructed. There was no action in place to guarantee participants took their capsules, and they were simply asked to record when they did not take a dose and return the remaining capsules at the end of the study. In the mirtazapine group, no participants reported missing the evening mirtazapine dose, but six participants missed three or fewer doses of the placebo capsules. In the hydroxyzine group, four participants reported missing three or fewer hydroxyzine doses, and one participant in the placebo group reported missing one dose. These missed doses did not alter the above results when added as covariates into the analysis.

As previously noted, mirtazapine has a variety of pharmacological actions. Hence, while it may be possible, for example, to exclude H_1 receptor blockade in the ability

of mirtazapine to produce a positive bias emotional memory as such was found in the present study, pharmacological properties other than 5-$HT_{2A/2C}$ receptor blockade may be involved in this effect. For example, the α_2-adrenoceptor antagonist properties of mirtazapine would be expected to facilitate noradrenaline neurotransmission and a similar effect on emotional memory was seen following seven days treatment with the noradrenaline re-uptake blocker, reboxetine (Harmer et al. 2004).

5.5 Conclusions

Overall, the antidepressant effects generally seen through the recognition of fearful faces and in the increased recall of positive emotional information are found with mirtazapine in the recall task, but only following exploratory analysis with the facial recognition task. Additionally, anxiolytic effects of drugs, as picked up by the emotion-potentiated startle task, are seen to some extent with hydroxyzine but not mirtazapine. This may reflect the anxiolytic properties of hydroxyzine and its use in GAD.

It may be useful to conduct a similar study on the effect of repeated mirtazapine on emotion processing again, this time with a higher mirtazapine dose, perhaps at 30 mg per day, to see whether more robust effects can be produced on emotional processing. In addition, it will be useful to examine the effect of other agents acting more specifically on the 5-HT_{2C} receptor in the future, in order to learn more about the role of this receptor in decreasing fearful processing. With this in mind,

the next chapter investigates a new antidepressant with putative 5-HT_{2C} antagonistic effects, agomelatine. The affinity of agomelatine for 5-HT_{2C} receptors is relatively low, so it is important to determine if the drug causes a functional 5-HT_{2C} blockade at clinical doses. This can be studied by examining changes in slow wave sleep, as this sleep parameter is reliably increased by 5-HT_{2C} antagonists.

Chapter 6

The effect of agomelatine on slow wave sleep

6.1 Introduction

In the previous chapters, the effects of the antidepressant mirtazapine on emotional processing were investigated with both neural and behavioural measures. Mirtazapine was found to alter emotional processing in healthy volunteers, specifically by reducing amygdala activation to fearful faces and increasing the recall of positive self-descriptors. As previously mentioned, mirtazapine acts as an antagonist at multiple receptors, including the 5-HT_{2C} receptor (Davis and Wilde 1996). However, it is an antagonist at histamine H_1 receptors, and H_1 receptor antagonists such as hydroxyzine are thought to have clinical anxiolytic effects. Therefore, the effect of hydroxyzine on emotional processing was studied, and it was found that this drug did not reproduce the effects of mirtazapine, suggesting that mirtazapine's affect on emotional processing may well be due to the 5-HT_{2C} actions rather than its H_1 receptor blockade.

Mirtazapine is an established antidepressant, and has been available for treatment of depression in the UK since 1997. It has been hypothesized that the effects of mirtazapine on emotional processing may be due to actions at 5-HT_{2C} receptors. To further extend this concept, it was planned to explore the effects of a new antidepressant with putative antagonistic properties at 5-HT_{2C} receptors. However, these antagonistic properties first needed to be verified using an

established sleep assay of 5-HT_{2C} receptor activity. The present study examined a recently licensed antidepressant which has also been suggested to be effective in depression treatment due to its actions at the 5-HT_{2C} receptor.

The pharmacological profile of agomelatine, detailed in Chapter 1, involves agonism of the melatonin MT_1 and MT_2 receptors and antagonism of the 5-HT_{2C} receptors (Millan et al. 2003; Dolder et al. 2008). However, the affinity of agomelatine for the 5-HT_{2C} receptors is around 100-fold less than that of the melatonin receptors (Millan et al. 2003), so it is important to test whether this new antidepressant does indeed cause a functional blockade of 5-HT_{2C} receptors in the human brain at standard clinical doses.

Previous work has shown that drugs which antagonize 5-HT_{2C} receptors increase SWS in healthy volunteers as measured by polysomnography (Sharpley et al. 1994; Sharpley and Cowen 1995). For example, the selective 5-HT_2 receptor antagonist, ritanserin, causes a dose related increase in SWS, and studies with the preferential 5-HT_{2A} receptor antagonist, ketanserin, suggest that this effect is mediated by 5-HT_{2C} receptor blockade (Sharpley et al. 1994). Therefore, if agomelatine produces an effective functional blockade of 5-HT_{2C} receptors in humans it would be expected to increase SWS in the polysomnogram.

The effect of a single dose of agomelatine on SWS has not been examined in healthy volunteers before, although a study of healthy older males given agomelatine for 15 days has been conducted (Leproult et al. 2005). Additionally, another study

investigated the effect of a course of treatment with agomelatine in individuals with major depression (Quera-Salva et al. 2007). The first study in older males found no change in sleep architecture or variables, including SWS, following repeated agomelatine administration. The study in individuals with major depression found an increase in SWS at six weeks of agomelatine treatment compared to baseline.

However, agomelatine has a short half-life (about two hours) and in neither of these studies was agomelatine administered at a time which would ensure maximal blockade of 5-HT_{2C} receptors during the time of peak SWS production in the early part of the night. The aim of the current study was to test the hypothesis that agomelatine would increase SWS in healthy human volunteers when administered as a single dose just prior to sleep onset.

6.2 Methods

6.2.1 Participants

Fifteen participants (eight female) were recruited into the study. All participants provided written informed consent prior to study enrolment, and received £100 reimbursement for participation. The study was approved by the Oxfordshire Research Ethics Committee. The mean age of participants at the time of participation was 25.9 years (range 19-47 years). For detailed information about participant recruitment and screening, see Chapter 2.1.

6.2.2 Experimental Design

The study was a double-blind, balanced order, crossover design, where participants took matching capsules of placebo and agomelatine (25 mg) once each, 30 minutes before retiring to sleep. The two sessions were separated by 7-14 days to allow for adequate washout period between each sleep polysomnogram. Subjective ratings of sleep (on a five-point scale) and side-effects (on a four-point scale) were completed the morning after each sleep study. Participants were asked not to drink alcohol the night before participation nor on the day of participation. Normal caffeine intake was maintained.

Each study night, polysomnograms were recorded as each participant slept at home, using the Embla A10 digital data recording system (Medcare, Broomfield, CO, USA). Participants went to bed and got up at their usual time, and this schedule was kept constant for all study nights and the night directly before the study night.

6.2.3 Analysis

Polysomnography involved a standard montage: four elecroencephalogram channels (C3/A2, C4/A1, O1/A2, O2/A1), two electro-oculogram channels, and a submentalis electromyogram. Polysomnograms were staged in 30-second epochs using the Embla diagnostic software, Somnologica Studio (Medcare, Broomfield, CO, USA). This software provides measures for all aspects of sleep architecture according to standard criteria. In addition, the polysomnograms were edited by an experienced sleep physiologist blind to treatment status. For detailed study design

and analysis information, see Chapter 2.5. The polysomnograms of two participants were excluded due to technical difficulties. All participants' subjective ratings were included for analysis.

6.2.4 Statistics

Subjective ratings of sleep and side-effects were assessed using Student's paired *t*-tests (two-tailed), as were the differences between the pairs of sleep nights.

6.3 Results

6.3.1 Polysomnogram

Agomelatine produced no significant changes in polysomnographic parameters including sleep continuity measures, non-rapid eye movement (NREM) sleep measures, and rapid eye movement (REM) sleep measures (Table 1). Specifically, agomelatine administration resulted in no change in amount of SWS compared to placebo ($t(12)=-0.928$, $p=0.372$) (Figure 1).

SWS was also examined in separate sequential cycles throughout the night, but no effect of agomelatine was found (all $p>0.3$) (Table 2, Figure 2).

Sleep Parameter	Group Mean ± SD	*t*	p-value
Sleep continuity measures			
Total sleep time Agomelatine Placebo	 469.96 ± 49.93 470.80 ± 48.17	-0.12	0.91
Sleep efficiency Agomelatine Placebo	 92.22 ± 4.90 92.35 ± 3.78	-0.11	0.92
Wake after sleep onset Agomelatine Placebo	 25.46 ± 26.48 28.15 ± 20.53	-0.48	0.64
Sleep onset latency Agomelatine Placebo	 15.19 ± 13.92 11.58 ± 6.11	0.88	0.40
Stage 1 Agomelatine Placebo	 57.27 ± 30.99 64.69 ± 28.92	-1.24	0.24
Stage 2 Agomelatine Placebo	 185.92 ± 40.69 181.35 ± 48.92	0.61	0.55
Slow-wave sleep Agomelatine Placebo	 114.35 ± 24.95 119.15 ± 21.09	-0.93	0.37
REM latency Agomelatine Placebo	 72.81 ± 14.03 88.69 ± 33.42	-1.58	0.14
REM sleep Agomelatine Placebo	 112.92 ± 25.80 105.58 ± 18.66	1.00	0.34

Table 1.
The effect of agomelatine, 25 mg orally, on polysomnogram (n=13) in healthy volunteers. Sleep parameters are expressed in minutes unless otherwise stated. [a]Sleep efficiency = actual sleep time/time in bed x 100

6.3.2 Sleep Ratings

Participants reported an improvement in sleep quality following administration of agomelatine ($t(14)=3.055$, $p=0.009$). Participants reported no other differences between the agomelatine and placebo administration in terms of subjective sleep (all $p>0.4$) (Table 3).

6.3.3 Side Effects

Participants reported a significant increase in nausea after administration of agomelatine ($t(14)=2.449$, $p=0.028$), but no other side effects (all $p>0.3$) (Table 3).

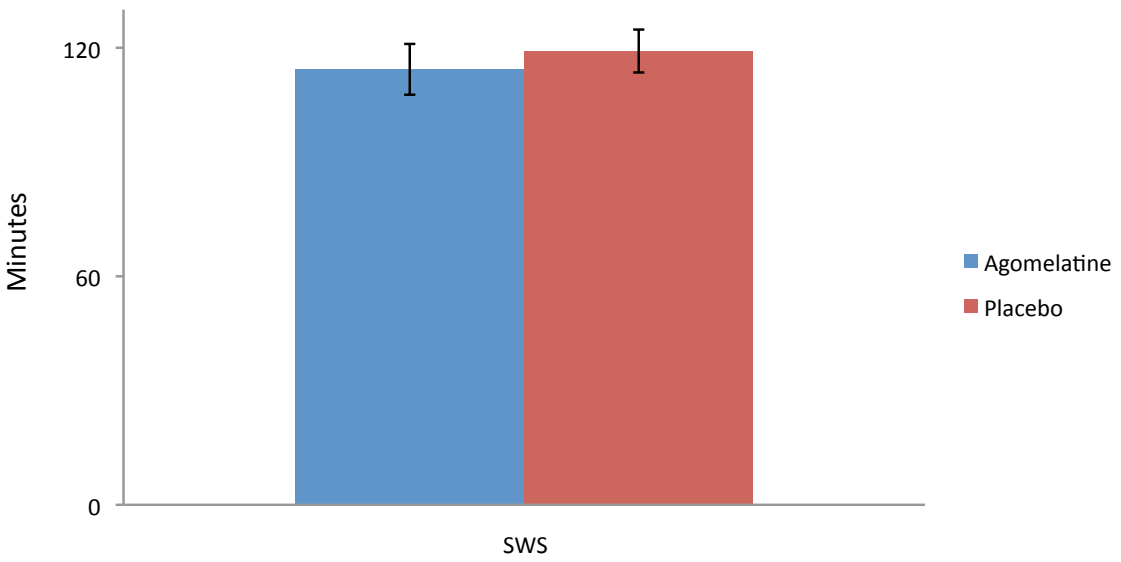


Figure 1.
Total amount of SWS (in minutes) under agomelatine and placebo. Data are means ± SEM.

