Testing the neurocognitive model of antidepressant treatment

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Submitted for the degree of DPhil in Psychiatry, Hilary Term 2016

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Abstract

The neurocognitive model of antidepressant treatment action states that antidepressants work by producing relatively immediate positive shifts in emotional processing, which translate into clinical improvement with time. Short-term or even acute doses of antidepressants can, for example, increase memory for positive self-referent words or decrease amygdala activation to fearful faces, and these early changes correlate with later clinical improvement. However, there are a number of ways in which the model needs further probing. The aim of this thesis was to test the neurocognitive model by: 1) investigating whether changes in emotional processing occur in an antidepressant with a novel mechanism of action, St John's wort, as the model predicts; and 2) examining whether there is a comparable pattern of neuropsychological changes to citalopram in a population of high neurotic volunteers, whose baseline emotional biases may make them a more ideal group in which to study drug effects. We found that seven days of St John's wort produced similar changes to other antidepressants, for example reducing recognition of disgusted faces and attention to fearful faces while increasing memory for positive words. The drug did not affect other aspects of cognition including working memory and reward learning. These findings support the theory that early psychological changes are a common feature of all antidepressants. On the other hand, four weeks of citalopram treatment produced apparently contradictory effects in high neurotics, increasing memory for positive words but also increasing recognition of *negative* facial expressions. Neuroimaging data showed that high neurotics had greater response to neutral faces in emotional processing areas compared to low neurotics, which was reduced with citalopram. High neurotics also showed increased resting state connectivity in default mode network areas and between amygdala and cortical areas, which was again reduced with citalopram. We suggest that in this group citalopram corrects general negative emotional processing biases, but also works to decrease a natural aversion to particularly threatening socially-relevant stimuli. Overall this thesis supports the idea that early changes in emotional processing are vital for antidepressant action, but also suggests that in certain groups such as high neurotics, some changes may be more nuanced than previously reported and warrant further scrutiny.

Acknowledgments

In 2011 I was fortunate enough to be able to travel halfway around the world to begin an MSc programme at Oxford. I would never have guessed that four years later, I would still be here, putting the finishing touches to a DPhil thesis. For giving me this opportunity, I will forever be grateful to my supervisor, Professor Catherine Harmer, who encouraged me to apply and has provided so much support and guidance throughout my time with PERL.

I am also hugely grateful to Martina Di Simplicio, who trusted me to take the reins of her project and provided help and advice even after having left to the Other Place. The project was a massive venture and so many people contributed to it. Thanks to Anna Tuenter and Caroline Figueroa for laying the groundwork and making it easy for me to step in, and to Maria Serra-Blasco for accompanying me on that initial plunge into the depths of imaging analysis. The project would have been impossible without the medical support provided by Beata Godlewska, for which I am incredibly appreciative. But most of all, I am lucky to have worked with Anna-Lena Frey, without whom I would still be frantically looking for participants and muddling through Matlab scripts.

It is a privilege to have worked alongside so many other fantastic people at PERL, all of whom have helped me along the way at some point or another. I couldn't have asked for better companions on the road to the DPhil than Maria Ironside and Charlotte Cooper. Thanks for sharing in all the highs and lows – I can't believe we are almost there! Even when busy, Kristin Schmidt and Nisha Singh were always there for advice or even just a beer. Thanks to Kristin and Annie Walsh for providing data for my resting state template, and to Mike Browning for the reward task. Thank you also to Phil Cowen and Abbie Pringle for all the help. And of course thanks to the best DGS ever, Phil Burnet, for kick-starting my caffeine habit.

I am grateful to the Clarendon Fund for funding my DPhil.

I would never have made it to Oxford without the support – emotional, intellectual and financial – of my parents, Liz and Paul. I am enormously thankful for everything you have done to get me here. Thank you to my partner Sana Suri for making Oxford feel like home for the last few years, and always knowing when I needed that little bit of encouragement to carry on. And also for taking time right in the middle of writing up to give me a crash course in resting-state analysis!

Finally, thank you to my grandparents, who, when they heard I was planning to come to Oxford, did everything in their power to help me out and who have made me feel so welcome in the UK. I would like to dedicate this thesis to the memory of my grandfather, Brian, who is sadly not around to see the day that it was completed. His love of knowledge and curiosity in the world around him is an inspiration.

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List of abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
AUN	Auditory network
AVIC	Acute Vascular Imaging Centre
BBR	Boundary-based registration
BDI	Beck Depression Inventory
BDNF	Brain-derived neurotrophic factor
BET	Brain Extraction Tool
BOLD	Blood oxygen level dependent
CORE	Clinical Outcomes in Routine Evaluation
CSF	Cerebrospinal fluid
DA	Dopamine
dl	Dorsolateral
dm	Dorsomedial
DMN	Default mode network
ECG	Electrocardiogram
ECN	Executive control network
EMG	Electromyography
EPI	Echo planar imaging
Epo	Erythropoietin
EPQ(-R)	Eysenck Personality Questionnaire (Revised)
ETB	Emotional Test Battery
EV	Explanatory Variable
FEAT	FMRI Expert Analysis Tool
FERT	Facial Expression Recognition Task
FILM	FMRIB's Improved Linear Model
FIX	FMRIB's ICA-based Xnoiseifier
FLAME	FMRIB's Local Analysis of Mixed Effects
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional magnetic resonance imaging
FMRIB	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FNIRT	FMRIB's Non-linear Image Registration Tool
FPN	Frontoparietal network
FSL	FMRIB Software Library
HAM-D	Hamilton Rating Scale for Depression
IAPS	International Affective Picture System
ICA	Independent components analysis
ITI	Intertrial interval

LSAS	Liebowitz Social Anxiety Scale
LVN	Lateral visual network
MAO	Monoamine oxidase
MDQ	Mood Disorders Questionnaire
MPRAGE	Magnetisation-prepared rapid acquisition with gradient echo
MRS	Magnetic resonance spectroscopy
MVN	Medial visual network
N	Neuroticism/neurotic
NaSSA	Noradrenergic and specific serotonergic antidepressant
NRI	Norepinephrine reuptake inhibitor
OCD	Obsessive compulsive disorder
OFC	Orbitofrontal cortex
PANAS	Positive and Negative Affect Schedule
PCC	Posterior cingulate cortex
PFC	Prefrontal cortex
ROI	Region of interest
SCID	Standard Clinical Interview for DSM-IV
SEM	Standard error of the mean
SHAPS	Snaith-Hamilton Pleasure Scale
SJW	St John's wort
SMC	Supplementary motor cortex
SMN	Sensorimotor network
SN	Salience network
SNRI	Serotonin and norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
STAI	State-Trait Anxiety Inventory
tDCS	Transcranial direct current stimulation
TE	Echo time
TR	Repetition time
TRPC	Transient receptor potential channel
VAS	Visual Analogue Scale
vl	Ventrolateral
vm	Ventromedial
WM	White matter

Chapter 1

Overview

The neurocognitive model of treatment action in depression states that the clinical effects of antidepressants can be directly attributed to positive changes in emotional processing early on in treatment (Warren, Pringle, & Harmer, 2015). The purpose of this thesis was to investigate the predictions made by the model, using two distinct lines of inquiry. Firstly, we investigated whether an antidepressant with a novel mechanism of action, St John's wort, produced similar changes in emotional processing to other antidepressants. Secondly, we aimed to characterise the pattern of changes to the antidepressant citalopram in highly neurotic volunteers, a group which may be a better model for investigating drug effects. This section summarises the chapters of the thesis.

In **Chapter 2** we provide a description of the neurocognitive model of treatment action in depression and review the evidence supporting the model. We then discuss some of the current limitations of the field and the rationale for the current studies. This leads to our two main research questions:

1) Do early neurocognitive changes generalise to other antidepressants?
2) Are the same neurocognitive effects present in clinically-relevant populations?

1.1 Do early neurocognitive changes generalise to other antidepressants?

In **Chapter 3,** we examined the effect of one week's administration of St John's wort (SJW) on emotional processing in healthy volunteers. SJW is an effective antidepressant with a novel mechanism of action, and thus represents an ideal drug with which to test the hypothesis that early changes in emotional biases should be common to a range of different antidepressants.We found that SJW did indeed produce a positive shift in emotional processing, with volunteers on the drug performing worse at recognising disgusted faces and attending to fearful faces, and

showing increased memory for positive words. Despite its novel mechanism of action, SJW therefore seemed to have similar effects to other antidepressant drugs.

In **Chapter 4,** we were interested in whether one week's administration of SJW had any other effects on cognition: animal studies have suggested that SJW could affect memory, while the putative effects of the drug on dopamine transmission may be expected to affect reward learning. We failed to find any effects of the drug on working memory, assessed by an n-back task, or on a reward learning task. This suggests that SJW does not modulate these aspects of cognition.

1.2 Are the same neurocognitive effects present in clinically-relevant populations?

High neurotic volunteers (high Ns) may be a particularly useful group of participants in which to study the neuropsychological effects of antidepressant treatments, as they display baseline biases in emotional processing without the clinical impairments associated with depression. However, emotional processing effects have not been fully characterised in this population, with some research suggesting that short-term doses of citalopram actually produce *negative* emotional biases (Di Simplicio, Norbury, Reinecke, & Harmer, 2014).

In **Chapter 5,** we were interested in whether these early paradoxical effects of citalopram on emotional processing persisted after longer-term treatment, and whether the pattern of results differed between high and low neurotics. We found some evidence of these negative effects after four weeks of treatment, in certain tasks: citalopram increased recognition of negative facial expressions in high Ns while decreasing recognition in low Ns. On the other hand, for both groups citalopram increased memory for positive words. We suggest that treatment effects in high Ns might reflect two distinct processes: the production of a general positive shift in emotional biases, but also the reversal of an innate avoidance to socially-relevant, threatening

information which manifests as apparent increased recognition of certain negative information such as faces.

In **Chapter 6,** we had a similar aim, investigating whether the previously-reported increases in neural response to threatening faces after citalopram treatment in high Ns persisted after four weeks. In fact, we failed to find any effects of neuroticism or citalopram on neural response to fearful faces; rather, we found effects specific to *neutral* faces. High Ns had a baseline increase in response to neutral faces in areas associated with emotional processing such as anterior cingulate, insula and dorsolateral prefrontal cortex, which was reduced with citalopram. These findings appear to reflect a hyperactive response to emotionally ambiguous information in high Ns, which is corrected by treatment.

In **Chapter 7** we were interested in whether resting state connectivity would be differently modulated by citalopram in high and low Ns. High Ns showed greater baseline connectivity between dorsomedial prefrontal cortex and a number of default mode network areas, similar to results reported in depressed populations and potentially reflecting heightened levels of rumination and negative thinking about the self. Citalopram reduced this connectivity, while increasing connectivity in the same areas for low Ns. Interestingly, a similar pattern of results was found for connectivity between amygdala and a number of cortical and subcortical areas, suggesting that the amygdala may have increased bottom-up output at rest amongst high Ns.

Finally, in **Chapter 8** we discuss our results in the context of our two original questions, concluding that our studies support the idea that early changes in emotional processing are a fundamental feature of diverse antidepressant treatments, and that individual differences such as neuroticism can have quite nuanced influences on treatment effects. We discuss some of the limitations of our studies as a whole, and consider how the studies can inform future directions for research.

Chapter 2

Introduction

An extended version of this chapter was previously published as a review article: Warren et al. (2015). A neurocognitive model for understanding treatment action in depression. *Phil. Trans R. Soc. B., published online July 2015.*

2.1 Affective neuroscience and pharmacological interventions

The field of affective neuroscience considers how the brain represents and processes emotion. This usually involves employing tasks designed to tap into the processing of emotional information, including aspects of cognition such as memory, attention and perception, as well as more elaborative processes. These tasks will often be paired with neuroimaging methods, such as functional magnetic resonance imaging (fMRI), in order to ascertain how regions and networks of the brain represent this information.

In the absence of any intervention, these studies can be valuable in understanding how the brain processes emotional information. However, interfering with neural functioning through the use of pharmacological interventions can provide additional insight. Researchers can examine the effects of drugs which are known to generate or reduce a given emotion and relate these to behavioural and neural changes. For example, the increased amygdala response to fearful faces after administration of anxiogenic drugs like amphetamines (Hariri et al., 2002) and decreased response after anxiolytic drugs like benzodiazepines (Del-Ben et al., 2012) reinforces the central role of this structure for attending to negative, particularly fear-related, information.

With an increased understanding of how pharmacological interventions affect the behavioural and neural processing of emotional information, we can also gain a unique perspective into how clinically useful drugs might exert their effects. Rather than being secondary to the clinical effects

of these drugs, the neuropsychological changes may in fact *produce* these clinical effects. To take the example above, perhaps the reduction of amygdala response to fearful faces after benzodiazepine administration is in fact instrumental in decreasing anxiety. This is the basis for the neurocognitive model for understanding treatment action in depression.

2.2 The neurocognitive model of treatment action in depression

Models of treatment action in depression have generally focussed on the molecular and cellular changes thought to underlie the clinical response. Because improvement in depressive symptoms is traditionally thought to take several weeks to emerge (Frazer & Benmansour, 2002), these models often concern slow, adaptive processes in the brain. One of the more common forms of antidepressant, the serotonin reuptake inhibitor (SSRI), works by blocking the serotonin reuptake transporter, increasing availability of serotonin in the synapse. A popular theory is that clinical effects are not seen immediately due to the existence of negative feedback from autoreceptors, and it is not until these receptors are desensitised after chronic treatment that improvements in mood emerge (Stahl, 1998). More recently, hippocampal neurogenesis has been suggested to be fundamental to the clinical effects of antidepressant drugs. In animal models, neurogenesis is stimulated by antidepressant treatment, and some of the behavioural effects of these treatments are blocked by ablating neurogenesis (Perera et al., 2011). The maturation of new cells takes several weeks, in line with the delay in treatment response (Becker & Wojtowicz, 2007).

One of the challenges for these models is to explain how molecular and cellular-level changes produce improvements in mood. The neurocognitive model provides an alternate approach to understanding treatment action, which places more of an emphasis on how clinical effects emerge. There is growing evidence that antidepressant interventions produce relatively immediate neural and behavioural changes in relation to emotional processing. Specifically, antidepressants

appear to bias emotional processing in favour of more positive stimuli and away from negative stimuli (Harmer, Bhagwagar, et al., 2003; Harmer, Hill, Taylor, Cowen, & Goodwin, 2003).

Patients suffering from depression display baseline negative biases in emotional processing, which may produce and maintain lowered mood (Harmer et al., 2009). The effects of antidepressants on emotional processing thus serve to remediate these biases. After commencing antidepressant treatment, a patient begins to see the world around them in a more positive way, for example attending less to negative information, or becoming better at remembering positive events. With more and more experience of their environment in this new, more positive way, the patient feels increasingly better. Thus cognitive responses to affective situations and experiences will be altered straightaway and will culminate in symptomatic improvement which becomes evident over time, consistent with recent studies into the time course of clinical effects (M. J. Taylor, Freemantle, Geddes, & Bhagwagar, 2006).

Below we describe the neurocognitive model in more detail. First we examine evidence for the presence of baseline negative cognitive biases in depression. Then we describe studies demonstrating that antidepressant drugs remediate these biases early on in treatment, and showing that these early neuropsychological changes can actually predict how a patient will respond to treatment. Finally, we discuss two key ways in which the model needs further characterisation: it is necessary to show that neuropsychological changes are common to a range of antidepressant drugs with different mechanisms of action, and it is also important to establish the presence and time-course of these effects in clinically-relevant populations. These two areas are the focus of this thesis.

2.3 Negative cognitive biases are present in depression

The presence of emotional biases amongst patients suffering from depression is well-established (Roiser, Elliott, & Sahakian, 2012). Behaviourally, depressed patients show increased processing of negative versus positive emotional information. These biases are apparent in a range of tasks measuring attention, perception and memory for emotional stimuli: for example, compared to healthy controls, depressed patients are slower at categorising positive self-referent personality words, and later worse at remembering these (Harmer et al., 2009) (see Figure 2.1a). In contrast, they are better at recalling negative words (Bradley, Mogg, & Williams, 1995). They are also worse at recognising happy facial expressions, and interpret ambiguous expressions as more sad than healthy controls (Bourke, Douglas, & Porter, 2010; Harmer et al., 2009).

These differences are mirrored at the neural level. In functional imaging studies, depressed patients show greater blood oxygenation level dependent (BOLD) response to negative stimuli in a network of areas thought to be involved in detecting and responding to salient emotional information, including the amygdala, insula and anterior cingulate cortex (ACC; Disner, Beevers, Haigh, & Beck, 2011; Hamilton et al., 2012a). In parallel with this hyperactivity in limbic areas, there is also reduced activity in dorslolateral prefrontal cortex (dlPFC) to both positive and negative stimuli, as well as lower resting blood flow (Gonul, Kula, Bilgin, Tutus, & Oguz, 2004; Hamilton et al., 2012a). Thus the model to emerge from neuroimaging literature involves a hyperactive limbic system which biases emotional processing towards negative stimuli at an early stage, while a hypoactive dlPFC is in turn less able to provide top-down regulation of the limbic system (Disner et al., 2011; Elliott, Zahn, Deakin, & Anderson, 2011; Suslow et al., 2010).

These neuropsychological biases are thought to play a fundamental role in producing a depressed mood. Processing of information about the self and the surrounding world in a more negative fashion produces depressive symptoms, through increased attention and memory for

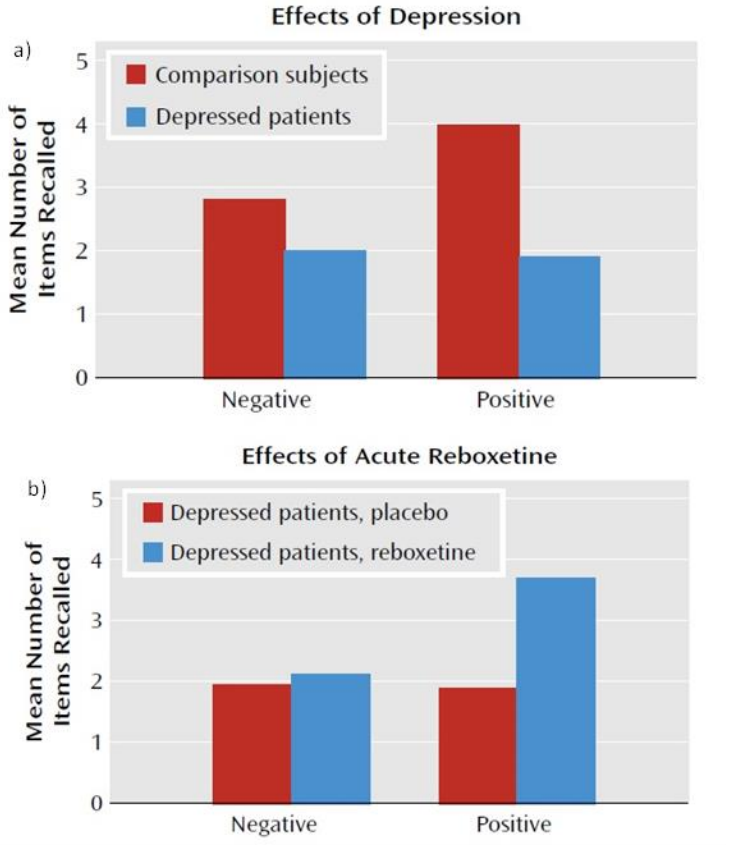

Figure 2.1 Effects of depression and reboxetine treatment on word recall
a) Number of words correctly recalled for depressed and healthy volunteers given a placebo. Depressed patients show significantly reduced recall of positive words, $p < .05$.
b) Effect of an acute dose of reboxetine on word recall. Patients given reboxetine remember more positive words than those taking placebo, $p < .01$. From Harmer et al. (2009)

negative information, as well as through more elaborative processes like rumination and negative interpretations (Disner et al., 2011; Teasdale, 1988). A number of studies provide evidence for an explicitly causative role of emotional processing biases on depressed mood: heightened processing of negative facial expressions has been shown to predict relapse amongst remitted depressed patients (Bouhuys, Geerts, & Gordijn, 1999), while generating or reducing bias with cognitive bias modification techniques can affect responses to emotional information and markers of depressive relapse (Browning, Holmes, Charles, Cowen, & Harmer, 2012; MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002). Once established, the symptoms of

depression then reinforce the original negative biases, serving to maintain the depressed mood (Disner et al., 2011).

2.4 Antidepressant drug treatment produces positive changes in cognitive biases

The normalisation of biases in emotional processing is central to the neurocognitive model of antidepressant treatment. By producing a more positive way of processing emotional information, antidepressant treatments effectively break this cycle and relieve symptoms of depression. The following sections examine evidence for such changes after antidepressant treatment.

2.4.1 Healthy volunteer studies

Healthy volunteer studies have been vital in establishing the neurocognitive effects of antidepressant treatments. Examining the effects of these drugs in healthy volunteers demonstrates that any neurocognitive changes cannot be attributed to early improvements in depressive symptoms, as these do not exist in the first place. Unless specified, the results described below (and throughout the rest of this chapter) compare drug effects to a placebo control group. Further information about doses given can be found in the Appendix, Table 9.1 and Table 9.2, these tended to be at the lower end of the normal clinical range.

Short-term administration

Short-term antidepressant treatment produces biases in the processing of emotional facial expressions (see Appendix Table 9.1 for a summary of behavioural results). For example, in response to seven days' administration of the norepinephrine reuptake inhibitor (NRI) reboxetine, recognition of both fearful and angry faces decreased (Harmer, Shelley, Cowen, & Goodwin, 2004). Seven days of the SSRI citalopram produced broader changes, reducing

recognition of fearful and angry faces but also disgusted and surprised faces (see also Harmer, Mackay, Reid, Cowen, & Goodwin, 2006).

These effects are reflected in changes in neural processing. Seven days' administration of both citalopram (Harmer et al., 2006) and reboxetine (Norbury, Mackay, Cowen, Goodwin, & Harmer, 2007) reduced BOLD activation in the amygdala in response to fearful faces. Increases in neural response to happy faces have also been seen, both in the amygdala (Norbury et al., 2009) and the right fusiform (Norbury et al., 2007) (see Appendix Table 9.2 for a summary of neural effects relating to face processing). These patterns of neural activity, restricted to areas involved in relatively low-level processing of emotional stimuli, suggest that early effects of antidepressants may be working in a "bottom-up" fashion, affecting the automatic evaluation of emotional stimuli (Harmer, 2012). Indeed, a recent meta-analysis found that for a range of different cognitive tasks, short-term antidepressant treatment increased activation to positive emotional information, and decreased activation to negative information, across a network including the amygdala, putamen, ACC, parahippocampal gyrus, and medial prefrontal cortex (Ma, 2014). These limbic and paralimbic structures are involved in detecting and responding to salient emotional information, supporting this "bottom-up" interpretation of early antidepressant effects.

Short-term administration of antidepressants also affects processing of self-referent words and subsequent memory for these. Seven days of reboxetine reduced the reaction time to classify positive self-referent words, and both reboxetine and citalopram increased later recall of positive vs negative words (Harmer et al., 2004). At the neural level, seven days of reboxetine increased activity to positive relative to negative words in the inferior frontal gyrus and precuneus during categorisation, while decreasing activity in medial frontal gyrus and precuneus during correct recognition of positive words (Norbury, Mackay, Cowen, Goodwin, & Harmer, 2008). Increased

activity to positive words during categorisation may reflect heightened attentional processing of these words. Conversely, reduced activity during the subsequent memory task may be attributed to decreased retrieval effort for positive words.

Finally, citalopram but not reboxetine reduced attentional vigilance to fearful faces (Murphy, Yiend, Lester, Cowen, & Harmer, 2009), and reduced the eyeblink startle response to a short, loud burst of sound when viewing negative pictures (Harmer et al., 2004). These effects may be related specifically to citalopram's anxiolytic effects, and both heightened startle response and negative attentional biases have been related more consistently to anxiety than depression (Craske et al., 2012; Mogg & Bradley, 2005).

Acute administration

The processing of emotional facial expressions is also altered by acute doses of antidepressants. After acute doses of citalopram (Harmer, Bhagwagar, et al., 2003; Murphy, Norbury, O'Sullivan, Cowen, & Harmer, 2009), reboxetine (Harmer, Hill, et al., 2003; Harmer et al., 2009) and duloxetine (Harmer, Heinzen, O'Sullivan, Ayres, & Cowen, 2008), healthy participants were better at recognising happy faces. The noradrenergic and specific serotonergic antidepressant (NaSSA) mirtazapine has also been shown to decrease recognition of fearful faces (Arnone, Horder, Cowen, & Harmer, 2009).

These early effects of antidepressants on facial expression processing are related to neural changes in fMRI studies (see Appendix Table 9.2). Again, a network of limbic and paralimbic structures shows altered activation after acute doses, with a key role for the amygdala. For example, participants who took a dose of mirtazapine showed decreased response in the amygdala to fearful faces presented for 100ms, and an increased amygdala response to happy faces (Rawlings, Norbury, Cowen, & Harmer, 2010). Several studies of acute SSRI administration

also found decreased amygdala response to fearful faces (Anderson et al., 2007; Anderson et al., 2011; Del-Ben et al., 2005; Murphy, Norbury, et al., 2009).

At acute doses, noradrenergic drugs appear to have particular effects on the categorisation of personality characteristic words, as well as later memory for these words. Reboxetine decreased the time taken to categorise self-referent personality words as positive (Harmer, Hill, et al., 2003; Harmer et al., 2009), and also increased recognition of positive words (Miskowiak, Papadatou-Pastou, et al., 2007) and decreased recall of negative words (Harmer, Hill, et al., 2003). These effects are not as consistently seen in acute SSRI treatment, although they are seen later, after short-term SSRI treatment (Harmer et al., 2004), so it may be that potentiation of noradrenaline has earlier effects on memory than serotonin. Indeed, while acute studies using citalopram have failed to find acute effects on word categorisation or memory (Browning, Reid, Cowen, Goodwin, & Harmer, 2007), duloxetine, a serotonin *and* norepinephrine reuptake inhibitor (SNRI), increased the number of positive, but not negative, words falsely recalled in a memory task (Harmer et al., 2008).

At the neural level, an acute dose of reboxetine decreased activation in a right fronto-parietal network including the medial frontal gyrus when recognising previously seen positive words (Miskowiak, Papadatou-Pastou, et al., 2007). This again appears consistent with reduced retrieval effort for positive, but not negative, words.

2.4.2 **Depressed patient studies**

One of the limitations of studying depressed populations is that they display cognitive deficits such as impairment in working memory, executive dysfunction and psychomotor problems (Castaneda, Tuulio-Henriksson, Marttunen, Suvisaari, & Lonnqvist, 2008; Golinkoff & Sweeney, 1989). This makes it hard to separate the effects of antidepressants on emotional processing *per se*

from these possible confounding factors. In addition, early effects of the drugs on patients' mood could influence the results.

Nevertheless, it is important to confirm that the findings from healthy volunteer studies are replicated in depressed populations. A number of studies have examined changes in emotional processing after long-term treatment. After chronic SSRI administration, depressed patients show reduced response in the amygdala, ventral striatum and frontal-parietal cortex to negative faces (Fu et al., 2004; Sheline et al., 2001), as well as increased response in extra-striate cortex to happy faces (Fu et al., 2007). However, because these changes were only examined after several weeks of treatment, it is possible that they were the result, rather than cause, of improvements in mood.

A number of studies have examined emotional processing in patients after short-term or acute doses. These changes occur in the absence of any significant effects on mood, and so cannot be attributed to individuals feeling happier. One study found that seven days of citalopram treatment improved recognition of happy facial expressions compared to baseline, although the lack of a placebo control condition means that this could be the result of practice effects (Shiroma, Thuras, Johns, & Lim, 2014). However, another study found that seven days of escitalopram reduced right amygdala response to fearful faces, compared to those given a placebo (Godlewska, Norbury, Selvaraj, Cowen, & Harmer, 2012).

After an acute dose of reboxetine, patients showed an increase in ability to recognise happy faces (Harmer et al., 2009). They also showed a decrease in time to categorise words as positive, and increased memory for positive words at a later recall test (see Figure 2.1b). The effect of acute antidepressant treatment on neural activity in depressed participants remains to be explored.

2.5 Early neurocognitive changes predict later treatment response

One of the most important findings in support of the neurocognitive model is that the early production of a more positive bias in emotional processing is actually predictive of ultimate improvement in symptoms in depressed patients.

In one study, a group of patients suffering from depression were assigned to either 20mg/day citalopram or 4mg/day reboxetine for six weeks (Tranter et al., 2009). Both antidepressants increased recognition of happy faces at two weeks compared to baseline, as well as recognition of disgust and surprise. But importantly, the increase in recognition of happy faces at two weeks was significantly positively associated with improvement in clinical outcomes (measured by the Clinical Outcomes in Routine Evaluation; CORE) at six weeks (R^2 = 0.21; see Figure 2.2).

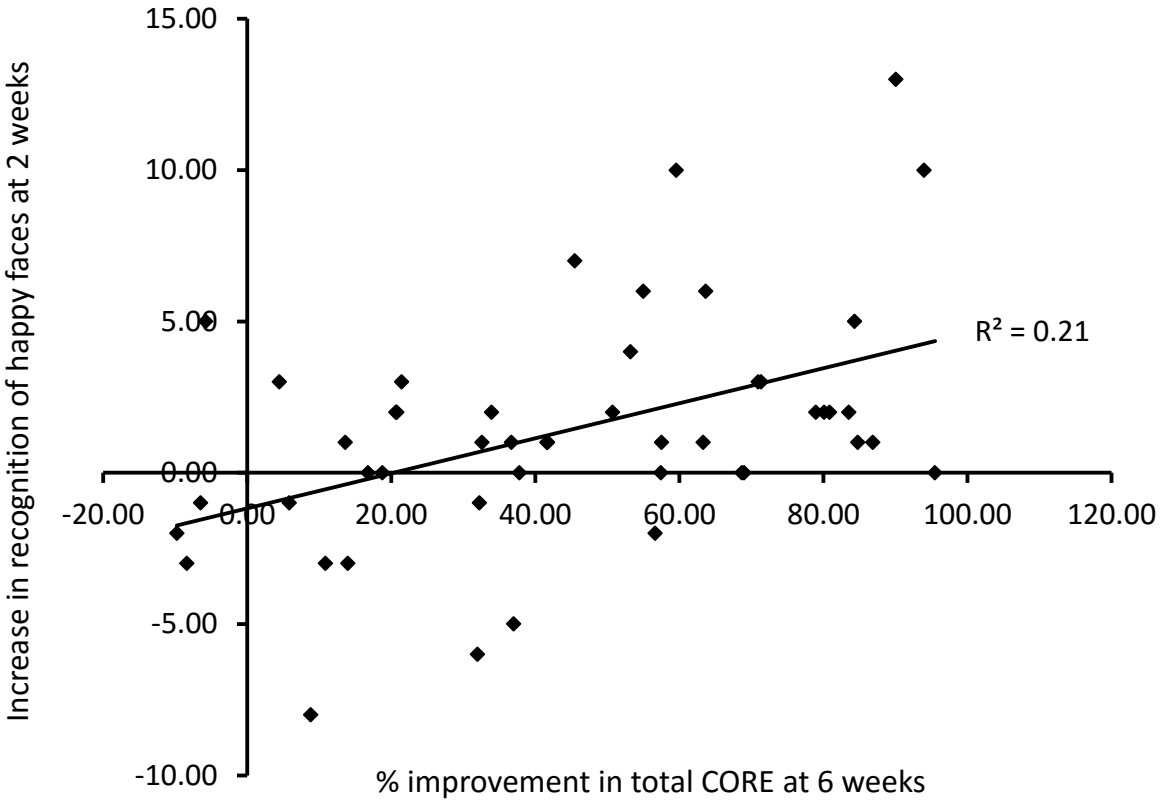

Figure 2.2 Predictive value of early neurocognitive changes
Change in recognition of happy faces from baseline to 2 weeks against percentage improvement in clinical outcome (CORE score) from baseline to 6 weeks of treatment. Adapted from Tranter et al. (2009)

This finding is important, as it demonstrates that early changes towards more positive emotional processing are directly associated with later symptom improvement – a fundamental assumption of the neurocognitive model. On the other hand, given evidence that improvement in mood occurs during the first weeks of antidepressant treatment (M. J. Taylor et al., 2006), changes in emotional processing after two weeks could be the product of symptom change. Research has yet to examine whether similar changes in emotional processing after an acute dose predict clinical response. However, two recent papers have examined changes in emotional processing at an earlier time point during short-term administration.

In one study, 27 older patients suffering from depression were given open-label citalopram treatment for eight weeks (Shiroma et al., 2014). Before beginning treatment and after seven days of treatment, participants were presented with neutral faces and happy faces of different intensities and had to indicate whether or not a face was happy. In line with the neurocognitive model, recognition of happy faces improved between baseline and day 7, and the extent of this improvement was a predictor of improvement on depression scores on the Hamilton Depression Rating Scale at eight weeks.

Early neural changes can also predict later treatment response. A recent study found that changes in neural activity in a network of brain regions after seven days of escitalopram treatment can be used to differentiate responders and non-responders to the treatment six weeks later. Specifically, responders showed more of a reduction than non-responders in BOLD activity to fearful vs happy faces in a network which included the ACC, insula, thalamus and amygdala (Godlewska, Browning, Norbury, Cowen, & Harmer, 2014).

2.6 Further defining the neurocognitive model

As can be seen from the above literature review, over the past decade a large number of studies have supported the neurocognitive model of antidepressant treatment. Patients with depression show clear cognitive biases, and in both patients and healthy volunteers, common antidepressant drugs produce positive shifts in emotional processing. Furthermore, these early changes can predict later improvement in mood, suggesting that they have a direct causative role.

However, there are currently a number of limitations of the model which remain to be explored. This thesis follows two major lines of inquiry in order to further characterise the neurocognitive model. Firstly, we test the prediction made by the model that early neurocognitive changes should be a common feature of a variety of antidepressant treatments, by examining their presence in a novel drug not considered so far, St John's wort. Secondly, we seek to overcome the limitations imposed by using samples of healthy controls or depressed patients, by characterising the effects of the SSRI citalopram in a clinically-relevant, healthy population group of high-neurotic volunteers. In the following sections we summarise the rationale behind each study.

2.7 Do early neurocognitive changes generalise to other antidepressant treatments?

As outlined in the literature review, the early neuropsychological effects of antidepressants have been demonstrated in only a select few drugs: mainly SSRIs and SNRIs, with a few instances of other classes such as mirtazapine. Yet the cognitive neuropsychological model of antidepressant action states that early changes in emotional processing are fundamental to the mechanism of the drugs: that is, they should be produced by *all* antidepressants, rather than being restricted to a select few. It is therefore necessary to examine whether other interventions useful in depression also produce similar neurocognitive changes.

There is preliminary evidence from a small number of studies that other pharmacological interventions useful in treating mood disorders also produce early changes in emotional processing. The serotonin precursor L-tryptophan appears to have antidepressant effects, although it is not widely used as there is limited data on the safety of the drug (Shaw, Turner, & Del Mar, 2002). However, 14 days of 3g/day tryptophan induced emotional processing changes in healthy female participants similar to those of other antidepressants (Murphy, Longhitano, Ayres, Cowen, & Harmer, 2006). Participants were worse at recognising facial expressions of disgust, and better at recognising happy faces than those who took a placebo, and also showed reduced attentional vigilance to negative words on a dot-probe task.

Agomelatine is a novel antidepressant which acts on the melatonin system, as an agonist at M1 and M2 receptors, and on the serotonin system, as a 5-HT2C antagonist. The drug is thought to exert antidepressant effects in part via the correction of disturbances in circadian rhythms (Quera Salva et al., 2007). Despite these novel mechanisms, the drug appears to have similar psychological effects to the conventional antidepressants. Seven days of 25mg/day agomelatine reduced recognition of sad facial expressions, and increased recall of positive vs negative self-referent words (Harmer et al., 2011). It also reduced the acoustic startle response when viewing negative pictures, similar to the SSRIs (Harmer et al., 2004).

Non-pharmacological treatments for depression may also produce early neurocognitive changes. Transcranial direct current stimulation (tDCS), a form of non-invasive brain stimulation which involves applying an electric current to the head in order to increase or decrease neuronal excitability, is effective at treating depression (Shiozawa et al., 2014), and acute stimulation with tDCS affects emotional processing. A single session of tDCS over the dlPFC reduced attentional vigilance to fearful faces in healthy controls (Ironside, O'Shea, Cowen, & Harmer, 2015). Similar results have been found in depressed populations: depressed patients were slower to name the

colours of negative words than positive words in an emotional Stroop task; however, a single session of tDCS abolished this effect, reducing response times to negative words (Brunoni et al., 2014). Thus participants appeared to be better able to supress the emotional content of negative words after stimulation.