SWS Cycle	Group Mean ± SD	*t*	p-value
Cycle 1 Agomelatine Placebo	 48.58 ± 9.59 46.08 ± 16.27	0.61	0.55
Cycle 2 Agomelatine Placebo	 26.85 ± 20.60 34.77 ± 14.24	-1.10	0.29
Cycle 3 Agomelatine Placebo	 21.31 ± 12.13 16.15 ± 15.77	1.07	0.31
Cycle 4 Agomelatine Placebo	 12.81 ± 9.99 14.73 ± 12.70	-0.42	0.68

Table 2.
The effect of agomelatine, 25 mg orally, on individual cycles of SWS in healthy volunteers.

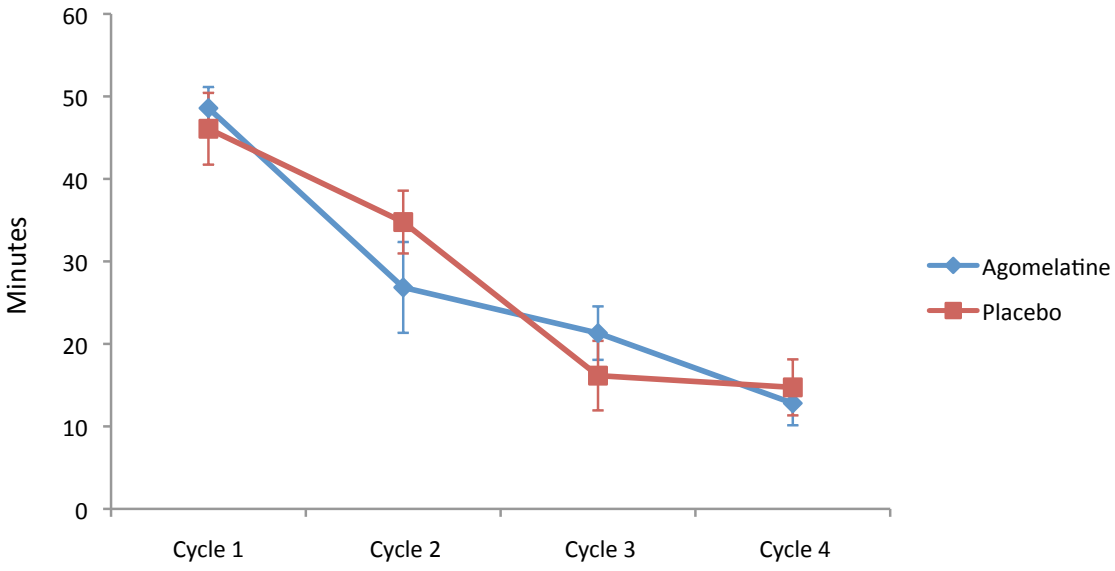


Figure 2.
Individual cycles of SWS (in minutes) in healthy volunteers under agomelatine and placebo. Data are means ± SEM.

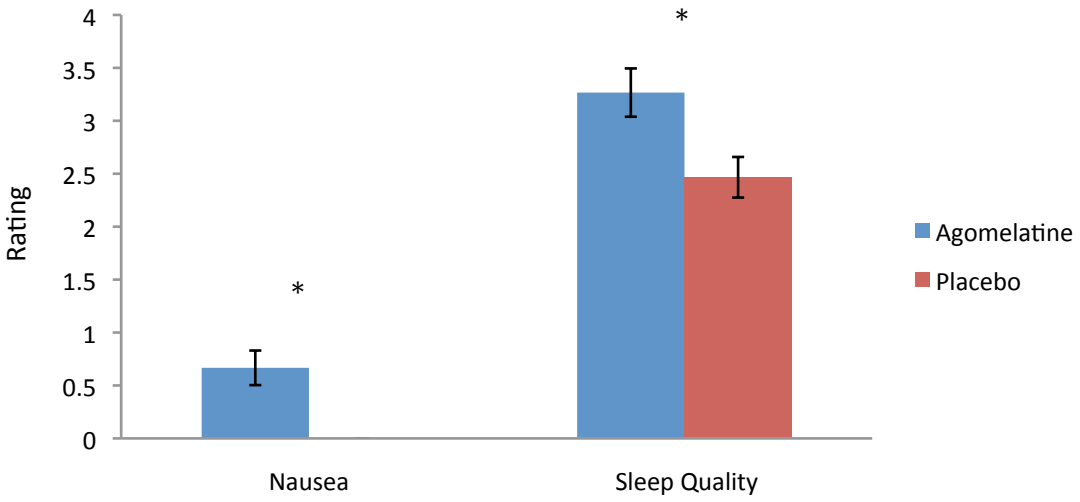


Figure 3.
The subjective effects of nausea ratings and sleep quality ratings in participants following agomelatine and placebo administration. Data are means ± SEM. *p<0.05.

Subjective Sleep Measures	Group mean ± SD	*t*	p-value
Sleep Onset Agomelatine Placebo	 3.13 ± 0.92 2.93 ± 0.88	0.82	0.42
Total sleep Agomelatine Placebo	 3.00 ± 0.76 3.13 ± 0.74	-0.44	0.67
Sleep quality Agomelatine Placebo	 3.27 ± 0.88 2.47 ± 0.74	3.06	0.01
Sleep disturbance Agomelatine Placebo	 3.20 ± 0.77 3.13 ± 0.64	0.27	0.79
Nausea Agomelatine Placebo	 0.40 ± 0.63 0.00 ± 0.00	2.45	0.03
Vomit Agomelatine Placebo	 0.07 ± 0.26 0.00 ± 0.00	1.00	0.33
Dizziness Agomelatine Placebo	 0.07 ± 0.26 0.13 ± 0.52	-1.00	0.33
Headache Agomelatine Placebo	 0.13 ± 0.35 0.13 ± 0.35	0.00	1.00
Alertness Agomelatine Placebo	 0.00 ± 0.00 0.13 ± 0.52	-1.00	0.33
Drowsiness Agomelatine Placebo	 0.07 ± 0.26 0.13 ± 0.52	-0.44	0.67
Increased Appetite Agomelatine Placebo	 0.00 ± 0.00 0.00 ± 0.00	.	.

Table 3.
The effect of agomelatine, 25 mg orally, subjective measures of sleep and side-effects in healthy volunteers.

6.4 Discussion

In this study, the effect of a single dose of agomelatine on SWS was assessed to determine if agomelatine causes a functional blockade of brain 5-HT_{2C} receptors in healthy humans when administered just prior to sleep onset. Agomelatine is reported to be an antagonist at the 5-HT_{2C} receptor and an agonist at the MT_1 and

MT_2 melatonin receptors (Millan et al. 2003; Dolder et al. 2008). However, no increase in SWS following a single standard clinical dose of agomelatine was found, although participants reported their sleep quality as improved and experienced more nausea. This finding indicates that agomelatine probably does not cause a functional blockade of 5-HT_{2C} receptors at an initial standard clinical dose of 25 mg, as reflected in SWS changes. Agomelatine has a short half-life and is rapidly absorbed (peak plasma concentration is reached within one to two hours) (Dubovsky and Warren 2009). Hence, participants should be exposed to maximal levels of agomelatine during the first SWS cycle, which occurs within 30-40 minutes of sleep onset in healthy adults (Parkes 1985), as participants took their capsules at least 30 minutes prior to retiring to sleep.

The fact that participants felt their sleep was improved and that they experienced some nausea following agomelatine does suggest, however, that agomelatine did have an effect on subjective physiological state. This increase in reported sleep quality is difficult to explain. The strongest predictors of a sleep quality index measured by Keklund and Akerstedt (1997) was SWS, sleep efficiency, and measures related to sleep continuity such as total sleep time. The authors suggest that depth of sleep and sleep continuity appear to be the most important elements of sleep quality. These variables did not differ between agomelatine and placebo treatment in the present study, so cannot account for the increase in sleep quality following agomelatine. It may be that the melatonergic properties of agomelatine influenced subjective sleep quality, although a single dose administration of melatonin before retiring to sleep has been shown to increase actual sleep time,

sleep efficiency, non-REM sleep, and REM sleep latency (Attenburrow et al. 1996), again, measures not altered in the present study. However, it is possible that the combination of 5-HT_{2C} antagonism and melatonergic agonism may have acted in such a way as to increase subjective sleep quality.

Previous studies examining single doses of drugs acting as antagonists at the 5-HT_{2C} receptors have shown that increases in SWS appear to be a reliable means of identifying drugs with a significant affinity for brain 5-HT_{2C} receptors in humans. Single doses of ritanserin (10 mg) and cyproheptadine (4 mg), for example, cause approximately 50% increases in SWS, as do olanzapine (5 mg) and mirtazapine (30 mg) (Solomon et al. 1989; Sharpley et al. 2000; Aslan et al. 2002). Ritanserin, which is a selective 5-$HT_{2A/2C}$ receptor antagonist with a preferential affinity for the 5-HT_{2C} receptor, was shown to increase SWS in a double-blind placebo controlled study conducted in a sleep laboratory (Idzikowski et al. 1986). 10 mg of ritanserin was given each testing day either in the morning (8 am) or evening (10 pm) and volunteers were studied for 12 nights divided into four periods of three consecutive nights each. The ritanserin conditions produced large increases in SWS. Another study investigated the effect of ritanserin, cyproheptadine, and a histamine H_1 antagonist mepyramine on SWS (Solomon et al. 1989). This study used ambulatory recording where participants slept in their own home. Both ritanserin and cyprophetadine have a high affinity for 5-HT_{2C} receptors, and increased SWS. While these drugs also have antihistaminic properties, the H_1 antagonist mepyramine did not increase SWS.