This initial evidence for the presence of emotional processing effects in a number of other antidepressant interventions bolsters the neurocognitive theory. However, data is still restricted to a small number of interventions and it is vital that more evidence from a larger sample of drugs is obtained.

2.7.1 **Study 1: Investigation into the neurocognitive effects of St John's wort**

One of the aims of this thesis was therefore to characterise the early neurocognitive effects of the drug *Hypericum perforatum* or St John's wort (SJW). SJW is one of the few – perhaps only – so-called "herbal remedies" for which there exists a body of evidence in support of its antidepressant efficacy (Linde, Berner, & Kriston, 2008). It also appears to have a different mechanism of action from the antidepressants so far examined, most likely working to reduce reuptake of a range of neurotransmitters by increasing intracellular levels of sodium (Leuner et al., 2007). However, SJW has received no attention in the cognitive psychopharmacology literature.

We hypothesised that seven days of SJW treatment in healthy controls would produce a positive shift in emotional processing similar to those seen as the result of other antidepressant drugs. If these changes were found, then this would support the theory that such changes are common across a range of antidepressants with diverse mechanisms. The effects of SJW on emotional processing are examined in Chapter 3.

We also sought to examine other non-emotional cognitive effects that could be caused by SJW. A number of animal studies suggest a beneficial effect of the drug on memory (Trofimiuk, Walesiuk, & Braszko, 2005), though the few human studies conducted so far have not been so positive (Ellis, Stough, Vitetta, Heinrich, & Nathan, 2001). We were also interested in the drug's effect on reward processing, given that it is thought to have an effect on dopamine transmission. In addition, it is important to disentangle any effects on cognition *per se* from those on emotional processing. These non-emotional effects of SJW are examined in Chapter 4.

2.8 Are the same neurocognitive effects present in clinically-relevant populations?

As discussed above, both general healthy volunteer populations and depressed patient populations present certain limitations when it comes to investigating the neuropsychological effects of antidepressants. Healthy volunteers do not display the same baseline biases in emotional processing present in depressed patients, and so it is not possible to confidently state that the effects of antidepressant treatment in such a population are the same as they would be for depressed patients. On the other hand, depressed patients themselves may display other cognitive deficits such as memory impairments or psychomotor dysfunction (Castaneda et al., 2008; Golinkoff & Sweeney, 1989), making it difficult to separate the effects of antidepressants on emotional processing *per se* from other possible cognitive effects.

An alternative approach has recently been utilised: to select participants from the population who score highly on a measure of neuroticism (high Ns). Neuroticism has long been considered a risk factor for depression (e.g. Hirschfeld et al., 1989; Saklofske, Kelly, & Janzen, 1995), and people with a high N score also show similar cognitive and neural biases to depressed subjects (Chan, Goodwin, & Harmer, 2007; Chan, Harmer, Goodwin, & Norbury, 2008a). However, these participants typically do not display the deficits in memory or executive functioning that may be present in a clinical population, nor do they present with the confounding factor of clinical levels

of depressed mood. They may therefore represent a more ideal population in which to study the neuropsychological effects of antidepressants.

Studies of short-term antidepressant administration in high Ns have in part been consistent with those in other populations. For example, seven days of citalopram treatment increased recognition of positive facial expressions in a sample of high Ns (Di Simplicio, Doallo, et al., 2014), and also decreased activation in ventromedial prefrontal cortex while categorising negative words (Di Simplicio, Norbury, & Harmer, 2012).

However, there have been some contradictory findings. The same study as above showed that those in the citalopram group maintained their gaze at facial expressions *longer* than those in the placebo group, and also showed an *increased* amygdala response to fearful faces, as well as to happy and neutral faces (Di Simplicio, Norbury, et al., 2014).

These apparently paradoxical findings imply that the neuropsychological changes produced by antidepressants may be more nuanced than previous studies have suggested. In particular, the studies' authors have suggested that early on, treatment may work to reverse a baseline avoidance of threatening or socially-relevant emotional information that is present in populations who are highly neurotic. That is, baseline emotional biases might consist of both increased processing of general negative information and avoidance specifically of threatening information. Rather than solely decreasing negative emotional processing, antidepressants may also initially increase attention to threatening stimuli. However, it is as yet unclear what happens to these biases as treatment progresses, and these findings have not been confirmed by directly comparing a high N group with a low N group.

2.8.1 **Study 2: Characterising the pattern and timecourse of neurocognitive changes in high Ns**

The second major aim of this thesis was to further describe the neurocognitive effects of citalopram in a high N group. If this group represents a more accurate model for understanding antidepressant drug effects, then it is vital to fully characterise the pattern of neurocognitive response to antidepressants in this population – particularly if these changes might be subtly different from those seen in other groups.

In Chapter 5 and Chapter 6 we investigated the behavioural and neural effects of four weeks of citalopram treatment in high and low N volunteers. We predicted that, as reported previously, high Ns would show both behavioural and neural biases towards negative information compared to low Ns – but would also show biases *away from* threatening information in particular. We expected that the early "paradoxical" increases in threat processing would be restricted to early on in treatment, and that by the time we tested our participants after four weeks, both high and low Ns would show similar reductions in processing of negative information, regardless of how threatening it was.

Finally, treatment effects on resting state functional connectivity have so far not been studied in high neurotic volunteers. Indeed, even in healthy volunteers not selected for neuroticism levels, such studies have tended to be restricted to short-term doses of antidepressant drugs, and there is a need to examine resting state effects during longer treatment courses. In Chapter 7 we therefore conducted a more exploratory study, examining how four weeks of citalopram treatment modulated resting state functional connectivity in high and low N populations.

Chapter 3

St John's wort and emotional processing

3.1 Introduction

To date, the drug *Hypericum perforatum*, or St John's wort, has not received any attention in the literature on emotional processing. SJW is traditionally used to treat depression, and is one of the only herbal remedies for which there is a body of research that largely supports its clinical use. Nevertheless, it has been a divisive drug: doubt was shed on the positive results of early trials due to methodological issues such as short time-courses of treatment and heterogeneous patient groups (De Smet & Nolen, 1996). However, over the past two decades, further research has been conducted, which generally suggests that SJW is an effective antidepressant (Fava et al., 2005; Linde et al., 2008).

3.1.1 Evidence for the antidepressant efficacy of SJW

A number of studies have found that depressed individuals taking SJW respond better than individuals taking a placebo. For example, in outpatients with mild-to-moderate depression, 300mg/day SJW for six weeks produced a greater reduction in scores on the Hamilton Rating Scale for Depression (HAM-D) and higher response and remission rates than a placebo (Lecrubier, Clerc, Didi, & Kieser, 2002). Patients with atypical features of depression also benefitted more from an eight week course of SJW than placebo (Mannel, Kuhn, Schmidt, Ploch, & Murck, 2010). Additionally, SJW compares favourably with other antidepressants: there was no significant difference between response rates for patients taking SJW and patients taking sertraline (Brenner, Azbel, Madhusoodanan, & Pawlowska, 2000) or imipramine (Woelk, 2000b), and one study found that SJW produced *greater* reduction in HAM-D scores than fluoxetine (Fava et al., 2005).

However, not all studies have been positive. SJW did not produce greater response rates than placebo amongst severely depressed patients (Hypericum Depression Trial Study, 2002), and another study failed to find any benefit amongst patients with mild depression (Rapaport et al., 2011). On the other hand, in these studies standard antidepressants, sertraline and citalopram respectively, also failed to show a clinical effect above placebo, raising doubts about the validity of the findings - though another well-controlled study also found that an eight week course of SJW did not have any effect greater than placebo on a number of measures (Shelton, Keller, Gelenberg, & et al., 2001).

However, when all studies are considered together, the data support the antidepressant efficacy of SJW. A meta-analysis by the Cochrane Collaboration in 2008 analysed data from 29 studies comparing the efficacy of SJW to a placebo and/or an existing antidepressant, amongst patients suffering from major depression (Linde et al., 2008). Compared to patients taking a placebo, those taking SJW were more likely to respond to treatment. There was no difference in clinical response between those taking SJW and those taking other antidepressants, though the SJW group were less likely to drop out of the study because of adverse effects. Although the authors do raise some concerns about methodological issues in some of the studies, they conclude that the best available evidence suggests that SJW is an effective antidepressant, particularly for mild-to-moderate depression.

It should be noted that in contrast to the many trials conducted on the use of SJW in depression, very few trials have investigated the efficacy of the drug for treating anxiety disorders. An open-label trial found that SJW produced improvement in obsessive-compulsive disorder (OCD; L. H. Taylor & Kobak, 2000), but a subsequent placebo-controlled trial did not (Kobak, Taylor, Bystritsky, et al., 2005). Similarly, a study failed to find any differences between SJW and placebo for treating social phobia (Kobak, Taylor, Warner, & Futterer, 2005), although one trial found

that SJW was superior to a placebo for treating somatoform disorders (Volz, Murck, Kasper, & Moller, 2002). There is thus currently very little evidence either supporting or refuting the use of SJW for treating anxiety disorders.

Despite the positive findings in the depression literature, many are reluctant to embrace the possibility that SJW could be an effective treatment for depression, or to further assess its clinical potential. Some of this reluctance may be justified, as SJW is not without its problems: the drug interacts with a number of other medications by inducing activity of CYP enzymes, which increases metabolism of drugs such as oral contraceptives, anticoagulants and antiretrovirals (Russo et al., 2014). Of course, these issues do not rule out the drug as an antidepressant, but do mean that care must be taken when prescribing it. On the other hand, SJW has minimal side-effects, and tends to be tolerated better than other antidepressants (Linde et al., 2008), so may confer some advantages over other drugs.

3.1.2 **Mechanism of action of SJW**

Another reason that people have had reservations about the clinical utility of SJW is that its mechanism of action has not been unequivocally established. Extracts of SJW contain a range of bioactive substances such as flavonoids and various amino acids and essential oils (Wurglics & Schubert-Zsilavecz, 2006). These may contribute to the antidepressant effect of the drug. However, there are two substances present in SJW that are rarely found elsewhere: hypericin and hyperforin. These compounds have received by far the most attention in the literature, and it is likely that one or both of these are the main producers of the antidepressant effect of the drug.

An early study found that hypericin inhibited monoamine oxidase (MAO; Suzuki, Katsumata, Oya, Bladt, & Wagner, 1984), similar to the first generation antidepressants, providing a possible mechanism of action. However, subsequent studies failed to replicate these findings; instead, they

found that hypericin only had *in vitro* inhibitory effects at concentrations much higher than those given to humans, and failed to find any effects on MAO when extracts were administered to rodents (Bladt, 1994; Thiede & Walper, 1994). These studies found that other components of SJW such as flavonoids had a much greater effect on MAO, but these were present at such low quantities within the drug that they could not account for the clinical effects.

Current consensus is that hyperforin is the more likely candidate for producing the clinical effects of SJW. Hyperforin produces much broader inhibition of neurotransmitter reuptake than other antidepressants: both *in vivo* (Müller, 2003) and *in vitro* studies (Chatterjee, Bhattacharya, Wonnemann, Singer, & Müller, 1998) have demonstrated that the substance inhibits reuptake of serotonin, dopamine and noradrenaline. In addition to its monoaminergic effects, hyperforin also inhibits reuptake of GABA and glutamate in *in vitro* studies (Wonnemann, Singer, & Muller, 2000).

Consistent with its broad inhibition of neurotransmitter reuptake, and unlike the selective reuptake inhibitors commonly used to treat depression, hyperforin does not seem to bind to any specific sites: one *in vitro* study found that although hyperforin clearly inhibited reuptake of serotonin, it bound only weakly at the reuptake transporter in mouse synaptosomes (Singer, Wonnemann, & Müller, 1999). However, hyperforin increased intracellular sodium levels. It appears to do this by binding to and activating a transient receptor potential channel (TRPC) that is permeable to sodium on the presynaptic neuron. Specifically, hyperforin activates the channel TRPC6, resulting in an increase in sodium flowing into the neuron through the channel. Because neurotransmitter reuptake transporters are reliant on the sodium gradient, reducing the difference in sodium concentration between intra- and extra-cellular fluid in this way produces a broad-acting decline of neurotransmitter reuptake that is not limited to any particular transmitter (Harteneck & Gollasch, 2011; Leuner et al., 2007).

It should be noted that a few clinical trials have found beneficial effects of SJW extracts with very low hyperforin content, suggesting that there are other components of the drug that also contribute to their clinical effect (Schrader, 2000; Woelk, 2000a). However, the evidence is equivocal: the methodology of these studies has been criticised (Müller, 2003) and other studies have found only beneficial effects of hyperforin-rich, but not hyperforin-poor, SJW extracts (Laakmann, Schule, Baghai, & Kieser, 1998). Thus the best evidence remains that hyperforin produces the clinical effects of SJW via its effects on neurotransmitter reuptake – though there may well be other mechanisms that work alongside or in tandem with hyperforin.

3.1.3 **Current study**

The cognitive neuropsychological model of antidepressant action highlights the fundamental importance of early positive changes in emotional bias in producing the antidepressant effects of medications. Yet so far, this model has only been tested on a small number of drugs. With its clear antidepressant effects and novel mechanism of action, SJW is a prime candidate to test the hypothesis that these early changes in emotional bias are key features of a range of different antidepressants. The current study therefore sought to examine whether short-term administration of SJW produced changes in emotional processing similar to those found in studies using other antidepressants. Specifically, we predicted that seven days of SJW compared to placebo would produce changes in emotional bias towards positive and away from negative information.

3.2 Materials and Methods

Ethical permission for this study was obtained from the University Research Ethics Committee. Healthy participants aged between 19 and 43 were screened for the study. Screening included a medical history and screening for axis 1 psychiatric disorders (using the Standard Clinical

Interview for DSM-IV; SCID). Exclusion criteria included concurrent use of other medications or hormonal contraception, a current or past psychological disorder, current pregnancy or breast-feeding, and use of psychotropic drugs or participation in a drug trial within the previous three months. Participants were also excluded if they had completed the emotional processing tasks before. During the screening session participants also completed a number of mood questionnaires (see below for details).

A total of 48 participants met criteria and were assigned to take part in the study. One participant exhibited unexpected responses across a range of tasks; this participant was excluded from all analyses, leaving 47 participants in total (SJW = 23; placebo = 24). Demographic information is reported in Table 3.1; t-tests found no significant differences between the groups (*p*s > .05).

Table 3.1 Demographic characteristics of volunteers

	SJW (*n* = 23)		Placebo (*n* = 24)	
Gender	*N*	*%*	*N*	*%*
Male	11	47.83	12	50
Female	12	52.17	12	50
	Mean	*SD*	*Mean*	*SD*
Age	25.43	3.88	24.04	4.97
Years of education*	18.09	2.34	17.67	2.33

*Not available for one participant in the SJW group

Participants were randomly assigned to receive either SJW or placebo. The SJW treatment consisted of 300mg tablets (Jarsin® 300mg; Klosterfrau), containing a standard preparation, LI 160, standardised to contain 0.3% hypericin and 3-5% hyperforin. The placebo consisted of 200mg lactose tablets (Rayotabs; RAYONEX), which were encapsulated in gelatin capsules (CapsuleDepot). The study followed a double blind design; to maintain blinding, capsules were stored in opaque containers and handed to participants by another member of the lab.

Participants took three pills per day for six days, with their meals. On the seventh day, participants took a single pill two hours before the testing session. Throughout the week, participants also completed daily visual analogue scales and side-effects questionnaires.

In order to avoid any confounding effects of time of day, the testing session always began between 9am and 11am, with the majority of participants beginning at 10am. Testing sessions lasted approximately 2.5 hours, and testing was scheduled to avoid testing female participants in their pre-menstrual week. Participants first filled in mood questionnaires and then completed a number of computer-based tasks examining emotional processing, explained below.

3.2.1 **Questionnaires**

Beck Depression Inventory (BDI)

The BDI (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) is a standard self-report questionnaire used to measure levels of depression. It contains 21 items referring to feelings over the past week, and for each item participants have to choose which of four options applies to them. Answers increase in severity from 0 (e.g. "I do not feel like a failure") to 3 (e.g. "I feel I am a complete failure as a person"); a higher overall score indicates more depressed mood. The BDI was filled in at baseline and testing.

Snaith-Hamilton Pleasure Scale (SHAPS)

The SHAPS (Snaith et al., 1995) is a 14-item self-report scale that measures the ability to experience pleasure over the past few days. Questions include "I would find pleasure in my hobbies and pastimes" and "I would be able to enjoy a beautiful landscape or view", and participants are required to respond on a four-point scale from 1 (Definitely agree) to 4 (Definitely disagree). "Agree" and "Definitely agree" responses are scored as 0, "Disagree" and

"Definitely disagree" responses are scored as 1; a higher overall score indicates greater anhedonia. The SHAPS was filled in at baseline and testing.

State-Trait Anxiety Inventory (STAI)

The STAI (Spielberger, Gorsuch, & Lushene, 1970) consists of two self-report scales measuring anxiety. Each questionnaire consists of 20 questions regarding how participants are feeling currently (State Anxiety scale) or how they generally feel (Trait Anxiety scale). Questions include "I feel upset" and "I feel like a failure". Participants have to respond on a four-point scale from 1 (Not at all) to 4 (Very much). Some questions are reverse worded (e.g. "I feel pleasant"); these are reversed in analysis so that a higher overall score indicates greater anxiety. The State Anxiety scale was filled in at baseline and testing, the Trait Anxiety only at baseline.

Positive and Negative Affect Schedule (PANAS)

The PANAS (Watson, Clark, & Tellegen, 1988) is a self-report scale in which participants have to indicate the extent to which they have felt each of 20 emotions that day. Ten emotions are positive (e.g. "Proud") and ten are negative (e.g. "Distressed"), and participants rate these on a scale from 1 (Very slightly or not at all) to 5 (Extremely). Two scores are derived from the scale – one for overall positive emotions and one for negative emotions. The PANAS was filled in at baseline and testing.

Eysenck Personality Questionnaire (EPQ)

The EPQ (H. J. Eysenck & Eysenck, 1975) is a 90-question self-report measure which assesses personality traits. The questionnaire is designed to measure levels of extraversion, neuroticism and psychoticism, and also contains a "lie" subscale to control for participants deliberately trying to respond in a socially-desirable way. Each question (e.g. "Do you worry about things you should not have done or said?") requires a yes/no reply. "Yes" responses are scored as 1 and

"No" responses as 0, giving a total score for each subscale. The EPQ was filled in only at baseline.

Visual Analogue Scales

The VAS scales consist of 10cm lines measuring current emotions. In this study these emotions were happy, sad, hostile, alert, anxious and calm. Participants have to put a mark on the line where they fall between 0 (Not at all) and 10 (Extremely). The distance of this mark from 0 is then measured to determine the participant's score on each scale. VAS scales were completed at baseline and testing, as well as daily throughout the study period.

Side-Effects

Side-effects questionnaires asked whether participants had experienced any of five side-effects: nausea, dizziness, dry mouth, headache or sensitivity to light. Participants could score each side-effect as absent (scored as 0), mild (1), moderate (2) or severe (3). Total side-effect scores were then calculated for each day.

3.2.2 **Emotional processing tasks**

Emotional Test Battery (ETB)

a) Facial Expression Recognition Task (FERT)

In the FERT, participants were presented with facial expressions of the six basic emotions (anger, disgust, fear, happiness, sadness and surprise) taken from ten individuals from the Pictures of Facial Affect series (Ekman & Friesen, 1976). Each photograph was morphed to provide different intensities of the expression, from 10% to 100% in 10% steps (for details on the morphing process see Murphy, Downham, Cowen, and Harmer (2008); Young et al. (1997)).

Faces were presented in a random order for 500ms each, and participants were required to identify the facial expression as quickly and as accurately as possible by pressing the correspondingly labelled button on a keyboard. Participants were required to make a response for every expression. For each expression, participants saw a total of forty faces (four of each intensity) as well as one neutral expression for each of the ten individuals, meaning 250 faces were presented in total. Accuracy and reaction time were recorded for each trial.

b) Emotional Categorisation

In the emotional categorisation task, participants were presented with 60 personality characteristics, 30 chosen to be positive and 30 to be negative, and matched for length, frequency and meaningfulness. Words were presented for 500ms and participants were required to indicate whether they would like or dislike to be described in that way, as quickly and as accurately as possible.

c) Emotional Recall

A reward task was conducted following emotional categorisation (see Chapter 4), which lasted approximately 15 minutes. After this participants were given a surprise recall task. In this task they had to recall as many words as they could from the emotional categorisation task. They were given two minutes in which to write these words down.

d) Emotional Recognition

In the emotional recognition task, participants were presented with 120 personality characteristic words. Sixty of these had been presented in the emotional categorisation task, and 60 were new words (30 positive, 30 negative). Words were presented for 500ms and participants were required

to indicate whether or not the word had been presented in the categorisation task, as quickly and as accurately as possible.

Dot Probe

Each trial of the attentional dot probe began with a fixation cross in the centre of the screen, which was immediately followed by the presentation of two faces, one towards the top and one towards the bottom of the screen. These faces were taken from 20 individuals in the JACFEE/JACNeuF sets (Matsumoto & Ekman, 1988). A pair consisted of two faces from the same individual, in of one of three combinations of expressions: neutral-neutral, neutral-happy, or neutral-fearful. In unmasked trials, these faces were presented for 100ms; in masked trials they were presented for 16ms followed by a mask, consisting of a jumbled face, for a further 84ms. There were 192 trials in total, consisting of 32 trials of each of the three combinations of faces for both the masked and unmasked conditions. These were presented in eight blocks, with masked and unmasked faces being presented in separate, alternating blocks.

Immediately after the faces had been presented, a probe appeared in the previous location of one of the faces. This probe consisted of two dots aligned vertically (:) or horizontally (. .). The participant was required to indicate the orientation of the dots as quickly and as accurately as possible. For neutral-happy and neutral-fearful trials, attentional vigilance scores could subsequently be calculated as the difference in reaction time to the probe when it was behind an emotional face and reaction time when the probe was behind a neutral face. Higher scores indicate faster reaction times for the emotional face, and hence attentional bias towards that face, while negative scores indicate attentional bias *away* from that face.

Emotion-Potentiated Startle

Participants were presented with pictures from the International Affective Picture System (IAPS; (Lang, Bradley, & Cuthbert, 2008)). These pictures were chosen to elicit either a positive or

negative response, or to be neutral. The positive and negative pictures were selected to be opposite in valence but similar in arousal, while the neutral pictures were low in arousal and average in valence. A few pictures were different for male and female participants.

The task consisted of three blocks. A block consisted of seven of each kind of picture, each presented for 13s with an intertrial interval (ITI) of 11-15s (mean = 13s). Pictures were presented in a fixed random order, with the limitation that two pictures of the same type were never presented consecutively. An acoustic startle probe (white noise) was delivered binaurally for 50ms at 100dB either 1.5s, 4.5s, or 7.5s after the onset of the picture. The noise occurred during five presentations of each kind of picture in each block: it was absent for two presentations of each kind of picture, and also occurred during three ITIs in each block, to avoid participants developing an expectation regarding its occurrence. A shorter practice trial preceded the main three blocks of the task so that the participant could habituate to the task.

Eyeblink in response to the startle probe was recorded using electromyography (EMG) to record from the *orbicularis oculi* (EMG startle response system, San Diego Instruments, San Diego, CA, USA). Three electrodes were placed around the right eye: in the right hand corner and immediately below the eye, as well as on the right cheekbone.

The EMG signal was filtered between 0.5 and 100 Hz. Maximum eye-blink amplitude for each trial was calculated by subtracting baseline signal (average amplitude in the first 20ms after the probe) from the peak amplitude occurring between 30-80ms. Trials with poor signal to noise ratio, defined as peak eyeblink as less than three times the baseline, were removed.

3.3 Results

3.3.1 Baseline group characteristics

Scores on baseline measures of mood, anxiety and personality are displayed in Table 3.2. Independent samples t-tests were used to investigate any possible differences between groups; these were all non-significant.

Table 3.2 Pre-treatment scores on measures of mood and personality

	SJW mean (SD)	Placebo mean (SD)	p-value
BDI	2.70 (3.54)	2.21 (2.75)	0.60
SHAPS	0.22 (0.52)	0.29 (0.75)	0.70
State Anxiety	31.00 (6.58)	30.56 (3.97)	0.73
Trait Anxiety	34.17 (9.29)	32.71 (6.93)	0.54
PANAS			
Positive	28.87 (6.68)	27.67 (5.33)	0.50
Negative	11.43 (1.38)	11.75 (1.78)	0.50
EPQ			
Neuroticism	6.61 (4.44)	5.75 (3.03)	0.44
Psychoticism	3.04 (2.18)	3.00 (1.72)	0.94
Lie	8.52 (3.72)	8.08 (3.78)	0.69
Extraversion	14.30 (4.79)	12.92 (5.00)	0.34
VAS			
Happy	7.12 (1.27)	6.94 (1.07)	0.60
Sad	1.13 (1.49)	0.66 (0.85)	0.19
Hostile	0.44 (0.65)	0.58 (0.88)	0.55
Alert	5.58 (2.67)	6.28 (2.43)	0.35
Anxious	1.66 (1.65)	1.38 (1.36)	0.53
Calm	7.57 (1.67)	7.46 (1.89)	0.84

3.3.2 **Effect of treatment on mood, anxiety and side-effects**

The BDI, State Anxiety Inventory, PANAS (positive and negative) and SHAPS were repeated at the final testing session, in order to ascertain whether the week's course of SJW influenced mood. For each measure, a 2 (timepoint) x 2 (treatment) mixed effects analysis of variance (ANOVA) failed to find any main effects or interactions (all *p*s > .05). ANOVAs were also run to compare

baseline and testing scores on each of the VAS scales: again, there were no significant interactions or main effects, with the exception of a reduced 'calmness' rating for all participants at testing, $F(1, 45)=6.11$, $p < .05$. There was therefore no evidence that SJW affected individuals' mood.

Side-effect data was missing for one participant in the SJW group. Overall, SJW appeared to be well-tolerated. Only one participant reported any "severe" side-effects, and these were restricted to just one day. Ten participants (2 on placebo, 8 on SJW) reported experiencing a "moderate" side-effect at least once. A 7 (day) x 2 (treatment) ANOVA was run on total daily side-effect scores averaged across groups. The treatment effect approached significance, $F(1, 44) = 4.07$, $p = .05$, suggesting that the SJW group did experience greater side-effects than the placebo group. Nevertheless, it should be noted that the highest average daily side-effect score in the SJW group was only 1.05 (roughly equivalent to a single "mild" side-effect), indicating that side-effects were still very low overall. There was no main effect of day, $F(6, 264) = .80$, $p = .57$, and no interaction between day and treatment group, $F(6, 264) = 1.20$, $p = .31$,

3.3.3 **Participants' guesses**

The number of participants who guessed that they had been given SJW or placebo for each group is displayed in Table 3.3. A chi-square test found no evidence for an association between the group the participants were in and the guess they made, $\chi^2(1) = .70$, $p = .53$. Thus despite the apparent increase in side-effects in this group, there was no evidence that they were aware they were taking the active substance.

Table 3.3 Participants' guesses regarding treatment group

		Guess	
		SJW	Placebo
Group	SJW	6	17
	Placebo	9	15

3.3.4 **ETB**

FERT

a) Accuracy

As we were concerned specifically with emotional facial expressions, data from trials in which faces were classified as neutral were excluded from all analyses for the facial expression recognition task. Where the assumptions of sphericity or equality of variances were violated, we report corrected results; for ease of reading we report uncorrected degrees of freedom.

Figure 3.1 shows the number of faces correctly recognised. A 6 (emotion) x 2 (treatment) mixed effects ANOVA was used to examine whether SJW affected categorisation of emotional facial expressions. There was no significant effect of treatment, $F(1, 45) = .06$, $p = .80$. However, there was a significant interaction between treatment and emotion, $F(5, 225) = 2.90$, $p < .05$. Individual t-tests revealed that the groups differed significantly on recognition of disgust faces, with the SJW group showing reduced recognition, $t(45) = 2.38$, $p < .05$. There was also a non-significant trend for SJW to increase recognition of happiness ($p = .09$).

The ANOVA also showed a significant effect of emotion, $F(5, 225) = 17.42$, $p < .001$. Post-hoc comparisons with Bonferonni correction indicated that participants were significantly better at recognising happy faces than any other emotion (ps $< .05$). Surprised faces were also better recognised than other emotions (except happy; ps $< .05$).

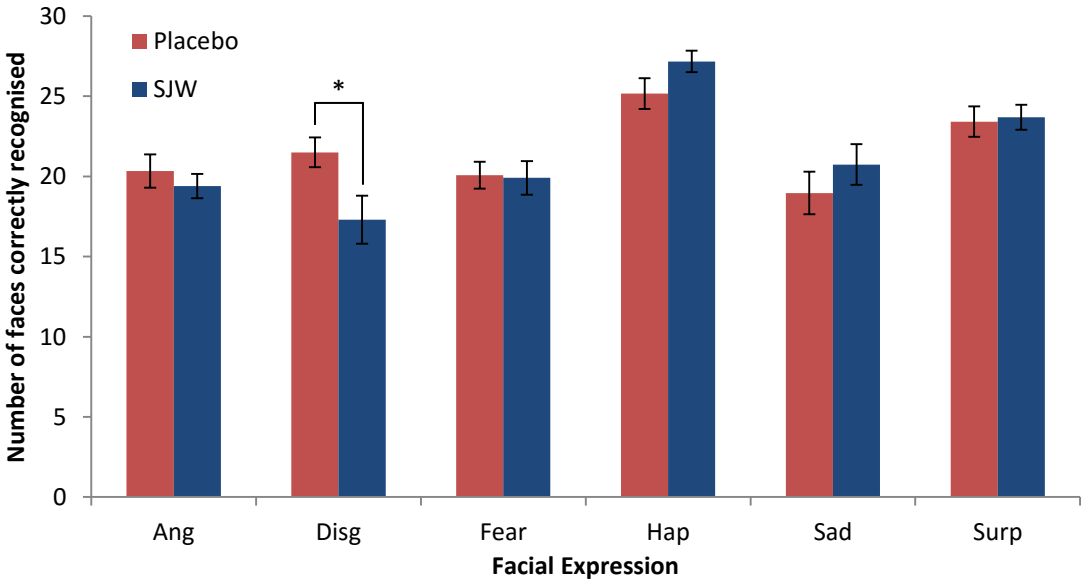

Figure 3.1 Accuracy in the FERT
Mean number of faces correctly recognised (out of 30) for each facial expression. *$p < .05$. Error bars represent standard error of the mean (SEM)

Signal detection theory provides an alternative way to measure accuracy, which takes into account both hit rate (i.e. correct recognition) and false alarms (i.e. misclassifications). Two parameters, *d'* and *ß*, can be calculated as follows:

$$d' = 0.5 + ((y - x)(1 + y - x)/4y\,(1 - x))$$

$$ß = y(1 - y) - x(1 - x)\ /\ y(1 - y) + x(1 - x)$$

where x is the proportion of all responses that were falsely classed as that expression, and y is the proportion of facial expressions correctly recognised.

d' represents accuracy in responding in which false are alarms are taken into account: i.e. a higher value represents greater accuracy with fewer false alarms. *ß* represents the criterion value that participants use to judge a stimulus as being "present" – in this case, as being a given expression. A higher value represents a more conservative response style, in which participants will make fewer false alarms, but also will miss more instances of the facial expression.

Figure 3.2 shows *d'* for each of the facial expressions. Again, there was no significant effect of treatment, $F(1, 45) = .22$, $p > .05$. There was a significant interaction between emotion and treatment, $F(5, 225) = 3.35$, $p < .05$. As before, t-tests revealed that the groups differed significant on d' for disgust faces, with SJW showing reduced recognition, $t(45) = 2.1$, $p < .05$. There was also a trend for increased recognition of happy faces ($p = .08$). That is, the above findings continued to be found when false alarms were taken into account.

Finally, there was a significant effect of emotion, $F(5, 225) = 30.27$, $p < .001$. Post-hoc comparisons with Bonferonni correction again indicated that participants were significantly better at recognising happy faces than any other emotion (ps < .05). Surprised faces were also better recognised than other emotions (except happy; ps < .05).

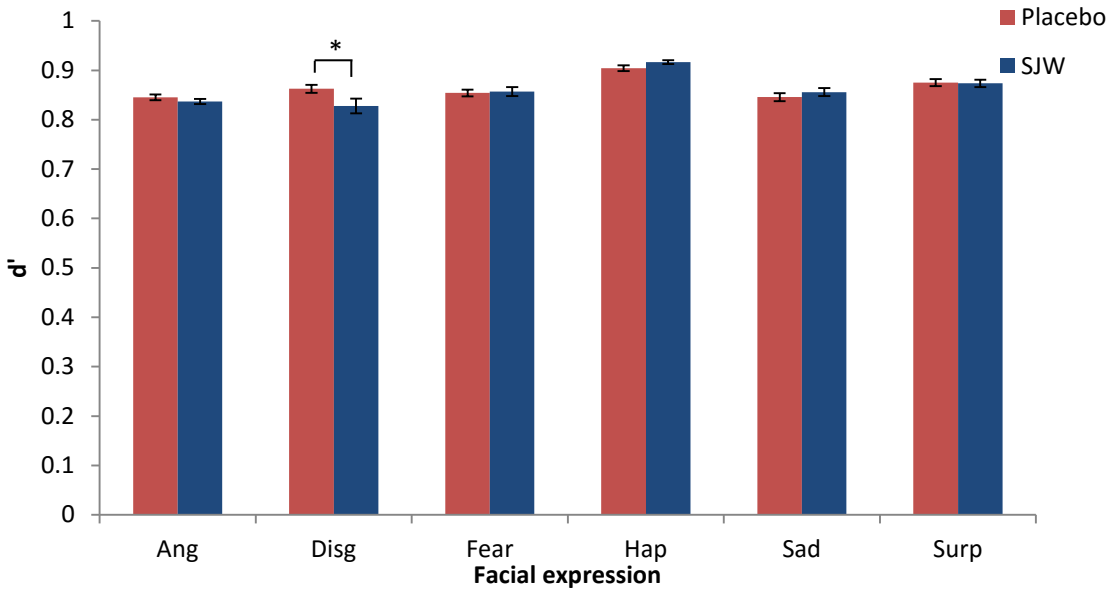

Figure 3.2 *d'* calculated for the FERT
Mean *d'* measure for responses to each facial expression. *$p < .05$. Error bars represent SEM.

Figure 3.3 shows *ß* for each of the facial expressions. There was no significant effect of treatment, $F(1, 45) = .08$, $p = .70$, and no significant interaction between emotion and treatment, $F(5, 255) = .32$, $p = .90$. There was therefore no suggestion that *ß* differed between groups on any of the expressions. There was a significant effect of emotion, $F(5, 255) = 17.41$, $p < .001$. Pairwise comparisons with Bonferonni correction indicated that participants had significantly higher *ß* for happy faces than other emotions (*p*s < .05). There was also a significant difference between anger and fear.

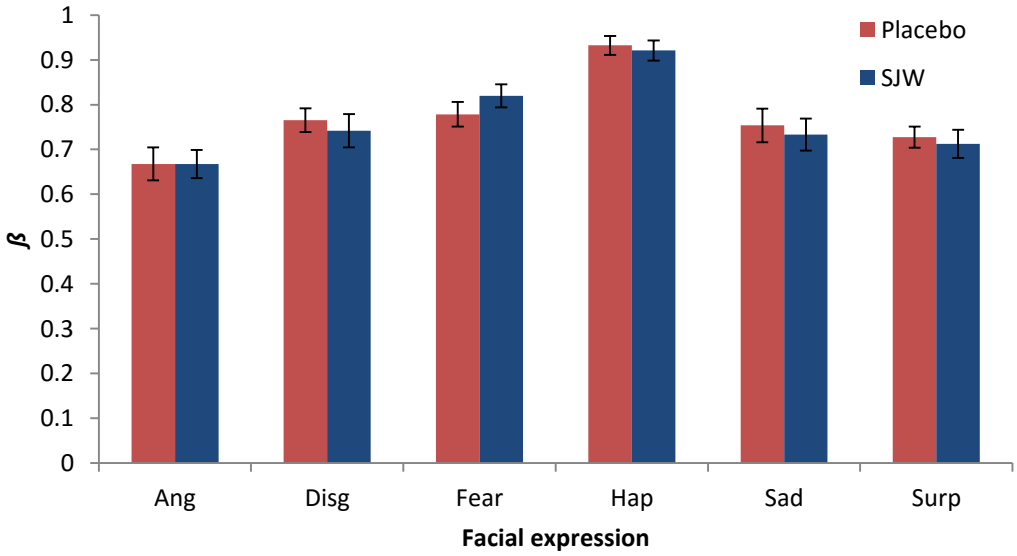

Figure 3.3 *ß* calculated for the FERT
Mean *ß* measure for responses to each facial expression. There was no significant effect of treatment or interaction effect. Error bars represent SEM

In order to determine whether the intensity of expression influenced ability to recognise the face, 2 (treatment) x 10 (intensity) mixed effect ANOVAs were carried out on recognition data for disgusted and happy faces only. As expected, there was a main effect of intensity: participants were better at recognising more intense faces for both happy faces, $F(9, 405) = 248.19$, $p < .001$, and disgusted faces, $F(9, 405) = 144.47$, $p < .001$. However, there was no treatment x intensity interaction for either happy faces, $F(9, 405) = .35$, $p = .89$, or disgusted faces $F(9, 405) = 1.03$, p

= .40), providing no evidence that the effect of SJW differed depending on the intensity of the facial expression.

b) Reaction times

Figure 3.4 shows reaction times for classifying different facial expressions. There was no significant effect of treatment, $F(1, 45) = 2.31$, $p = .14$ and no interaction between treatment and emotion, $F(5, 255) = .54$, $p = .74$. However, given the findings from the accuracy data, a t-test was conducted to see whether the two groups differed on reaction times to disgust faces. There was a trend for the SJW group to take longer to classify faces as disgusted, but this did not reach significance ($p = .07$). This suggests that the impaired perception of disgust is not confounded by a speed-accuracy trade off.