Other 5-HT_{2C} receptor antagonists have also been shown to increase SWS with polysomnography, while agonists of the 5-HT_{2C} receptor decrease SWS. Olanzapine is an atypical antipsychotic and has antagonistic actions at $5HT_{2A}$ receptors, dopamine D_2 receptors, 5-HT_{2C} receptors, muscarinic cholinergic receptors, α_2-adrenoceptors, and H_1 histamine receptors (Rues 1997). A single dose of 5 mg of olanzapine significantly increased SWS in healthy volunteers in a double-blind, placebo-controlled, cross-over design (Sharpley et al. 2000). Olanzapine has a high affinity for the 5-HT_{2C} receptor, and previous studies have shown that the range of other receptors upon which olazapine acts do not appear to modify SWS, so the SWS increase has been attributed to the 5-HT_{2C} blockade (Solomon et al. 1989; Sharpley et al. 1990 and 1991; Nicholson and Pascoe 1990). Mirtazapine, discussed earlier, also acts as an antagonist at a variety of receptors, such as 5-HT_{2C}, $5HT_{2A}$, $5HT_3$, H_1, and noradrenergic α_2-adrenoceptors (Davis and Wilde 1996). In a double-blind, placebo-controlled study, 20 healthy volunteers were given a single 30 mg dose of mirtazapine (n=10) or placebo (Aslan et al. 2002). Sleep was then assessed on three consecutive nights: the adaptation night, the baseline sleep measurement night, and drug/placebo sleep measurement night. It was found that mirtazapine significantly increased SWS. Conversely, mCPP, a 5-HT_{2C} receptor agonist, has been shown to decrease SWS in healthy volunteers following a single 7.5 mg dose and a single 15 mg dose (Katsuda et al. 1993). Overall, the literature suggests that drugs that have a significant ability to block 5-HT_{2C} receptors increase SWS even where their effects on 5-HT_{2C} receptors are part of a more complex pharmacological profile.

The effect of agomelatine on SWS has been investigated previously in older healthy males in a double-blind, two-period cross-over study (Leproult et al. 2005). Eight elderly males (mean 60 years) were treated with agomelatine (50 mg) or placebo daily at 6:30 pm for 15 days. Following the treatment period, sleep polysomnography was conducted. There was no effect of agomelatine treatment on SWS duration, nor on any other sleep measures in that study, similar to the current findings. The authors suggest that the lack of sleep changes may be due to the advanced age of the population sample, the dosage, or the duration of drug administration.

Changes in SWS following agomelatine treatment have also been studied in depressed patients, with differing results. Quera Salva et al. (2007) treated 15 outpatients with MDD (seven males, mean age 36.5, mean HAM-D score 21.8) for six weeks with 25 mg per day of agomelatine, taken in the evening. The patients were assessed with polysomnography at baseline, week 1, week 2, and week 6. SWS duration and percentage of sleep period time in SWS were increased at the final week 6 assessment compared to baseline.

These results contrast with those found in the study in older males, as well as the present findings. Both the current study and the older adult study included healthy volunteers without a history or current episode of depression, whereas the third study examined individuals with MDD. It may be that the change in SWS is due to a change in underlying clinical state, not due to direct pharmacological effects of agomelatine on 5-HT_{2C} receptors. The fact that SWS changes were not

significant until the end of treatment at six weeks supports this proposal, as the pharmacological effect of 5-HT_{2C} receptor blockade would be assumed to occur soon after agomelatine administration. Additional elements such as dose timing and duration vary across each of the three studies, and may contribute to the opposing findings.

Given that 5-HT_{2C} antagonism has been shown to increase SWS in healthy volunteers, it is of note, then, that this study did not find any significant changes in SWS following agomelatine administration. There are several possibilities that may address the lack of significant changes in SWS in this study besides the relatively low affinity of agomelatine for 5-HT_{2C} receptors.

Agomelatine has been shown to have a relatively low bioavailability (about 5-10%) because of a high first-pass effect (Dolder et al. 2008). Additionally, the mean terminal elimination half-life of agomelatine is 2.3 hours (Zupancic and Guilleminault 2006). These fast effects may mean that the actions of agomelatine on the 5-HT_{2C} receptors may be variable and short-lived, and not suited to an overnight measure such as polsomnography, which in this case, began recording 30 minutes following agomelatine administration and continued over multiple hours. It may be that the 5-HT_{2C} receptor antagonistic actions of agomelatine are too transient to demonstrate a robust effect on SWS. To determine if that may have been the case, SWS throughout the night was divided into separate cycles to see if agomelatine had an effect during one of the earlier cycles when its blood

levels should be at a maximum. There were, however, no differential effects of agomelatine across the SWS cycles.

The agonist action of agomelatine on the melatonin MT_1 and MT_2 receptors may also have a role in the lack of increase in SWS following agomelatine administration. The effect of varying doses of melatonin alone on SWS has been studied with polysomnography, and it does not appear to have an effect on SWS (Attenburrow et al. 1996). However, it may be possible that the melatoninergic properties of agomelatine could interact with 5-HT_{2C} receptor blockade to prevent increases in SWS that 5-HT_{2C} antagonists alone produce.

6.5 Conclusions

In conclusion, the present study showed that there was no effect of a single dose of agomelatine (25 mg) on SWS in healthy volunteers, which suggests that at this standard clinical dose, agomelatine does not cause functional blockade of brain 5-HT_{2C} receptors. The pharmacological profile of agomelatine has been suggested to involve the activation of the MT_1 and MT_2 melatonin receptors as well as blockade of the 5-HT_{2C} receptor. It is this blockade of the 5-HT_{2C} receptors, together with melatonin agonism, that has been implicated in the antidepressant and anxiolytic profile of agomelatine (Millan 2003; Papp et al. 2006), so the current result needs further study. Agomelatine has been shown through controlled trials to be an effective antidepressant, so clarifying its mode of action in humans is very important. It is also important to characterize the functional properties of

agomelatine further, such as the effect of agomelatine on emotional processing, which will be explored in the following study.

Chapter 7

The effect of agomelatine and mCPP on emotional processing

7.1 Introduction

Previous chapters have shown that mirtazapine has consistent effects on emotional processing in healthy volunteers across a single dose and a weeklong administration, possibly due to the immediate effects of blocking the 5-HT_{2C} receptor. In order to more fully implicate the 5-HT_{2C} receptors' involvement in emotion processing, the final study moved focus from the antidepressant and anxiolytic effects of mirtazapine to another antidepressant, agomelatine. This study was designed to investigate if other drugs acting at the 5-HT_{2C} receptor would also alter the processing of emotional information after a single dose. Following from the study examining the effect of agomelatine on SWS, this chapter tested the effect of agomelatine and its 5-HT_{2C} antagonistic actions on emotional processing. This study also included an important additional facet - the effect of agomelatine was compared to a compound that has potent 5-HT_{2C} agonist actions, mCPP. The addition of mCPP also allows for the comparison of effects on emotional processing between two agents with diametrically opposing actions, a 5-HT_{2C} antagonist and a 5-HT_{2C} agonist. Another outcome measure in this study was the participants' level of salivary cortisol, taken at hourly intervals. Cortisol levels were measured as an index of 5-HT_{2C} receptor activation with mCPP, to determine the efficacy of the drug dose.

As discussed in Chapter 1, agomelatine is a licensed antidepressant, marketed as

targeting the MT_1 and MT_2 melatonin receptors as an agonist and the 5-HT_{2C} receptor as an antagonist, making it a novel drug treatment for depression. The investigation of SWS following agomelatine administration reported in Chapter 6 did not reveal the expected difference in SWS between agomelatine and placebo treated volunteers, which suggests the initiation of a standard clinical dose of agomelatine does not cause functional blockade of brain 5-HT_{2C} receptors in humans. This finding is interesting given the emotion processing difference found in a seven-day study comparing placebo to agomelatine (Harmer et al. 2010a). This study found that agomelatine decreased recognition of sad facial expressions, improved positive affective memory, and reduced the emotion-potentiated startle effect after a week. The effect of agomelatine on cortisol levels has not been characterized previously, but as agomelatine is a putative 5-HT_{2C} receptor antagonist, administration of the drug may lower cortisol levels. Other drugs with putative 5-HT_{2C} receptor antagonist actions, such as mirtazapine, have been shown to decrease cortisol levels (Laakmann et al. 1999).

The compound mCPP, also discussed in Chapter 1, is an established probe of serotonin function in psychiatric research, often used to characterize the role of serotonin in anxiety and other psychiatric disorders (Gatch 2003). It has potent agonist actions at the 5-HT_{2C} receptor, but also has actions at other 5-HT receptors (Hamik and Peroutka 1989; Middlemiss and Hutson 1990; Schoeffter and Hoyer 1989). Administration of mCPP increases anxiety in human and animal models (Kahn and Wetzler 1991) and these effects are blocked by the 5-$HT_{2A/2C}$ receptor antagonist, ritanserin, at doses that would be expected to preferentially occupy 5-

HT_{2C} receptors. The effect of mCPP on emotion processing has not previously been tested using the ETB. As mentioned previously, mCPP reliably elevates levels of cortisol (Kahn and Wetzler, 1991), and this effect on cortisol is likely mediated via 5-HT_{2C} receptors (Hamik and Peroutka, 1989).

For this final study, participants were given a single dose of either agomelatine, mCPP, or placebo followed by a behavioural emotion testing battery consisting of the facial expression recognition test, the emotional categorization, recall and recognition tests, the dot-probe and the emotion-potentiated startle test. Hourly samples of salivary cortisol were also taken. Based on the known pharmacological properties of mCPP and its anxiogenic effects, as well as the fact that mCPP and agomelatine have opposing actions on 5-HT_{2C} receptors, we hypothesized that the mCPP group would have an increased emotion-potentiated startle response, increased fearful face recognition, and increased cortisol levels and the agomelatine group would have decreased recognition of fearful faces, increased recall of positive words, and decreased cortisol levels relative to placebo.

7.2 Methods

7.2.1 Participants

Forty-five participants were recruiting into the study. All participants provided written informed consent prior to study enrolment, and received £75 reimbursement for participation. The study was approved by the Oxfordshire Research Ethics Committee. The mean age of participants at the time of participation was 22.87 years (range 18-33). Participants had a mean predicted

full-scale intelligence quotient of 119.8 (as indexed by the National Adult Reading Test (Nelson and Willison 1991)).

7.2.2 Experimental Design

The study was a double-blind, randomized, placebo-controlled between-groups design. Subjects were randomly assigned to receive either a single dose of agomelatine (n=15, 25 mg), mCPP (n=15, 15 mg), or placebo (n=15). Due to the differing absorption rates of agomelatine and mCPP, participants received two capsules during the study to remain blinding, one at approximately 9:30 am and one two hours later (one hour before behavioural testing commenced). The aim was to assess, as far as possible, that the testing took place around the time of peak plasma levels of the respective drugs. For the agomelatine group, the first capsule was a placebo, and the second was the agomelatine (capsules were matched to maintain blinding). The mCPP group received mCPP first, followed by a matched placebo capsule. All capsules were given to participants by a third party to ensure researcher blinding.

Participants completed the following baseline measurements in the laboratory prior to taking their first capsule: the State and Trait Anxiety Inventory (Spielberger et al. 1970), the BDI (Beck et al. 1961), EPQ (Eysenck and Eysenck 1975), BFS (von Zerssen et al. 1974), PANAS (Watson et al. 1988), and VAS (Bond and Lader 1974). Following completion of baseline questionnaires, participants gave their first cortisol sample with salivette and took their first capsule.

Participants were then free to relax and read or do work, except for hourly questionnaires (state, VAS, PANAS, BFS) and cortisol samples, until 11:30 am. At 11:30 am participants took their second capsule and again completed questionnaires and gave a cortisol sample. After repeating at 12:30 pm, the computerized testing session including the facial expression recognition, emotional categorization and memory, attentional dot-probe, and emotion-potentiated startle began, with the final cortisol sample taken at 1:30 pm.

7.2.3 Psychological Tests

Please see Chapter 2.6 for information on the ETB.

7.2.4. Salivary Cortisol

Saliva for cortisol estimation was collected in salivettes (Sarstedt AG &Co., Nümbrecht, Germany). Samples were spun down following the experimental session and stored at -23 °C. Salivary cortisol was measured by a direct radioimmunoassay using 125-iodine labelled cortisol with solid-phase second antibody separation of free from bound hormone. The coefficients of variation for both inter- and intra-assay variation were within 5%.

7.2.5 Statistical Analyses

Demographic characteristics, baseline questionnaire scores, and measures of subjective state were analyzed using one-way ANOVA with group (agomelatine, mCPP, placebo) as the factor.

Signal detection analysis was performed for the facial recognition task and emotional word recognition. Target sensitivity (d') and response bias (ß) were calculated by the formulae: $d' = 0.5 + [(y - x)(1 + y - x)]/[4y(1 - x)]$, $0 < d' < 1$, where a higher value is associated with increased accuracy. $ß = [y(1 - y) - x(1 - x)]/[y(1 - y) + x(1 - x)]$, $-1 < ß < 1$, where a higher value is associated a conservative response style (few false alarms). Here, x = the probability of a false alarm [number of false alarms / (number of false alarms + number of correct rejections)] and y = the probability of a hit [number of hits / number of targets]. Note that number of targets = (hits+misses) (Green and Swets 1966; Grier 1971).

Cortisol data were analysed using repeated-measures ANOVA with group (agomelatine, mCPP, placebo) as the between-subjects factor. The within-subjects factor was time (Time Point 1-5). For more information on general statistical analyses for ETB tasks, please see Chapter 2.7.

Extreme outlying data (data lying at more than ± 2.5 times the standard deviation) were removed from psychological tasks. Outliers were defined by factoring by group in each measure (i.e. accuracy, reaction time, misclassifications). Of the 45 participants, seven were not included in the emotion-potentiated startle task

analysis because of electrode interference or because they displayed fewer than 25% satisfactory blink responses in the paradigm (four in the mCPP group, two in the placebo group, and one in the agomelatine group). Where assumptions of equality of variances were broken, an ε factor was calculated and used to correct the degrees of freedom using the Greenhouse-Geisser procedure (Howell 2002). For clarity, uncorrected degrees of freedom are reported.

7.3 Results

7.3.1 Baseline Characteristics

The three groups were comparable at baseline for age, gender, verbal IQ, BDI score, measures of anxiety, and measures of personality (Table 1).

7.3.2 Mood and Anxiety Scales

To allow change in mood over the participation day to be quantified, baseline ratings were subtracted from scores at the end of the day for each measure. There were no significant effects of treatment group on state anxiety, visual analogue ratings, the total and energy BFS scores, and the negative scale of the PANAS (Table 2). There was, however, a significant difference between the groups on two measures. The positive scale of the PANAS was different between the treatment groups ($F(2,44)=3.31$, $p=0.046$). This difference was driven by a decrease in positive ratings by the mCPP group compared to the agomelatine group ($F(1,29)=5.68$, $p=0.02$), with no significant differences between either drug group

and placebo (all $p>0.16$). The negative scale of the PANAS, though, did not differ significantly between the groups. The mood BFS scores were also significantly different between groups, ($F(2,44)=4.56$, $p=0.02$), which was driven by an increase in scores (indicating worse mood) by the mCPP group compared to the agomelatine group ($F(1,29)=6.64$, $p=0.02$), with no difference between the mCPP group and placebo ($F(1,29)=2.33$, $p=0.14$), and a trend for a difference between the agomelatine group and placebo ($F(1,29)=3.50$, $p=0.07$).

Pearson's one-tailed correlations were conducted between the tasks' main outcomes of interest and PANAS positive and BFS mood scores for each group. In the facial recognition task, there were significant positive correlations in the placebo group. The PANAS positive score was significantly related to anger recognition ($r=0.53$, $p=0.03$) and happy recognition ($r=0.60$, $p=0.01$). In the recall task, there were significant negative correlations in the agomelatine group. Improved mood on the BFS mood scores were associated with increased accuracy for positive information ($r=-0.52$, $p=0.02$) in the agomelatine group.

	Agomelatine (n=15)	mCPP (n=15)	Placebo (n=15)	Significance p-value
Age	22.60 (4.26)	23.73 (7.51)	22.27 (3.20)	0.73
Gender	8M:7F	8M:7F	8M:7F	N/A
Verbal IQ	120.86 (3.03)	119.87 (3.34)	118.56 (3.76)	0.20
BDI	1.67 (1.94)	2.53 (2.50)	2.23 (2.91)	0.63
Trait Anxiety	33.00 (6.34)	32.40 (7.33)	33.33 (5.67)	0.92
State Anxiety	30. 00 (6.90)	29.87 (7.58)	27.80 (5.24)	0.60
EPQ-N	7.47 (6.29)	7.27 (4.35)	7.20 (4.23)	0.99
EPQ-P	2.93 (1.98)	2.80 (1.90)	3.13 (2.36)	0.91
EPQ-L	8.40 (4.01)	7.60 (2.50)	9.33 (3.13)	0.36
EPQ-E	15.33 (3.24)	14.73 (3.28)	14.53 (4.31)	0.82
BFS-T	16.93 (11.49)	19.60 (12.36)	22.00 (20.96)	0.67
BFS-E	5.33 (5.29)	7.80 (4.52)	6.27 (5.50)	0.42
BFS-M	13.20 (9.29)	11.80 (8.89)	15.73 (16.13)	0.66

Table 1.

Demographic, mood, and personality measures pre-treatment. Means (± standard deviations). BDI: Beck Depression Inventory; EPQ: Eysenck Personality Questionnaire (N= neuroticism, P= psychoticism, L= lie, E= extraversion); BFS: the Befindlichkeits scale of mood and energy (T= total, E= energy, M= mood).

	Agomelatine (n=15)	mCPP (n=15)	Placebo (n=15)	Significance p-value
BFS-T	-3.53 (13.67)	5.53 (14.45)	0.53 (7.62)	0.14
BFS-E	-1.00 (4.80)	1.33 (5.47)	4.20 (9.78)	0.44
BFS-M	-4.13 (7.83)	4.20 (9.78)	0.067 (3.79)	0.02
State Anxiety	-1.60 (5.07)	0.80 (3.71)	-0.13 (3.29)	0.28
PANAS-P	-0.60 (5.46)	-6.20 (7.28)	-2.87 (5.01)	0.046
PANAS-N	-0.87 (0.99)	-0.80 (1.61)	-0.07 (0.88)	0.14
VAS-nausea	-4.87 (13.36)	-2.33 (13.70)	0.80 (13.62)	0.52
VAS-dizziness	1.73 (10.57)	2.53 (7.64)	1.47 (5.57)	0.93
VAS-hunger	20.53 (35.20)	21.47 (25.72)	26.73 (24.01)	0.82
VAS-anxiety	1.47 (7.93)	-3.80 (18.48)	-0.27 (6.79)	0.49
VAS-happy	-0.13 (13.29)	-4.87 (14.28)	2.53 (8.28)	0.26
VAS-sad	3.53 (9.34)	-2.40 (9.48)	-0.27 (8.88)	0.22
VAS-alert	3.40 (14.31)	-8.87 (22.61)	-1.67 (10.16)	0.14

Table 2.

Subjective state changes over time. Values are ratings at the end of treatment minus baseline ratings. Means (± standard deviations). BFS: the Befindlichkeits scale of mood and energy (T= total, E= energy, M= mood); PANAS: Positive and Negative Affective Schedules (P= positive, N= negative); VAS: Visual Analogue Scale.

7.3.3 ETB

Facial expression recognition

Reaction time

There was no main effect of treatment group or interaction between treatment group and facial expression for reaction time in the facial expression recognition task (main effect of group: $F(2,38)=0.14$, $p=0.65$; group x expression: $F(12,228)=0.90$, $p=0.52$).

Misclassifications

There were also no significant interactions or effect of treatment group on the number of misclassifications of facial expressions (main effect of group: $F(2,39)=2.35$, $p=0.11$; group x expression: $F(12,234)=0.52$, $p=0.80$).

Accuracy

There was a main effect of treatment group for accuracy of facial expression recognition across all emotions, but no interaction in this task (main effect of group: $F(2,38)=4.64$, $p=0.02$; group x expression: $F(12,228)=1.01$, $p=0.44$). This main effect was driven by the agomelatine group, which was more accurate compared to both the placebo group across all emotions (main effect of group: $F(1,25)=7.30$, $p=0.01$) and the mCPP group ($F(1,25)=5.01$, $p=0.03$). (Figure 1).

Thus, volunteers receiving agomelatine were generally better at recognising emotions, though this was only significant for surprise and disgust facial

expressions in exploratory post-hoc simple main effect analyses between agomelatine and placebo [surprise: (t(24)=1.14, p=0.00), disgust: (t(24)=1.89, p=0.05)], and for happy facial expression between agomelatine and mCPP (t(25)=0.86, p=0.02) (Figure 2). These results should not, however, be over-interpreted.

Signal detection analysis also revealed a trend for main effect of treatment group on target sensitivity (d') (main effect of group: F(2,36)=3.00, p=0.06; group x expression: F(12,216)=0.66, p=0.76). Exploratory post-hoc tests show this trend was also driven by the agomelatine group, which differed significantly from the placebo (main effect of group: F(1,23)=4.80, p=0.04) and mCPP group (F(1,24)=4.36, p=0.048).

Emotional word categorisation

There was no main effect of treatment group and no interaction between word valence and treatment group on accuracy or reaction time in the categorisation of self-referent personality characteristics as positive or negative (accuracy: main effect of group F(1,39)=1.89, p=0.16; group x valence F(2,39)=0.01, p=0.10; reaction time: main effect of group F(2,42)=0.03, p=0.98; valence x group F(2,42)=0.01, p=0.99).

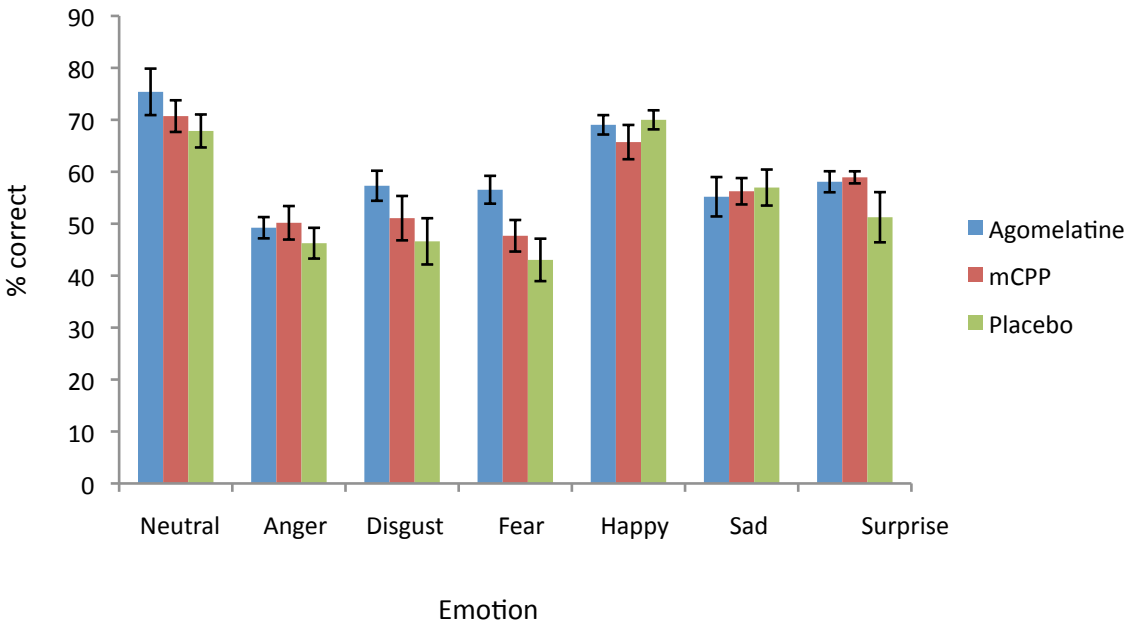


Figure 1.