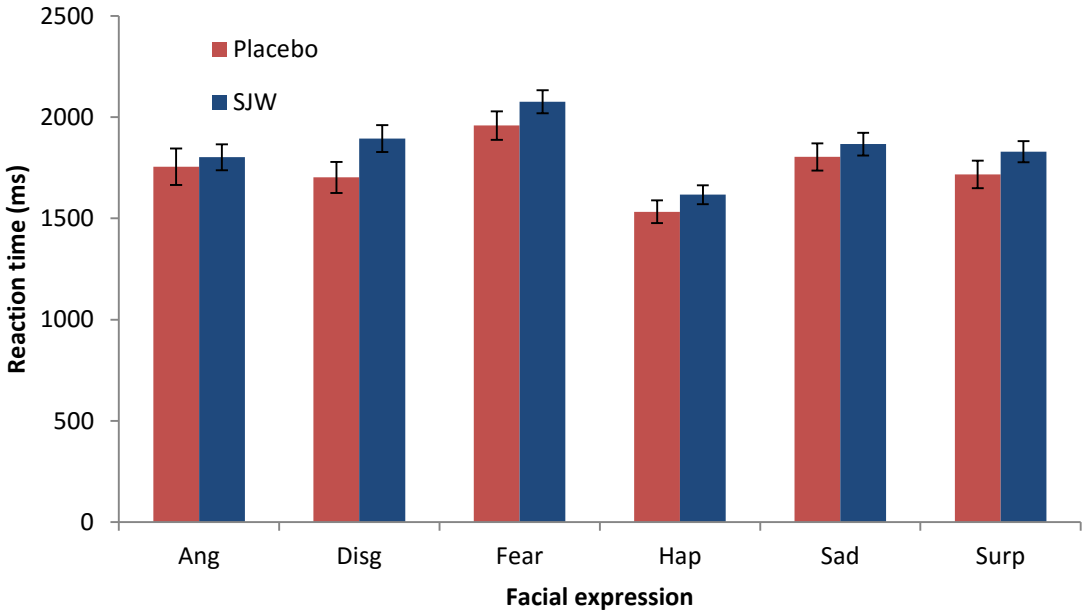

Figure 3.4 Reaction times in the FERT
Mean reaction time to classify different facial expressions. There was no significant effect of treatment or interaction effect. Error bars represent SEM

There was also a significant effect of emotion, $F(5, 225) = 16.30$, $p < .001$. Post-hoc comparisons with Bonferonni correction indicated that participants were significantly faster at responding to happy faces than any other emotion, and slower at responding to fearful faces than other emotions ($ps < .05$).

c) Misclassifications

The number of faces misclassified as each emotion is displayed in Figure 3.5. There was no effect of treatment and no interaction effect, but there was again a significant main effect of emotion, $F(5, 225) = 17.07$, $p < .001$. Post-hoc comparisons with Bonferonni correction indicated that participants misclassified faces as happy less than any other emotion, and as angry more than disgust or fear ($ps < .05$).

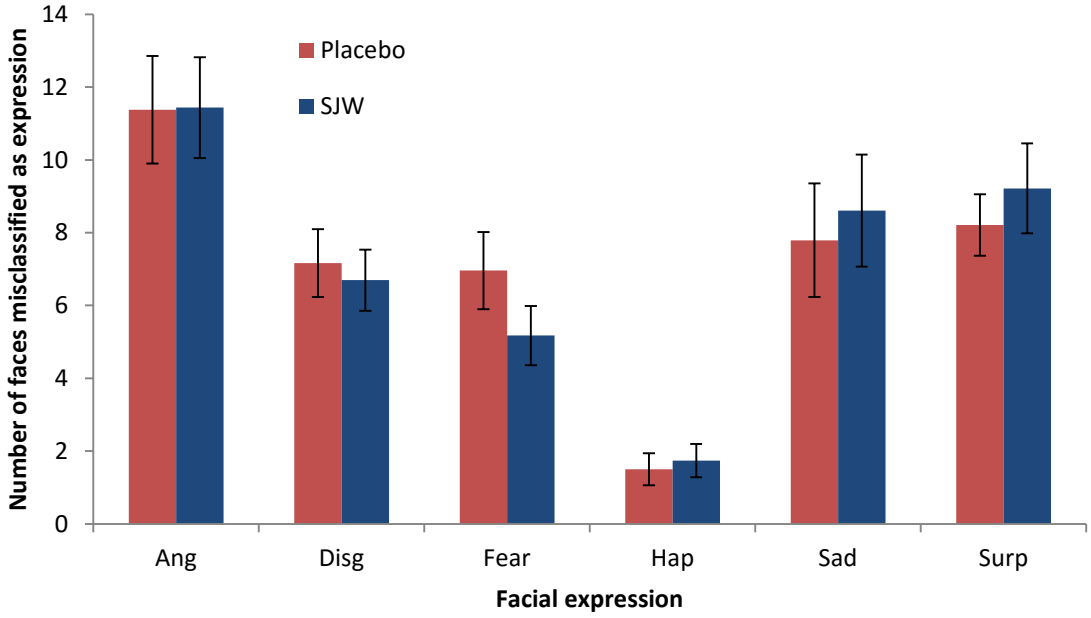

Figure 3.5 Misclassifications in the FERT
Mean misclassifications by emotion for each group. There was no significant effect of treatment or interaction effect. Error bars represent SEM.

Categorisation

Data from one subject from the SJW group was excluded due to technological problems. This left placebo n = 24 and SJW n = 22. Figure 3.6 shows accuracy at categorising words. A 2 (valence of word) x 2 (treatment) mixed effects ANOVA was used to see whether SJW affected accuracy at categorising words. There was no main effect of treatment, $F(1, 44) = 1.95$, $p = .17$, and no interaction between treatment and word valence, $F(1, 44) = .72$, $p = .40$, providing no evidence that St John's wort had any effect on accuracy. There was also no effect of word valence, $F(1, 44) = 2.21$, $p = .14$, suggesting that overall participants were equally accurate at categorising positive and negative stimuli.

Figure 3.7 shows reaction time for categorising words. Again, there was no effect of treatment on reaction times, $F(1, 44) = 1.08$, $p = .30$, nor an interaction between treatment and stimulus, $F(1, 44) = .01$, $p = .91$, indicating that SJW had no effect on reaction times. However, there was a significant main effect of stimulus type, $F(1, 44) = 7.00$, $p < .05$, indicating that overall participants were slightly faster at categorising positive words (M=1007.52 ms) than negative words (M=1039.55).

Recall

Data from one participant in the placebo group was excluded as they had been given the wrong instructions; data from one participant in the SJW group was also excluded as their writing was illegible. This left placebo n = 23 and SJW n = 22.

Figure 3.8 shows the number of words correctly recalled by each group. A 2 (word valence) x 2 (treatment) mixed effects ANOVA was used to examine whether the groups differed on recall. There was no main effect of treatment, $F(1, 43) = 2.83$, $p = .10$, and no interaction between treatment and word valence, $F(1, 43) = .15$, $p = .70$, indicating that SJW did not affect correct

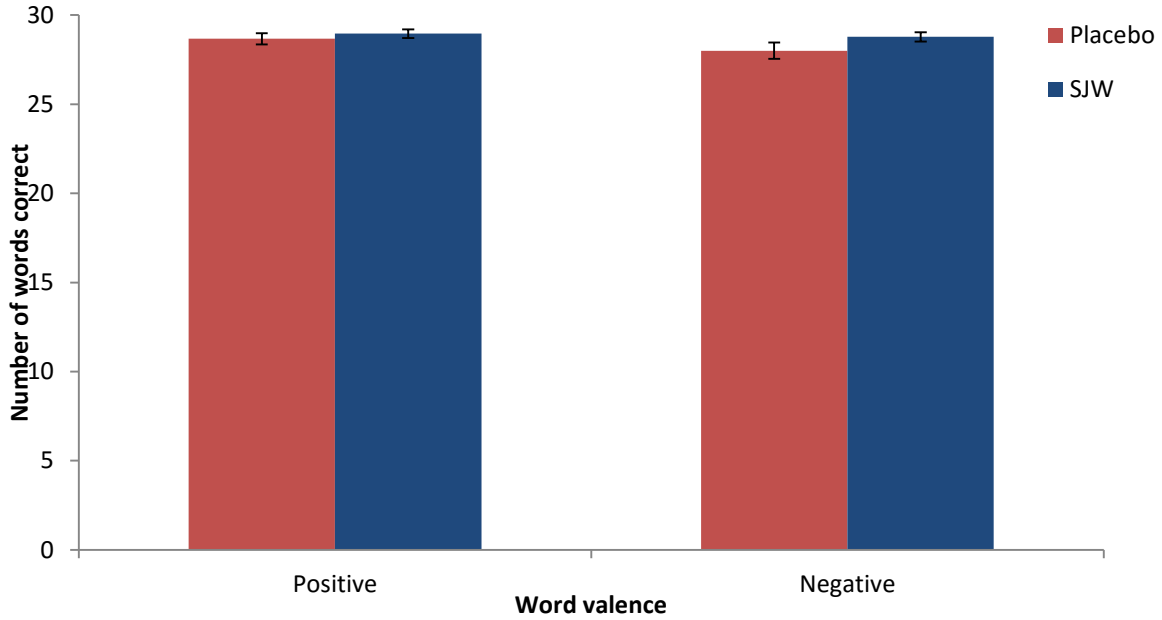

Figure 3.6 Accuracy for word categorisation
Mean number of words (out of 30) correctly categorised as positive or negative. There were no significant effects. Error bars represent SEM.

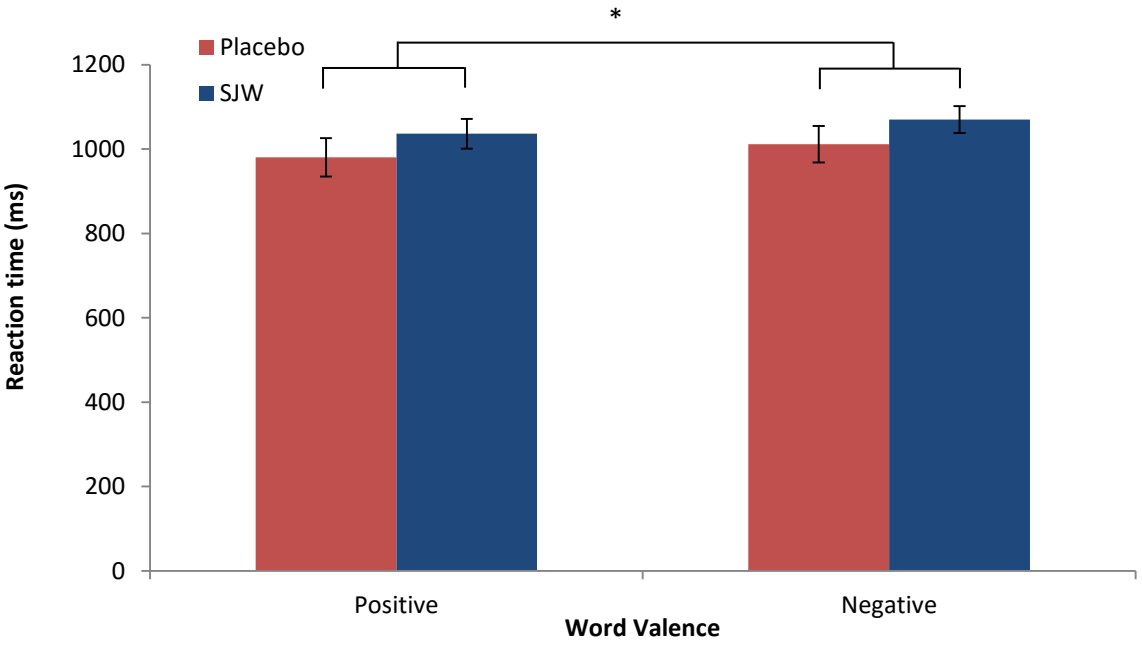

Figure 3.7 Reaction times for word categorisation
Mean reaction time to correctly categorise words as positive or negative. There was no significant effect of treatment or interaction effect. * $p < .05$. Error bars represent SEM.

recall of words. There was also no effect of stimulus type, $F(1, 43) = 0.98$, $p = 0.33$, suggesting that participants remembered equal numbers of positive and negative words.

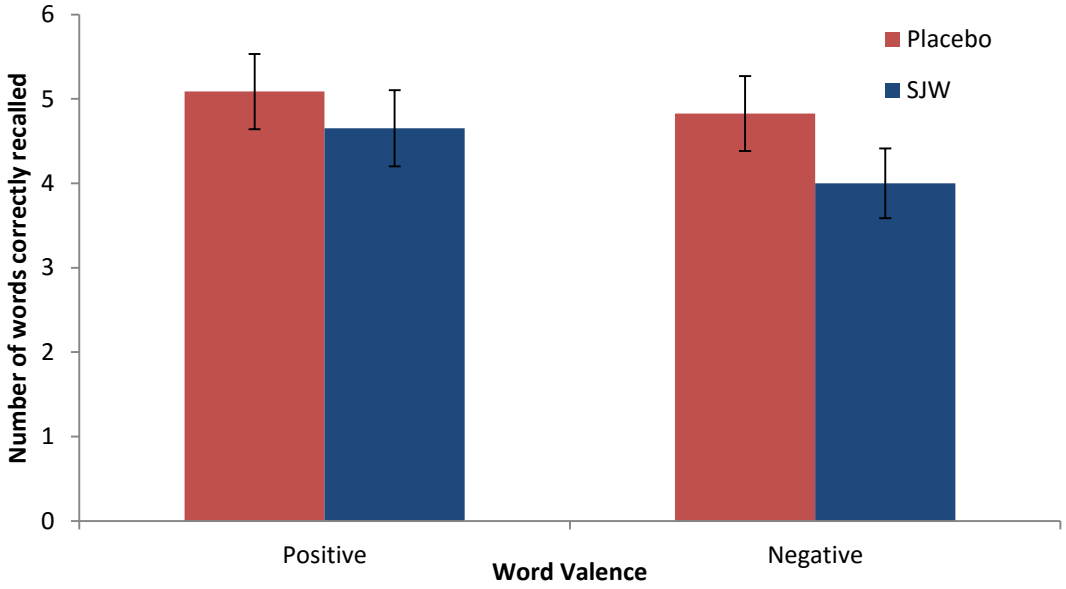

Figure 3.8 Correct recall of words
Mean number of positive and negative words correctly recalled. There were no significant effects. Error bars represent SEM.

Figure 3.9 shows the number of positive and negative intruder words – i.e. words that were "recalled" but which were not on the original list. A 2 (word valence) x 2 (treatment) mixed effects ANOVA was again used to investigate potential group differences. There was a significant effect of treatment, $F(1, 43) = 6.31$, $p < .02$, indicating that the SJW group recalled more words than the placebo group. There was also a main effect of stimulus, $F(1, 43) = 19.43$, $p < .001$, indicating that overall participants recalled more false positive words than false negative.

There was no significant interaction between treatment and stimulus type, $F(1, 44) = 2.42$, $p = .13$; however, given the significant main effects, coupled with the *a priori* hypothesis that St John's wort would increase recall of positive words, t-tests were conducted on each valence of stimuli to examine the specific influence of the drug on positive and negative stimuli. These revealed a significant effect of St John's wort on false positive recollections, $t(43) = 2.22$, $p < .05$, but not

false negative recollections, $t(43) = 1.18$, $p = .25$. This suggests that the main effect of group was driven largely by increased false positive recollection by the SJW group.

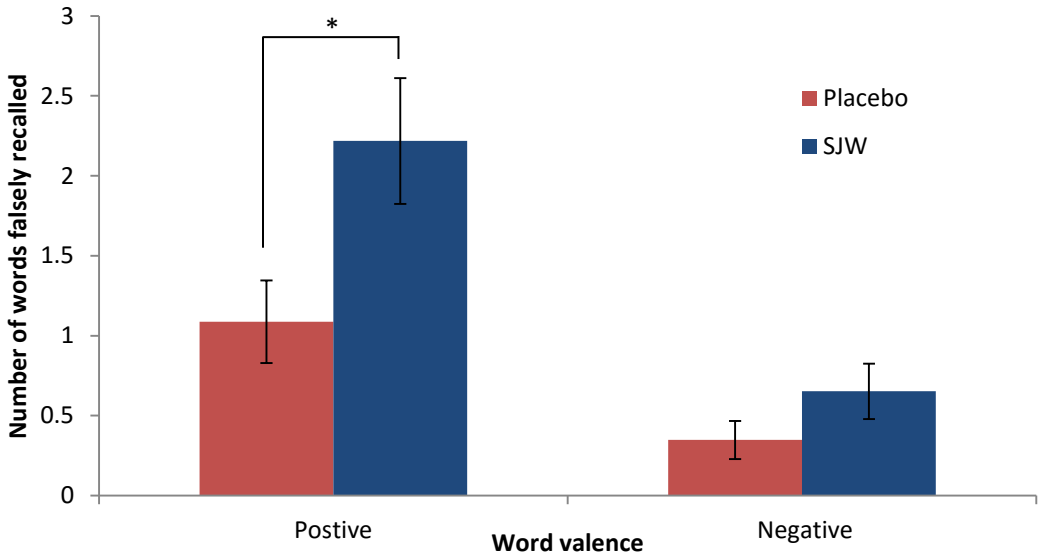

Figure 3.9 False recall of words
Mean number of positive and negative words falsely recalled. *$p < .05$. Error bars represent SEM.

Recognition

Data from one participant in the placebo group was excluded as they appeared to perform at floor. This may have resulted from incorrect placement of response buttons. This left $n = 23$ in each group.

Figure 3.10 shows the number of positive and negatively words correctly recognised for each group. A 2 (word valence) x 2 (treatment) ANOVA was conducted to see whether there were any significant differences between group. There was a main effect of valence, $F(1,44) = 32.50$, $p < .001$, indicating that overall, subjects correctly recognised more positive than negative words. The main effect of treatment approached significance, $F(1,44) = 3.09$, $p = .09$, and there was no interaction between treatment and word valence, $F(1,44) = 1.43$, $p = .24$.

Given the trend towards a group difference in the task, as well as the *a priori* hypothesis that St John's wort would increase recognition of positive emotional information, individual t-tests were conducted to further examine group differences in recognition. These found a significant effect of treatment on positive word recognition, $t(44) = 2.45$, $p < .05$, but not negative recognition, $t(44) = .61$, $p = .55$. Thus the SJW group seemed to be better at recognising positive, but not negative, words.

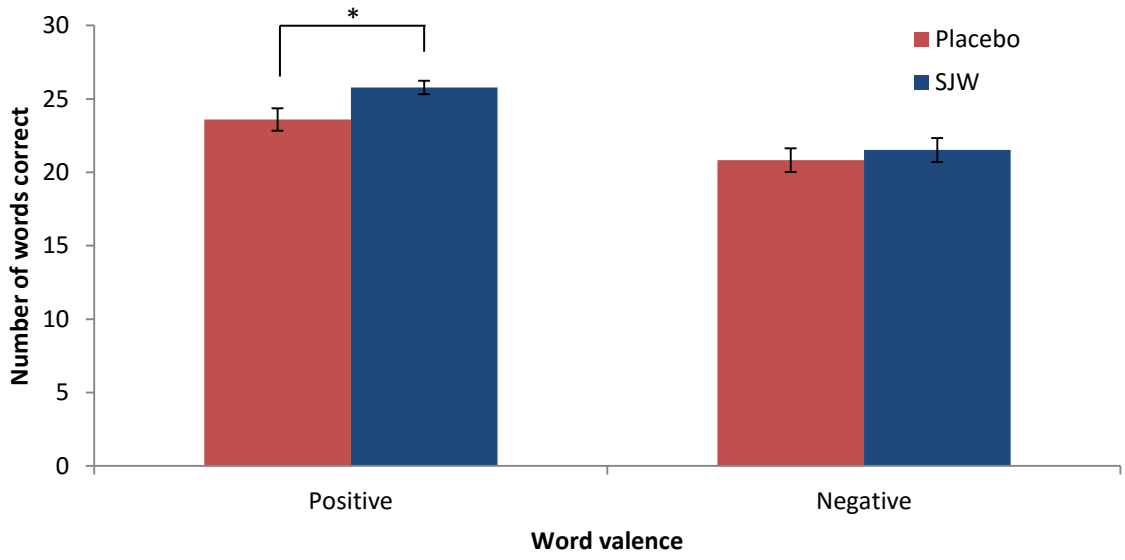

Figure 3.10 Correct recognition of words
Mean number of words (out of 30) correctly recognised. *$p < .05$. Error bars represent SEM.

As for the FERT, signal detection theory was used to calculate *d'* and *ß* for each group as an alternative measure of accuracy. For *d'* there was no main effect of valence, $F(1, 44) = 2.70$, $p = .11$, no effect of treatment, $F(1, 44) = .67$, $p = .42$, and no interaction, $F(1, 44) = .25$, $p = .62$.

For *ß* there was a main effect of valence, $F(1, 44) = 22.05$, $p < .001$, indicating that as a group, participants showed a less conservative response style towards positive words (*ß* = .02) than negative words (*ß* = .32). However, there was no effect of treatment, $F(1, 44) = 1.70$, $p = .20$, and no treatment x valence interaction, $F(1, 44) = 1.42$, $p = .24$.

Reaction times to correctly recognise positive and negative words as having been present in the original task are presented in Figure 3.11. There was a significant effect of valence, $F(1, 44) = 4.53$, $p < .05$, indicating that subjects were faster at recognising positive words. However, there was no effect of treatment, $F(1, 44) = .74$, $p = .39$, and no interaction between treatment and valence, $F(1, 44) = 1.32$, $p = .26$, providing no evidence that SJW affected speed to recognise positive or negative words.

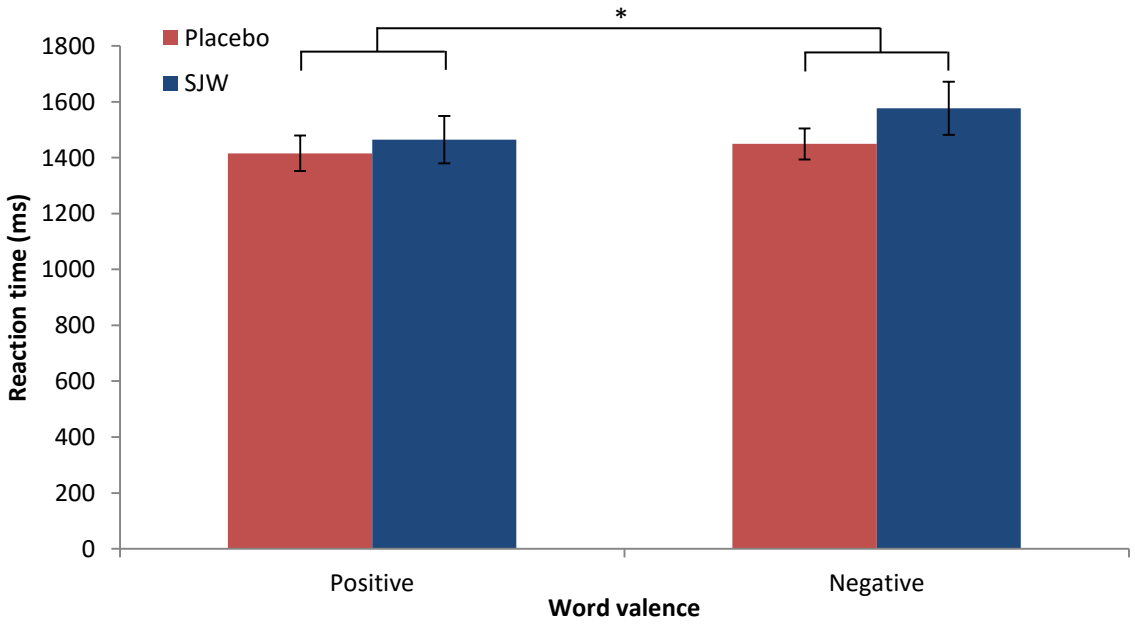

Figure 3.11 Reaction times for word recognition
Mean reaction times to correctly recognise positive and negative words as having been present in the previous tasks. There was no significant effect of treatment or interaction effect. * $p < .05$. Error bars represent SEM.

Words incorrectly recognised as familiar ("false alarms") are presented in Figure 3.12. Again, the only significant effect was one of valence, $F(1, 44) = 12.90$, $p < .01$, indicating that participants falsely recognised more positive than negative words. There was no effect of treatment, $F(1, 44) = 0.44$, $p = .51$, and no interaction between treatment and valence, $F(1,44) = .88$, $p = .35$, providing no evidence that SJW affected the number of falsely recognised positive or negative words.

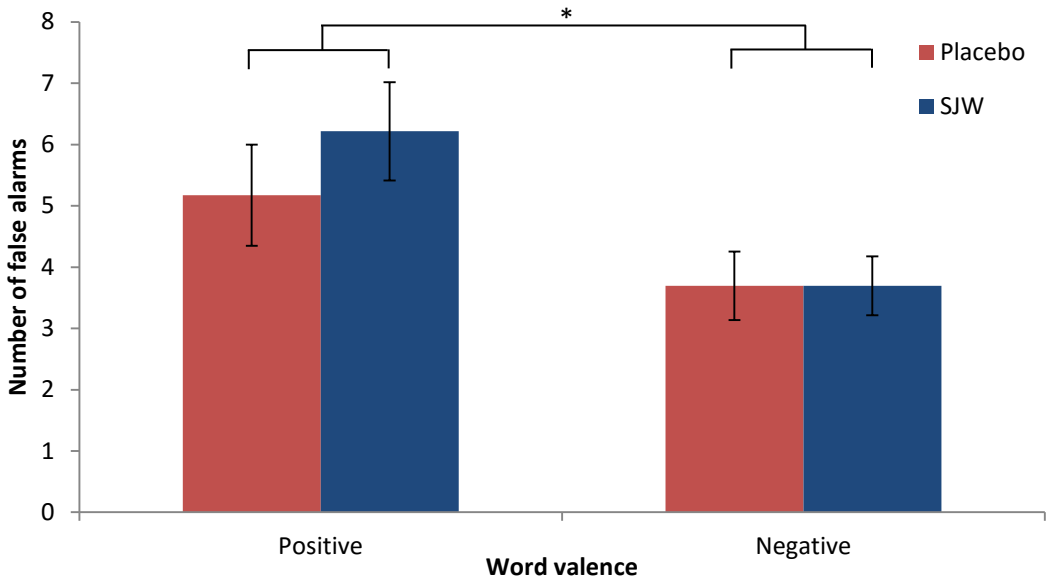

Figure 3.12 False word recognition
Mean number of positive and negative false alarms (out of a possible 30). There was no significant effect of treatment or interaction effect. * $p < .05$. Error bars represent SEM.

3.3.5 Dot Probe

Data from two participants was missing, leaving placebo $n = 22$ and SJW $n = 23$. All trials in which participants failed to correctly respond to the orientation of the dots were excluded. Median reaction times were calculated for responses to dots appearing after fearful and neutral faces in fear-neutral trials, and happy and neutral faces in happy-neutral trials. Attentional vigilance scores for happy and fearful faces were calculated for each participant by subtracting the median time taken to respond when dots were behind a neutral face from median time taken to respond when dots were behind a happy/fearful face. A higher value represents attentional vigilance towards the emotional face.

Results for the unmasked trials are presented in Figure 3.13. An ANOVA found no significant effect of valence, $F(1, 43) = 1.07$, $p = .31$. However, there was a significant effect of treatment, $F(1, 43) = 8.21$, $p < .01$, indicating that overall, those in the SJW group showed less vigilance to emotional faces. There was also an interaction between treatment and valence, $F(1, 43) = 7.73$, p

< .01, suggesting that SJW affected vigilance differently depending on the kind of expression. Post-hoc t-tests found a significant difference between groups for vigilance to fearful faces, $t(43) = 3.84$, $p < .001$, but not happy faces, $t(43) = .36$, $p = .72$. Thus the placebo group showed more of a vigilance towards fearful faces than the SJW group, who appeared to show vigilance away from fearful faces.

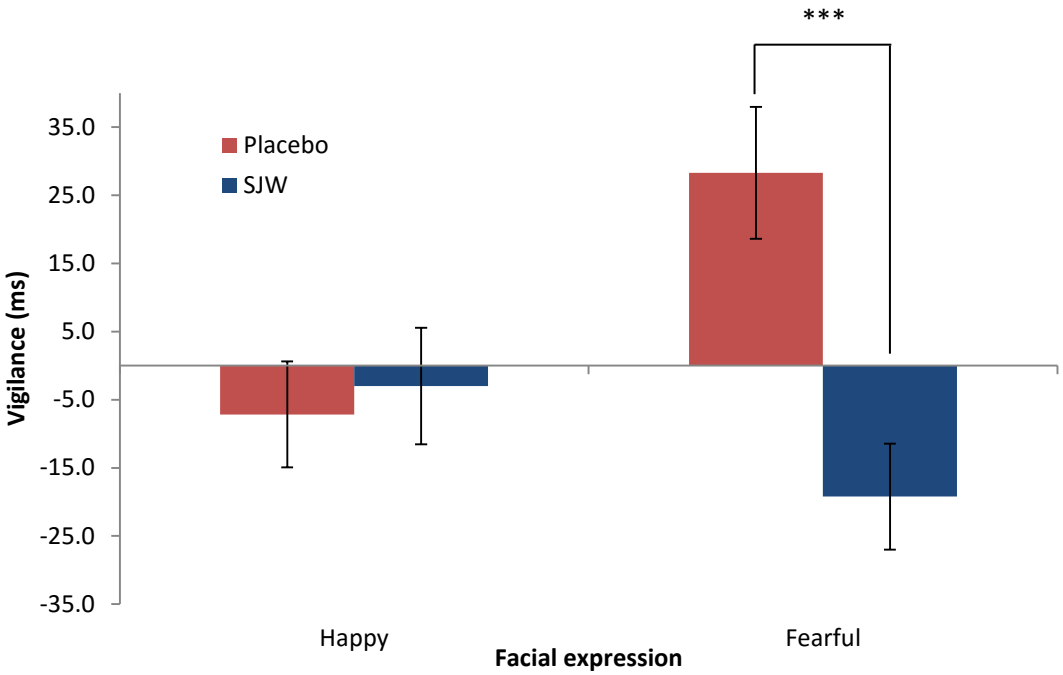

Figure 3.13 Emotional vigilance in unmasked trials
Group mean of individuals' median vigilance towards (positive values) or away from (negative values) happy and fearful faces in unmasked trials. ***$p < .001$. Error bars represent SEM.

Individual one-sample t-tests were conducted for each group, to determine whether the mean vigilance values for fearful faces were significantly different from zero. The placebo group mean differed significantly from zero, $t(21) = 2.92$, $p < .01$, demonstrating that this group did indeed have a bias towards fearful faces. The SJW group mean also differed significantly from zero, $t(22) = 2.47$, $p < .05$, demonstrating that this group did have a bias away from fearful faces.

Figure 3.14 displays median vigilance scores for masked trials. An ANOVA found no significant main effects of valence, $F(1,43) = 1.14$, $p = .29$, nor of treatment, $F(1,43) = .99$, $p = .33$. There

was also no interaction effect, $F(1,43) = .00$, $p = .98$. Thus there was no evidence that SJW affected attentional vigilance to masked faces.

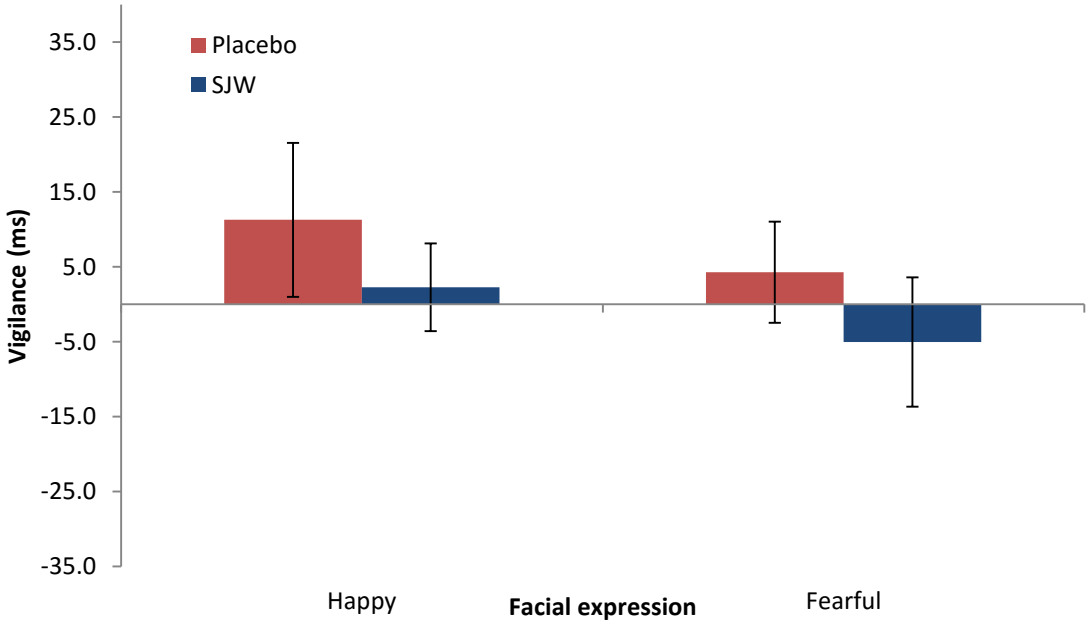

Figure 3.14 Emotional vigilance in masked trials
Group mean of individuals' median vigilance towards (positive values) or away from (negative values) happy and fearful faces in masked trials. There were no significant effects. Error bars represent SEM.

3.3.6 Emotion-potentiated startle

The data from seven participants were excluded for having poor signal-to-noise ratio in at least 50% of the trials (no signal or a baseline greater than a third of the amplitude of the peak). Data from a further six participants were excluded due to recording artifacts. Additionally, four participants did not complete the task: poor signal-to-noise ratio was detected during set-up for three of these participants, and one participant found the task too aversive to complete.

Data from 17 participants in the SJW group and 13 participants in the placebo group were analysed. On average, 7 out of the 45 trials were excluded for each participant due to noise or other artifacts.

Figure 3.15 displays the mean maximum eyeblink amplitude in response to the acoustic startle probe presented during neutral, pleasant and unpleasant pictures. There was a significant effect of valence, $F(2,56) = 4.90$, $p < .05$. Post-hoc comparisons with Bonferroni correction found a significant difference in eyeblink amplitude between neutral and unpleasant pictures, $p < .05$, but no significant difference between pleasant and unpleasant pictures, $p = .09$, nor neutral and pleasant pictures, $p = .96$. Participants therefore showed a greater eyeblink amplitude when viewing unpleasant pictures than neutral pictures.