The effect of agomelatine, mCPP, and placebo on accuracy of facial expression recognition. Percent correct for all emotions summed over the different intensity levels used in the task. Values are mean % correct ±SEM.

Emotional word recall

There was a trend for a main effect of treatment group in the accuracy for words recalled (main effect of group: $F(2,42)=2.83$, $p=0.07$; group x valence: $F(2,42)=0.98$, $p=0.38$), with the mCPP group recalling the most words. Exploratory post-hoc tests were used to further explore this trend, and the agomelatine vs. mCPP comparison was found to be significant (main effect of group: $F(1,28)=4.66$, $p=0.04$), with mCPP recalling more negative words ($F(1,29)=6.31$, $p=0.02$) and no difference between the two groups in positive words ($F(1,29)=1.76$, $p=0.20$). Although there was a trend for a group difference in the total number of words

correctly recalled, this finding may be explained by a significant difference in the total number of words written ($F(2,44)=3.35$, $p=0.045$), as mCPP wrote more words than the other groups, and this was driven by a difference between the agomelatine group and the mCPP group ($F(1,29)=5.37$, $p=0.03$). Additionally, there was no significant difference between the groups when the ratio between the number of correct words to the number of words written was analysed ($F(2,44)=0.46$, $p=0.64$).

Emotional word recognition

Recognition of positive and negative personality characteristics were not significantly affected by treatment group (main effect of group: $F(2,41)=2.34$, $p=0.11$; group × valence: $F(2,41)=2.69$, $p=0.08$). There was a trend for an interaction between group and valence in the accuracy for the recognition of emotional words, which was investigated further. Treatment groups were compared in separate ANOVAs with group and valence as factors. There was a significant difference between the mCPP and agomelatine groups ($F(1,27)=5.18$, $p=0.03$). The simple main effects analysis showed that volunteers receiving agomelatine showed reduced recognition of negative words compared to mCPP ($F(1,28)=5.81$, $p=0.02$) (Figure 2).

Reaction time was not significantly affected by treatment group (main effect of group: $F(2,41)=0.24$, $p=0.79$; group x valence: $F(2,41)=1.79$, $p=0.18$), but there was a significant effect of treatment group on false alarms (main effect of group:

$F(2,40)=3.87$, $p=0.03$; group x valence: $F(2,40)=1.12$, $p=0.34$), which appears to be driven by an increase in false alarms in the agomelatine group. This main effect was further explored with post-hoc tests, and there was a significant difference between the agomelatine and mCPP group (main effect of group: $F(1,27)=7.16$, $p=0.01$). Signal detection analysis did not show any significant differences (all $p>0.11$).

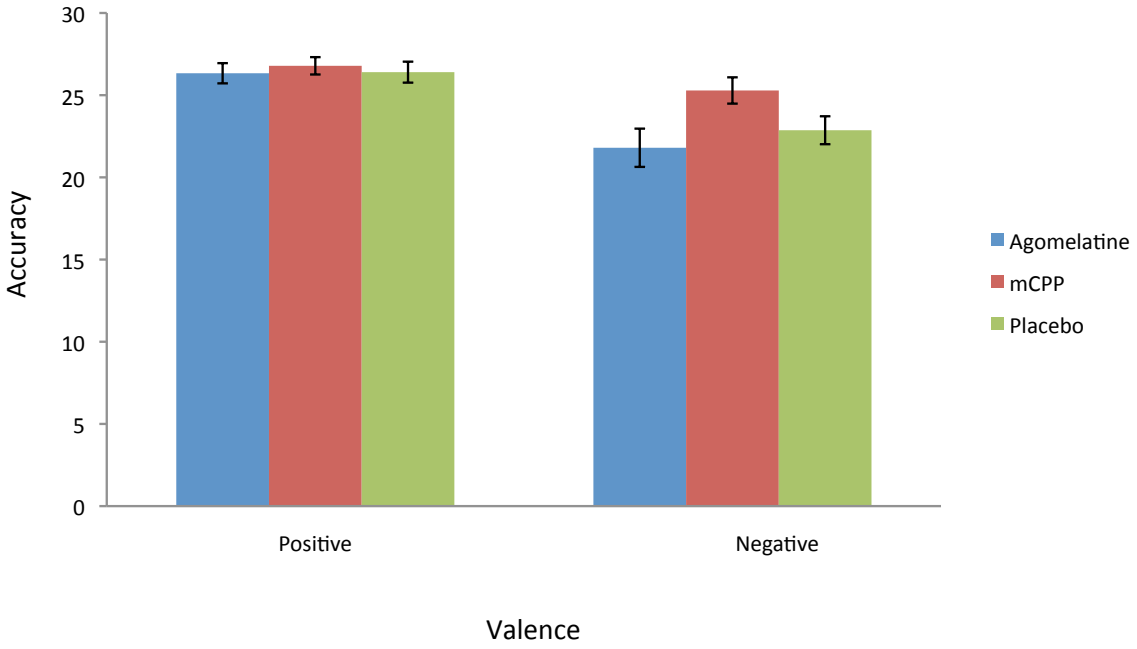


Figure 2.

The effect of agomelatine, mCPP, and placebo on accuracy of emotional word recognition (A total of 30 words were presented for each valence). Data are means ± SEM.

Dot probe

All groups had high accuracy (agomelatine mean: 94.21%, mCPP mean: 94.70%, placebo mean: 95.13%), which suggests a ceiling effect with the accuracy measure. There were also no significant effects on the vigilance scores of reaction time

(main effect of group: $F(2,39)=1.31$, $p=0.28$; group x valence x mask: $F(2,39)=0.71$, $p=0.50$). This was also true when only unmasked trials were considered (main effect of group: $F(2,39)=1.21$, $p=0.31$: valence x group: $F(2,39)=0.62$, $p=0.54$) (Table 3).

		Agomelatine	mCPP	Placebo
Masked condition				
Reaction time vigilance (ms)	Positive Negative	-9.16 (43.32) 7.52 (48.66)	4.59 (28.98) 11.84 (43.99)	-7.03 (34.73) -8.05 (63.02)
Unmasked condition				
Reaction time vigilance (ms)	Positive Negative	7.75 (37.92) 5.31 (44.68)	-3.92 (44.47) -6.90 (50.00)	-19.24 (31.42) 4.05 (48.35)

Table 3.
Attentional bias as measured by the dot-probe task under masked and unmasked conditions. Values represent group means ± standard deviations.

Emotion-potentiated startle

There was a significant modulation of the relative startle amplitude (z-values) by the valence ($F(2,70)=17.72$, $p<0.01$), with the expected increase in startle amplitude in all groups in the negatively valenced condition relative to the neutral condition (Agomelatine: $t(13)=-3.10$, $p=0.01$; mCPP: $t(10)=-4.01$, $p=0.002$; Placebo: $t(12)=-3.60$, $p=0.004$) (Figure 4). There was however, no significant main effect of treatment group ($F(2,35)=0.18$, $p=0.84$), or interaction between picture valence and treatment group ($F(4,70)=0.53$, $p=0.72$). These results remain

when also considering the raw amplitude of startle responses (main effect of valence: $F(2,70)=9.29$, $p=0.00$; valence x group: $F(4,70)=0.62$, $p=0.61$; main effect of group: $F(2,35)= 0.18$, $p=0.84$).

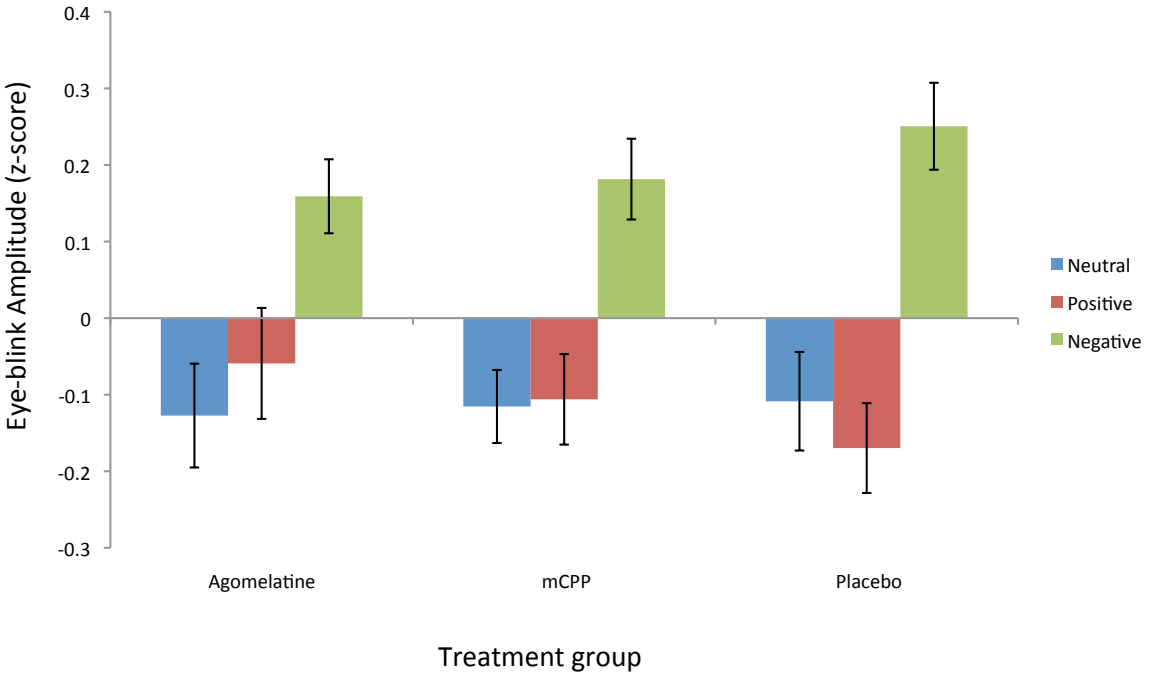


Figure 3.

Effect of agomelatine, mCPP, and placebo on emotion-potentiated startle responses. Values are relative startle response (z-transformed) to the white noise stimulus in the context of neutral, positive, and negative picture stimuli. Values are mean responses with standard errors in the three groups of volunteers.

7.3.4 Salivary Cortisol

There were no significant differences in cortisol levels between the groups throughout the experimental session (main effect of group: $F(2,37)=0.57$, $p=0.57$; group x time: $F(8,148)=0.71$, $p=0.57$) (Figure 4).

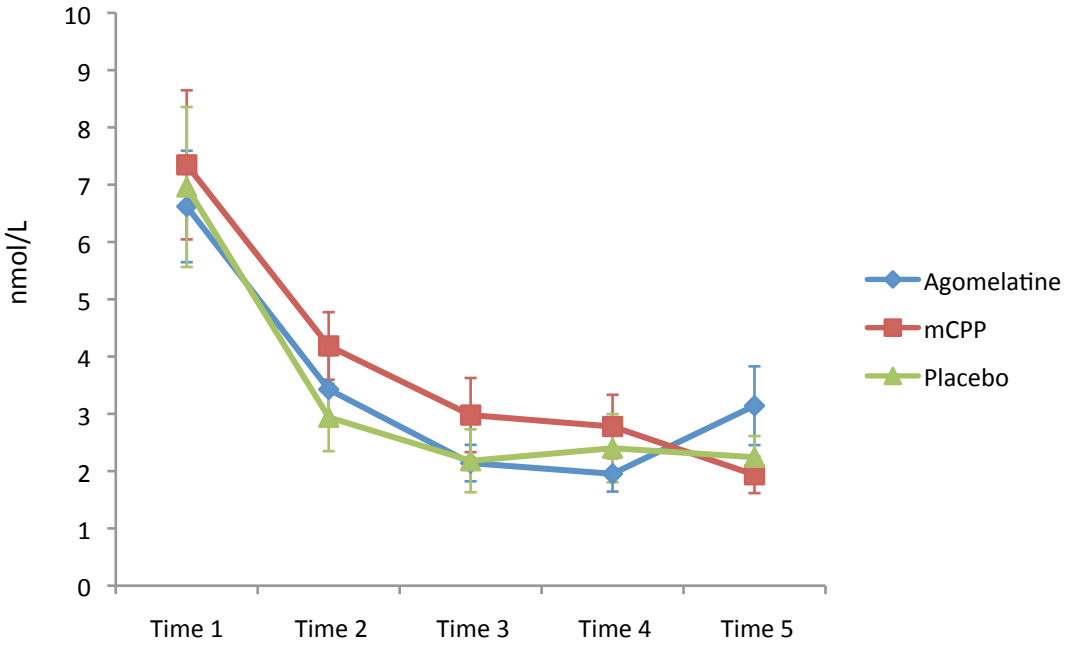


Figure 4.
Effect of agomelatine, mCPP, and placebo on cortisol values over the course of the experimental session. Data are means ± SEM.

7.4 Discussion

The effects of a single dose of agomelatine, the serotonergic agent mCPP, and placebo were compared in the present study. There were few significant results, but the presence of several trends suggests a tendency for agomelatine to increase memory for positive stimuli, and for mCPP to increase memory for negative stimuli. Additionally, there were changes in the subjective measures of mood and anxiety between the three groups over the course of the testing day. There was a decrease in ratings of positive mood as measured by the positive scale of the PANAS and an increase in mood ratings, indicating more negative feelings, in the BFS following 15 mg of mCPP administration relative to 25 mg of agomelatine. The significant correlations between the main outcomes of interest and mood scores

suggest that generally, as mood increases in participants, so does performance. For example, in the recall task, the agomelatine group had an increased accuracy for positive words, which was associated with positive mood.

Here, there were few trends in the emotional memory tasks, whose modulation seems to be particularly relevant to antidepressant drug action (Harmer et al. 2010b). There was a trend for a main effect of treatment group in the recall task, with the mCPP group recalling more correct words, although this is possibly due to the fact that the mCPP group also wrote more words overall, as when the ratio between the number of correct words to the number of words written is analysed, there is no significant difference between the groups. There was also a trend toward differences in the recognition task, which is consistent with previous findings of agomelatine's action on emotion processing. Volunteers receiving agomelatine vs. mCPP recognised fewer negative words. Participants who received seven days of 25 mg of agomelatine in a previous study showed increased recall of positive versus negative items compared to participants in the placebo group (Harmer et al. 2010). This improved positive affective memory has also been demonstrated after both a single dose of mirtazapine and after a repeated dose (Arnone et al. 2009, Chapters 3 and 5). Again, this change in bias appears to be a consistent finding of antidepressant action, regardless of the antidepressant immediate mechanism of action. The fact that the significant changes here are only evident when comparing mCPP and agomelatine suggests that the two drugs are acting in opposite directions. Perhaps mCPP tends to increase 5-HT_{2C} activation

while agomelatine tends to decrease it. Therefore, the difference between the two drugs is greater than the difference between drug and placebo.

In the facial expression recognition test, there was no treatment group by facial expression interaction, but there was a main effect of treatment group. The agomelatine group were generally better at recognising facial expressions overall. There is not an immediate explanation for this group difference. It could be suggested that the agomelatine group maintained alertness over the study day, while the other two groups became more tired and less alert, however, subjective reports of alertness do not support this. The only difference between the groups in ratings of mood was the rating of positive mood as measured by the PANAS, which decreased over the study day in the mCPP group. This overall increase in recognition does not fit with the studies of mirtazapine, which specifically decreased the recognition of fearful facial expressions.

Also in contrast to the current results, a previous study on the effects of agomelatine on emotion processing found an interaction effect in facial expression recognition (Harmer et al. 2010a). A seven-day administration of a 25 mg dose of agomelatine decreased recognition of facial expressions of sadness compared to placebo. This decrease in bias for sad facial expressions has not been seen with previous antidepressant drugs, including mirtazapine and citalopram, which tend to decrease other negative facial expressions such as fear and anger (Arnone et al. 2009; Harmer et al. 2004). It is of interest, however, that agomelatine decreases sad facial processing because individuals with depression are more likely to label

neutral faces as sad (Gur et al. 1992; Surguladze et al. 2004). It was suggested that this specific decrease in sad facial expression recognition rather than a general negative expression decrease may relate to a different effect on emotion perception sometimes seen in SSRI treatment, such as a general blunting of affect. Further studies are required to first replicate the result of seven days of treatment with agomelatine. Agomelatine has a very short half-life so it would seem unlikely that the accumulation of the drug would occur over seven days. This suggests that if there is a real difference between the effects of acute and repeated agomelatine on facial expression recognition it is likely to reflect neuroadaptive changes in relevant brain mechanisms. These changes could occur at the level of the 5-HT_{2C} receptor or, in view of the negative results found in the sleep study (Chapter 6), might be due to the melatonergic properties of agomelatine.

It is interesting that a single dose of agomelatine did not show the expected modulation of the emotion-potentiated startle effect. There were no group effects or interactions in the analysis of the startle task in the present study. The mCPP group also did not show the expected modulation of the emotion-potentiated startle effect. Both a seven-day administration of 25 mg and 50 mg of agomelatine previously decreased relative blink amplitudes in the negative picture condition and increased relative blink responses during the positive picture condition (Harmer et al. 2010a). These findings were assumed to be due to the 5-HT_{2C} antagonistic properties of agomelatine, but again the current results suggest this may not be the case.

Indeed, these findings demonstrated that neither a single dose of a 5-HT_{2C} agonist nor a 5-HT_{2C} antagonist altered emotion-potentiated startle. Mirtazapine has had inconsistent effects on the modulation of the emotion-potentiated startle task. One study found a decrease in overall startle responses after a single dose of mirtazapine while the study presented in Chapter 5 found no change following seven days of mirtazapine administration, which itself does not help clarify the role of 5-HT_{2C} receptors in emotion. The current findings, however, together with the negative findings in the sleep study, suggest that another pharmacological action may contribute to the reduced responses in the negative picture condition in the emotion-potentiated startle task following repeated agomelatine. It is possible that melatonin receptor-induced chronobiotic activity following repeated agomelatine may have been key to startle modulation rather than the 5-HT_{2C} receptor blockade.

As discussed in Chapter 1, in addition to its 5-HT_{2C} antagonistic properties, agomelatine has a more potent action at the MT_1 and MT_2 melatonin receptors. Agomelatine has been shown to reset the electrical activity of the suprachiasmatic nucleus (SCN) (Ying et al. 1996) and resynchronize disrupted circadian rhythms (Armstrong et al. 1993; Redman et al. 1995), which is related to the effect of melatonin receptors in the SCN (Ying et al. 1998). The notion that chronobiotic shifts might influence emotional processing is an interesting one and deserves further study.

7.4.1 Limitations

There are several methodological issues that may have affected the current results, and as a consequence, may have influenced the fact that there were only a number of trends for significance in the study. The dose of mCPP may have been too low to have a meaningful impact on emotion processing, as measured by the ETB. The differences found in this study were mainly between the mCPP and agomelatine groups, which may indicate a potential dose problem. Prior studies in our laboratory have used both a 7.5 mg and a 15 mg dose of mCPP, which was sufficient to decrease measures of SWS and were well-tolerated, without a disruptive level of side-effects (Katsuda et al. 1993). As both doses altered SWS, this suggests they are sufficient to activate brain 5-HT_{2C} receptors. Higher doses of mCPP (0.4 mg/kg) have been used to demonstrate a difference in food intake in healthy female volunteers, but ratings of nausea and light-headedness were significantly increased. In order to try and balance the action of mCPP with its tolerability, a 15 mg dose was chosen for this study. This dose, however, may not have been sufficient to cause significant changes in emotion processing. Previous studies have also only shown a marginal effect of 7.5 mg and 15 mg doses of mCPP on plasma prolactin and cortisol, suggesting that the doses may lie at the lower end of the dose response curve (Smith et al. 1994). The cortisol data from the current study also indicate a potential dose problem, as there were no significant differences in cortisol levels between the groups throughout the experimental session. It was expected that the mCPP group would have increased salivary cortisol levels (Kahn and Wetzler, 1991; Smith et al. 1994), but this lack of change from the placebo group suggests the dose may have been too low.

Another issue may be the relatively small number of participants in each of the three treatment groups. Previous studies of agomelatine and emotion processing have found significant differences with sixteen participants in each of the three treatment groups, and this number is what the sample size in the present study was based on. This number probably represents the lower bound of acceptable power, however, and having fifteen participants in each group may have contributed to the lack of statistically significant results in this study.

7.5 Conclusions

In conclusion, this study examined the effects of a 5-HT_{2C} agonist and 5-HT_{2C} antagonist on the ETB, a measure of emotional processing. While most of the results were trends, exploratory analysis reflects that agomelatine and mCPP may have an effect on emotional memory, particularly emotional word recall but not recognition. Unlike mirtazapine, agomelatine here increased overall recognition of emotional faces, which as mentioned above, is not easily explained. Taken together with previous research also discussed above, these findings suggest further study is warranted, perhaps with higher doses of mCPP. It is important further explore the contribution of these drugs to emotional processing, as previous studies have found differing effects of agomelatine on emotional processing depending on the dose. A lower 25mg dose facilitated positive versus negative affective memory recall and reduced the perception of sadness, but a higher 50mg dose had no effect (Harmer et al. 2010a). One possible explanation for this difference is an inverted U dose-response curve, which is often found in pharmacological studies (Harmer et al. 2010a). This dose-response curve may

involve non-specific side effects negating the effects seen at lower doses or an interaction of processes with an optimal balance of neurotransmitters required. This concept could potentially involve 5-HT_{2C} antagonism increasing prefrontal catecholamine function, but other effects such as non-specific side-effects cannot be ruled out (Harmer et al. 2010a). Thus, the contributions of the 5-HT_{2C} receptor and melatonin to the effect of agomelatine on emotional processing are as of yet unclear (Harmer et al. 2010a). The significance and implications of the present single dose of agomelatine and mCPP study will be discussed alongside the previous studies in the final chapter.