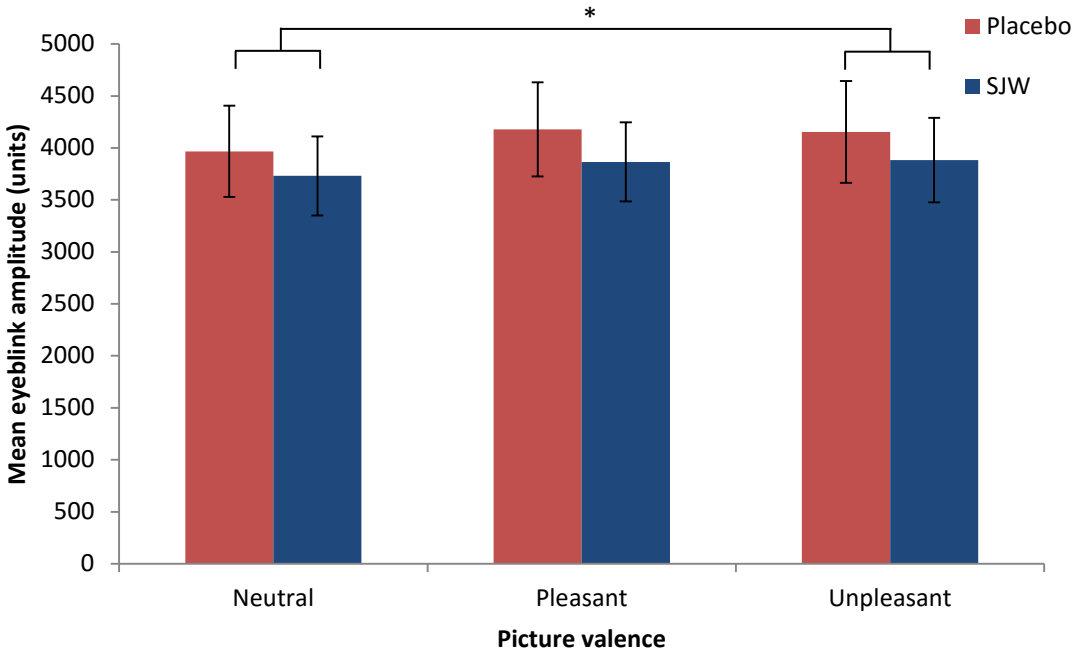

Figure 3.15 Maximum eyeblink amplitude to startle probe
Mean maximum eyeblink amplitude to an acoustic startle probe presented while viewing neutral, pleasant and unpleasant pictures. *$p < .05$. Error bars represent SEM.

In contrast, there was no main effect of treatment, $F(1,28) = 2.34$, $p = .14$, and no interaction between treatment and valence, $F(1,28) = .19$, $p = .66$. There was thus no evidence that SJW altered the pattern of startle response while viewing emotional stimuli.

Because there was a high degree of variability in maximum eyeblink amplitude between different participants, data were converted into z-scores. These z-scores are displayed in Figure 3.16. Again, there was a significant effect of valence, $F(2, 56) = 4.8$, $p < .05$. Post-hoc comparisons with Bonferroni correction found a significant difference between z-scores for neutral and unpleasant stimuli, $p < .05$, while the difference between pleasant and unpleasant pictures bordered on significance, $p = .05$. There was no significant difference between z-scores for neutral and pleasant pictures, $p = 1.00$. Participants therefore showed a greater startle blink response when viewing unpleasant pictures than neutral pictures, and a trend towards a similar

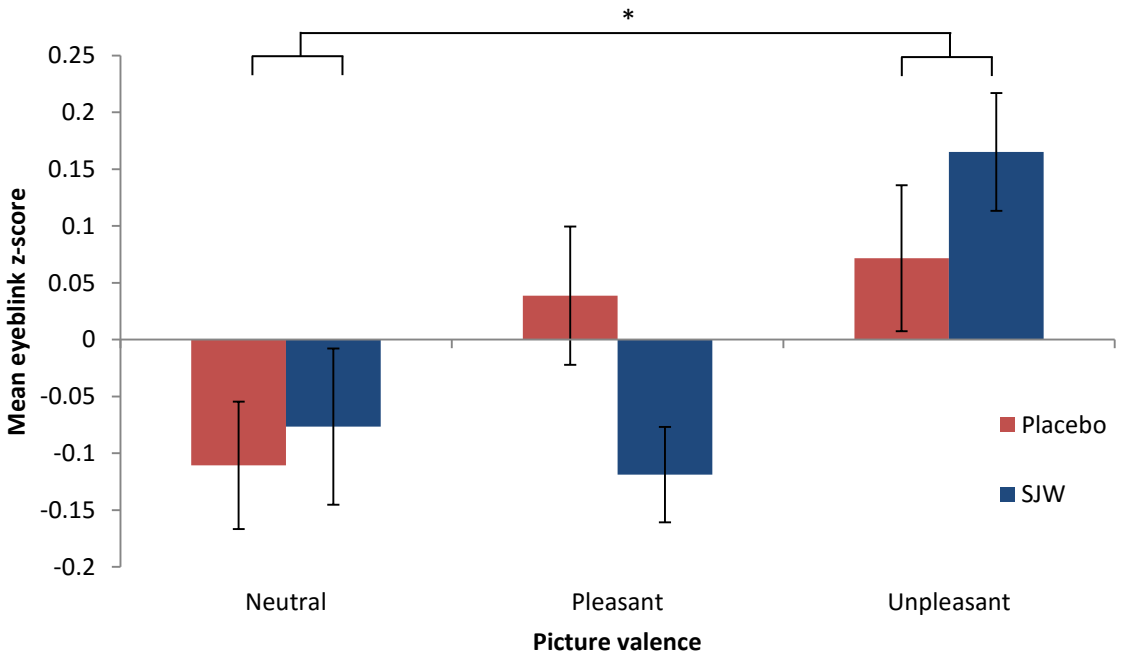

Figure 3.16 Maximum eyeblink amplitude to startle probe (z-scores)
Mean maximum eyeblink amplitude z-score to an acoustic startle probe presented while viewing neutral, pleasant and unpleasant pictures. *$p < .05$. Error bars represent SEM.

difference between unpleasant and pleasant pictures.

In contrast, there was no main effect of treatment, $F(1,28) = 2.12$, $p = .16$, and no interaction between treatment and valence, $F(1,28) = .15$, $p = .70$. As in the non-transformed data, there was thus no evidence that SJW altered the pattern of startle response while viewing emotional stimuli.

3.4 Discussion

Our study is the first to investigate how short-term treatment with St John's wort affects the processing of emotional information in healthy volunteers. We have found that after taking 900mg/day SJW for seven days, participants display changes in emotional processing very similar to those found as the result of other antidepressants, including reduced attention to unmasked fearful faces, reduced recognition of disgusted faces, and a positive bias in memory for self-referent words. On the other hand, SJW did not affect responses to masked faces or startle response to an acoustic probe.

3.4.1 SJW reduces processing of negative information

The most striking result we found was a clear reduction in attentional vigilance to unmasked fearful faces for participants taking SJW compared to those taking placebo. The placebo group displayed the vigilance towards fearful faces typically seen in healthy volunteers (Murphy, Yiend, et al., 2009). However, after taking SJW, this bias was reversed.

Heightened attention to threat-related information is associated with depression and anxiety, and is reduced by treatment with antidepressants. Patients with depression show a greater bias towards threat-related words presented within awareness than controls (Mogg, Bradley, & Williams, 1995), and the SSRI citalopram can reduce attentional bias towards unmasked fearful

faces (Murphy, Yiend, et al., 2009). The reduction in attentional bias towards unmasked fearful faces with SJW is therefore consistent with its antidepressant effect.

We also found that SJW reduced the recognition of disgusted facial expressions. Until recently, disgust recognition in depression has not been closely examined, and the literature remains equivocal. One study found that participants with major depression showed a specific deficit in recognising disgust faces on a modified version of the Facial Expression Recognition Task (Douglas & Porter, 2010). However, another study found that depressed patients had greater activity to disgust faces than healthy controls in left frontal-temporal regions including the insula, as well as right middle/inferior temporal regions (Surguladze et al., 2010).

The authors of this study suggest that increased activity to disgust faces might relate to heightened processing of cues relating to social rejection in depressed populations. Our findings of a reduced recognition of disgust may therefore reflect reduced processing of such cues with SJW treatment. Consistent with this interpretation, a number of studies have found reduced recognition of disgust following antidepressant treatment: both seven days of citalopram and 14 days of tryptophan reduced recognition of disgust in healthy volunteers (Harmer et al., 2004; Murphy et al., 2006).

However, reduced recognition of disgust has not been consistently found following antidepressant treatment. One study found that citalopram and reboxetine both *increased* disgust processing after 2 and 6 weeks (Tranter et al., 2009), and a number of studies have failed to find any effect of antidepressant treatment on recognition of disgust (Harmer, Bhagwagar, et al., 2003; Harmer et al., 2009).

Another possibility is that the broader neurochemical changes produced by SJW have a specific effect on disgust recognition not consistently seen with other antidepressants. In particular, the

dopaminergic effects of the drug may be important: disgust recognition is reduced in disorders associated with dysfunctional dopamine activity such as Parkinson's disease and Huntington's disease (Sprengelmeyer et al., 1996; Sprengelmeyer et al., 2003). It would be interesting to see whether other dopaminergic antidepressants such as bupropion have any specific effects on disgust recognition.

3.4.2 **SJW increases memory bias for positive words**

We also found that SJW affected memory for positive, but not negative, words. Specifically, 15 mins after a categorisation task, SJW increased the number of positive words incorrectly recalled in a surprise recall task, and increased the number of positive words correctly recognised amongst novel distractor words in a recognition task.

It should be noted that in both cases, these effects were only found in post-hoc t-tests: in neither case was there a significant interaction between treatment group and valence of word. These results should therefore be interpreted cautiously. However, previous research has specifically found effects of antidepressants on positive word memory, but very few studies have shown negative word memory to be affected (see below). It was therefore deemed suitable to conduct analyses specifically on the positive words, to identify small effects that might be obscured when negative words were also included in the analysis.

A number of studies have found that recall of positive words is influenced by antidepressant drugs. Seven days of citalopram and reboxetine both increased recall of positive words in healthy controls (Harmer et al., 2004), as did an acute dose of mirtazapine (Arnone et al., 2009), although at acute doses citalopram may not produce effects on memory (Browning et al., 2007). While we did not find an increase in correct recall *per se*, we did see an increase in false recall of positive words, an effect also seen after an acute dose of duloxetine (Harmer et al., 2008). None of these

studies found any effects on negative memory; in fact, only one study has provided any suggestion that an antidepressant – in this case reboxetine - could reduce recall of negative words (Harmer, Hill, et al., 2003). Effects of antidepressants on recognition memory have not been as consistent, but again when effects have been found, they have been involved increasing recognition accuracy or speed for positive words only (Harmer et al., 2009; Miskowiak, Papadatou-Pastou, et al., 2007). The effects of SJW on memory for positive, but not negative, words seem consistent with effects seen with other antidepressants, especially at subchronic doses.

3.4.3 **SJW does not modulate performance during more "automatic" processing**

We found that our sample displayed the normal emotion-potentiated startle response (Lang, Davis, & Öhman, 2000): participants' eye-blink response to an acoustic probe was greater when viewing unpleasant pictures than neutral pictures, while a similar difference between unpleasant and pleasant pictures also bordered on significance. However, seven days of SJW did not affect the magnitude of this eye-blink. In contrast, seven days of citalopram reduced the potentiation of the startle response during negative pictures (Harmer et al., 2004).

It has been suggested that, in contrast to the other more elaborative tasks that we used, the startle response may particularly reflect automatic processing of emotional information, and as such may be more relevant to anxiety-related mechanisms than depression-related ones (Pringle, Browning, Cowen, & Harmer, 2011). Indeed, other anxiolytics also reduce the startle response (Arnone et al., 2009; Murphy et al., 2008), while drugs which are not effective anxiolytics, such as SNRIs, do not (Harmer et al., 2004). Given that there is currently little evidence that SJW is an effective anxiolytic (Kobak, Taylor, Warner, et al., 2005), it is perhaps unsurprising that we failed to find any effect of the drug on startle response.

It should be noted that due to a large amount of noise in the data, we had to exclude a third of our participants from the analysis of the startle data. Our study may therefore be underpowered to detect any differences between the two groups. For example, there is a trend towards a decrease in eye-blink response to pleasant faces for the SJW group, which may have become significant with a larger sample. Although there is little sense in speculating about effects that could be seen with more power, it would be useful to conduct another study with a larger group to determine whether SJW really does have no effect on the startle response

The lack of any anxiolytic effect of SJW may also go some way to explaining why there was no effect in the masked faces dot probe, despite the clear results for the unmasked faces. It has been suggested that attention towards stimuli presented for very short periods of time and/or subliminally may be particularly related to anxiety-relevant processes: indeed, patients with anxiety, but not depression, showed a bias to threat-related words presented subliminally (Mogg et al., 1995), and patients with panic disorder show a bias towards fearful faces in the masked but not unmasked condition (Reinecke, Cooper, Favaron, Massey-Chase, & Harmer, 2011). While it seems likely that, with a presentation time of only 100ms, the unmasked faces in our study also tap into the immediate, automatic processes relevant to anxiety, the masked faces, presented subliminally and for an even shorter time, may do so more exclusively.

Taken together, our results suggest that subchronic treatment with SJW produces a positive shift in the processing of emotional information. Importantly, these effects occur in the absence of any changes in mood or anxiety, and in a healthy population that would be unlikely to experience any psychological benefit from taking the drug. They lend support to the neuropsychological model of antidepressant mechanism by demonstrating that these kinds of early, psychological changes are present not only in SSRIs or SNRIs, but also in an as yet unstudied antidepressant with a novel mechanism of action.

Chapter 4

St John's wort and cognition

4.1 Introduction

In the previous chapter we found that SJW produced a positive bias in the way in which people responded to emotional information. However, it may be that SJW also produces other changes in cognition which are not directly related to emotional processing. The presence of these changes would be interesting in itself, but it is also important to disentangle any effects of SJW on cognition from those on emotional processing *per se* in order to be confident in our findings in Chapter 3. We therefore looked at how SJW affected working memory as well as response to reward.

4.1.1 SJW and memory

A number of *in vivo* studies have suggested that SJW extracts could improve memory. Extracts of SJW have been found to counteract the negative effects of chronic stress on spatial working memory in rats. Chronically-stressed rats which were pretreated with SJW extracts showed superior performance on measures of spatial memory including the Barnes maze and Morris water maze compared to those treated with vehicle (Trofimiuk & Braszko, 2008; Trofimiuk et al., 2005; Widy-Tyszkiewicz, Piechal, Joniec, & Blecharz-Klin, 2002). SJW-treated animals also showed increased levels of molecular markers of neurogenesis, suggesting a possible mechanism for these learning effects (Trofimiuk, Holownia, & Braszko, 2011). Importantly, SJW improved memory even in unstressed rats, suggesting that these effects generalised to a broader population.

SJW has also been suggested to protect against memory impairments in Alzheimer's disease. In mouse models of Alzheimer's, both acute and chronic administration of hyperforin appeared to protect against the deleterious effects on spatial working memory, as well as reduce expression of

molecular markers of the disease (Dinamarca, Cerpa, Garrido, Hancke, & Inestrosa, 2006; Hofrichter et al., 2013). Positive effects of SJW have also been found in longer-term measures of memory. SJW-treated rats were faster to learn and showed better memory retrieval in a passive avoidance test (Hasanein & Shahidi, 2011; Khalifa, 2001; Trofimiuk, Walesiuk, & Braszko, 2006). Finally, chronic administration of SJW extracts reduced age-related impairments on spatial working memory (Trofimiuk, Holownia, & Braszko, 2010) and passive avoidance retrieval (Trofimiuk & Braszko, 2010).

Despite the number of studies showing a beneficial effect of SJW extracts in rodent models, the few studies examining the effects of the drug on human memory have not been so positive. An acute dose of 900mg SJW had no effects on spatial or numeric working memory, word recall or recognition, or picture recognition, while an 1800mg dose actually impaired numeric working memory and picture recognition (Ellis et al., 2001). Similarly, neither 14 days (Siepmann, Krause, Joraschky, Muck-Weymann, & Kirch, 2002) nor 10 weeks (Camfield et al., 2013) of SJW treatment affected spatial working memory. This lack of effects on memory in human participants is surprising given the animal findings and the fact that SJW modulates a number of neurotransmitters with a key role in learning and memory (Müller, 2003; Wonnemann et al., 2000). However, the literature has tended to focus on either acute or long-term dosing, and few studies have examined subchronic doses. Examining short-term SJW treatment is therefore necessary for a fuller picture of the effects, if any, of the drug on human memory.

4.1.2 **SJW and reward learning**

The modulation of dopamine transmission by SJW suggests that the drug could affect reward learning. Dopamine (DA) plays a fundamental role in learning about reward. Prediction error theory suggests that mid-brain dopamine neurons signal the difference between expected reward and received reward after a behaviour is performed in response to a stimulus. The greater the

received reward is compared to the predicted reward, the greater the activation of DA neurons will be. These neurons therefore provide a "teaching signal" about the reward value of a stimulus, sending this information on to higher-level areas involved in goal-directed behaviour such as striatum and frontal cortex, influencing future behaviour in response to the stimulus (Schultz, 1998; Schultz, Dayan, & Montague, 1997).

This theory is supported by studies showing that animals have strong activation of midbrain DA neurons when the first reward is given in response to a behaviour towards a stimulus (e.g. receiving a treat after choosing the "correct" picture out of two options). However, with further trials, activation of these neurons decreases as the animals learn the reward value of the stimulus (Bayer & Glimcher, 2005; Hollerman & Schultz, 1998).

Human imaging studies have similarly found that midbrain structures within the reward system, such as ventral striatum, encode prediction errors (Pagnoni, Zink, Montague, & Berns, 2002). Importantly, pharmacological manipulation of dopamine transmission affects both behavioural and neural responses to reward. Enhancing dopaminergic activity improves performance in an instrumental learning task (Pessiglione, Seymour, Flandin, Dolan, & Frith, 2006). Compared to participants given the DA antagonist haloperidol, participants given the DA precursor L-DOPA became better at choosing a stimulus with a higher probability of winning money over a stimulus with a lower probability of winning. Moreover, BOLD response in the striatum was enhanced to reward under L-DOPA, and using BOLD response in this area as a measure of prediction error, participant's behaviour could be accurately modelled. That is, it seemed that enhancing DA transmission with L-DOPA increased the magnitude of prediction error as represented in the striatum, which led to improved decisions regarding the reward.

On the other hand dopamine antagonists have been shown to impair reward learning and reduce prediction error signals in the striatum (Jocham, Klein, & Ullsperger, 2014). Similarly, 0.5mg of

the DA agonist pramipexole, which actually works to inhibit DA release at such a low dose, impaired probabilistic reward learning (Pizzagalli et al., 2008).

The broad acting effects of hyperforin on neurotransmitter systems include inhibition of dopamine reuptake (Nathan, 2001; Yoshitake et al., 2004); indeed, DA reuptake inhibition by SJW extracts has been demonstrated specifically in the striatum both *in vivo* and in rodent brain slices (Di Matteo, Di Giovanni, Di Mascio, & Esposito, 2000; Ruedeberg, Wiesmann, Brattstroem, & Honegger, 2010) . This has led to suggestions that SJW could potentially be used to treat disorders which are mediated by reward pathways, such as substance addiction (Rezvani, Overstreet, Yang, & Clark, 1999; Ruedeberg et al., 2010). However, to our knowledge, no research to date has examined the effect of SJW on reward processing in humans.

4.1.3 **Current study**

In the current study we therefore examined whether seven days of SJW affected working memory or performance in a reward task in healthy volunteers. Given the lack of positive findings in human participants, despite promising evidence from animal studies, we did not expect SJW to have a beneficial effect in numerical working memory during an n-back task. On the other hand, given SJW's demonstrated modulation of dopaminergic transmission, and the fundamental role of that neurotransmitter system in reward learning, we predicted that SJW would improve performance on a reward task.

4.2 Method

The cognitive tasks were conducted in the same study as in Chapter 3; methods are identical to that chapter. N-back and reward tasks were completed on the test day and are described below.

4.2.1 **Tasks**

N-back working memory

In the n-back task, participants were presented with a series of upper- and lower-case letters, and were instructed to indicate whether or not each letter was the same or different from a previously presented letter. The letter they were required to use for comparison was dependent on the block of the task: in the one-back blocks they had to compare each letter to the one previously presented, in the two-back blocks they had to compare each letter to the one presented two letters previously, and in the three-back to the letter presented three letters previously. The case of the letter was irrelevant. There was also a zero-back block, in which participants simply had to indicate whether or not each letter was an *X*.

A block began with instructions (e.g. "One-back"), and when participants were ready to begin they pressed the space bar. A series of 10 letters would then appear sequentially, each letter presented for 500ms, with an ITI of 1500ms. Reaction times and accuracy of participants' responses to each letter were recorded. The four block types each occurred four times during the experiment, resulting in a total of 16 blocks.

Reward task

In each trial of the reward task, participants were presented with a pair of symbols, each of which was associated with a likelihood of winning or losing 50p, or resulting in no change in earnings. There were two types of trial, a "win" trial, and a "lose" trial. In the win trial, symbol A and B were always presented. A was associated with a 70% chance of winning and 30% chance of no change, and B was associated with a 70% chance of no change and a 30% chance of winning. In the lose trial, symbol C and D were always presented. C was associated with a 70% chance of losing and 30% chance of no change, while D was associated with a 70% chance of no change

and 30% chance of losing. The optimal choices would therefore be A in the win trials and D in the lose trials. Participants were simply told that they should try and win as much money as possible, and that they would receive this money in addition to their reimbursement; no further information about the nature of the task or the probabilities behind it was supplied.

The procedure for each trial is displayed in Figure 4.1. Participants began the task with £5. Each trial began with a fixation cross for 1s, after which the two symbols were displayed side by side. Participants were required to choose one of the two by pressing the corresponding key on the keyboard; symbols remained on the screen until participants had made their decision. After making their decision, the symbol that they chose was highlighted for 1s, followed by a screen giving feedback, indicating whether they had won 50p, lost 50p or neither. The feedback lasted 1.5s, after which the next trial began.

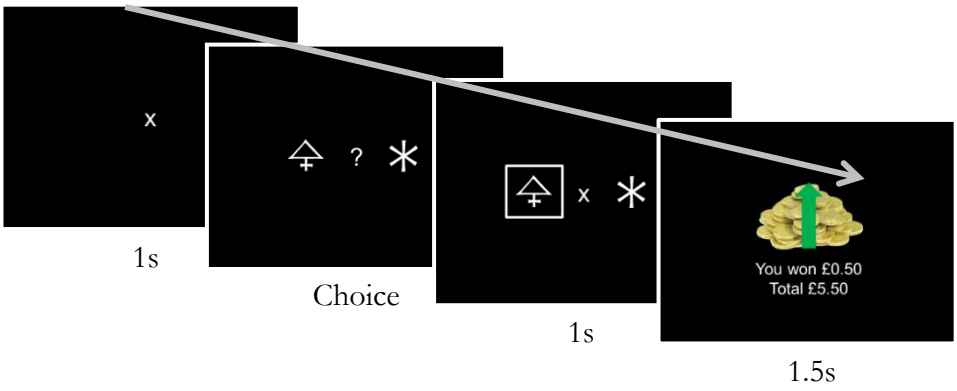

Figure 4.1 Procedure for a trial of the reward task

The task began with a short example to ensure participants understood the instructions. This consisted of only 10 trials. The main task included two blocks, each consisting of 60 trials (30 "win"; 30 "lose"). Trials were presented in a fixed random order, with the limitation that there were never more than two trials of the same type (i.e. "win" or "lose") in a row. The second block was exactly the same as the first, but the symbols used were different.

4.3 Results

4.3.1 N-back

Accuracy

Accuracy was calculated for each block of the task for each participant. This was calculated as total number correct/total number of trials for each block. Mean accuracy for each block was then calculated for each group. Results are displayed in Figure 4.2.

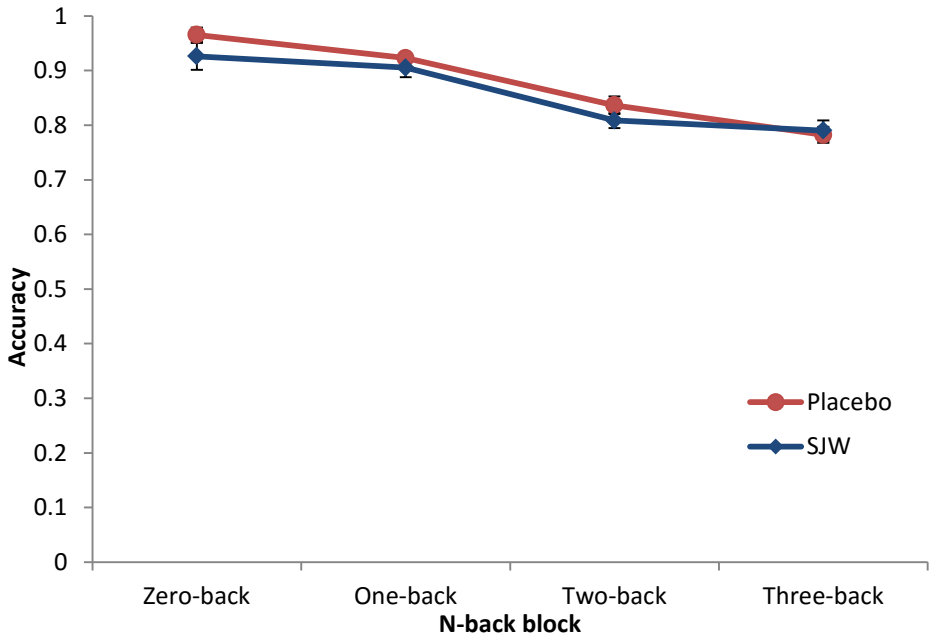

Figure 4.2 Accuracy in n-back task
Accuracy at classifying letters as same/different for each block of the task. Participants overall were less accurate at later blocks; there was no significant effect of treatment or interaction effect. Error bars represent SEM.

A 2 (treatment) x 4 (task block) mixed effects ANOVA was used to examine whether SJW affected accuracy at responding to stimuli. There was no significant effect of treatment, $F(1, 45) = 1.99$, $p = .17$, and no significant interaction between treatment and task block, $F(3, 135) = 1.30$, $p = .28$, providing no evidence that SJW had affected accuracy on the task.

There was a significant effect of task block, $F(3,135) = 71.63$, $p < .001$. Pairwise comparisons with Bonferonni correction found significant differences between all blocks ($p < .01$) except

between zero-back and one-back (p = .13). Thus participants were most accurate for zero- and one-back, followed by two-back, and were least accurate for three-back.

To further investigate possible group differences in accuracy, signal detection theory was used to calculate *d'* and β individually for "same" responses and "different" responses (see Chapter 3 for details on how these are calculated). For *d'*, results were exactly the same as in the raw accuracy data: there was no effect of treatment on "same" responses, $F(1, 45) = 2.26$, p = .14, and no block x treatment interaction, $F(3, 135) = .50$, p = .68. There was a main effect of task block, $F(3,135) = 55.09$, $p < .001$, with pairwise comparisons showing significant differences between all blocks except zero- and one-back. For "different" responses there was no main effect of treatment, $F(1, 45) = 1.79$, p = .19, and no treatment x task block interaction $F(3, 135) = .28$, p = .84. A main effect of task block, $F(3,135) = 44.15$, $p < .001$, was again driven by significant differences between all blocks except zero- and one-back.

For β, there was no effect of treatment on "same" responses, $F(1,45) = 2.18$, p = .15 and no significant interaction between treatment and task block, $F(3,135) = 1.92$, p = .13. There was a main effect of task block, $F(3, 135) = 10.36$, $p < .001$, with pairwise comparisons showing that β was significantly higher for the zero back than any other block. For "different" responses there was no main effect of treatment, $F(1,45) = 1.48$, p = .23 and no significant interaction between treatment and task block, $F(3,135) = 1.23$, p = .30. There was a main effect of task block, $F(3,135) = 6.97$, $p < .001$, with pairwise comparisons again showing that β had a significantly higher absolute value for the zero-back than any other block. Therefore it seemed that participants had a stricter criterion for making a decision during zero-back trials than during the other task blocks, but this was not affected by SJW.

Reaction times

Reaction time data was calculated for correct trials only: incorrect trials or trials in which no response was given were excluded. From the correct trial reaction times, the mean and standard deviation was calculated for each version of the task for each participant. These were then used to calculate outliers for each version for each participant (values more than 3 standard deviations away from the mean).

The means were recalculated with outliers removed. A 2 (treatment) x 4 (task block) mixed effects ANOVA was used to examine whether SJW affected reaction time in correct responses to stimuli. There was no significant main effect of treatment, $F(1, 45) = .10$, $p = .76$, and no interaction between treatment and block, $F(3, 135) = .74$, $p = .53$, indicating that SJW had no effect on reaction times.

There was a significant effect of block, $F(3, 135) = 63.80$, $p < .001$. Pairwise comparisons with Bonferonni correction indicated that RTs were significantly different between all conditions ($p < .001$), apart from between two- and three-back conditions ($p = 1.00$). As a group, participants

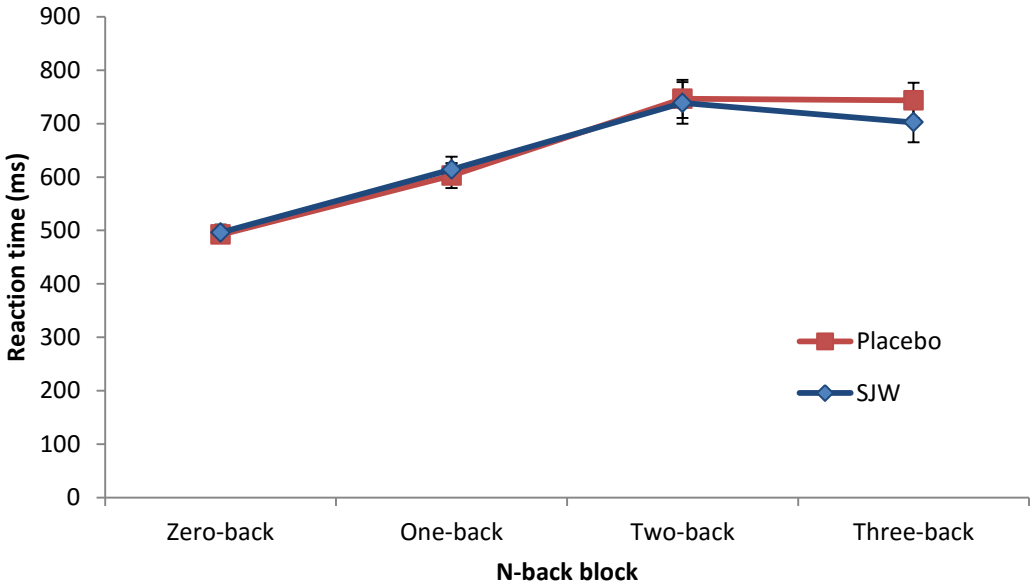

Figure 4.3 Reaction times in n-back task
Reaction time to classify letters as same/different for correct trials only. Participants overall were faster for earlier blocks; there was no significant effect of treatment or interaction effect. Error bars represent SEM.

were therefore fastest at the zero-back, then the one-back, and were slowest at the two- and three-back (see Figure 4.3).

4.3.2 **Reward task**

The data from one participant was removed from analysis as they had mentioned after the task that they had not understood the instructions, and behavioural results showed that they had simply chosen the same symbol throughout the entire task. This left 23 participants in each group.

Accuracy - Overall

Overall accuracy for each block and each kind of trial (win and lose) was recorded for every participant. A "correct" choice was defined as choosing the symbol associated with a higher probability of winning money (win trials) or a lower probability of losing money (lose trials). Figure 4.4 shows accuracy for win and lose trials.

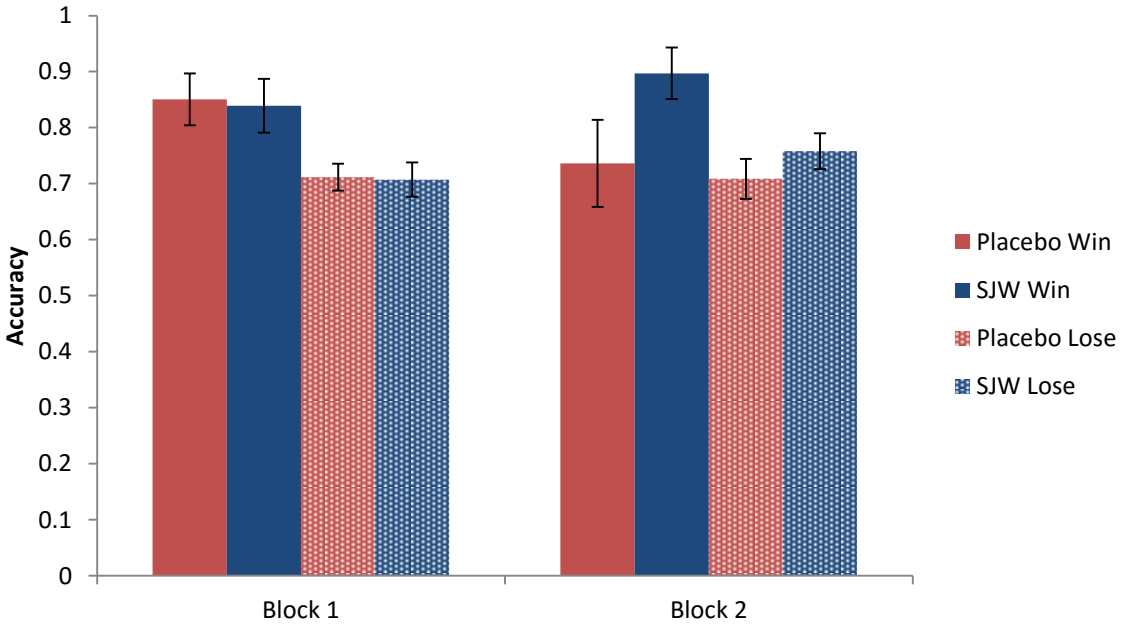

Figure 4.4 Accuracy in the reward task
Proportion of "win" trials in which the stimulus associated with a higher probability of winning money was chosen, and proportion of "lose" trials in which stimulus associated with a lower probability of losing money was chosen. Participants were more accurate at win than lose trials; there was no significant effect of treatment or interaction effect. Error bars represent SEM.

A 2 (block) x 2 (trial type) x 2 (treatment) mixed effects ANOVA was run to see whether there were any group differences in accuracy. There was a main effect of trial type, $F(1, 44) < .001$, indicating that participants made more accurate decisions during win trials than lose trials. However there was no significant effect of treatment or block, and no interactions between any of the variables (*p*s > .05), providing no evidence that SJW modulated accuracy at choosing rewarding or avoiding losing stimuli in the task.

As can be seen in Figure 4.4, the placebo group in block 2 performed numerically worse in win trials that the SJW group, although this difference was not significant. They also show a much higher variance than any other group, as evidenced by the error bars. In order to examine this in more detail, we plotted out a scatter plot with all individual values for the win trials. This is displayed in Figure 4.5.

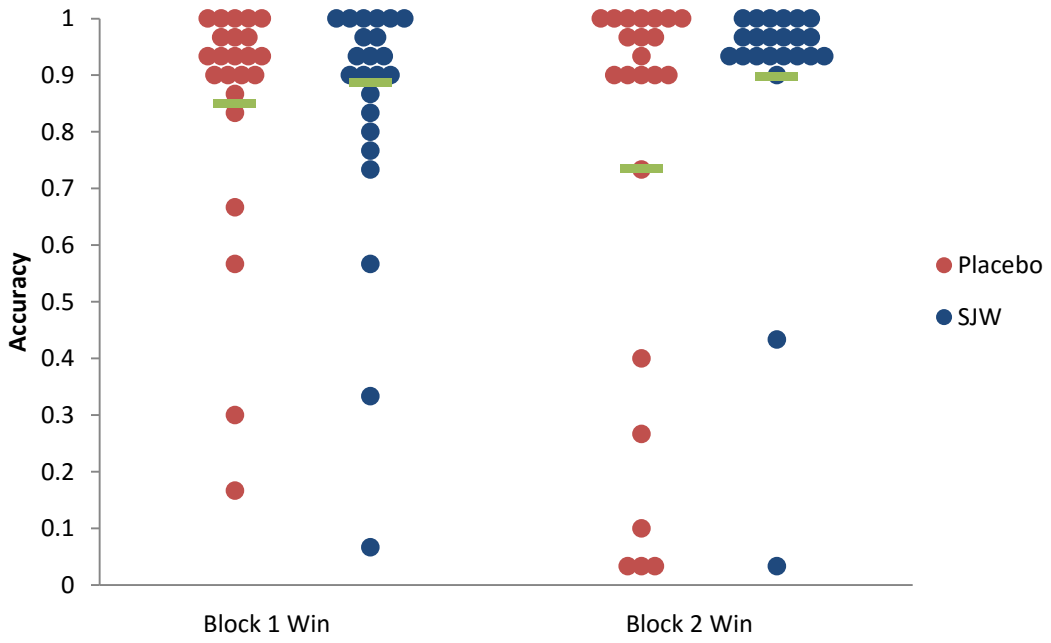

Figure 4.5 Scatterplot of correct win trials
Green bars represent means. Several placebo participants perform at floor in block 2.

This figure shows that the placebo group in block 2 had a number of participants performing near floor. The reason behind this poor performance is not entirely clear: the same participants performed well in block 1, suggesting that they understood the task and there were no problems

in the set up. However, it seems that the apparent trend for improved performance in the SJW group in block 2 is in fact produced by poor performance in a few individuals in the placebo group.