Chapter 8

General discussion

8.1 Summary of Findings

The series of studies detailed in this thesis have examined the impact that drugs with actions at 5-HT_{2C} receptors may have on emotional processing in healthy volunteers. To develop a more complete understanding of both the role of 5-HT_{2C} receptors in emotional processing and of antidepressants with putative 5-HT_{2C} receptor antagonist properties, studies utilizing a variety of methods, from neuroimaging to behavioural testing, were conducted. Through these and other studies described here, there is converging evidence that antidepressants may act early after administration to bias emotional processing in a positive manner, which may be important in treatment of depression.

The first chapter introduced the 5-HT system, and its association with depression, and offered a brief background on depression before turning to focus on the 5-HT_{2C} receptor. The evidence from work in humans and animals implicating 5-HT_{2C} receptor antagonism in antidepressant and anxiolytic action was detailed. Next, the general methods for each experimental chapter were introduced and explained. Following this background, the first two experimental chapters (3 and 4) were presented. These chapters addressed the neural processing of emotional information following a single dose of an antidepressant, mirtazapine, which has amongst its actions, 5-HT_{2C} receptor antagonism. This research was designed to extend an earlier study examining the effect of a single dose of mirtazapine on

behavioural emotional processing (Arnone et al. 2009). In that study, mirtazapine was found to reduce fear processing and increase memory for positive self-referent words. Similarly, the results of Chapter 3 demonstrated a single dose of mirtazapine reduces activation to fearful faces in several brain regions, including the amygdala and fusiform gyrus, as hypothesized. In Chapter 4, mirtazapine was found to increase the number of positive words recalled in a self- referent memory task as hypothesized, but had no effect on neural processing during the encoding phase of the task.

Following this corroboration of effects of mirtazapine on emotional processing after a single dose, Chapter 5 considered the effect of a seven-day administration of mirtazapine on emotional processing. Contrary to the hypothesized findings, mirtazapine did not decrease recognition of fearful facial expressions, although this effect was found to be significant in exploratory analyses, and did not alter the emotion-potentiated startle response. Mirtazapine did, however, increase the recall of positive versus negative personality characteristics. These results suggest that repeated administration of mirtazapine has some similarities to a single dose, and to the repeated administration of SSRIs such as citalopram. Next, in Chapter 6, another antidepressant with putative 5-HT_{2C} actions was considered. This chapter examined the ability of the new antidepressant agomelatine to produce a functional 5-HT_{2C} blockade in the brain, as measured by increases in SWS. Using this SWS measure, the study was unable to detect an effect of 5-HT_{2C} antagonism, which was contrary to the hypothesized findings. Chapter 7 again investigated the action of agomelatine on a different measure, that of emotional processing. In this

final experimental chapter, agomelatine was compared to mCPP, a 5-HT_{2C} agonist, although contrary to the hypothesized findings, there were no significant differences between the groups. Exploratory results did suggest that these drugs with diametrically opposing actions at the 5-HT_{2C} receptor may have opposing actions on emotional processing. Although the results may suggest a role for 5-HT_{2C} receptors in emotional processing and may emphasize the potential importance of antidepressants with putative 5-HT_{2C} receptor antagonist properties, other explanations are also possible. Given that mirtazapine and reboxetine have similar effects on emotional processing tasks such as emotional memory recall task, there is also potential for enhanced noradrenaline neurotransmission to be an important contributor to the antidepressant effect on emotional processing. If the single dose of agomelatine and mCPP study is not considered a failed study due to dosage or sample size, then the lack of results could suggest that 5-HT_{2C} receptors may not be an important contributor to the antidepressant effect on emotional processing. When put in the larger context of antidepressant action and emotion processing research, this set of studies is an important extension of previous research and raises key questions for the future. In the following sections, the implications of these findings will be discussed and the limitations evaluated.

8.2 Emotional Memory and the Effect of Antidepressants

The most consistent finding across the studies presented here is the effect of antidepressants with 5-HT_{2C} receptor antagonism to increase positive recall of emotional words. This finding is echoed in other research addressing the effect of

antidepressants on emotional processing. Antidepressants appear to reduce the negative bias in emotional word memory, as measured by word recall or recognition in most of the antidepressants tested with the ETB. For example, mirtazapine, agomelatine, reboxetine, citalopram, and duloxetine have all been shown to improve positive vs. negative emotional memory (Arnone et al. 2009; Harmer et al. 2010a; Harmer et al. 2003b; Harmer et al. 2004; Harmer et al. 2008).

This finding is congruent with the emotional impairment of memory reported in depression, where depressed individuals are more likely to remember negative vs. positive information (Bradley et al. 1995). Negative memory bias is suggested to be a core component of depression, as this process will support negative thinking about themselves, the world and their future (Williams et al. 1997; Bower 1981). The robust effect found after antidepressant administration suggests that the increase in positive memory might be a means for recognizing effective antidepressant action regardless of the mechanism of action of a drug. This measure could potentially be used to help identify new targets of antidepressant action or help screen agents earlier in the development process (Harmer et al. 2010b). Overall, the findings suggest that while 5-HT_{2C} receptor antagonism (or downregulation, in the case of SSRIs) may contribute to the effects of some antidepressants on emotional processing, drugs with no apparent effects on 5-HT_{2C} receptors (for example, reboxetine) can produce a similar effect. Future research, therefore, is needed to examine to what extent direct action at 5-HT_{2C} receptors or downstream effects on PFC catecholamine function, known to be important in other types of memory, are involved in this effect.

Further research is also required to separate the specific aspects of emotional memory that are altered by antidepressants. In these types of emotional processing studies, memory tends to be considered together rather than differentiating between recognition and recall. It is not always consistent, however, which form of memory is affected. In this thesis, recall memory is the only form of memory that was found to be significantly different between the drug and placebo groups, but recognition memory has less frequently been found to differ between the drug and placebo groups in previous studies, such as an increased speed to recognize positive compared to negative words following a single dose of reboxetine (Miskowiak et al. 2007).

It is also important to note that each is a different method of accessing memory. Recognition involves comparing a memory with a piece of information, whereas recall involves remembering information without any cue. Along with this methodological difference, the pattern of neural activation during different aspects of emotional memory has also been shown to differ. Both recognition and recall activate similar areas such as the right prefrontal cortex and the anterior cingulate when compared to a task such as reading, but there are activation differences when contrasted with each other (Cabeza et al. 1997). Recognition compared to recall is associated with increased activation in the right inferior parietal cortex, and recall compared to recognition is associated with increased activation in the anterior cingulate, globus pallidus, thalamus, and cerebellum (Cabeza et al. 1997). Based on the differences in these regions, it has been suggested that recognition

has a larger perceptual component than recall (Cabeza et al. 1997). Taken together, these studies suggest it will be important to further clarify the type of emotional memory and the particular neural circuitry involved in antidepressant action.

8.3 Agomelatine and Mechanism of Action

The results of the studies addressing the effects of agomelatine, first on SWS, and then on emotion processing have raised interesting questions that remain to be answered. Agomelatine is a recently licensed antidepressant, released for treatment of depression in the UK in 2009. Clinical studies have shown it is an efficacious antidepressant, as effective as paroxetine and more effective than placebo (Loo et al. 2002; Olie and Kasper 2007; Kennedy and Emsley 2006). As discussed, agomelatine has a novel pharmacological antidepressant profile as an agonist at MT_1 and MT_2 receptors and an antagonist at 5-HT_{2C} receptors.

The clinical efficacy of agomelatine is attributed to several mechanisms, such as the restoration of circadian rhythms and increased release of noradrenaline and dopamine in the PFC through 5-HT_{2C} receptor blockade (Millan et al. 2003). For these reasons, it was unexpected to find no effect of agomelatine on SWS. Previous studies have shown SWS to be a sensitive measure of 5-HT_{2C} receptor antagonism properties (Sharpley et al. 1994; Sharpley and Cowen 1995).

There are a number of reasons for the lack of effect of agomelatine on SWS which were discussed in Chapter 6. One likely possibility is that the affinity of

agomelatine for 5-HT_{2C} receptors is substantially less than that for the melatonin receptors, and that in humans at therapeutic doses agomelatine does not block the 5-HT_{2C} receptors involved in SWS regulation effectively. Another possible contributor to this result may be the fact that agomelatine is actually a neutral antagonist at the human 5-HT_{2C} receptor (Millan et al. 2010) and there is lack of endogenous 5-HT 'tone' onto the 5-HT_{2C} receptors that control SWS. However, drugs that typically increase SWS, such as ritanserin and mirtazapine, are inverse agonists which could increase SWS by constituent activity at the 5-HT_{2C} receptor without any need to block endogenous 5-HT.

The results of the single dose agomelatine study also raise questions regarding the role of 5-HT_{2C} receptor action in agomelatine's antidepressant effects, at least as measured by the ETB. While there were few significant effects, several trends suggest a tendency for agomelatine to increase memory for positive stimuli. A previous study, however, did demonstrate significant effects of agomelatine on emotional processing tasks after seven days of treatment, such as a decrease in sad facial expression recognition, an increase in positive emotional memory, and a decrease in the emotion-potentiated startle response (Harmer et al. 2010a).

It is interesting to note that agomelatine has a short half-life, and therefore, plasma levels would not be expected to increase over the seven days of the study. To account for the discrepancy in findings between the two studies, it can be hypothesized that repeated dosing of agomelatine produces neuroadaptive changes in brain mechanisms, perhaps at the 5-HT_{2C} receptor level. This

hypothesis would depend on the assumption that agomelatine does indeed occupy 5-HT_{2C} receptors in relevant brain regions. Alternatively, fitting with the sleep study results in Chapter 6, the changes in emotional processing after seven days of agomelatine treatment may be due to the agonism of agomelatine at the MT_1 and MT_2 receptors. It is possible that melatonin receptor-induced chronobiotic changes in circadian activity following repeated agomelatine given at 9 pm may have been more important in the effect on emotional processing than 5-HT_{2C} receptor blockade. Options to further explore the specific effects of agomelatine on 5-HT_{2C} receptors include a neuroendocrine approach. For example, a mCPP challenge study could be used to see if agomelatine is able to block the anxiogenic effects of mCPP. If agomelatine did block these effects, it would suggest agomelatine had functional action at the 5-HT_{2C} receptor. Additionally, pharmacological MRI could be used in combination with pharmacological challenge to examine agomelatine's effect on brain function. This approach has been utilized previously to show an increase in BOLD signal following mCPP infusion in areas corresponding to 5-HT_{2C}- enriched regions (Anderson et al. 2002).

8.4 Neuroimaging and the Effects of Antidepressants

The effects of antidepressants on neural circuitry involved in depression is far from understood. The effects of antidepressants on neural responses to emotional information may therefore provide an important way of assessing these processes. In Chapter 3 of this thesis, a single dose of mirtazapine decreased BOLD response to fearful faces and increased response to happy faces in a network of areas involved in emotional processing including the amygdala-hippocampal complex

and, fusiform face area. This finding fits with previous studies of facial processing with healthy volunteers, where attenuation of activity in the right amygdala-hippocampal region and specifically the amygdala during the presentation of aversive facial expressions was found after both treatment with acute citalopram and seven days of citalopram (Harmer et al. 2006a; Del-Ben et al. 2005; Murphy et al. 2009; though see Bigos et al. 2008). Additionally, the amygdala has been shown to be hyperactive to negative emotional stimuli in individuals with depression (Sheline et al. 2001; Fu et al. 2004; Siegle et al. 2007), perhaps representing an increased salience to negative stimuli in depression. Imaging studies of antidepressant treatment in depression have provided evidence that a key action of antidepressant drugs involves decreasing the hyperactivity of the amygdala (Fu et al. 2004; Sheline et al. 2001; Davidson et al. 2003).

The current finding suggests that mirtazapine modulates amygdala-hippocampal activity to affective stimuli in healthy volunteers hours after a single dose. On the other hand, the effect of acute citalopram on amygdala function is in need of clarification. Based on behavioural studies, there is an increase in fear processing after a single dose of citalopram (Browning et al. 2007; Harmer et al. 2003a); however, imaging studies have shown mixed results with increased amygdala responses to fear with a relatively high dose citalopram (20 mg I.V., Bigos et al. 2008) but decreased amygdale responses to fear with lower doses (20 mg oral, Murphy et al. 2009; 7 mg I.V., Del-Ben et al. 2005; 7.5 mg I.V., Anderson et al. 2010). These findings therefore do not provide straightforward support for the notion that increased activity in the amygdala may underlie the acute anxiogenic

effects of SSRIs, and this discrepancy requires more study. It has been suggested that these inconsistent findings may be related to factors such as genotype and gender of participants (Attenburrow et al. 2003; Neumeister et al. 2006; Murphy et al. 2009), and perhaps participants in some of these studies were not susceptible to the anxiogenic effects of acute citalopram administration for those reasons.

The fusiform face area is another brain region affected by antidepressant treatment, a result that has been found across several studies. In this thesis, a single dose of mirtazapine decreased BOLD response to fearful faces and increased response to happy faces in the fusiform face area, and similar effects have been seen with repeated reboxetine administration in healthy volunteers (Norbury et al. 2007). Activity in the fusiform gyrus, which has direct feedback connections with the amygdala, is known to be increased when the face presented is fearful, in line with increased attention and visual processing of these salient stimuli (Vuillemier et al. 2005). Additionally, right fusiform activation to happy faces is decreased in depressed individuals compared to controls (Surguladze et al. 2005). These findings suggest antidepressants may modulate activity in the fusiform, which may reflect increased attentional processing of positive and affiliative cues.

8.5 Limitations

As discussed, above, the study of emotional processing in individuals with depression is critical to the cognitive neuropsychological model of antidepressant drug action. All the work in this thesis was conducted on healthy individuals, screened to be free of current or past axis-1 disorder (including anxiety disorders,

depression, eating disorder, psychosis and substance abuse) by assessment using the Structured Clinical Interview for DSM-IV (First et al. 1997). These studies, therefore, form an important base to understand the effects of antidepressants on emotional processing. The use of healthy volunteers is needed to help clarify the results of emotional processing studies. For example, studying the effects of antidepressants on healthy individuals allows for the investigation of the direct action of antidepressants themselves without being confounded by changes in mood or symptom remission, which may occur in a depressed sample. In order to directly address the ability of these antidepressants with 5-HT_{2C} receptor actions to ameliorate the negative bias seen in depression, however, studies with a depressed sample must be conducted. Due to constraints on time and resources, it was beyond the scope of these projects to begin this process.

Confirmation of the hypothesis would be require a demonstration that the initial change in emotional processing strongly predicted subsequent clinical response to treatment. There is preliminary evidence for this (Tranter et al. 2009); however, further studies are needed. One study has recently examined the possibility that the negative bias in depressed patients is resolved by antidepressant treatment after a single dose (Harmer et al. 2009b). Depressed individuals in the placebo group demonstrated a decrease in positive facial recognition, a decrease in speed to categorise positive self-referent words, and a reduction in positive emotional memory compared to healthy volunteers in the placebo group. This negative effect was reversed in individuals with depression in the antidepressant-treated group. Future studies are therefore required to assess, for example, whether mirtazapine

is able to reverse the hyperactivity of the amygdala very early in treatment in depressed patients and whether this effect correlates with subsequent clinical response.

It is important to address the issue of drug and compound specificity when considering this research. One of the general limitations in 5-HT_{2C} receptor research has been the difficulty in finding specific 5-HT_{2C} agonists and antagonists for use in humans. mCPP is considered the prototypical 5-HT_{2C} agonist (Dekeyne et al. 2008), and numerous research studies have described and utilized it as such. As previously mentioned, however, this compound acts at several different 5-HT sites including the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_3 receptors. This non-specificity makes results more difficult to interpret. For example, anxiogenic effects of 5-HT_{2C} receptor agonism could theoretically be modified by 5-HT_{2B} and 5-HT_3 activity (Kennett et al. 1996; Duxon et al. 1997; Olivier et al. 2000; Harmer et al. 2006b). However, it is well established that at sufficient doses, mCPP can cause anxiety in healthy volunteers.

Additionally, some antidepressants may act on multiple receptors and subtypes. Mirtazapine, in particular, has actions on a number of different receptor types. Thus α_2-adrenoceptors, H_1 receptors, 5-HT_{2A} receptors, 5-HT_{2C} receptors, and 5-HT_3 receptors are all blocked by mirtazapine; in fact mirtazapine has the highest affinity for the H_1 receptor and H_1 receptor antagonists may have anxiolytic properties. For this reason, I attempted to control for the effects of H_1 receptor with the inclusion of hydroxyzine in the week-long study; however clearly actions

of mirtazapine at other receptors were not controlled for. Agomelatine is a 5-HT_{2C} antagonist, but its action at MT_1 and MT_2 may also play a role in the drug's antidepressant action. The development of drugs with specific 5-HT_{2C} properties for human use is necessary to clarify the role of 5-HT_{2C} receptors in emotional processing and antidepressant drug action.

The overall design of these studies could also be improved by quantitatively determining sample size rather than qualitatively assessing previous studies' sample size. Power calculations were not conducted to determine appropriate sample sizes or effect sizes. The imaging study resulted in findings from the gender discrimination task, suggesting that study was adequately powered, and the effect size of the extracted percent signal change to fear faces in the amygdala was r=0.74, similar to previous imaging studies conducted in the lab. It is possible, however, that some of the behavioural results were null due to inadequate powering. The sample size of these studies may be on the lower end of the acceptable range and as the group sizes were lower than ideal, it may mean we were not able to detect real effects. The group sizes were based on previous studies, and were of a convenient size, considering studies were limited by time and available resources. Had the sample sizes in the behavioural studies been larger, it may have been possible to detect smaller differences.

Effect sizes between the significant results found here and those from previous studies are generally comparable. For example, the initial mirtazapine study (Arnone et al. 2009) and the study from Chapter 5 both had medium to large effect

sizes for the difference in recall of positive versus negative words (r=0.80 and r=0.60).

Another limitation to several of the studies presented here is in the design of the experimental session: the dose strength and time of administration. In Chapter 5, both the timing of drug administration and dose strength were potentially factors in the lack of results and small effects found in the study. Thus, the doses of mirtazapine and hydroxyzine were at the lower end of their respective clinical ranges, and plasma levels of mirtazapine 12 hours after the final dose may have been sub-optimal.

Dosing can be difficult to set-up correctly, especially due to the nature of pharmacological studies in human volunteers. All decisions regarding dosage and drug administration were made with reference to previous work with these drugs, particularly those performed in our laboratory. It is also important to balance the drug action with side-effects. For example, powerful subjective effects of medication are likely to influence emotional processing (Harmer et al. 2008). This was the challenge in Chapter 7 when mCPP was utilized. The side-effects of mCPP can be difficult for participants to tolerate, especially in higher doses. Overall, the results from these chapters highlight the need for careful consideration of the issues of administration and dosage of antidepressants and other compounds. The experience gained here will be of use for future studies in this area of research.

8.6 Future Research

The need for further work in this field is clear, as these results can only contribute to the larger story, which remains incomplete. Some of the specific areas of future research have been mentioned earlier, but will be summarized now. The effect of antidepressants on emotional memory is a consistent finding across studies, but the neural effects underpinning this effect is not clear in all antidepressants. A neuroimaging study investigating the effect of mirtazapine on emotional self-referent word recognition and recall may be useful. Additionally, the effect of antidepressant drugs on the encoding phase of emotional words is needs further study, as findings are unclear across antidepressants. There are several directions that further imaging studies could take in investigating the effect of antidepressants with 5-HT_{2C} actions. For example, an fMRI study utilizing connectivity analysis could examine functional circuits and how distributed networks are coupled together during certain functions. Connectivity could be investigated in task-related activations, similar to tasks presented in Chapters 3 and 4, or connectivity could be analysed in resting conditions to examine the default mode network (Rogers et al. 2007). Additionally, it is important to move towards an ability to predict an individual's response to antidepressant medication prospectively, and it may be possible that this prediction could be made from fMRI measures. A study similar to Tranter et al. (2009) could be conducted in depressed patients, where changes in amygdala activation to emotional stimuli (such as fear faces) are compared to clinical outcome after 6 weeks of antidepressant treatment.

It is also important to continue the investigation of agomelatine, and to study which actions of the drug contribute to its antidepressant effect. First, it would be useful to carry out another study on the effect of agomelatine on SWS with a higher dose, perhaps at 50 mg. Both the 25 mg dose and 50 mg doses are currently being prescribed for antidepressant treatment. It would be interesting to see if a higher dose, still within clinical range, would alter the measurement of SWS following agomelatine administration. Additionally, to help differentiate between the role of the MT receptors and 5-HT_{2C} receptors in agomelatine's effect, a study of emotional processing and melatonin itself should be undertaken. More generally, these emotional processing studies, both those involving behavioural and neuroimaging methods, need to be conducted in a group of individuals with depression, as the current studies have all included healthy volunteers.

Another important consideration is the effect factors such as gender or genotype may have in the above findings. It is possible that individual differences, such as genetic differences, contributed to the non-significant results. It has been shown that gender and genotype can contribute to the results of acute studies involving serotonin manipulation (Attenburrow et al. 2003; Neumeister et al. 2006). Additionally, genetic studies have found associations between gene variants and response to antidepressant drugs (Uher et al. 2009). It may be that the groups tested here were not susceptible to the effects of the drugs used. Future work should consider the possible contribution of individual differences to these types of studies, and may want to include measures to account for these differences.

8.7 Conclusions

In conclusion, the series of studies presented here have provided evidence that 5-HT_{2C} receptors may contribute to the modulation of emotional processing by some psychotropic drugs. From imaging studies, it has been demonstrated that a single dose of the antidepressant mirtazapine reduces activation in regions important in emotional processing, such as the amygdala, to threat-relevant stimuli. These effects may be important generally in the therapeutic actions of antidepressants in depression. The behavioural studies have utilized drugs acting, at least in part, as 5-HT_{2C} antagonists and agonists to show that these drugs are able to alter emotional processing, particularly emotional memory. These findings suggest that antidepressants may work by altering the bias in emotional processing. Overall, the results of this exploration of the role of the 5-HT_{2C} receptor in emotional processing have contributed to the understanding of antidepressant treatment, and raise new possibilities for the continuation of study in this field.

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