Accuracy – Trial by Trial

Examining accuracy on a trial-by-trial basis could reveal more specific effects that are obscured when accuracy is examined in a block as a whole. Therefore we plotted learning curves illustrating how accuracy changed as the task progressed. These are shown in Figure 4.6 for block 1 (top) and block 2 (bottom). Note that in the graph accuracy is inversed for the lose trials for ease of displaying the data.

Two 2 (treatment) x 2 (block) x 30 (trial) ANOVAs were conducted for win and lose trials separately, to determine whether the drug influenced learning across trials. For win trials, there was a main effect of trial, $F(29, 1276) = 9.53$, $p < .001$; pairwise comparsions with bonferonni correction revealed that this effect was driven mainly by differences between the first trial and all but one subsequent trials, and between the second trial and a few trials in the 20s. There was no main effect of block, $F(1,44) = 2.40$, $p = .63$, and no block x trial interaction, $F(29, 1276) = .60$, $p = .96$. There was no main effect of treatment, $F(1, 44) = 1.99$, $p = .17$, treatment x block interaction, $F(1, 44) = 2.13$, $p = .15$, or treatment x trial interaction, $F(29, 1276) = 1.22$, $p = .19$. However, the block x trial x treatment interaction just reached significance, $F(29, 1276) = 1.48$, $p = .05$.

In order to examine this interaction in more depth, 2 (treatment) x 30 (trial) ANOVAs were run on the win trial data for each block separately. For block 1, the only significant effect was that of trial, $F(29, 1276) = 4.98$, $p < .001$; the main effect of treatment and treatment x trial interaction

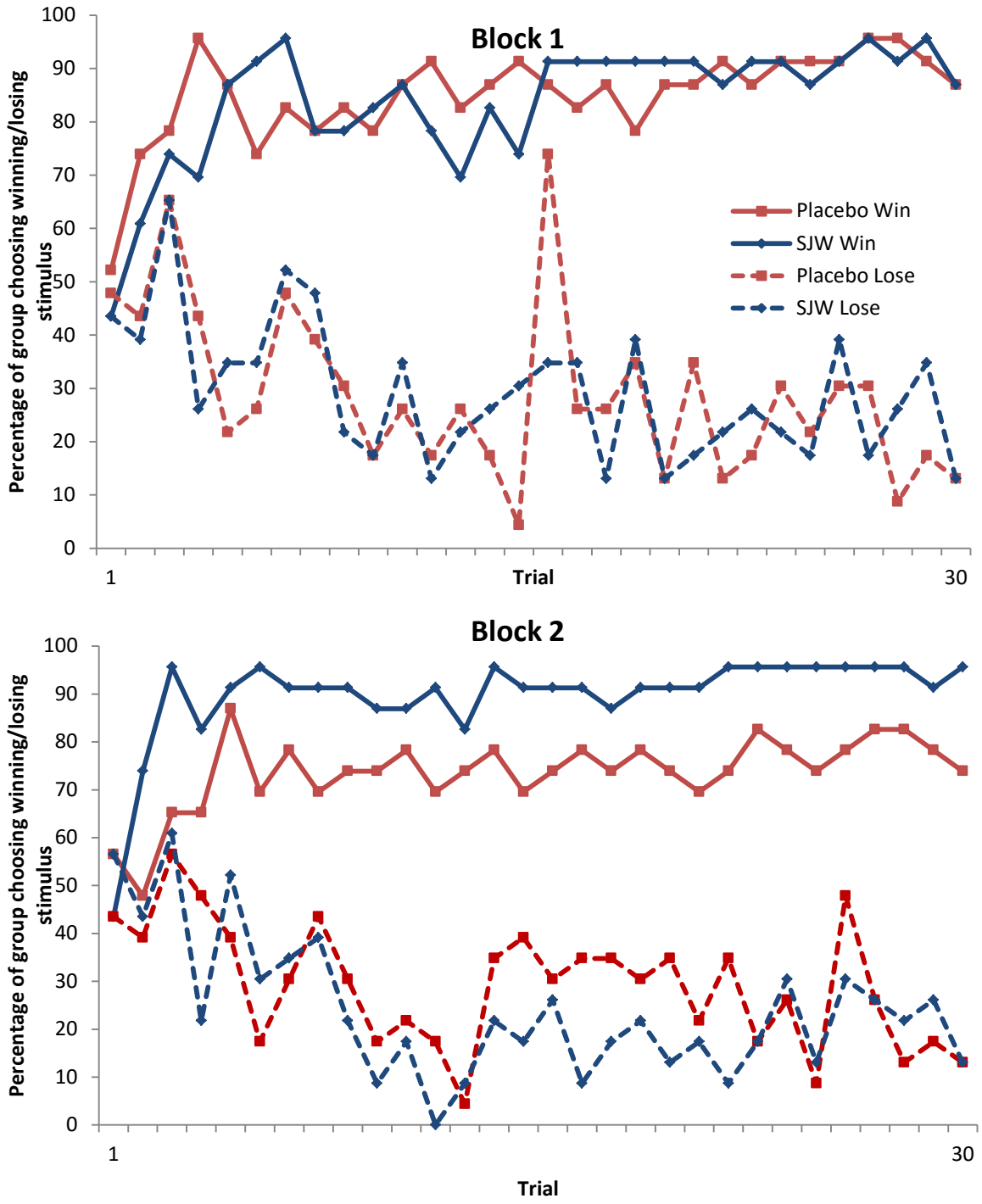

Figure 4.6 Learning curves plotted for the reward task
Learning curves illustrate the percentage of each group choosing the winning stimulus in win trials (solid lines) and losing stimulus in lose trials (dashed lines). *Top*: block 1; *bottom*: block 2.

were not significant (*p*s > .05). For block 2, there was a significant effect of trial, $F(29, 1276) = 6.02$, $p < .001$, but the effects of treatment ($p = .08$) and treatment x trial interaction ($p = .06$) did not quite reach significance. Therefore the block x trial x treatment interaction failed to provide clear results when interogated further. Again, it seems likely that any apparent effects are the result of poor performance in a select few placebo participants in block 2: these effects will be particularly prominent in later trials, where most other participants have learned which symbol to choose, but these participants are still consistently choosing the wrong symbol.

For lose trials, the 2 (treatment) x 2 (block) x 30 (trial) ANOVA revealed a significant effect of trial, $F(29, 1276) = 7.00$, $p < .001$; pairwise comparsions with bonferonni correction revealed that this effect was driven mainly by differences between trials 1, 3 and 8 and a selection of later trials, as well as between trial 16 and a number of other trials. There was no main effect of block, $F(1, 44) = 1.13$, $p = .29$ and no trial x block interaction, $F(29, 1276) = 1.14$, $p = .28$. There was no main effect of treatment, $F(1, 44) = .36$, $p = .55$, no treatment x block interaction, $F(1, 44) = 1.42$, $p = .24$, no treatment x trial interaction, $F(29, 1276) = 1.07$, $p = .37$ and no treatment x block x trial interaction, $F(29, 1276) = 1.04$, $p = .41$.

Reaction times

For each participant, mean reaction time and standard deviation were calculated for each trial type in each block (i.e. block 1 win, block 1 lose, block 2 win, block 2 lose). These were then used to exclude outliers, defined as reaction times that were further than three standard deviations from the mean. Mean reaction times were then recalculated for each trial type in each block. These are displayed in Figure 4.7.

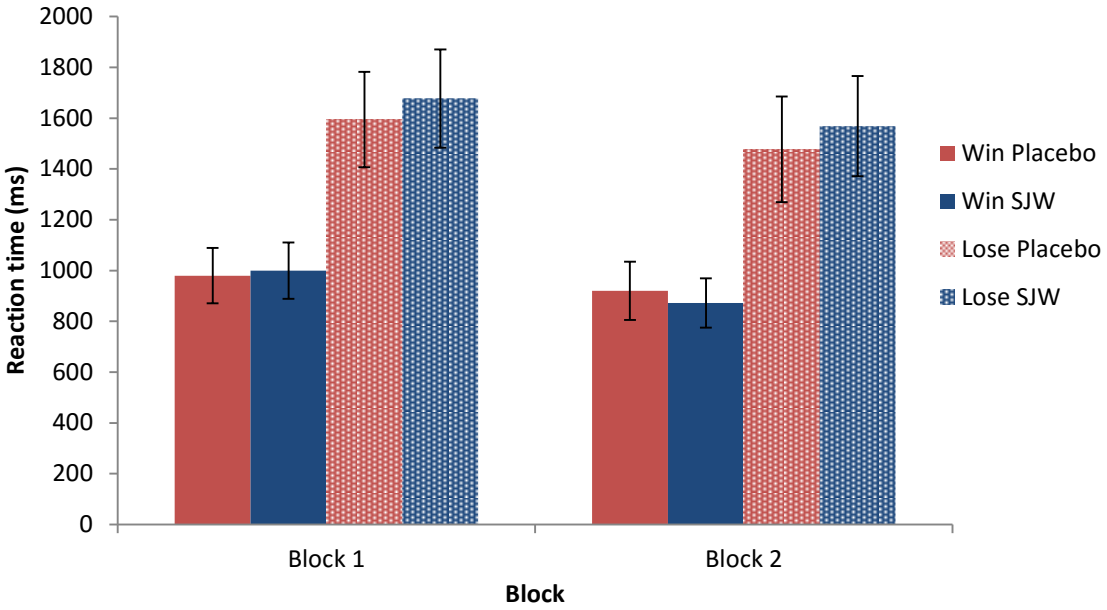

Figure 4.7 Reaction times in the reward task
Mean reaction time to make a choice in the reward task. Participants overall were faster for win trials; there was no significant effect of treatment or interaction effect. Error bars represent SEM.

A 2 (block) x 2 (trial type) x 2 (treatment) mixed effects ANOVA was run to see whether there were any group differences in reaction times. There was a significant effect of trial type, $F(1, 44) = 123.50$, $p < .001$, indicating that participants were slower to make a decision in lose trials than win trials. There was also a significant effect of block, $F(1, 44) = 4.49$, $p < .05$, indicating that participants were faster overall in block two than block one. However there was no main effect of treatment and no interaction between any of the variables (ps > .05), providing no evidence that SJW affected speed to make decisions in the task.

4.4 Discussion

We found that administration of 900mg/day SJW to healthy volunteers for seven days had no effect on cognitive performance. SJW did not affect numerical working memory: in an n-back task participants showed increased reaction times and reduced accuracy with increasing task difficulty, but this pattern of response was not influenced by the drug. Similarly, SJW did not

modulate performance on a reward learning task. Overall accuracy and reaction times in this task were unaffected by administration of SJW, and when data were examined on a trial-by-trial basis, patterns of learning were similar to those reported elsewhere (Pessiglione et al., 2006), but again unaffected by SJW administration.

4.4.1 **SJW does not affect numerical working memory**

This study adds to a growing body of literature that has found no effects of SJW on working memory in human participants: studies examining both numerical working memory (Ellis et al., 2001) and spatial working memory (Camfield et al., 2013; Siepmann et al., 2002) have failed to find any benefit of the drug. Despite consistent negative findings in human research, animal studies have generally found beneficial effects of SJW on working memory (e.g. Hasanein & Shahidi, 2011; Khalifa, 2001; Trofimiuk et al., 2011). It is worth attempting to understand the reason behind these discrepancies. One potential explanation is that the doses used in animal studies are far higher than those given to humans: indeed, some of the above rodent studies (e.g. Trofimiuk & Braszko, 2010; Trofimiuk et al., 2011) used doses 20-30 times higher than those given in human studies. It may be that the doses used in human studies are simply too low to produce any positive effects on memory; however, doses comparable to those in animal studies would be unacceptable to give to a human volunteer.

It is noteworthy that despite failing to find any memory-related effects in the n-back task, we did find that SJW resulted in increased false recall of positive words and increased recognition memory for positive words (see Chapter 3). This lends further support to the idea that these changes in emotional word memory are related to the emotional content of the words *per se*, and do not simply reflect a more general modification of memory.

4.4.2 **SJW does not affect reward learning**

We also failed to find any effect of SJW on the reward task. This is perhaps surprising given the striking effects found in response to L-DOPA in a previous study (Pessiglione et al., 2006). The discrepancy in the findings could be due to differences in the task itself: while our task was based on the task in that study, the reward magnitude in our study was lower (50p vs £1) and the difference in probabilities between stimuli was smaller (0.7/0.3 vs 0.8/0.2). It may be, for example, that with a lower reward value participants are less engaged or motivated, although it is not entirely clear how the drug would differentially affect the task depending on the reward value. More importantly, it may be that the comparison of a dopamine agonist with a placebo is not enough to produce results in this task: Pessiglione and colleagues found differences between L-DOPA and haloperidol (a dopamine antagonist), but do not report significant group differences between L-DOPA and placebo groups.

Alternatively, these differences in findings may be due to the specific pharmacology of SJW. Some researchers have suggested that SJW's potency for blocking reuptake transmission is simply too small to translate to any detectable cognitive effects in humans (Timoshanko, Stough, Vitetta, & Nathan, 2001), although potency for blocking monoamine reuptake is considerably larger than that for GABA or glutamate (Nathan, 2001). Perhaps more problematic is the broad-acting effects of the drug. While inhibition of dopamine reuptake (and perhaps serotonin reuptake; Palminteri, Clair, Mallet, & Pessiglione, 2012; Seymour, Daw, Roiser, Dayan, & Dolan, 2012) may facilitate reward learning, facilitation of other neurotransmitter systems may have an opposing effect. For example, there is evidence that midbrain GABAergic neurons provide regulatory control over DA neurons in the area (Parker et al., 2011; van Zessen, Phillips, Budygin, & Stuber, 2012). Increasing GABA transmission could therefore negate any effects of the increase in DA on reward learning.

Based on animal studies finding that SJW increases dopamine concentrations, a number of studies have put forward SJW as a candidate for treating disorders implicating reward pathways such as nicotine and alcohol addiction (Rezvani et al., 1999; Ruedeberg et al., 2010). However, the few studies that have examined SJW as a smoking cessation aid have generally found negative results (Parsons et al., 2009; Sood et al., 2010). The current study provided no evidence that SJW modulates reward pathways, and so casts further doubt on the utility of SJW as a treatment for substance addiction.

4.4.3 **Future directions**

Future research should examine a broader range of cognitive tasks. Numeric working memory is only one facet of memory, and given the positive findings in animal studies particularly in spatial memory tasks (Trofimiuk & Braszko, 2008; Trofimiuk et al., 2005) it would be interesting to explore the effects of the drug on spatial memory in humans in more depth (although preliminary evidence for effects in humans has not been positive; Camfield et al., 2013; Siepmann et al., 2002). Given that many of the animal studies suggest that SJW protects against negative effects of stress on memory (Trofimiuk et al., 2011; Trofimiuk et al., 2005), it would also be interesting to incorporate a model of stress in human research. It may be that beneficial effects of SJW on cognition only emerge in a more demanding environment, or in individuals who are experiencing impairment in memory.

Research could also examine the neural effects of SJW. Almost all of the evidence for the pharmacology of SJW comes from rodent studies, but neuroimaging could be used to provide direct or indirect indicators of how the drug effects neurotransmitter concentrations in humans. Magnetic resonance spectroscopy (MRS) could be used to examine how the drug affects GABA and glutamate levels. Although monoamine concentrations cannot be measured by MRS, it would be interesting to see whether participants given SJW and placebo differ in BOLD response

to reward in midbrain areas such as the striatum, as in the study by Pessiglione et al. (2006). There may be subtle neural changes that are not large enough to translate into behavioural effects, that could provide an indication of modulation of dopaminergic transmission.

In summary, our study failed to find any cognitive effects of SJW, adding to an increasing body of research that suggests SJW does not alter human cognition. On the one hand this illustrates the dangers of generalising from animal research to human cognition, especially when doses used in animal studies are considerably different from those considered safe for human consumption. However, the lack of effects on human cognition also serves to bolster the findings that SJW does affect emotional processing (Chapter 3). Like other antidepressants, SJW seems to affect behavioural responses to emotional content *per se*, rather than having broader cognitive effects.

Chapter 5

Neuroticism and emotional processing

5.1 Introduction

One of the major limitations of investigating the neurocognitive effects of antidepressant treatment in healthy populations is that these participants do not display baseline negative cognitive biases. That is, the effect of an antidepressant on measures of emotional processing might be different for patients, who already display a bias in favour of negative information, than for healthy controls. While the results from studies such as the St John's wort study in Chapter 3 suggest that antidepressants do produce positive shifts in the processing of emotional information even in healthy controls, it is important to directly compare these two groups to confirm these effects.

On the other hand, as well as biases in emotional processing, depressed patients may also display cognitive deficits such as impairment in working memory, executive dysfunction and psychomotor problems (Castaneda et al., 2008; Golinkoff & Sweeney, 1989). This makes it hard to separate the effects of antidepressants on emotional processing *per se* from these possible confounding factors. In addition, early effects of the drugs on patients' mood could influence the results.

5.1.1 **Defining neuroticism**

An alternative approach is to investigate antidepressant effects in a subgroup of the healthy population who may display neurocognitive biases without actually suffering from a mood disorder. One such group of people are those who score highly on a measure of the personality trait neuroticism (N). N refers to a tendency to experience negative emotions, and people who score highly on measures of N display exaggerated emotional responses: they are both quicker to

evaluate situations as stressful and less adept at handling such situations (Barlow, Ellard, Sauer-Zavala, Bullis, & Carl, 2014; Lahey, 2009; Ormel et al., 2013). In addition, high Ns tend to show excessive rumination and negative thinking about the self (Barlow et al., 2014).

N is a risk factor for a number of mental health disorders, including depression (Kotov, Gamez, Schmidt, & Watson, 2010). Prospective studies have demonstrated that higher N is not simply the result of the development of a mood disorder, but rather precedes the onset of the disorder (Vink et al., 2009). A number of different theories attempt to explain the link between the trait and depression or other disorders, and while part of this association may be attributable to some common (genetic or environmental) causal factor, it also appears likely that high N itself represents a vulnerability for developing mood disorders (Ormel et al., 2013).

5.1.2 **Emotional biases in neuroticism**

People with a high N score also show similar emotional processing biases to depressed subjects. A number of studies have shown that high N is associated with increased recall of negative or unpleasant words (Bradley & Mogg, 1994; Desrosiers & Robinson, 1992; Rijsdijk et al., 2009), and possibly also of negative autobiographical memories (Mayo, 1989). High Ns have also been found to be faster at classifying negative vs positive words and to show fewer positive memory intrusions than low Ns, as well as to perform more poorly at recognition of low intensity happy faces in a facial expression recognition task (Chan et al., 2007).

In addition to these biases towards negative emotional information, in some instances high Ns exhibit an overall pattern of emotional avoidance. For example, high Ns showed reduced gaze maintenance at the eyes of emotional faces, regardless of whether they were happy or fearful (Di Simplicio, Doallo, et al., 2014). These avoidance effects may relate particularly to socially-relevant or potentially threatening stimuli such as fearful faces. Indeed, other studies have found that high

neurotics are prone to using avoidance strategies, for example showing greater avoidance of (aversive) conditioned stimuli (Lommen, Engelhard, & van den Hout, 2010)

5.1.3 **Antidepressant effects in neuroticism**

Very few studies have investigated the effects of antidepressant administration on emotional processing in high Ns. Nevertheless, there is preliminary evidence that antidepressant treatment normalises the cognitive biases seen in this sample. For example, high Ns given seven days' citalopram treatment showed increased recognition memory for positive vs negative words compared to those given a placebo (Di Simplicio, unpublished findings), as well as increased recognition of low intensity positive vs negative faces (Di Simplicio, Doallo, et al., 2014).

Additionally, studies have suggested that citalopram may reverse the natural avoidance to socially-relevant and/or threatening stimuli seen in high Ns. Seven days of citalopram increased gaze maintenance at facial expressions, regardless of valence (Di Simplicio, Doallo, et al., 2014). Similarly, citalopram *increased* BOLD response in the amygdala and other emotional processing areas to fearful vs happy faces in this group (Di Simplicio, Norbury, et al., 2014; this chapter will focus solely on cognitive behavioural effects, but see Chapter 6 for a detailed examination of the neuroimaging literature). Interestingly, citalopram has occasionally been found to increase fear recognition and amygdala activity at acute doses even in unselected healthy controls (Bigos et al., 2008; Browning et al., 2007; Harmer, Bhagwagar, et al., 2003). It may be that high Ns represent a group of subjects who are particularly prone to this initial increase in processing of threatening information, due to the baseline biases present.

5.1.4 **Current study**

These results suggest that to a certain extent antidepressant effects in high Ns are similar to those seen in unselected healthy controls (Harmer et al., 2004) and depressed patients (Harmer et al.,

2009), increasing processing of positive vs negative information. On the other hand, it is clear that there are some more nuanced effects, and that drugs may actually increase the processing of negative socially-relevant or threatening stimuli. However, to date no studies have compared the effects of antidepressants on emotional processing in both high and low Ns. Given the baseline differences previously reported in these groups, it is vital to directly compare the two populations in order to be able to fully characterise antidepressant effects in high Ns.

Furthermore, past studies have only examined emotional processing changes after one week of treatment. It is unknown whether these changes are only present early on in treatment, or persist after several weeks. This is especially important given the presence of early increases in processing of threatening facial expressions. Indeed, it has been predicted that these early effects will only be present subchronically: with an increase in processing of these stimuli due to decreased avoidance, participants should learn that the stimuli are in fact not aversive and so with longer-term treatment they will ultimately show a decrease in processing (Di Simplicio, Norbury, et al., 2014).

The aim of the present study was therefore to further characterise the effects of citalopram on emotional processing in high Ns. We examined performance on a number of tasks related to emotional processing after four weeks' administration of either citalopram or placebo in both high and low Ns. We predicted that high Ns on placebo would show a negative bias in emotional processing compared to low Ns on placebo. However, given the long timecourse of treatment, we predicted that both groups would display a shift in emotional processing towards positive vs negative information when given citalopram, even in the case of threatening facial expressions.

5.2 Methods

Ethical permission for this study was obtained from the NHS National Research Ethics Service Committee Oxford B (reference 12/SC/0118).

5.2.1 **Pre-Screening**

Potential participants aged between 18 and 40 were asked to complete an online version of the full revised version of the Eysenck Personality Questionnaires (EPQ-R; S. B. G. Eysenck, Eysenck, & Barrett, 1985). Scores on the neuroticism subscale were calculated, and participants were invited to take part in a screening session to determine eligibility if they scored less than six (low N) or more than 15 (high N).

5.2.2 **Screening**

Participants were screened for axis 1 psychiatric disorders using the SCID, and a medical history and basic medical examination were conducted. Exclusion criteria included concurrent use of other psychotropic medications, a current or past psychological disorder, current pregnancy or breast-feeding, use of psychotropic drugs or participation in a drug trial within the past three months, family history of bipolar disorder, contraindication to MRI scanning and QT interval prolongation as determined by an electrocardiogram (ECG). A total of 81 participants met criteria and were assigned to take part in the study.

Participants who met criteria completed a number of mood questionnaires (see below). They also underwent a five minute resting ECG measurement and completed a short interview designed to examine interpersonal communication styles (not examined in this thesis). Participants were randomly assigned to receive either citalopram (20mg) or placebo (lactose tablets). Both citalopram and placebo were encapsulated in gelatine capsules to maintain blinding.

5.2.3 **Treatment period**

Participants took one pill per day, with breakfast, for 28 days. After one, two and three weeks of treatment, participants completed a number of mood questionnaires (BDI, STAI, VAS) as well as a questionnaire asking about the experience of side-effects.

5.2.4 **Testing**

Day 7

On the seventh day of treatment, participants came in for an hour-long testing session, which involved the completion of an eye-tracking task and an emotional regulation task. These tasks are not examined in this thesis.

Day 28

On the final day of treatment, participants again came in for testing. Participants filled in mood questionnaires, and completed task-based and resting state fMRI scans (see Chapter 6 and Chapter 7 respectively). They also again completed the interpersonal interview and emotional regulation task (not examined here).

Finally, participants also completed the emotional test battery (ETB). The tasks were as described in Chapter 3, except the order was slightly altered: the ETB started with the emotional categorisation task, followed by the Facial Expression Recognition Task (FERT) then emotional recall and emotional recognition. In addition, during emotional recall participants were only given one minute to write down as many words as they could remember.

5.2.5 **Questionnaires**

Many questionnaires were the same as in the St John's wort chapter, including the Beck Depression Inventory (BDI; Beck et al., 1961), State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970), Snaith-Hamilton Pleasure Scale (SHAPS; Snaith et al., 1995) and Visual Analogue

Scales. Side-effects questionnaires included four possible side-effects: nausea, dizziness, dry mouth and headache, scored as in the SJW study. Two additional questionnaires were used:

Liebowitz Social Anxiety Scale (LSAS)

The LSAS (Liebowitz, 1987) consists of 24 situations, 13 related to performance (e.g. "Speaking up at a meeting") and 11 related to social interaction (e.g. "Going to a party"). Participants are required to rate each item in terms of how much anxiety that situation produces, from 0 ("None") to 3 ("Severe"), as well as how much they avoid that situation, from 0 ("Never") to 3 ("Usually"). The scale produces four scores, measuring performance anxiety, performance avoidance, social anxiety, and social avoidance.

Mood Disorders Questionnaire (MDQ)

The MDQ (Hirschfeld et al., 2000) consists of 13 yes/no items related to bipolar disorder (e.g. "Has there ever been a period of time when you were not your usual self and you were much more talkative or spoke faster than usual?"), as well as two questions concerning whether any symptoms happened simultaneously, and whether they caused any problems. A score of 7 or more, combined with an indication that these symptoms happened simultaneously and caused at least moderate problems, has shown to be sensitive to bipolar disorder.

5.3 Results

Out of the original 81 participants who were assigned to take part after the screening session, a total of 69 participants completed the study. Reasons for dropping out included side-effects (N = 3 [1 citalopram, 2 placebo]) and other work/life commitments (N = 9). For one participant there were incidental findings in the MRI scan; this participant was excluded from further behavioural or MRI analysis, leaving a total of 68 participants.

5.3.1 **Demographic characteristics**

Demographic data for participants is displayed in Table 5.1. A Pearson chi-square test found no evidence of an association between neuroticism and treatment groups, $\chi^2(1) = 0.96$, $p = .33$. A chi-square test across all four combined neuroticism/treatment groups found that gender distribution did not differ between groups, $\chi^2(3) = .28$, $p = .97$. To compare age and years of education, 2 x 2 ANOVAs were conducted. There were no effects of neuroticism or treatment, and no interaction, suggesting that the groups did not differ on these variables (ps > .05).

Table 5.1 Demographic characteristics of volunteers

	High Neurotics				Low Neurotics			
	Citalopram (*n*=14)		Placebo (*n*=19)		Citalopram (*n*=19)		Placebo (*n*=16)	
Gender	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Male	5	35.71	7	36.84	7	36.84	7	43.75
Female	9	64.29	12	63.16	12	63.16	9	56.25
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Age	21.07	3.05	22.53	3.12	24.16	4.56	22.56	3.86
Years of education[1]	15.35	2.01	16.05	2.01	16.63	2.75	16.13	1.89

[1]Not available for one participant in the high N citalopram group

5.3.2 **Baseline questionnaires**

Screening questionnaire data were analysed in 2 x 2 ANOVAs to investigate any baseline between-group differences (Table 5.2). High neurotics scored more highly on the neuroticism subscale of the EPQ, as well as on several measures of mood and anxiety: BDI, trait anxiety, state anxiety, and all subscales of the LSAS. Low neurotics scored higher on the extroversion subscale of the EPQ. There were no effects of treatment group and no interaction between treatment group and neuroticism group, suggesting that participants were matched across treatment groups for these measures.

Table 5.2 Baseline mean (SD) scores on measures of mood and anxiety

	High Neurotics		Low Neurotics		
	Citalopram	Placebo	Citalopram	Placebo	Sig effects
BDI[1]	6.57 (4.89)	7.47 (6.56)	0.89 (1.33)	1.00 (1.07)	High > Low
MDQ[2]	4.07 (3.22)	2.84 (2.98)	3.00 (2.98)	2.86 (3.66)	-
SHAPS[3]	0.30 (0.64)	0.53 (1.07)	0.11 (0.32)	0.13 (0.35)	-
EPQ					
P	7.43 (3.57)	6.21 (2.46)	8.37 (3.52)	7.01 (4.50)	-
E	14.01 (5.65)	11.42 (5.25)	17.78 (3.29)	18.06 (3.17)	Low > High
N	18.93 (2.30)	19.00 (2.56)	3.05 (1.65)	2.94 (1.57)	High > Low
L	5.79 (3.66)	6.22 (3.62)	7.37 (2.95)	7.20 (2.89)	
Trait anxiety[3]	40.93(10.59)	43.32 (9.23)	30.27 (5.28)	30.83 (5.71)	High > Low
State anxiety[3]	38.68 (6.27)	42.75 (7.17)	36.94 (3.52)	36.33 (2.87)	High > Low
LSAS[3]					
Social Fear	7.77 (4.76)	9.16 (5.26)	3.79 (3.69)	4.75 (4.04)	High > Low
Social Avoid.	8.00 (5.77)	7.84 (4.60)	3.00 (3.45)	4.25 (3.59)	High > Low
Perform. Fear	10.31 (3.97)	9.42 (5.01)	6.21 (4.64)	5.56 (5.10)	High > Low
Perform. Avoid.	6.92 (5.31)	7.37 (3.72)	3.74 (3.72)	4.06 (4.11)	High > Low

[1]Data missing from 3 participants
[2] Data missing from 2 participants; no participant met criteria for bipolar on the MDQ
[3] Data missing from one participant

5.3.3 **Effect of treatment on mood, anxiety and side-effects**

We conducted 2 (treatment group) x 2 (neuroticism) x 2 (time point) ANOVAs to examine whether citalopram treatment affected scores on BDI, state anxiety and SHAPS questionnaires, as well as on the neuroticism subscale on the EPQ and each of the VAS scales. Data is displayed in Figure 5.1; below we consider each measure in turn. Due to technical problems with electronic questionnaires at week 4, data is missing from several participants, as noted in the text. Statistics and graphs are displayed only for participants for whom there is both screening and week 4 data.

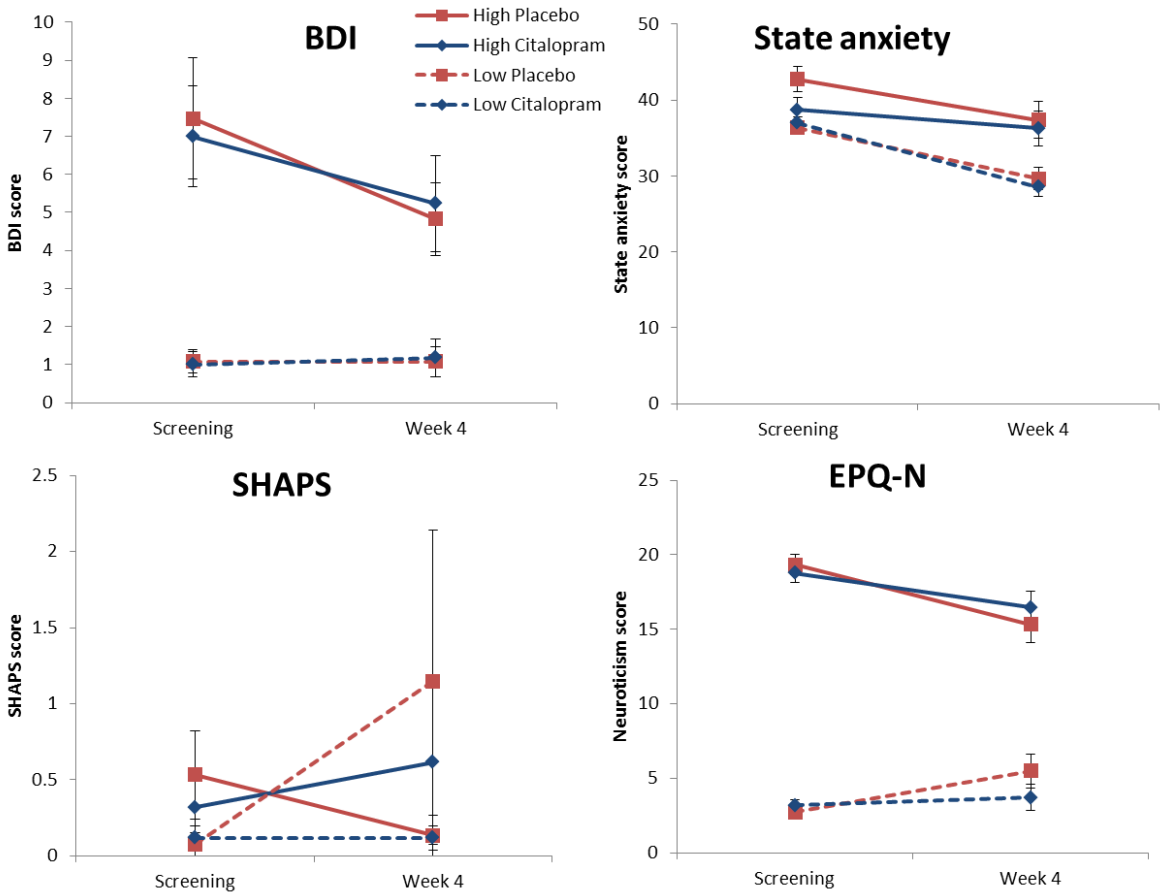

Figure 5.1 Screening and week 4 questionnaire scores
Error bars represent SEM

BDI

Screening and week 4 BDI data were available for 60 participants. There was a main effect of neuroticism, $F(1, 56) = 37.55$, $p < .001$. The effect of time approached significance, $F(1, 56) = 3.86$, $p = .06$, and there was a significant time x neuroticism interaction, $F(1, 56) = 38.85$, $p < .05$. No other effects were significant (ps > .05).

In order to explore the time x neuroticism interaction in more detail, individual t-tests were used to compare BDI scores for screening and week 4 for each neuroticism group. These showed that for high Ns, BDI decreased between screening and week 4, $t(29) = 2.23$, $p < .05$, but for low Ns there was no difference between the two time points, $t(29) = .36$, $p = .73$.

State Anxiety

Screening and week 4 BDI data were available for 66 participants. There was a significant effect of neuroticism, $F(1,62) = 16.46$, $p < .001$. There was also a significant effect of time, $F(1, 62) = 44.43$, $p < .001$, and a time x neuroticism interaction, $F(1, 62) = 4.55$, $p < .05$. No other effects were significant.

In order to explore the time x neuroticism interaction in more detail, individual t-tests were used to compare state anxiety scores for screening and week 4 for each neuroticism group. These showed that state anxiety decreased between screening and week 4 for both high Ns, $t(32) = 2.91$, $p < .01$, and low Ns, $t(32) = 8.03$, $p < .001$. Both neuroticism groups therefore showed less state anxiety after four weeks, however there appeared to be *more* of a reduction in the low neurotic group.

Neuroticism

Screening and week 4 EPQ-N data were available for 56 participants. There was a significant effect of neuroticism, $F(1,55) = 400.04$, $p < .001$, and a significant time x neuroticism interaction, $F(1,55) = 21.20$, $p < .001$. No other effects were significant.

In order to explore the time x neuroticism interaction in more detail, individual t-tests were used to compare neuroticism scores for screening and week 4 for each neuroticism group. These showed that for high Ns, neuroticism scores decreased between screening and week 4, $t(27) = 3.94$, $p < .01$, while for low Ns, neuroticism scores increased between screening and week 4, $t(30) = 2.27$, $p < .05$.

SHAPS

Screening and week 4 SHAPS data were available for 56 participants. There were no significant main effects or interactions (ps > .05).

VAS

We also examined scores on the visual analogue scales for the 63 participants who had screening and week 4 data. For ease of reporting these are not graphed and significant effects are reported at $p < .05$. We found that there was a main effect of neuroticism for four of the scales: overall high Ns were less happy and calm than low Ns, and also more anxious and sad. There was also a main effect of time for three of the scales: participants were more anxious and less calm at the test than at the screening. Participants were also more hostile at the test day, and a time x neuroticism interaction indicated that this was driven by an increase in hostility in high Ns but not low Ns.

The overall pattern is therefore of increased negative mood and anxiety and decreased positive mood in high neurotics. Interestingly, over the course of the study BDI, state anxiety and even neuroticism itself decreased for the high Ns, while for the low Ns state anxiety decreased and BDI and neuroticism increased. However, at week 4 the two neuroticism groups still differed profoundly along these dimensions. Treatment with citalopram had no effect on scores in either group.

Side-effects

Side-effects data recorded at week 4 was available for 61 participants. This is displayed in Figure 5.2 (right). A 2 (treatment) x 2 (neuroticism) ANOVA found no main effects of treatment or neuroticism, nor an interaction between the two, (ps > .05), providing no evidence that at week 4 side-effects participants in the citalopram group were suffering from any side-effects.

Due to anecdotal evidence from participants suggesting that side-effects were particularly common during the first week, we also examined side-effects data from week 1. Data were available from 61 participants, and are displayed in Figure 5.2 (left). The main effect of treatment approached significance, $F(1, 57) = 3.37$, $p = .07$. The results therefore suggest that there may be

early side-effects of citalopram, but that by four weeks these effects are no longer being experienced.

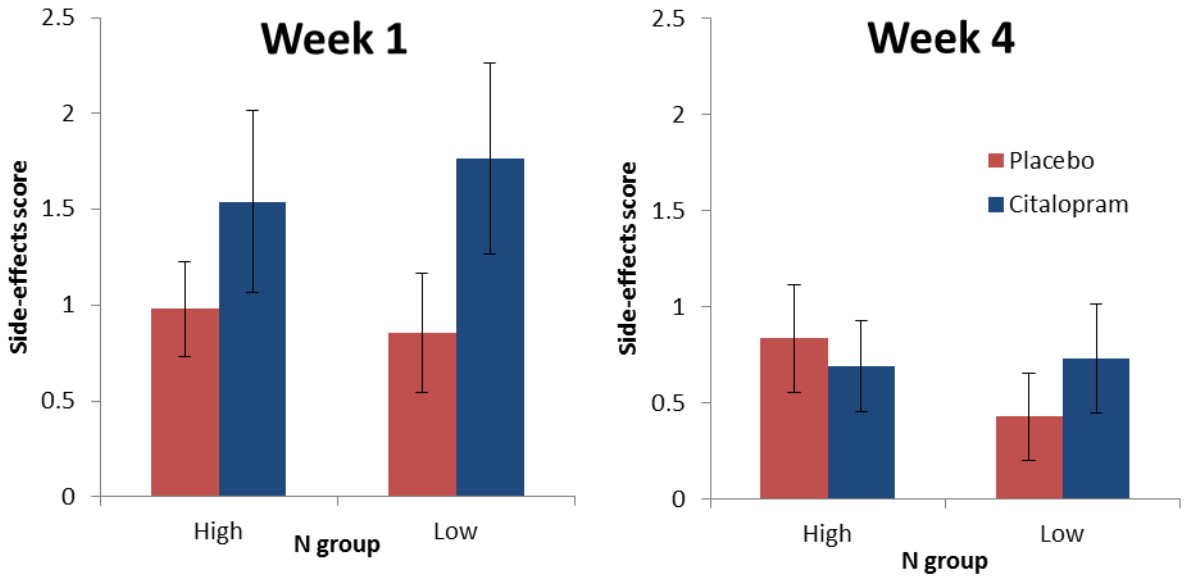

Figure 5.2 Self-reported side-effects at weeks 1 and 4
At week 4 there were no main effects or interaction; at week 1 there was a trend for increased side effects on citalopram.

5.3.4 Participants' guesses

Table 5.3 records participants' guesses, after the end of the final testing session, regarding whether they had been give citalopram or placebo. This data was available for 56 participants. A chi-square test found no evidence for an association between the group the participants were in and the guess they made, $\chi^2(1) = .96$, $p = .33$

Table 5.3 Participants' guesses regarding treatment group

		Guess	
		Citalopram	Placebo
Group	Citalopram	12	14
	Placebo	10	20

5.3.5 **ETB**

FERT

a) Accuracy

Data was missing from one participant in the high N citalopram group. Data from trials in which faces were classified as neutral were excluded from all accuracy and reaction time analyses. A first analysis was run as for the St John's wort study; in this analysis no effects came out significant (except for some effects of emotion: e.g. participants as a group were more accurate and faster at classifying happy faces). We decided to re-analyse the FERT collapsing across expressions of the same valence. As our groups were relatively small and there were two independent variables, combining data in this way could increase power to detect results which might be otherwise obscured. Average accuracy across anger, disgust, fear and sad emotions was classed as "negative", and average accuracy across happy and surprised emotions as "positive" (Post et al., 2015). These results are described below.

Accuracy data are displayed in Figure 5.3. A 2 (valence) x 2 (treatment) x 2 (neuroticism) mixed effects ANOVA was used to examine whether neuroticism and/or treatment affected categorisation of emotional facial expressions.

There was a main effect of valence, $F(1,63) = 93.59$, $p < .001$, indicating that overall, participants were more accurate for positive than negative facial expressions. There were no main effects of treatment, $F(1, 63) = .31$, $p = .58$, nor of neuroticism, $F(1, 63) = .17$, $p = .68$, and no interaction between valence and treatment, $F(1, 63) = .44$, $p = .51$, nor treatment and neuroticism, $F(1, 63) = 1.15$, $p = .29$. However, there was an interaction between valence and neuroticism, $F(1, 63) = 5.33$, $p < .05$, and a three-way interaction between all variables, $F(1, 63) = 4.61$, $p < .05$.

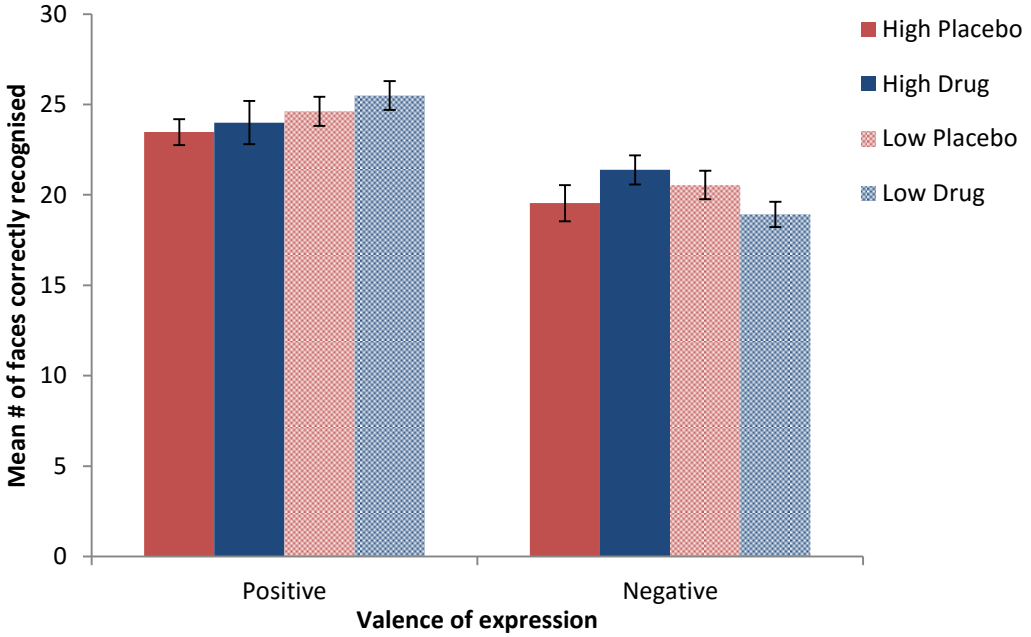

Figure 5.3 Accuracy on the FERT
Mean number of faces correctly recognised (out of 40), averaged across positive and negative facial expressions. There was a significant neuroticism x treatment interaction for negative but not positive faces ($p < .05$). Error bars represent SEM.

In order to explore these interactions in more detail, we conducted 2 (neuroticism) x 2 (treatment) ANOVAs for positive and negative faces separately. For positive faces, there were no significant main effects or interactions (ps > .05). For negative faces, there were no main effects, but there was a neuroticism x treatment interaction, $F(1,63) = 4.18$, $p < .05$. To further explore this effect, post-hoc t-tests between groups were carried out for the negative face accuracy data; however, none of these were significant (ps > .05). Therefore our results suggest that citalopram has contrasting effects between high and low Ns, increasing recognition of negative faces for high Ns while decreasing recognition of negative faces for low Ns - though it is important to note that the significant result is strictly the contrast between high and low Ns in the change caused by the drug: when the groups are considered individually, there is no significant effect of drug.

b) Reaction times

Reaction time data is displayed in Figure 5.4. A 2 x 2 x 2 mixed effects ANOVA was again conducted to examine the effects of treatment and neuroticism on reaction times.

There was again a main effect of valence, $F(1,63) = 38.78$, $p < .001$, with participants responding faster to positive than negative facial expressions. There were no main effects of treatment, $F(1, 63) = 2.22$, $p = .14$, nor of neuroticism, $F(1, 63) = 1.07$, $p = .30$, and no interaction between valence and treatment, $F(1, 63) = .02$, $p = .89$ nor between treatment and neuroticism, $F(1,63) = 3.18$, $p = .08$. In this case there was no three-way interaction, but as before there was an interaction between valence and neuroticism, $F(1, 63) = 5.83$, $p < .05$.

To further explore this interaction, post-hoc t-tests were carried out on the data collapsed across drug group. For ease of interpretation, collapsed data is presented in Figure 5.5. These t-tests were non-significant (ps > .05). Our results therefore suggest that the difference in speed to identify faces between high and low Ns is greater for negative faces than positive faces. That is, high Ns were faster than low Ns to identify negative faces when this was compared to speed for identifying positive faces. Again, it should be noted that it is strictly the contrast in the effects of neuroticism between positive and negative faces that is significant; there were no main effects of neuroticism when positive or negative faces were considered alone.

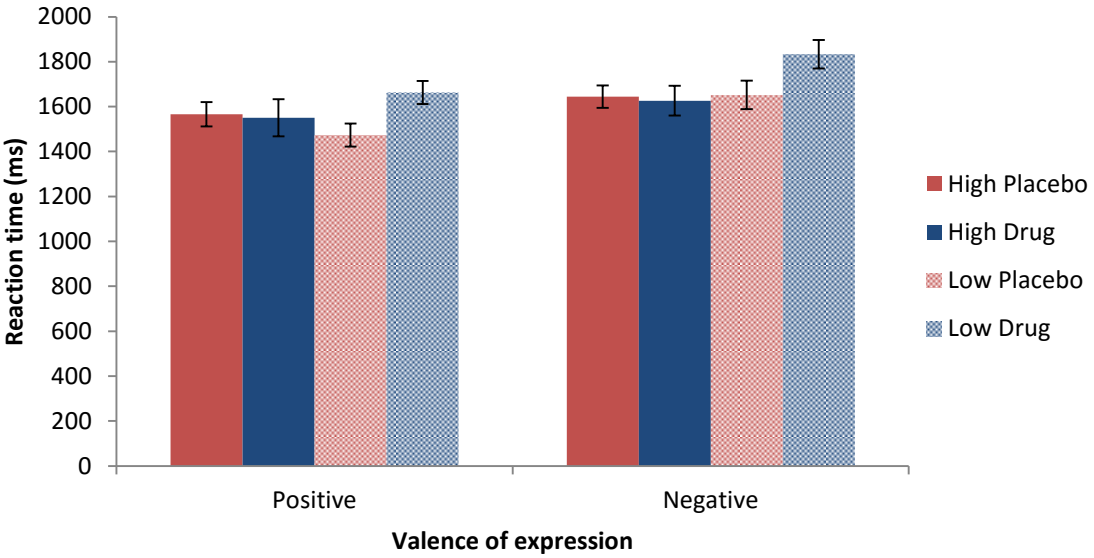

Figure 5.4 Reaction time on the FERT
Mean reaction time to classify faces, averaged across positive and negative facial expressions. There was a significant valence x neuroticism interaction. Error bars represent SEM.

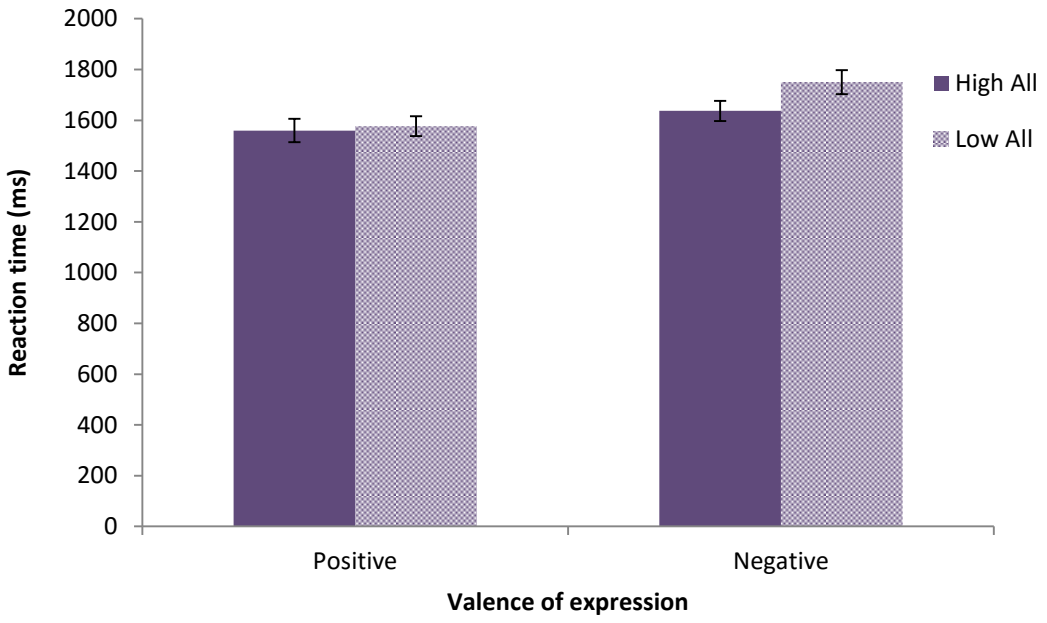

Figure 5.5 Reaction times on FERT collapsed across drug group
Mean reaction time to classify faces, averaged across positive and negative facial expressions and collapsed across drug group. Valence x neuroticism interaction can be seen more clearly. Error bars represent SEM.

c) Misclassifications

Neutral faces were included alongside positive and negative faces in misclassifications analysis, in order to avoid biasing the results. Number of faces misclassified as neutral, positive or negative is displayed in Figure 5.6. A 3 (valence) x 2 (treatment) x 2 (neuroticism) mixed effects ANOVA was again conducted to examine the data. The only main effect was one of valence, $F(2, 126) = 680.08$, $p < .001$. Pairwise comparisons indicated that participants misclassified expressions most often as neutral, then as negative and then positive. Given that there were more negative than positive options, it is perhaps unsurprising that participants more often made an incorrect negative than positive decision. There were no other significant effects ($ps > .05$).

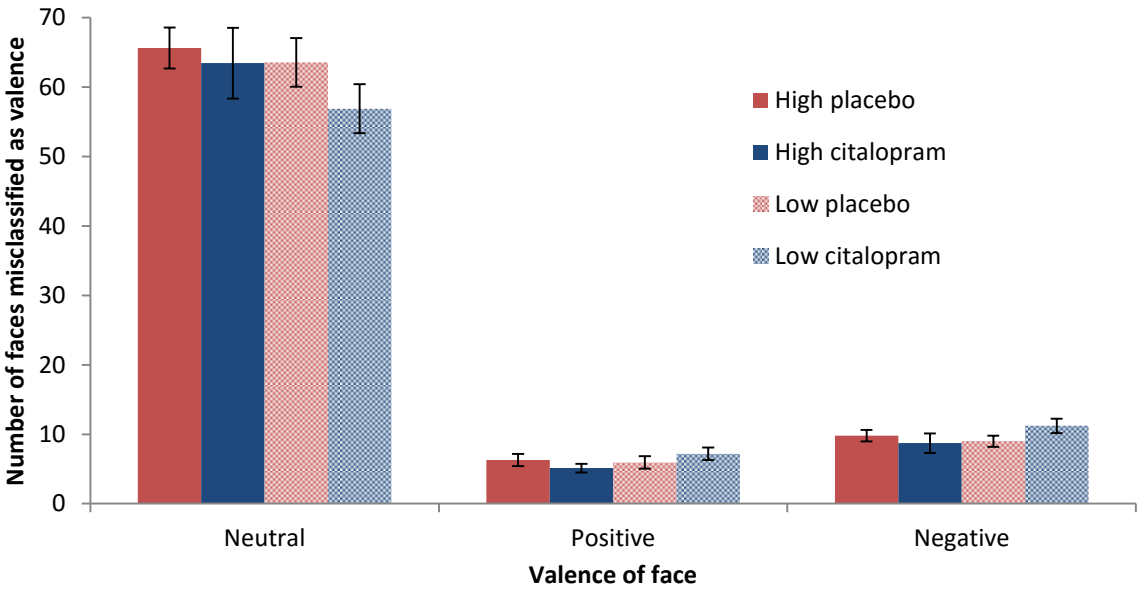

Figure 5.6 Misclassifications in the FERT
Mean number of faces incorrectly classified as neutral, positive or negative in the FERT. There were no effects of treatment or neuroticism. Error bars represent SEM.

Because neutral faces are by nature ambiguous, and may be assigned an element of emotionality in a task such as this, we also examined the percentage of neutral faces misclassified as negative or positive expressions. Data are presented in Figure 5.7. A 2 (valence) x 2 (treatment) x 2 (neuroticism) mixed effects ANOVA was conducted on the data. The only significant effect was that of valence, with participants making more misclassifications of neutral faces as negative than

positive, $F(1, 62) = 72.64$, $p < .001$. Again, given that there were more negative options, this is not surprising.

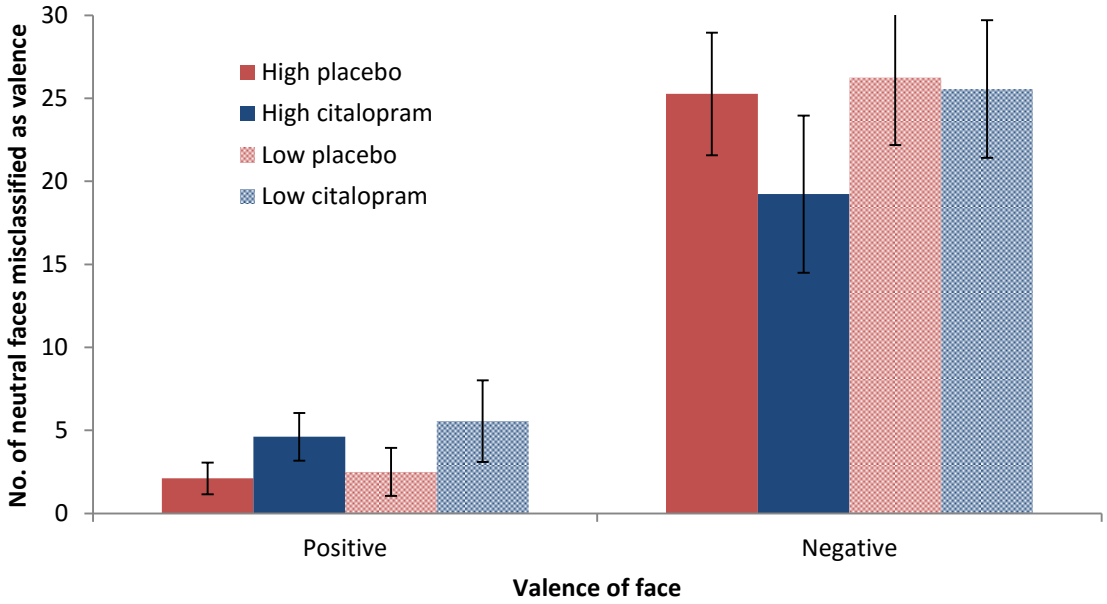

Figure 5.7 Number of neutral faces misclassified as positive or negative
Mean number of neutral faces misclassified as positive or negative expressions. Participants made more misclassifications of neutral faces as negative than as positive; there were no effects of neuroticism or treatment. Error bars represent SEM.

Categorisation

Figure 5.8 shows accuracy at categorising words. A 2 (valence of word) x 2 (neuroticism) x 2 (treatment) mixed effects ANOVA was used to see whether treatment and/or neuroticism affected accuracy at categorising words. There were no main effects and no interactions (ps > .05) providing no evidence that either variable had any effect on accuracy.

Figure 5.9 shows reaction time for categorising words. There was a main effect of valence, $F(1, 64) = 13.29$, $p < .01$, indicating that overall participants were faster at categorising positive words (M=1022.43 ms) than negative words (M=1073.96 ms). However, there were no other main effects and no significant interactions, again providing no indication that treatment or neuroticism affected reaction time to classify words.

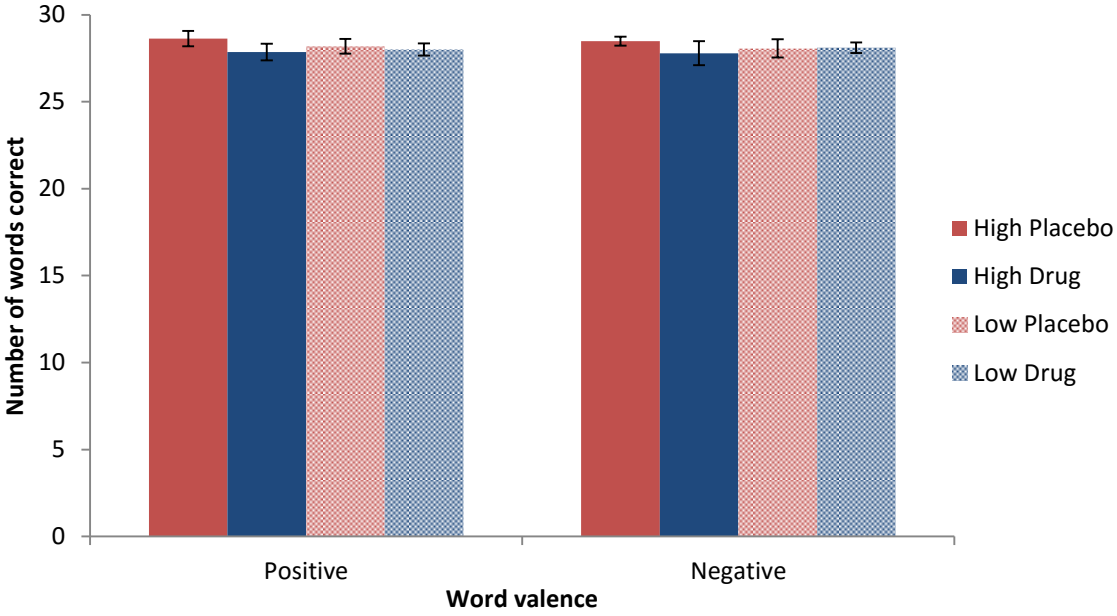

Figure 5.8 Accuracy for word categorisation
Mean number of words (out of 30) correctly categorised as positive or negative. There were no significant effects or interactions. Error bars represent SEM.

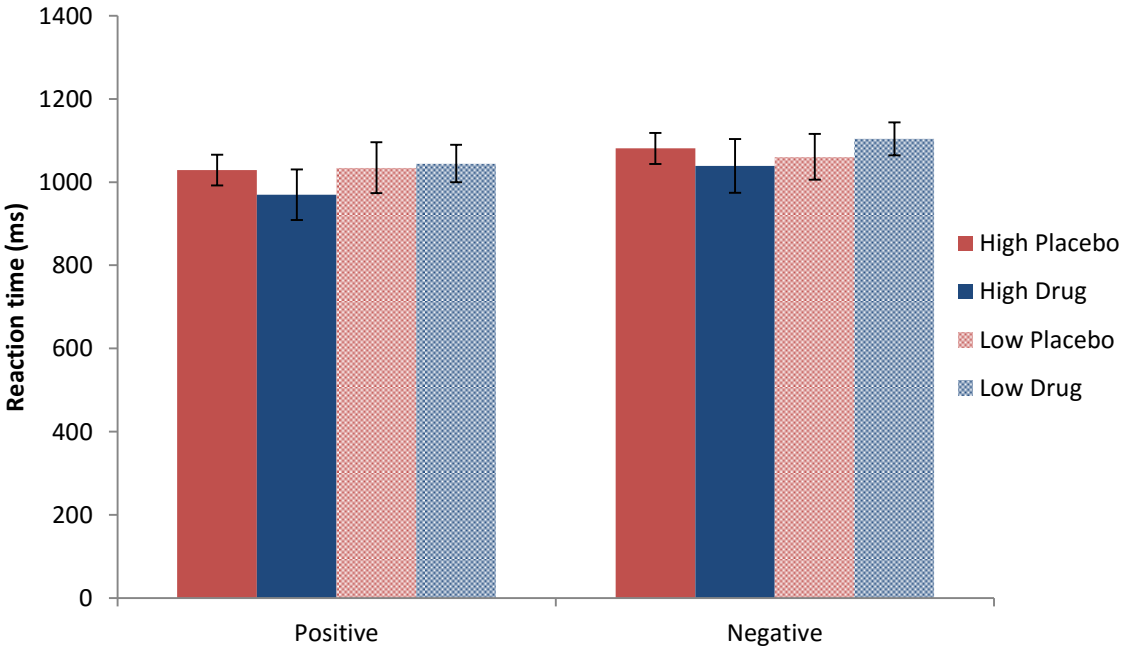

Figure 5.9 Reaction times for word categorisation
Mean number of words (out of 30) correctly categorised as positive or negative. Participants were faster at categorising positive words; there were no effects of neuroticism or treatment. Error bars represent SEM.

Recall

Due to experimenter error, a number of participants were given a longer time period in which to recall words (two minutes rather than one minute). Rather than exclude these participants, we opted to convert absolute numbers of positive and negative words recalled or falsely recalled into a percentage out of the total number recalled for each participant. We compared the results of this analysis with that of an analysis including only participants who had been given one minute to recall: there were no differences in findings, and so we report the whole group analysis below.

Figure 5.10 shows the number of words correctly recalled by each group as percentage of total words recalled. A 2 (valence) x 2 (treatment) x 2 (neuroticism) mixed effects ANOVA was used to examine whether the groups differed on recall. The only significant effect was that of valence, $F(1,64) = 7.41$, $p < .01$, indicating that participants were better at remembering positive words than negative words. The valence x treatment interaction was almost significant, $F(1, 64) = 3.96$, $p = .051$, suggesting that citalopram increased recall of positive words but decreased recall of negative words. However, individual t-tests showed no significant effect of treatment when positive or negative words were considered alone (ps > .05), and when only participants with one minute recall were considered, the interaction trend became slightly less significant ($p = .08$). There were no main effects of neuroticism or any other interactions.

Figure 5.11 shows the number of positive and negative intruder words – i.e. words that were "recalled" but which were not on the original list, again displayed as percentage of total recall. There was again a significant effect of valence, $F(1, 64) = 17.53$, $p < .001$, indicating that participants had a greater percentage of false recalls of positive than negative words. There were no other main effects or interactions, providing no evidence that citalopram treatment or neuroticism levels influenced false recall or words.

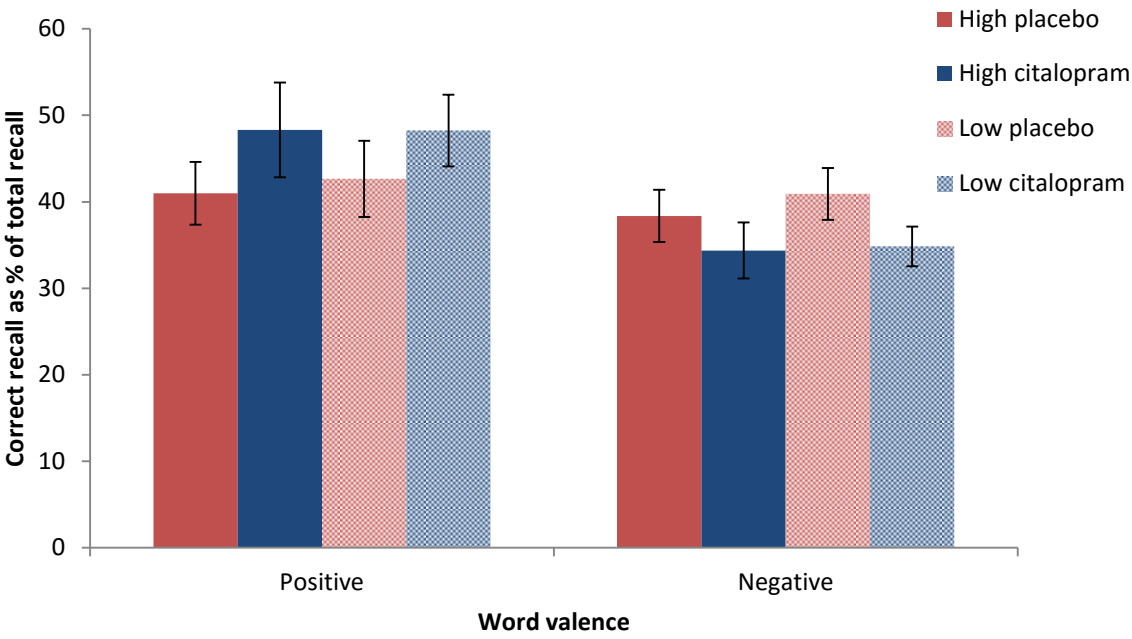

Figure 5.10 Accuracy in word recall
Mean number of positive and negative words correctly recalled, as percentage of total recall. Participants were better at recalling positive words; there were no effects of neuroticism or treatment. Error bars represent SEM.

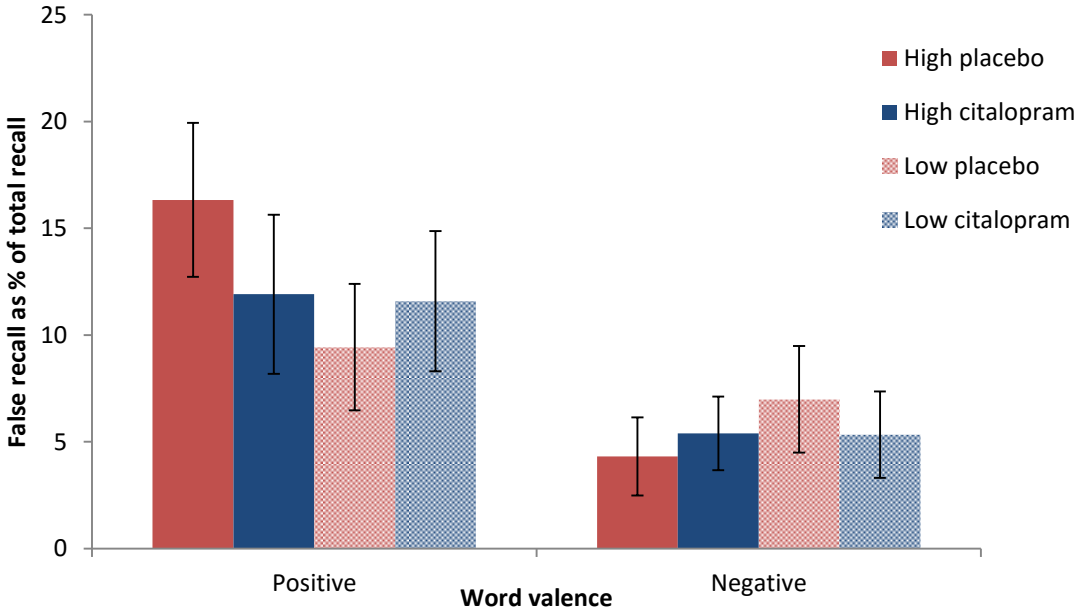

Figure 5.11 False recall of words
Mean number of positive and negative words falsely recalled, as percentage of total recall. Participants falsely recalled more positive words; there were no effects of neuroticism or treatment. Error bars represent SEM.

Recognition

Data was missing from two participants in the high N citalopram group. Figure 5.12 shows the number of positive and negative words correctly recognised for each group. A 2 (treatment) x 2 (neuroticism) x 2 (word valence) ANOVA was conducted to see whether there were any significant effects of treatment or neuroticism on correct recall.

There was a main effect of valence, $F(1,62) = 32.13$, $p < .001$, indicating that overall, subjects correctly recognised more positive than negative words. There was no main effect of treatment, $F(1, 62) = 1.96$, $p = .72$, and no interaction between treatment and any other variable (ps > .05), providing no evidence that citalopram affected accuracy in the task. However there was a significant valence x neuroticism interaction, $F(1, 62) = 6.05$, $p < .05$, suggesting that neuroticism affected accuracy depending on whether words were positive or negative.

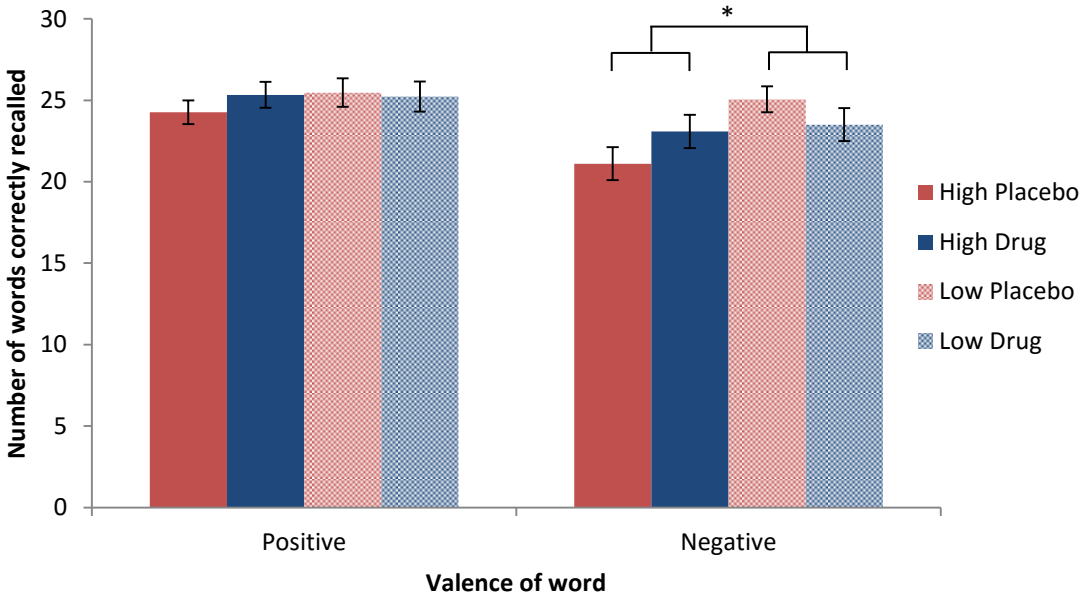

Figure 5.12 Accuracy in word recognition
Mean number of words (out of 30) correctly recognised. There was valence x neuroticism interaction: high Ns were worse than low Ns at recalling negative words but not positive words, *$p < .05$. Error bars represent SEM.

In order to further explore this interaction, we conducted individual t-tests between the high and low neurotic groups for each valence. There was no difference between high and low neurotics for the positive words, $t(64) = .79$, $p = .43$; however, for the negative words the high neurotics showed a reduced accuracy, $t(64) = 2.42$, $p < .05$.

As in Chapter 3, we also used signal detection theory to further examine accuracy on the recognition task. *d'* showed a very similar pattern to the accuracy data: there was again a main effect of valence, $F(1, 62) = 32.13$, $p < .001$, suggesting that participants were more accurate at recognising positive than negative words even when false alarms were taken into account. The valence x neuroticism interaction was also apparent, but only at a trend level, $F(1, 62) = 3.86$, $p = .054$.

For β, there was also a main effect of valence, $F(1, 62) = 13.20$, $p < .01$, demonstrating that participants had a more conservative response style for negative words ($\beta = 0.14$) than positive words ($\beta = -.02$). However there was also a treatment x neuroticism interaction, $F(1, 62) = 6.88$, $p < .05$; post-hoc tests on data collapsed across valence showed that high neurotics on placebo had a more conservative response style ($\beta = .21$) than low neurotics on placebo ($\beta = -.07$; $t(34) = 2.23$, $p < .05$), while treatment produced a more liberal response style for high neurotics ($\beta = -.08$; $t(29) = 2.45$, $p < .05$) but had no effect for low neurotics, $t(33) = 1.36$, $p = .19$. That is, regardless of valence, at baseline high neurotics appeared to use a stricter criterion for making a decision than low neurotics, while treatment produced a leftward shift, making the criterion more similar to that of the low neurotics.

Reaction times to correctly recognise positive and negative words as having been present in the original task are presented in Figure 5.13. A 2 x 2 x 2 ANOVA was again conducted to examine the effects of neuroticism and treatment on reaction times.

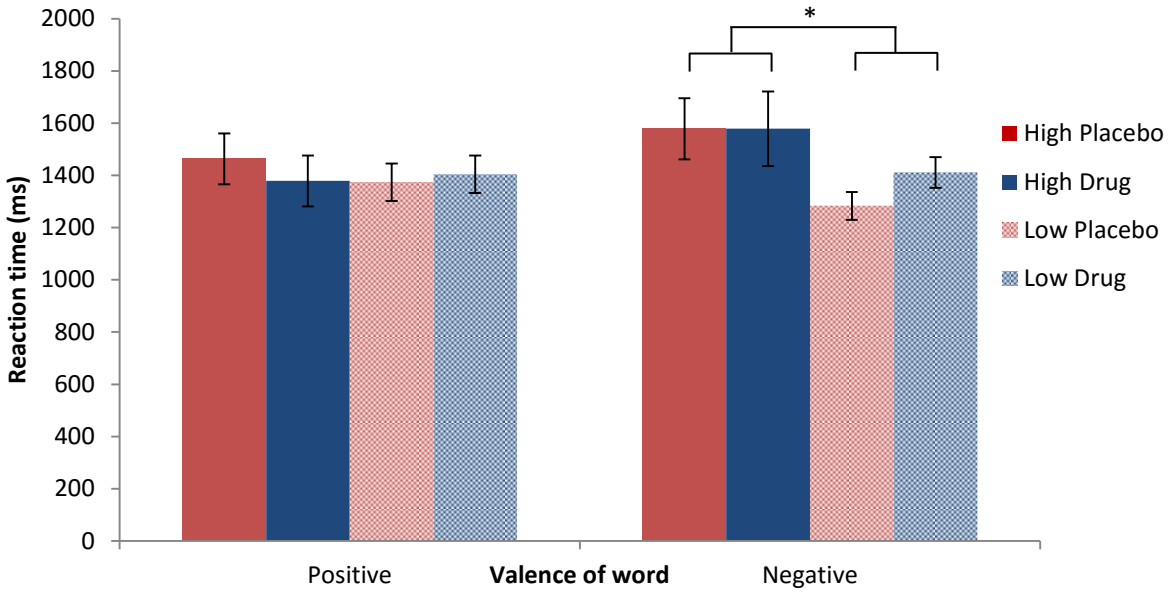

Figure 5.13 Reaction times for word recognition
Mean reaction times to correctly recognise positive and negative words as having been present in the previous task. There was valence x neuroticism interaction: high Ns were slower than low Ns at recalling negative words but not positive words *p < .05. Error bars represent SEM.

The main effect of valence did not reach significance, $F(1,62) = 2.89$, $p = .09$. There was no main effect of treatment, $F(1,62) = .05$, $p = .93$, and no interaction with treatment and any other variables (ps > .05), providing no evidence that treatment had any effect on reaction times. However, there was again a significant valence x neuroticism interaction, $F(1, 62) = 8.69$, $p < .01$.

Again, in order to further explore this interaction, we conducted individual t-tests between the high and low neurotic groups for each valence. There was no difference in reaction times between high and low neurotics for the positive words $t(64) = .49$, $p = .63$; however, for the negative words, the high neurotics showed an increased reaction time, $t(64) = 2.44$, $p < .05$. High neurotics therefore seemed to be both less accurate and slower at correctly recognising negative words than low neurotics.

Words incorrectly recognised as familiar ("false alarms") are presented in Figure 5.14. There was a significant effect of valence, $F(1, 62) = 5.28$, $p < .05$, indicating that participants falsely recognised more positive than negative words. There were no main effects of treatment, $F(1, 62) = 0.89$, $p = .77$, nor of neuroticism, $F(1, 62) = .01$, $p = .92$. However, there was a treatment x neuroticism interaction, $F(1,62) = 5.08$, $p < .05$, suggesting that the effect of treatment depended on neuroticism. Individual t-tests collapsed across valence indicated that although there was no difference between high and low neurotics on placebo, $t(34)=1.58$, $p = .12$, for high neurotics the drug increased overall false recognitions, $t(29) = 2.30$, $p < .05$, while for low neurotics, the drug had no effect, $t(33) = 1.23$, $p = .23$.

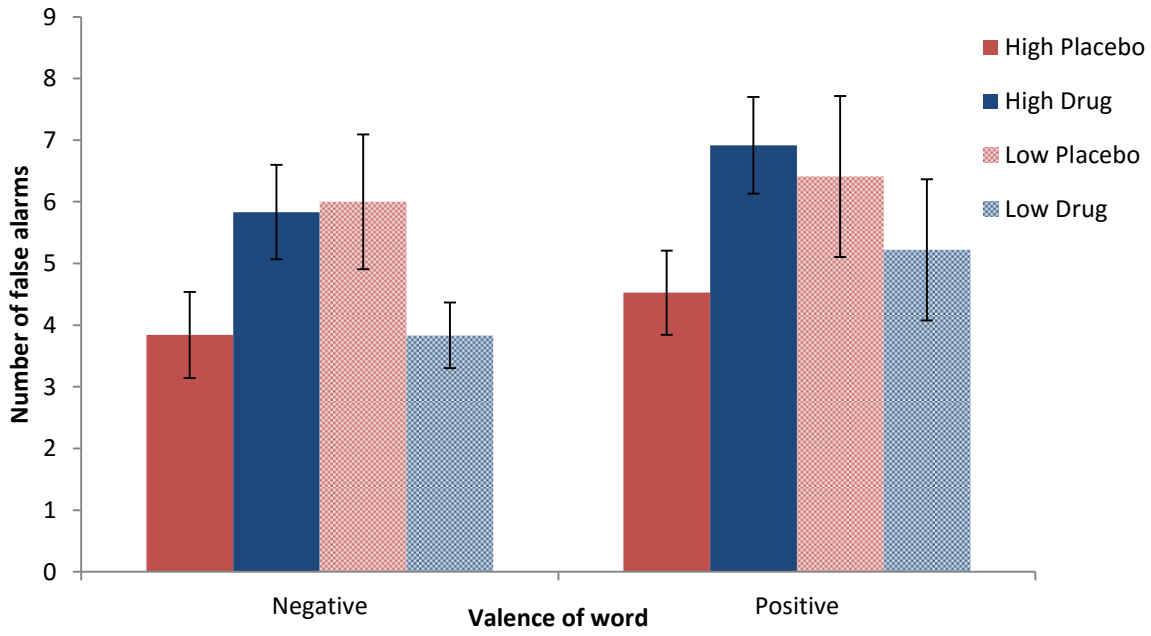

Figure 5.14 False word recognition
Mean number of positive and negative false alarms (out of a possible 30). There was an overall treatment x neuroticism interaction ($p < .05$). Error bars represent SEM.

5.4 Discussion

In our investigation into the effects of four weeks' citalopram treatment on emotional processing in high and low neurotics, we found a number of results, which can broadly be summarised as main effects of neuroticism, main effects of treatment, and interactions between the two.

Contrary to predictions, we did not find any baseline differences between high and low Ns (i.e. differences in the two placebo groups). However, we did find that overall high and low Ns performed differently on certain tasks. High Ns were faster to classify negative vs positive faces than low Ns, but also less accurate and slower at recognising negative words than low Ns. High Ns also showed reduced accuracy at recognising previously classified negative words compared to low Ns. None of these effects were influenced by treatment. As predicted, we found some treatment effects that were similar for both high and low Ns: in particular, citalopram appeared to increase correct recall of positive words vs negative words. On the other hand, in some cases treatment effects differed depending on N group: citalopram increased recognition of negative faces in high Ns compared to low Ns, and increased false word recognition for high Ns compared to low Ns regardless of valence. We consider these three types of effect below.

5.4.1 **Overall effects of neuroticism**

Increased memory for negative vs positive words is one of the most consistent emotional processing effects seen in high Ns (Bradley & Mogg, 1994; Chan et al., 2007). It is surprising, then, that we found *decreased* recognition memory and *increased* reaction times specifically to negative words for our high Ns compared to low Ns. It is possible that these findings reflect the aversion to negative emotional information sometimes reported in high Ns (see section 5.4.3 below); however, this explanation seems unlikely, as previous research appears to show specific aversion only to interpersonal or directly threatening cues such as faces (Di Simplicio, Doallo, et al., 2014).

An alternative explanation is that our high N sample does show some cognitive deficits in memory. While the use of a high N population might overcome some of the limitations of studying a depressed population, there is nevertheless some recent evidence that neuroticism is associated with memory impairment. For example, neuroticism has been associated with reduced

neuroplasticity in working memory networks during working the n-back task (Dima, Friston, Stephan, & Frangou, 2015), and one study found that the relationship between depression and memory complaints in older adults was mediated by neuroticism (Merema, Speelman, Foster, & Kaczmarek, 2013). Future research comparing high and low Ns should examine measures of memory as well as measures of emotional processing. As demonstrated in Chapter 4 of this thesis, including measures of cognition in this kind of research can help to provide a more complete understanding of group differences by separating out any differences that relate to emotional processing *per se* from those that relate to more general cognitive changes.

We found that, overall, high Ns were faster that low Ns to recognise negative faces, regardless of treatment condition. However, from Figure 5.4 it can clearly be seen that both high N groups were performing at a level almost identical to the low Ns on placebo. That is, the main effect of neuroticism on reaction times to negative faces appears to be driven almost entirely by increased reaction times in the low N citalopram group. This is somewhat consistent with previous research in healthy controls, which have tended to show worse recognition of negative faces with drug treatment (Arnone et al., 2009; Harmer et al., 2004; although few studies have reported effects of antidepressant medication specifically on reaction times). The interaction between treatment and neuroticism did not quite reach significance (p = .08); however, this may simply be due to a lack of power in the study. Future research with a larger sample size should investigate facial recognition in high and low Ns to confirm whether reaction time effects are indeed attributable to treatment effects in low Ns.

5.4.2 **Overall effects of citalopram treatment**

Unlike in the recognition task, self-referent word recall was not affected by neuroticism. Rather, we found effects very similar to those in other studies in healthy volunteers (Arnone et al., 2009; Harmer et al., 2011; Harmer et al., 2004): citalopram produced an increase in recall of positive vs

negative words, regardless of N group. As above, it is surprising that we did not find any baseline differences between neuroticism groups in word recall, given previous findings that neuroticism modulates memory for negative vs positive words (Chan et al., 2007). However, that citalopram increased recall of positive vs negative words for high Ns as well as low Ns is consistent with one other study finding the same effect in high Ns (Di Simplicio, unpublished findings).

5.4.3 **Modulation of treatment effects by neuroticism**

Past research has found that high Ns have reduced recognition of positive faces (in particular happy faces; Chan et al., 2007), which is corrected by citalopram treatment (Di Simplicio, Doallo, et al., 2014). While we failed to find baseline differences between high and low Ns when placebo groups were directly compared, we did find an interaction between N and treatment. This suggested that there were opposing treatment effects in the two groups, with citalopram increasing recognition of *negative* faces for high Ns compared to low Ns.

It may be that this effect relates to the reversal of an avoidant pattern of behaviour towards socially-relevant or threatening emotional information previously hypothesised to occur during antidepressant treatment in high Ns. Di Simplicio and colleagues found that one week of citalopram treatment increased gaze at emotional faces, regardless of expression, and suggest that the treatment is allowing this group to override their aversion to socially salient stimuli (Di Simplicio, Doallo, et al., 2014). In this context, increased recognition accuracy for negative faces may also reflect the reversal of avoidance. The low Ns, who do not have this same aversion, show the reduction in recognition of negative faces more consistently reported in the healthy control literature (Harmer et al., 2011; Harmer et al., 2004; as well as an increase in reaction times to negative faces as outlined above). This aversion may be too subtle to appear significant when placebo groups are considered alone, but begins to emerge when opposing treatment effects are included in the analysis.

These findings stand in contrast to the above results for self-referent word recall, in which both high and low Ns showed similar facilitation of positive vs negative word memory. This suggests that there may be separable processes in play. As above, for high Ns citalopram may remediate an aversion that this group specifically shows to stimuli that are particularly socially relevant and/or threatening, producing an apparent increase in processing of negative faces. However, for more general emotional information that is not directly social or threatening, such as self-referent emotionally-laden words, the drug produces a similar positive shift regardless of neuroticism group.

Finally, in the recognition memory task, citalopram administration increased false recognitions specifically for high Ns, regardless of the valence of word. Similarly, signal detection theory showed that citalopram shifted β for high Ns, producing a more liberal response style. An increase in false memory for positive words has previously been reported as the result of antidepressant administration (Harmer et al., 2008); however, a general increase in false recognition regardless of valence is unusual. Although it is unclear why antidepressant administration would alter response styles and increase false recognitions specifically for high Ns, these results nevertheless demonstrate that even in a sample of healthy controls, personality factors such as neuroticism can have an influence on the effect of drug administration on emotional processing tasks.

Overall then, we found a range of results that prove somewhat challenging to interpret. It appears that high neurotics show an impairment in recognition memory for negative words, which does not seem consistent with past research and could potentially be attributable to broader memory impairment. Regardless of neuroticism group, citalopram appears to improve recall of positive vs negative words, consistent with both the healthy control and high N literature. Finally, and perhaps most interestingly, the effects of citalopram on facial expression recognition depended

on neuroticism group: the drug appeared to increase recognition of negative faces in high Ns, while impairing recognition of the same faces in low Ns. This contrasting effect of citalopram specifically on facial processing in high and low Ns is examined in more detail in the following chapter, which investigates the effects of the drug on neural response to faces.

Chapter 6

Neuroticism and neural correlates of facial processing

6.1 Introduction

In the previous chapter we found some evidence that citalopram treatment may influence behavioural responses to facial expressions depending on neuroticism levels. Specifically, citalopram increased recognition of negative vs positive faces for high Ns compared to low Ns, while the drug also appeared to increase reaction times to negative faces for low Ns. These effects suggest that the drug may be reversing dysfunctional avoidance of threatening social information in high Ns, similar to effects found after seven days of treatment (Di Simplicio, Doallo, et al., 2014), while reducing the processing of the same information in low Ns.

However, these effects were small, and sometimes occurred only at a trend level of significance. An investigation into neural responses to emotional facial expressions could provide converging evidence to support this theory, or indeed reveal additional neuropsychological effects that are not apparent in purely behavioural measures.

6.1.1 Neural effects of neuroticism in emotional processing tasks

Neuroticism has been associated with a number of changes in BOLD response during the presentation of emotional information. The following section summarises some of the main findings.

Fronto-parietal hyperactivity in high N

Neuroticism has been associated with hyperactive BOLD response during the processing of negative emotional information. Studies have particularly implicated fronto-parietal structures. In a self-referential word categorisation task, high neuroticism was associated with greater BOLD response in right superior parietal cortex and left ACC when classifying negative, but not

positive, words (Chan, Harmer, Goodwin, & Norbury, 2008b). Similarly, high Ns showed greater activity than low Ns in middle frontal gyrus and superior parietal cortex when viewing medium intensity fearful vs happy faces; additionally, activity of left middle temporal gyrus and right fusiform gyrus increased linearly with intensity of fearful expressions (Chan, Norbury, Goodwin, & Harmer, 2009).

The association of fronto-parietal structures with neuroticism has been supported by a recent meta-analysis of 18 studies examining emotional processing of negative vs neutral material (Servaas et al., 2013). This found that neuroticism was significantly positively correlated with activity in a number of brain areas, including posterior ACC/paracingulate, superior medial frontal gyrus and parahippocampal and fusiform gyri. Subcortical areas including hippocampus and thalamus were also implicated. These areas are involved in emotion regulation, suggesting greater attentional bias to negative information and/or increased effort to regulate negative emotions in high Ns (Chan et al., 2008b; Servaas et al., 2013).

Amygdala hyperactivity in high N

The amygdala is known to have a key role in processing the salience of emotional information (Pessoa & Adolphs, 2010), and a number of studies have demonstrated greater BOLD response to negative stimuli in the amygdala in patients with depression (Hamilton et al., 2012b; Suslow et al., 2010). Similarly, there is some evidence that amygdala activity may also be dysfunctional in high neuroticism. Chan and colleagues found that the amygdala showed greater activity for medium intensity fearful faces vs happy faces in high Ns compared to low Ns (Chan et al., 2009). Others have found that high N is associated with increased amygdala response to negative stimuli only under conditions of stress (Everaerd, Klumpers, van Wingen, Tendolkar, & Fernández, 2015) and in incongruous trials of the Word-Face Stroop (Haas, Omura, Constable, & Canli,

2007). However it should be noted that the above meta-analysis failed to find any effects of neuroticism in the amygdala (Servaas et al., 2013).

Hypoactivity during threat-related tasks in high N

There is evidence that neuronal dysfunctions in neuroticism may be more complicated than simply heightened activation to all negative information. Some studies have failed to find that neuroticism modulates activity in fronto-parietal areas (Canli, Amin, Haas, Omura, & Constable, 2004); others have found that neuroticism actually *reduces* activation. One recent study showed that high Ns had reduced activation to high intensity fear faces vs high intensity happy faces compared to low Ns, in mPFC and temporo-parieto-occipital cortex (Di Simplicio, Norbury, Reinecke, & Harmer, 2013), an effect that appeared to be driven mainly by reduced response to fearful faces. Similarly, the above meta-analysis found a number of areas that were *negatively* correlated with neuroticism during negative emotional processing, including (anterior) ACC, PCC/precuneus, thalamus, striatum, hippocampus and middle temporal and occipital gyri (Servaas et al., 2013).

The studies that contributed to these effects within the meta-analysis largely involved the anticipation of a negative stimulus – for example, a cue signalling a negative image (Bruhl, Viebke, Baumgartner, Kaffenberger, & Herwig, 2011) or pain (Coen et al., 2011). The authors suggest that the reduction in activation amongst high Ns could relate to the use of avoidance strategies when faced with threatening information. The same could apply to the reduction in mPFC and temporo-parietal-occipital cortex activation in high Ns to fearful faces in the study by Di Simplicio and colleagues (Di Simplicio et al., 2013): fearful faces and the associated connotations of threat may be particularly relevant to the pattern of avoidance seen in high Ns. On the other hand, more general negative information that is not directly related to threat-

processing, such as classifying negative self-descriptors, produces increased BOLD response as the result of a more general negative bias (Chan et al., 2008b).

6.1.2 **Neuroticism and antidepressant treatment**

Despite the large number of studies investigating baseline differences in BOLD response amongst high Ns, very little research has examined the neural effects of antidepressant drugs in this population. Nevertheless, there is some evidence that antidepressants act to normalise the heightened BOLD response to negative stimuli in high Ns. After seven days' treatment with citalopram, high N participants show a decreased BOLD response to negative vs neutral words during a word classification task in the ventromedial prefrontal cortex and anterior cingulate cortex, compared to those on a placebo (Di Simplicio et al., 2012). That is, the drug appears to be normalising the heighted response seen in these areas at baseline (Chan et al., 2008b). These effects are similar to those seen in unselected healthy controls (Miskowiak, O'Sullivan, & Harmer, 2007; Norbury et al., 2008).

Similarly, in the group of high N participants who showed *reduced* mPFC and temporo-parietal-occipital cortex activation to fearful vs happy faces, seven days of citalopram treatment increased activity in these areas, as well as in the amygdala (Di Simplicio et al., 2013). The authors suggest that these apparently paradoxical findings of increased BOLD response to negative expressions with treatment may reflect short-term anxiogenic effects of antidepressants. Unmedicated, this group shows avoidance of threat-related emotional information. Antidepressant medication initially reverses this avoidance, producing a hyperactive neurocognitive response, similar to the early increases in anxiety sometimes seen in patients who have recently started antidepressant treatment (Sinclair et al., 2009). This reversal of avoidance appears consistent with apparent increases in behavioural responses to negative facial expressions with treatment, highlighted in the previous chapter (Di Simplicio, Doallo, et al., 2014).

6.1.3 **Current study**

As discussed in the previous chapter, the above interpretation predicts that the initial increase in vigilance to negative emotional information will eventually decrease: with reduced avoidance and hence longer-term exposure to threat-relevant stimuli, participants will learn that the stimuli are not in fact associated with aversive events. This should mean that with longer term treatment, BOLD response is ultimately reduced. The findings of the previous chapter cast some doubt on this interpretation, as even after four weeks there appeared to be some behavioural increases in response to negative facial expressions. However, the neural effects of treatment have yet to be studied.

The aim of the current study was therefore to characterise the longer term neural changes in relation to emotional face processing when taking citalopram. By examining neural changes after four weeks, we can start to build up a clearer picture of the timecourse of neural effects of antidepressants in high Ns. In addition, past research has not directly compared the neural effects of citalopram on high and low neurotics. In order to fully understand differences between the groups, they need to be directly compared.

6.2 Methods

6.2.1 **Participants**

fMRI scans were taken after four weeks of citalopram or placebo treatment in the same study as in the previous chapter. Imaging data was not available for seven participants: there were technical difficulties with five scans, a structural image was not obtained for one participant, and another participant was unable to remove jewellery and so was not safe to be scanned. Another participant was excluded from analyses due to incidental MRI findings. Further, seven participants were excluded due to excessive head movement.

Data from a total of 54 participants was included in the final analysis. Demographic data for these participants is displayed in Table 6.1. As in the previous chapter, a chi-square test found no evidence of association between neuroticism group and treatment group, $\chi^2(1) = 0.30$, $p = .59$. A chi-square test across all four combined neuroticism/treatment groups also found that gender distribution did not differ between groups, $\chi^2(3) = 0.46$, $p = .93$. To compare age and years of education, 2 x 2 ANOVAs were conducted; there were no main effects of neuroticism or treatment. For age, there was an interaction between neuroticism and treatment that just reached significance ($p = .05$), although subsequent t-tests between groups failed to find any significant differences between individual groups.

Table 6.1 Demographic data for participants included in task fMRI analysis

	High Neurotics				Low Neurotics			
	Citalopram (*n*=12)		Placebo (*n*=14)		Citalopram (*n*=15)		Placebo (*n*=13)	
Gender	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Male	4	33.33	6	42.86	6	40.00	6	46.15
Female	8	66.67	8	57.14	9	60.00	7	53.85
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Age	21.50	3.09	22.64	3.46	24.27	4.61	21.54	2.15
Years of education[1]	15.59	2.11	15.86	2.11	16.33	41	16.31	2.02

[1]Not available for one participant in the high N citalopram group

6.2.2 **Scanning protocol**

The scan took place on the 28th day of treatment. Scanning took place on two scanners, one at the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) and the other at the Acute Vascular Imaging Centre (AVIC) at the John Radcliffe hospital. Both scanners

were the same model: a 3T Siemens Magnetom Verio scanner with a 32-channel head coil. Identical acquisition parameters were used in each.

T1-weighted images were acquired using a magnetization-prepared rapid acquisition with gradient echo (MPRAGE) sequence. Repetition time (TR) was 2040 ms, echo time (TE) was 4.7ms, and the voxel dimension was $1mm^3$. Total acquisition time for the structural scan was 5m 56s.

6.2.3 **Task**

The study used an event-related design. Participants were presented with male and female faces that were neutral, happy or fearful, and were required to indicate the gender of the face using an MRI-safe button box. Happy and fearful facial expressions were morphed to three different intensities: low (30%), medium (60%), and high (100%) (Ekman & Friesen, 1976; Young et al., 1997). Stimuli were presented in a random order for 500ms, with an ITI that varied between 1.5-5.4s (*M* =3.45s); during the ITI, a fixation cross was presented. There were 24 presentations of each of the seven facial expressions/intensities, for a total of 168 presentations. Accuracy and reaction times for gender discrimination were recorded, and the task was coded and run in the software Presentation (NeuroBehavioural Systems).

While participants were completing the task in the scanner, $T2^*$-weighted gradient-echo echo planar imaging (EPI) slices were acquired with a TR of 3000ms and TE of 30ms. Voxel dimension was $3mm^3$. Images were acquired with a tilt angle of 30° in order to reduce susceptibility-induced signal losses in the frontal cortex (Deichmann, Gottfried, Hutton, & Turner, 2003). A total of 269 volumes were acquired and the task took 13m 33s in total.

6.2.4 **Analysis**

Analysis was carried out using FSL V5.08 (Smith, Jenkinson, Woolrich, Beckmann, Behrens, Johansen-Berg, Bannister, De Luca, Drobnjak, Flitney, et al., 2004). Pre-processing included slice-timing correction, motion correction using FMRIB's Linear Image Registration Tool (MCFLIRT; Jenkinson, Bannister, Brady, & Smith, 2002), deletion of non-brain tissue using the Brain Extraction Tool (BET; Smith, 2002), spatial smoothing with a Gaussian kernel of 6mm full-width-half-maximum and highpass temporal filtering using Gaussian-weighted least-squares straight line fitting, with a cut-off of 90s. Functional data were registered to an individual's structural scan using FMRIB's Linear Image Registration Tool (FLIRT; Jenkinson et al., 2002; Jenkinson & Smith, 2001) and optimised using the Boundary-Based Registration (BBR) technique (Greve & Fischl, 2009). Images were then normalised to the Montreal Neurological Institute template (MNI152) using FLIRT and the non-linear registration tool, FNIRT (Andersson, Jenkinson, & Smith, 2007).

First-level analysis was carried out for each individual subject's data using the FMRI Expert Analysis Tool (FEAT) v6.0, employing a general linear model (FMRIB's Improved Linear Model; FILM), with local autocorrelation correction (Woolrich, Ripley, Brady, & Smith, 2001). The fsl_motion_outliers script was run on data to identify timepoints associated with large movements; the resulting confound matrices were entered into the first-level analysis as confound explanatory variables (EVs). Activation maps were produced modelling the BOLD response to eight EVs: each intensity of happy and fearful faces and neutral faces, as well as fixation. Contrasts were modelled between happy faces and neutral faces, fearful faces and neutral faces, and happy and fearful faces overall and at each intensity. Areas of significant activation were identified using cluster-based thresholding with $Z > 2.3$ and a corrected cluster significance threshold of $p < .05$.

For the higher-level analysis, a mixed effects analysis was used to identify significant clusters of activation for each group: FMRIB's Local Analysis of Mixed Effects (FLAME) with automatic outlier detection (Beckmann, Jenkinson, & Smith, 2003; Woolrich, 2008; Woolrich et al., 2001). Contrasts examined the main effects of neuroticism and treatment, as well as the interaction between the two variables. Areas of significant activation were identified using cluster-based thresholding with $Z > 2.3$ and a corrected cluster significance threshold of $p < .05$. In order to control for confounding effects of scanner, this was entered as a demeaned covariate of no interest. Grey matter maps, prepared using the FSL feat_gm_prepare script, were entered as voxelwise covariates of no interest to ensure that any effects were not produced as the result of structural differences.

6.3 Results

6.3.1 Measures of mood, anxiety and personality

Baseline measures

As in the previous chapter, screening questionnaire data were analysed in 2 x 2 ANOVAs to investigate any baseline between-group differences. The pattern in this sample was exactly the same as that in the larger sample, with the addition of a significant difference between neuroticism groups for the lie subscale of the EPQ; details are given in Table 6.2.

Table 6.2 Baseline mean (SD) scores on measures of mood and anxiety for participants included in task fMRI analysis

	High Neurotics		Low Neurotics		
	Citalopram	Placebo	Citalopram	Placebo	Sig effects
BDI[2]	6.58 (5.21)	5.62 (3.64)	0.93 (1.33)	1.15 (1.07)	High > Low
MDQ[1,2]	3.75 (2.93)	2.14 (2.03)	3.40 (2.95)	2.67 (3.55)	-
SHAPS[2]	0.08 (0.29)	0.50 (1.02)	0.13 (0.35)	0.15 (0.38)	-
EPQ					
P	7.00 (3.67)	6.21 (2.58)	8.00 (3.76)	7.39 (4.66)	-
E	14.09 (5.24)	11.86 (6.02)	17.25 (3.39)	18.08 (3.33)	Low > High
N	18.92 (2.47)	17.93 (1.90)	3.27 (1.49)	3.00 (1.68)	High > Low
L	5.50 (3.12)	5.01 (2.65)	7.80 (3.00)	7.17 (3.06)	Low > High
Trait anxiety	40.75 (10.98)	43.00 (9.73)	29.81 (5.76)	31.50 (5.87)	High > Low
State anxiety	38.80 (6.47)	42.37 (8.02)	37.13 (3.48)	36.61 (2.99)	High > Low
LSAS[2]					
Social Fear	8.00 (5.06)	7.79 (4.44)	3.80 (3.95)	4.77 (4.42)	High > Low
Social Avoid.	8.09 (6.28)	7.21 (3.98)	3.07 (3.39)	4.62 (3.71)	High > Low
Perform. Fear	10.36 (4.34)	8.29 (4.07)	6.47 (5.15)	5.85 (5.60)	High > Low
Perform. Avoid.	7.09 (5.79)	6.14 (3.39)	3.53 (3.66)	4.46 (4.47)	High > Low

[1] No participant met criteria for bipolar
[2] Data missing from one participant

Effect of four weeks' citalopram treatment

We again examined whether four weeks' citalopram treatment affected scores on BDI, state anxiety, neuroticism, SHAPS, and VAS scales in this subsample of participants. The pattern of results was very similar to that found in Chapter 5, with a few minor exceptions: the interactions between time and neuroticism for BDI and state anxiety no longer quite reached significance, though the trends were still very close to significance (p = .07 and p = .05 respectively), while the main effect of time on BDI did become significant. There was also no longer a significant effect of time on the anxiety VAS (p = .07). As before, there were no effects of treatment on any of the measures.

6.3.2 **Behavioural results**

Due to technical problems, reaction time and accuracy data was not available for one participant. There were no significant main effects of neuroticism or treatment and no significant interaction for accuracy or reaction time during the gender classification task (ps > .05).

6.3.3 **Whole-brain analysis**

We examined the main effects of neuroticism and treatment, as well as the interaction between the two variables, for fearful faces vs neutral faces, happy faces vs neutral faces, and fearful vs happy faces at each intensity. The results of the whole brain analysis are summarised in Table 6.3. The following sections examine each contrast in turn.

Fear > Neutral contrast

Treatment x neuroticism interaction

In order to examine the overall effects of fearful faces, a contrast examined the combined fearful faces vs neutral faces. There were no main effects of treatment or neuroticism, but a number of clusters throughout the brain showed a treatment x neuroticism interaction.

Significant clusters of activation for this interaction are displayed in Figure 6.1a (left). Areas included bilateral dlPFC extending into anterior middle frontal gyrus and orbitofrontal cortex (OFC), bilateral insula and operculum, basal ganglia structures including bilateral caudate, putamen, thalamus, and right pallidum, and medial structures including ACC, paracingulate, superior frontal gyrus/supplementary motor cortex (SMC), and precuneous. There was also activation in the left supramarginal gyrus, as well as occipital pole and cerebellum.

Masks were created from the areas of significant activity, which were then warped back into individuals' functional space and used to extract percentage signal change from each subject for the fear > neutral contrast. These are plotted by group in Figure 6.1a (right). Post-hoc t-tests

demonstrated that high neurotics on placebo had reduced BOLD response to the fear > neutral contrast compared to low neurotics on placebo, $t(25) = 3.79$, $p < .01$. For high neurotics, citalopram increased activity, $t(24) = 4.82$, $p < .001$, while for low neurotics, citalopram decreased activity, $t(26) = 3.09$, $p < .01$.

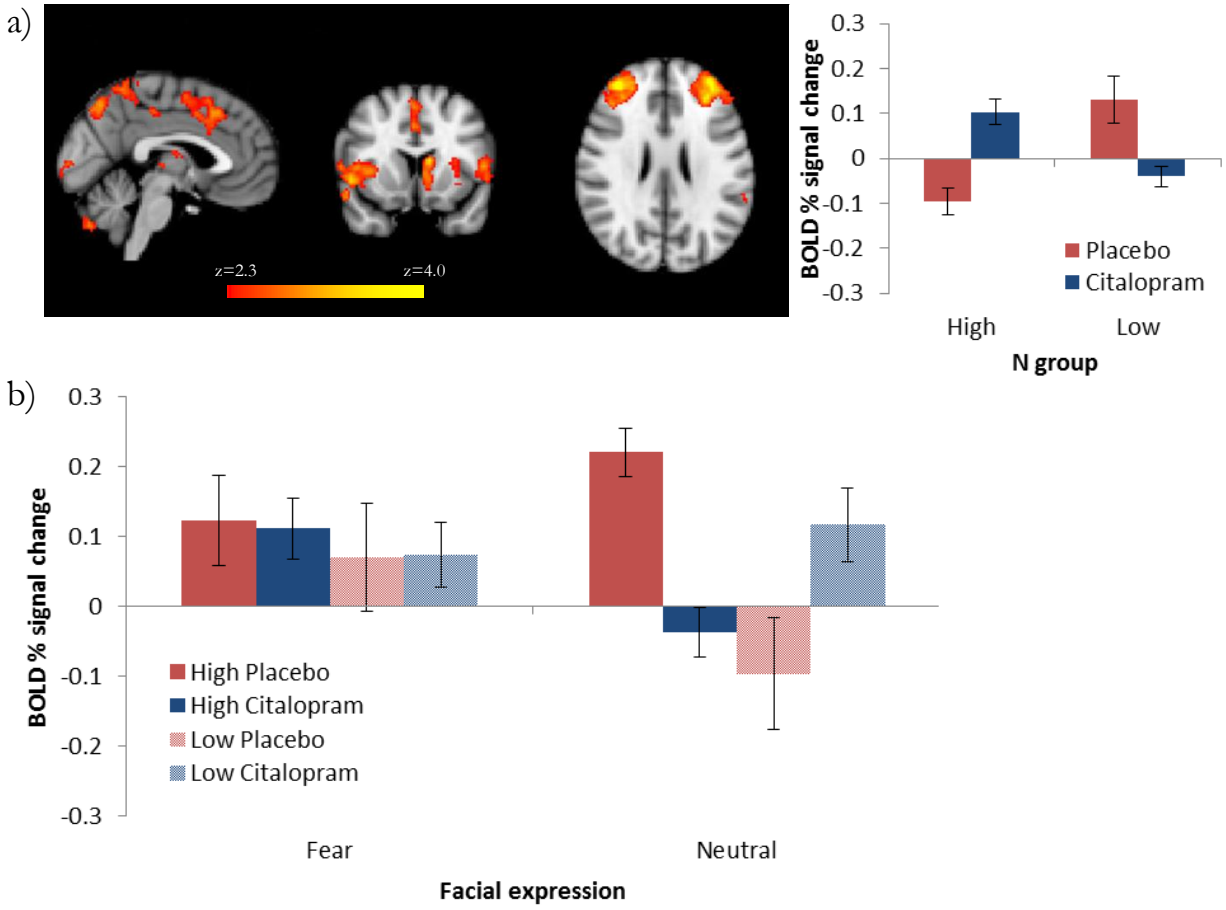

Figure 6.1 Treatment x neuroticism interaction for fear > neutral contrast
a) *Left*: Clusters of significant interaction; *Right*;: Mean percentage signal change within the clusters for each group for the fear > neutral contrast, showing the treatment x neuroticism interaction.
b) Mean percentage signal change within the clusters for each group, for fear and neutral faces (vs fixation baseline) separately. Neutral faces but not fearful faces showed a treatment x neuroticism interaction. Error bars represent SEM.

Table 6.3 Clusters showing significant group differences in BOLD response

Contrast	Anatomical location	MNI (x y z)			p-value
Fear>Neut					
T x N	R operculum, insular cortex, temporal pole, inferior frontal gyrus, putamen, pallidum, thalamus; L caudate	38	16	4	<0.001
	Bilateral cerebellum, occipital pole	2	-76	-44	<0.001
	L dlPFC, middle frontal gyrus, OFC	-36	48	24	<0.001
	R dlPFC, middle frontal gyrus, OFC	38	46	26	<0.001
	Bilateral precuneous cortex, PCC, L precentral gyrus	8	-72	48	<0.001
	L insular cortex, putamen, inferior frontal gyrus, precentral gyrus, operculum	-30	18	-2	<0.001
	ACC, paracingulate, SMC, superior frontal gyrus	2	16	38	0.003
	L supramarginal gyrus, angular gyrus, superior parietal lobule	-52	-42	38	0.036
Happy>Neut					
Cital. > Plac.	L dlPFC, OFC	-50	42	-8	0.005
T x N	Bilateral cerebellum, R occipital fusiform gyrus	36	-78	-28	<0.001
	Precuneuous, PCC, bilateral superior lateral occipital cortex	6	-68	46	<0.001
	R dlPFC, middle frontal gyrus, OFC	42	44	24	<0.001
	L dlPFC, middle frontal gyrus	-44	52	8	0.003
	L supramarginal gyrus, angular gyrus, superior parietal lobule	-50	-40	36	0.020
FM>HM					
Plac. > Cital.	R superior & inferior lateral occipital cortex, cuneal cortex	16	-80	34	0.039
FL>HL					
T x N	L caudate, putamen, thalamus, insular cortex, operculum, precentral gyrus, R thalamus	-32	-10	16	<0.001
	R insular cortex, operculum, OFC, dlPFC	30	12	12	<0.001
	SMC, ACC, paracingulate	18	2	44	0.003

PCC = posterior cingulate cortex; ACC = anterior cingulate cortex; OFC = orbitofrontal cortex; SMC = supplementary motor cortex; T = treatment

It is unclear from the contrast data itself whether this interaction is driven by differences in BOLD response to fearful faces, neutral faces, or a combination of the two. Therefore in order to further interrogate the data, the cluster mask was used to extract data from the fearful and neutral faces (versus the fixation baseline) individually. This data is displayed in Figure 6.1b. A 2 (emotion) x 2 (treatment) x 2 (neuroticism) mixed effects ANOVA was conducted on the extracted data. There was a significant treatment x neuroticism interaction, $F(1, 50) = 5.42$, $p < .05$, and a significant emotion x treatment x neuroticism interaction, $F(1, 50) = 24.54$, $p < .001$.

In order to investigate this three-way interaction further, we conducted separate 2 (treatment) x 2 (neuroticism) ANOVAs for each expression. For fearful faces, there were no main effects of neuroticism, $F(1, 50) = .59$, $p = .45$, nor of treatment, $F(1, 50) = .01$, $p = .94$, and no interaction between the two, $F(1, 50) = .02$, $p = .90$.

For neutral faces, there were no main effects of neuroticism, $F(1, 50) = 2.29$, $p = .13$, nor of treatment, $F(1, 50) = .17$, $p = .69$; however, there was a significant interaction, $F(1, 50) = 18.98$, $p < .01$. Post-hoc t-tests indicated that high Ns on placebo showed increased activity to neutral faces compared to low Ns on placebo, $t(25) = 3.75$, $p < .01$, and citalopram decreased activity for high Ns, $t(24) = 5.18$, $p < .001$, while increasing it for low Ns, $t(26) = 2.29$, $p < .05$. That is, the effects found in the fear > neutral contrast seemed to be driven almost entirely by changes in response specifically to neutral faces.

Finally, we reran the whole-brain analysis for the fear > neutral contrast separately for high neurotics and low neurotics, in order to check whether the inclusion of the interaction term with the combined groups could have artificially produced results which were mainly driven by only one of the neuroticism groups. For the high neurotics, the whole-brain analysis produced a similar pattern of activation as above, particularly in frontal and posterior medial and superior

parietal regions though with no basal ganglia or ACC activation and little insula activation, for the citalopram > placebo contrast. However, there were no significant areas of activation for the placebo > citalopram contrast for low Ns. This suggests that results may have been driven largely by the effects of citalopram in the high neurotic group.

Happy > Neutral contrast

1) Main effect of treatment

In order to examine the overall effects of happy faces, a contrast examined the combined happy faces vs neutral faces. There was a main effect of treatment, with participants on citalopram showing greater activity to happy vs neutral faces in left vlPFC compared to participants on placebo (Figure 6.2a).

In order to further investigate this effect, a mask of this cluster was created and used to extract BOLD percentage signal change for each participant for happy and neutral faces individually. This data is displayed in Figure 6.2b. A 2 (emotion) x 2 (treatment) ANOVA was conducted on this data, but the expected emotion x treatment interaction did not come out significant, $p > .05$. Rather, the only effect we found on the extracted data was a main effect of treatment, $F(1, 52) = 7.25$, $p < .01$, indicating that there was overall *reduced* activation in the citalopram group in this area. It therefore seems that the significant activation for the citalopram > placebo contrast reflects the fact that when viewing happy faces there is *slightly less negative activation* for the citalopram group and slightly more negative activation for the placebo group. However, due to differences in the treatment of statistics (for example, considering tests as two-tailed rather than one-tailed), these small effects are not significant when the data is extracted and run through SPSS. Results have been presented here for interest, however the lack of significant findings in extracted data limit possible interpretations of this effect, and so it will not be considered further.

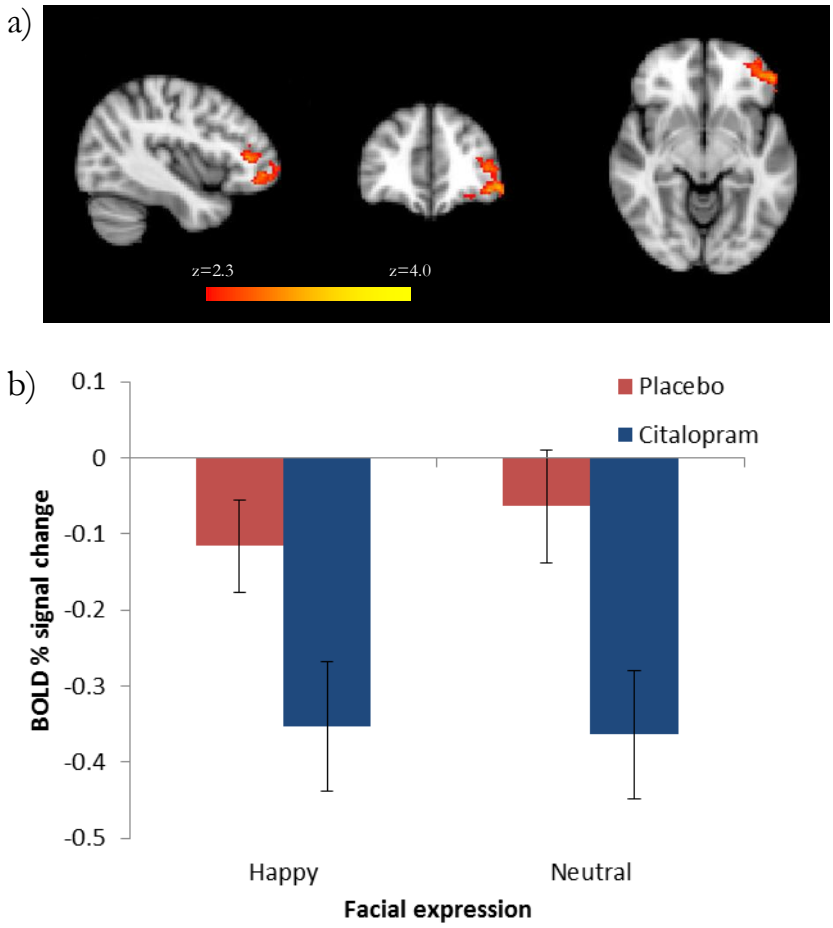

Figure 6.2 Main effect of treatment (citalopram > placebo) for happy > neutral contrast
a) Cluster of significant main effect of treatment
b) Mean percentage signal change within the cluster for each group, for happy and neutral faces (vs fixation baseline) separately. Error bars represent SEM.

2) Treatment x neuroticism interaction

A number of clusters throughout the brain showed a treatment x neuroticism interaction for the happy > neutral contrast. These areas are displayed in Figure 6.3a (left). Many of these were similar to those for the fear > neutral contrast: there was bilateral activation in dlPFC, anterior middle frontal gyrus and right OFC, precuneous/PCC and bilateral cerebellum, as well as left

superior parietal regions. Unlike the fear > neutral contrast, there was no basal ganglia, insula or ACC activation.

Masks were again created from the areas of significant activity and warped back into individuals' functional space to extract percentage signal change from each subject for the happy > neutral contrast. These are plotted in Figure 6.3a (left). Post-hoc t-tests indicated that there was a significant difference between low and high neurotics on placebo, $t(25) = 2.93$, $p < .01$. For high neurotics, activity was significantly higher on citalopram than on placebo, $t(24) = 4.29$, $p < .001$; while for low neurotics, activity was significant lower on citalopram than placebo, $t(26) = 3.31$, $p < .01$.

Again, to further interrogate the data, the cluster mask was used to extract data from the happy and neutral faces (versus fixation baseline) individually. This data is displayed in Figure 6.3b. A 2 (emotion) x 2 (treatment) x 2 (neuroticism) mixed effects ANOVA was conducted on the extracted data. There was a significant effect of neuroticism, indicating that overall, high neurotics showed greater activity in these areas compared to low neurotics, regardless of expression, $F(1, 50) = 4.15$, $p < .05$. There was also an emotion x treatment x neuroticism interaction, $F(1, 50) = 22.06$, $p < .001$.

In order to investigate this three-way interaction further, we conducted separate 2 (treatment) x 2 (neuroticism) ANOVAs for each expression. We found that for happy faces, there was a main effect of neuroticism, $F(1, 50) = 4.97$, $p < .05$, with high neurotics showing greater activation to happy faces compared to low neurotics. However, there was no main effect of treatment, $F(1, 50) = .45$, $p = .50$, and no interaction between the two variables, $F(1, 50) = .00$, $p = .97$.

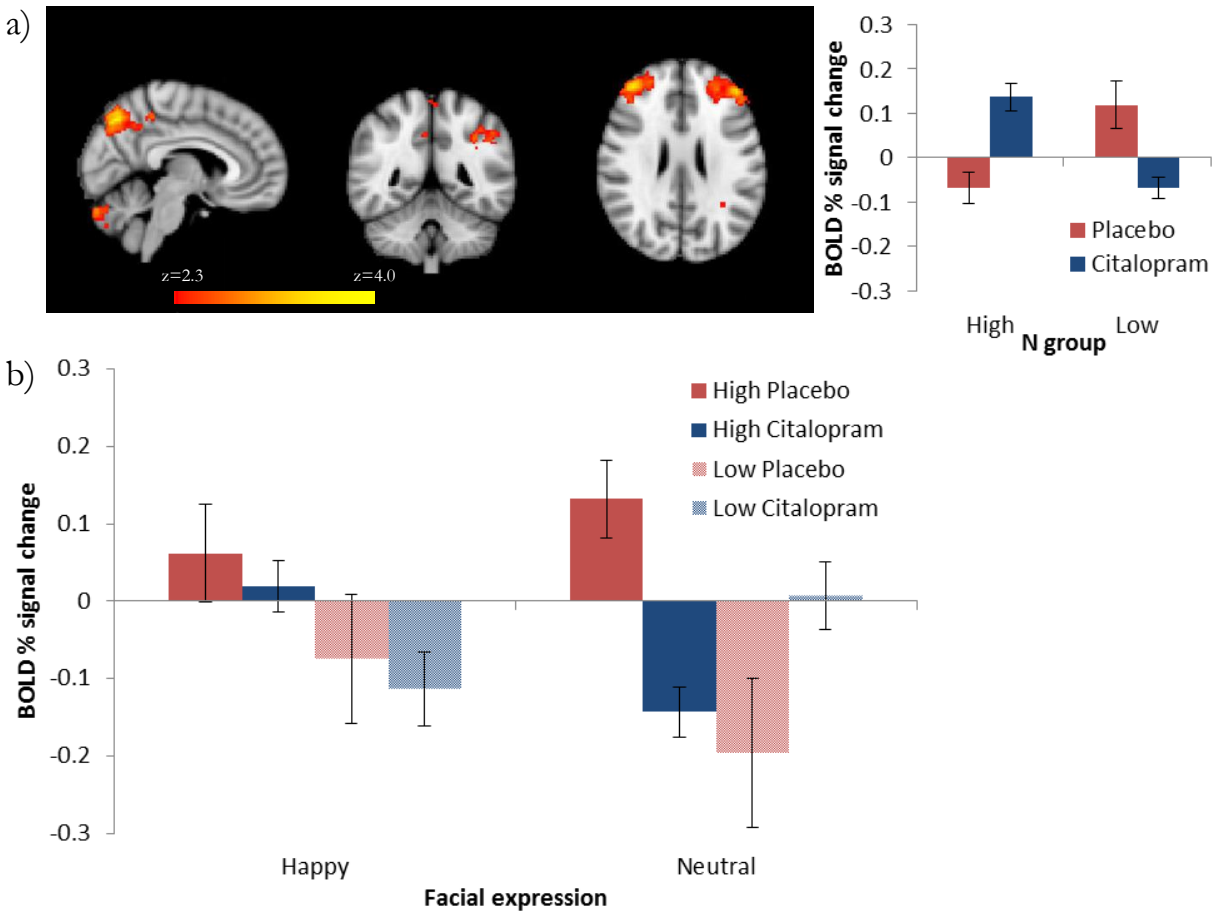

Figure 6.3 Treatment x neuroticism interaction for happy > neutral contrast
a) *Left*: Clusters of significant interaction; *Right*: Mean percentage signal change within the clusters for each group for the happy > neutral contrast, showing the treatment x neuroticism interaction.
b) Mean percentage signal change within the clusters for each group, for fear and neutral faces (vs fixation baseline) separately. For happy faces there was only a main effect of neuroticism; for neutral faces there was a treatment x neuroticism interaction.

For neutral faces, there were no main effects of neuroticism, $F(1, 50) = 2.17$, $p = .15$, nor of treatment, $F(1, 50) = .36$, $p = .55$; however, there was a significant interaction, $F(1, 50) = 15.74$, $p < .001$. Post-hoc t-tests indicated that high Ns on placebo showed increased activity to neutral faces compared to low Ns on placebo, $t(25) = 3.09$, $p < .01$, and citalopram decreased activity for high Ns, $t(24) = 4.40$, $p < .001$, while the difference for low neurotics bordered on significance, $t(26) = 2.02$, $p = .054$. Again, these results suggest that the interaction effect found in the happy vs neutral contrast is driven largely by changes in response to neutral faces.

Finally, we again reran the whole-brain analysis for the happy > neutral contrast separately for high neurotics and low neurotics, in order to check whether the inclusion of the interaction term with the combined groups may have artificially produced results which were mainly driven by only one of the neuroticism groups. For the high neurotics, the whole-brain analysis produced a very similar pattern of activation as above, with the addition of greater OFC activation and a small area of left temporal activation, for the citalopram > placebo contrast. However, there were no significant areas of activation for the placebo > citalopram contrast for low Ns. This again suggests that our results at the whole-group level may have been driven largely by the effects of citalopram in the high neurotic group.

Fear > Happy contrast

There were no main effects or significant interactions for the overall fear vs happy contrast. In order to explore further any effects we examined the contrasts between fear and happy faces at each intensity level.

Fear medium > Happy medium

Main effect of treatment

For the fear medium > happy medium contrast, there was a significant effect of treatment, with the citalopram group showing less activity in right lateral occipital cortex to fear medium vs happy medium faces (Figure 6.4a).

In order to further investigate this effect, a mask of this cluster was created and used to extract BOLD percentage signal change for each participant for fear medium and happy medium faces individually. This data is displayed in Figure 6.4b. A 2 (emotion) x 2 (treatment) ANOVA was conducted on this data. The emotion x treatment interaction was significant, $F(1, 52) = 11.03$, $p < .01$, and post-hoc t-tests indicated the difference between placebo and citalopram groups for

the happy medium expression approached significance, $t(52) = 1.97$, $p = .054$; no other contrasts were significant. Therefore the results suggest that this effect is largely driven by *increased* response to happy medium faces in the citalopram group.

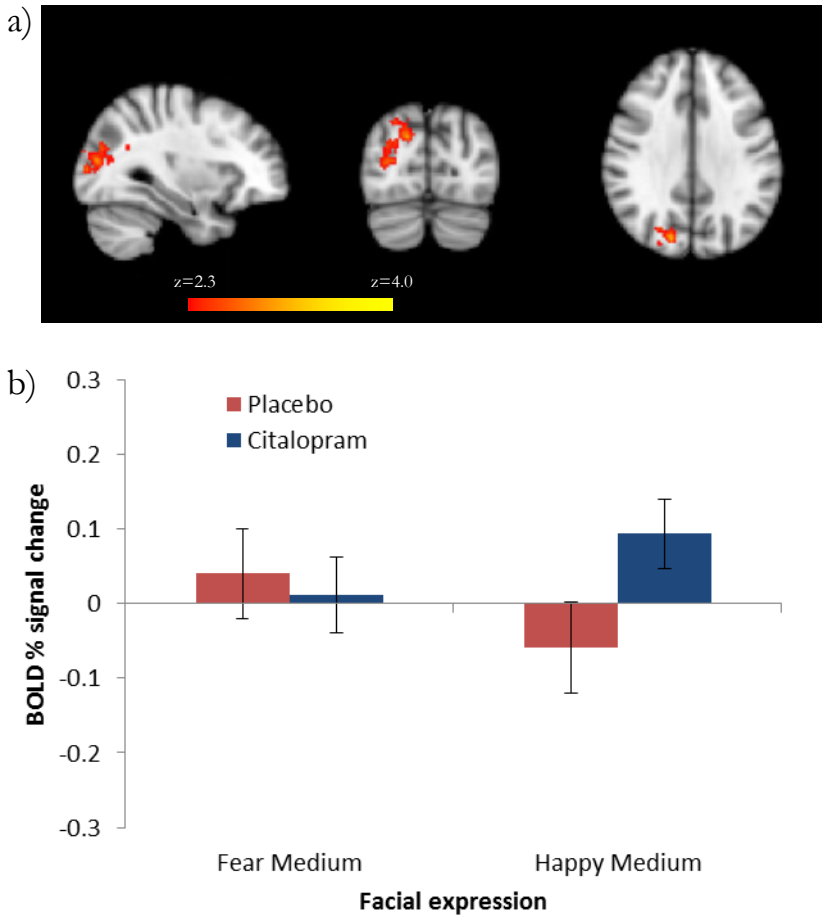

Figure 6.4 Main effect of treatment (placebo > citalopram) for the fear medium > happy medium contrast
a) Cluster showing the significant main effect of treatment
b) Mean percentage signal change within the cluster for each group, for fear medium and happy medium faces (vs fixation baseline) separately. The effect of treatment approaches significance for happy medium faces ($p = .054$), but not for fearful medium faces. Error bars represent SEM.

Fear low > happy low

Treatment x neuroticism interaction

For the fear low > happy low contrast, there was an interaction between treatment and neuroticism. Significant clusters of activation are displayed in Figure 6.5a (left). These included bilateral OFC, insula, operculum and thalamus, left basal ganglia regions including putamen and caudate, and ACC and paracingulate.

Masks were again created from the areas of significant activity and warped back into individuals' functional space to extract percentage signal change from each subject for the fear low > happy low contrast. These are plotted in Figure 6.5a (right). Post-hoc t-tests indicated that there was a significant difference between low and high neurotics on placebo, $t(25) = 3.72$, $p < .01$. For high neurotics, activity was significantly higher on citalopram than on placebo, $t(24) = 3.39$, $p < .01$, while for low neurotics, activity was significant lower on citalopram than placebo, $t(26) = 4.24$, $p < .001$.

Again, to further interrogate the data, the cluster mask was used to extract data from the fear low and happy low faces (versus fixation baseline) individually. These data are displayed in Figure 6.5b. A 2 (emotion) x 2 (treatment) x 2 (neuroticism) mixed effects ANOVA was conducted on the extracted data. There was a significant emotion x group x neuroticism interaction, $F(1, 50) = 28.65$, $p < .001$. The overall effect of emotion also approached significance, $F(1, 50) = 3.95$, $p = .052$.

In order to investigate this three-way interaction further, we conducted separate 2 (treatment) x 2 (neuroticism) ANOVAs for each expression. We found that for fear low faces, there were no main effects of neuroticism, $F(1, 50) = .04$, $p = .85$, nor of treatment, $F(1, 50) = .01$, $p = .94$, and no interaction, $F(1, 50) = .00$, $p = .96$.

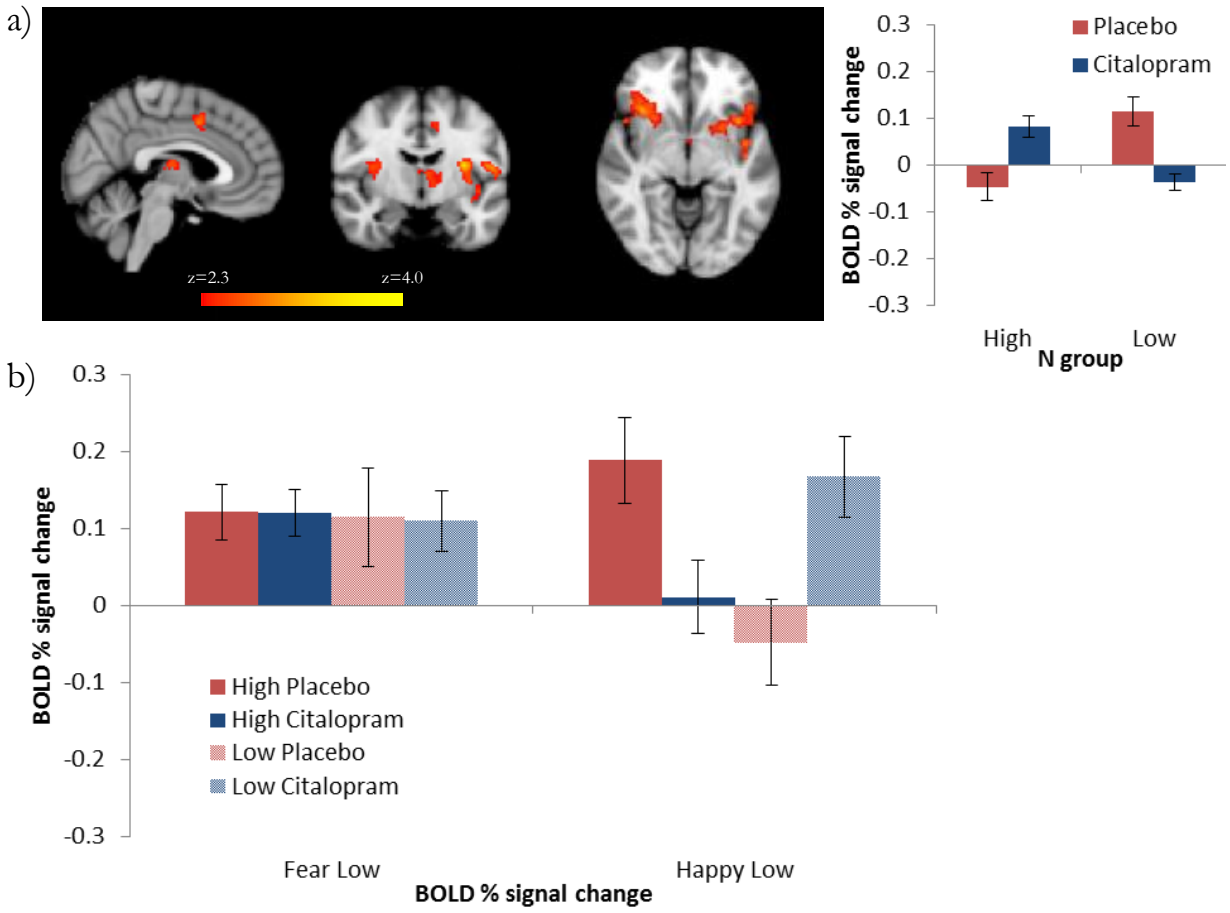

Figure 6.5 Treatment x neuroticism interaction for fear low > happy low contrast
a) *Left*: Clusters of significant interaction; *Right*: Mean percentage signal change within the clusters for each group for the fear low > happy low contrast, showing the treatment x neuroticism interaction.
b) Mean percentage signal change within the clusters for each group, for fear low and happy low (vs fixation baseline) separately. Happy low faces but not fear low faces showed a treatment x neuroticism interaction. Error bars represent SEM.

For happy low faces, there were no main effects of neuroticism, $F(1, 50) = .57$, $p = .46$, nor of treatment, $F(1, 50) = .12$, $p = .73$; however, there was a significant interaction, $F(1, 50) = 13.32$, $p < .01$. Post-hoc t-tests indicated that high Ns on placebo showed increased activity to happy low faces compared to low Ns on placebo, $t(25) = 2.99$, $p < .01$, and citalopram decreased activity for high Ns, $t(24) = 2.35$, $p < .05$, while increasing activity for low Ns, $t(26) = 2.82$, $p < .01$. These

results suggest that the effects found in the fear low vs happy low contrast are driven largely by changes in response to happy low faces.

Finally, we again reran the whole-brain analysis for the happy low > fear low contrast separately for high neurotics and low neurotics, in order to check whether the inclusion of the interaction term with the combined groups may have artificially produced results which were mainly driven by only one of the neuroticism groups. Unlike in the previous two contrasts, there was no significant difference between citalopram and placebo for the high neurotics. However, the low neurotics showed significant activation for the placebo > citalopram contrast, restricted to right insula and OFC. This suggests that for this contrast, our results at the whole-group level may have been driven largely by the effects of citalopram in the *low* neurotic group.

6.3.4 **Amygdala ROI analysis**

In order to examine specifically the effects of citalopram and/or neuroticism on amygdala activity, percent BOLD signal change was extracted from the right and left amygdala for each subject. Signal change was extracted for three contrasts: overall fearful faces, overall happy faces, and neutral faces. These data are displayed in Figure 6.6.

A 3 (emotion) x 2 (neuroticism) x 2 (treatment) mixed effects ANOVA was conducted on the data for each region of interest (ROI). There were no main effects or interaction effects for either ROI (*p*s > .05), providing no evidence that emotional expression, neuroticism, or treatment with citalopram had any effect on amygdala activation.

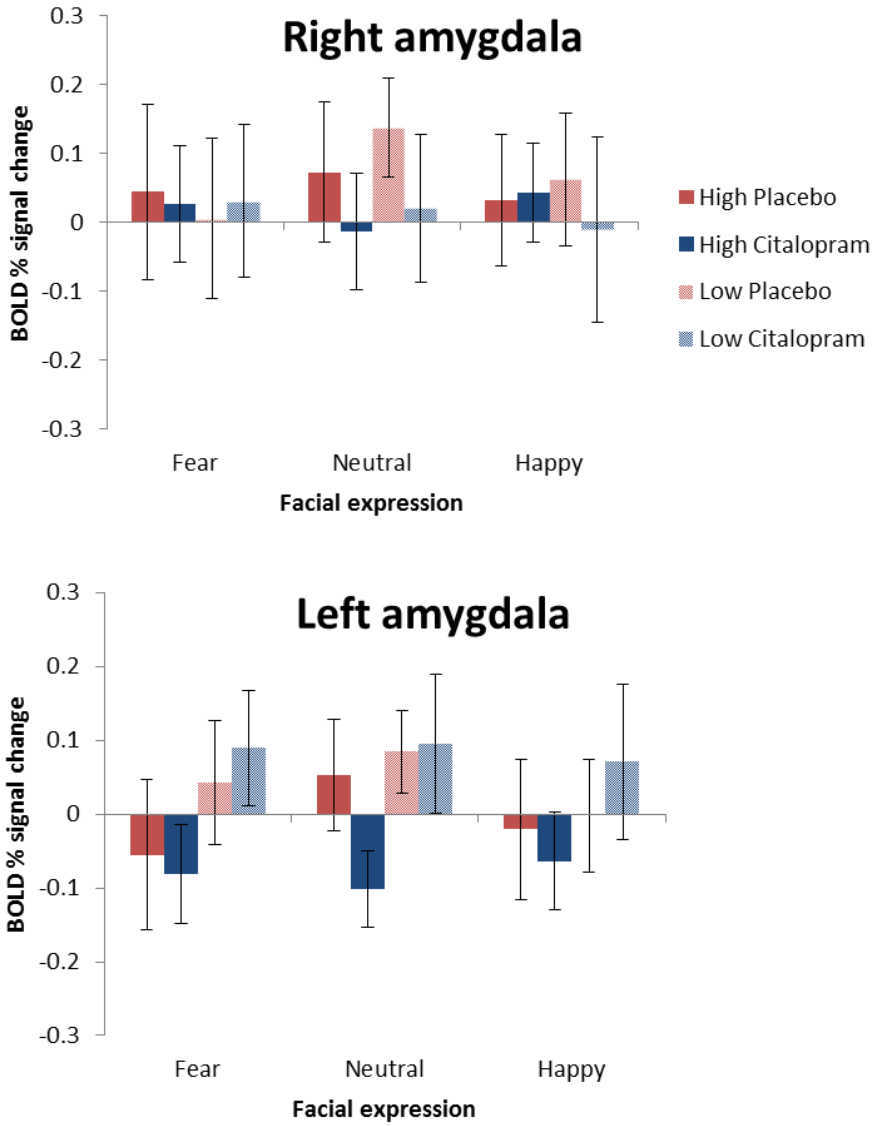

Figure 6.6 Mean percentage signal change in amygdala for each expression (vs baseline)
Top: Right amygdala ROI; *Bottom*: Left amygdala ROI. There were no significant effects or interactions. Error bars represent SEM.

6.4 Discussion

We found that high and low Ns on placebo show differences in neural activity when viewing both fearful and happy faces compared to neutral faces. High Ns showed reduced activity in frontal poles and frontal gyri, precuneous, superior parietal regions and cerebellum for both expressions, as well as insula cortex, basal ganglia and ACC for fearful vs neutral faces. Twenty-eight days of citalopram treatment had opposite effects on the two groups, increasing activation

in these areas for high Ns but decreasing activation for low Ns. However, when we parcelled out the relative contributions of activation to fearful and neutral or happy and neutral faces (versus baseline), we found that these effects were being driven by changes to neutral faces. That is, high Ns on placebo were showing increased activation to neutral faces compared to low Ns on placebo, and citalopram reduced activation to neutral faces amongst high Ns and increased activation amongst low Ns.

Our main research question was whether, as in a previous study (Di Simplicio et al., 2013), citalopram increased the hypoactive BOLD response to fearful faces in mPFC and temporo-parietal areas for high Ns, or whether after this longer time period this effect was reversed. In fact, as our effects were being driven by changes to neutral faces, we failed to find any difference between high and low Ns on placebo for fearful faces or any effect of citalopram for these faces, and so our results cannot directly confirm nor refute this hypothesis. However, our results add to a growing body of evidence that suggests there are key differences between high and low Ns, both at baseline and in terms of response to antidepressant treatment.

6.4.1 **Increased activity in emotion circuits to neutral faces in high N**

Firstly, we found *increased* activation for high Ns vs low Ns on placebo to neutral faces. There have been a number of studies suggesting that certain populations may interpret neutral faces in different ways. Behaviourally, depressed patients have been shown to falsely categorise neutral faces as sad more than controls, suggesting that they interpret supposedly neutral stimuli in a negative fashion (Leppänen, Milders, Bell, Terriere, & Hietanen, 2004). Differences are also seen in fMRI studies: individuals with social anxiety disorder have been shown to have greater right amygdala activity (and lower left amygdala activity) to neutral faces than controls (Cooney, Atlas, Joormann, Eugène, & Gotlib, 2006), and algorithms trained to recognise neural activity to neutral

faces in healthy controls subsequently perform more poorly at identifying activity in depressed patients (Oliveira, Ladouceur, Phillips, Brammer, & Mourao-Miranda, 2013).

As in our study, differences in response to neutral faces have also been found in healthy volunteers who vary on some dimension of anxiety or personality. Participants high on a social phobia scale respond fastest to a neutral face when it appears in a location previously associated with a negative face, suggesting that, like the depressed patients above, they were assigning negative emotional value to the face (Yoon & Zinbarg, 2008). Somerville and colleagues found that state anxiety was negatively associated with amygdala response to happy versus neutral faces (Somerville, Kim, Johnstone, Alexander, & Whalen, 2004). However, in a similar manner to our study, when the facial expressions were examined individually against baseline, it was clear that this effect was produced by a *positive* relationship between amygdala response and neutral faces. The authors suggest that this might be due to increased vigilance to ambiguous, potentially threatening stimuli such as neutral faces amongst more highly anxious people.

This idea is supported by a model put forward by Mogg and Bradley (1998). This suggests that, in their initial response to a stimulus, people low on anxiety tend to avoid ambiguous or mildly threatening stimuli, as it is not beneficial for an individual to waste resources attending to a stimulus that is most likely irrelevant. As threat levels increase, this avoidance will reverse and they will increasingly attend to the stimulus. However, a person who is more anxious may appraise this "mild" stimulus as more threatening, and so will attend to it rather than avoiding it. In our study, and those discussed above, it may be that neutral faces, with their inherent ambiguity, are being avoided by low Ns, but attended to by high Ns (see Figure 6.7). This could explain the differences found in the two placebo groups.

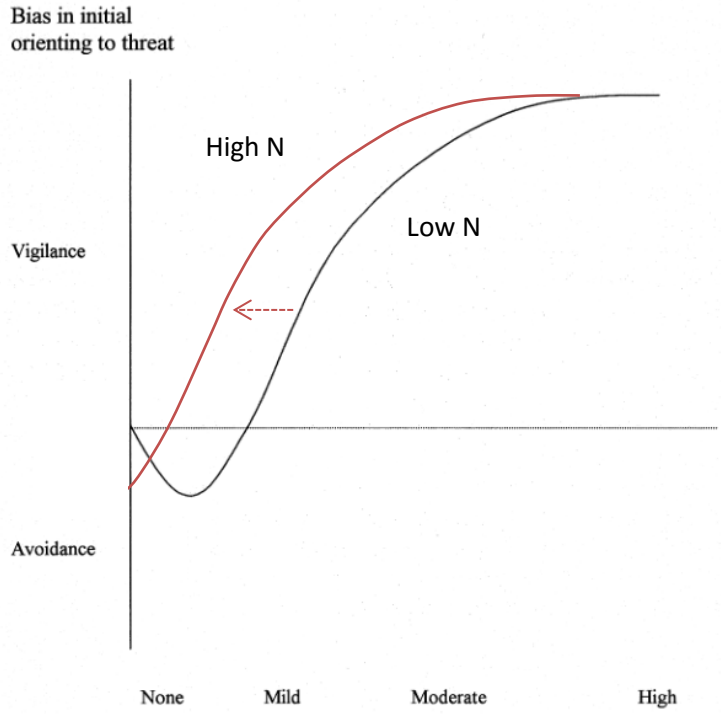

Figure 6.7 High Ns may show left-shifted curve for attending to threatening information
Adapted from Mogg and Bradley (1998)

Consistent with this hypothesis, the significant clusters implicated in the differential processing of neutral faces in our study included several structures involved in emotional processing. In particular, the dorsal ACC and insula have been implicated in the expression of fear and negative emotion (Etkin, Egner, & Kalisch, 2011; Reinecke, Thilo, Filippini, Croft, & Harmer, 2014), while dlPFC is involved in more top-down processes concerning emotional evaluation and regulation (Zwanzger et al., 2014). This pattern of response suggests that the high Ns are appraising and/or experiencing neutral faces as more emotional than the low Ns.

At first glance, this explanation would seem at odds with the study by Di Simplicio and colleagues (Di Simplicio, Norbury, et al., 2014), whose finding of decreased BOLD response to fearful faces in similar areas suggest that high Ns show an aversion to threatening information. However, it has been argued that in high anxious individuals, increased vigilance to threatening stimuli is quickly followed by avoidance (Booth, 2014), and that the "avoidance" part of this

vigilance-avoidance effect may only occur when levels of threat are relatively high (Mogg, Bradley, Miles, & Dixon, 2004). Thus high Ns may show increased neural response to neutral faces due to increased vigilance, while for unambiguously fearful faces there is a decreased neural response due to increased avoidance. This could explain why we found some evidence in the previous chapter that citalopram increased recognition of negative faces in high Ns, rather than decreasing recognition - although it is still unclear why in the present study we failed to show any group differences to fearful faces.

6.4.2 **Effect of citalopram is dependent on neuroticism**

In our study the baseline increase in BOLD response to neutral faces in high Ns was reduced after 28 days' citalopram treatment. This response suggests that citalopram treatment acts to "reset" this baseline pattern of hyperactive response. Given that similar effects in response to neutral faces have not been reported at seven days' treatment, it is hard to say whether these changes emerge early on in treatment and persist for several weeks, or whether they represent late-emerging effects. Future research should examine the modulation of responses specifically to neutral faces in more detail. Whatever the case, it is clear that effects of antidepressant drugs on BOLD response in high Ns persist – and remain distinguishable from effects on low Ns – even after four weeks.

Our study is the first to directly compare the effects of citalopram on a low and high N group. We have demonstrated that the drug has opposite effects for high and low Ns: our low N group actually showed an increase in response to neutral faces with citalopram treatment. It is difficult to explain why low neurotics would show this increase: previous studies on healthy controls have found increase in BOLD response to positive faces (Norbury et al., 2009; Rawlings et al., 2010) but similar increases to neutral faces have not been reported. However, it should be noted that when we examined the fear and happy vs neutral contrasts for high and low Ns individually, only

the high N group showed any effect of treatment. The presence of drug-induced changes in BOLD response to neutral faces in low Ns may be something of an artefact, which only appears once this group is compared directly to high Ns. In fact, the one effect we did see when low Ns were considered separately was increased insula response with citalopram to low intensity happy vs fear faces. In this case, there were no effects for high Ns when the groups were considered separately.

In one instance we did find an overall effect of citalopram that was not influenced by neuroticism: for the fear medium > happy medium contrast there was reduced activity in right lateral occipital cortex, which was driven by an increased response to happy medium faces in the citalopram group. Inferior occipital gyri have been implicated in early processing of faces (Haxby, Hoffman, & Gobbini, 2000), and increased activity in this area could represent increased attention towards medium intensity happy facial expressions. Similar effects in early processing regions have been found in previous research: for example, reboxetine increased BOLD response to happy faces in fusiform gyrus, an area also involved in facial processing and with reciprocal connections with occipital gyri (Norbury et al., 2007). These results suggest that for higher intensity happy faces, citalopram has similar effects regardless of neuroticism group, and so treatment differences between groups may only be apparent in response to negative or ambiguous emotional information.

6.4.3 **Amygdala activity not modulated by N or treatment**

Finally, we failed to find any effects of neuroticism or treatment in response to emotional faces in an amygdala ROI analysis. The lack of an effect of neuroticism is not entirely surprising, given a recent meta-analysis which failed to find any consistent effects of neuroticism in amygdala response (Servaas et al., 2013). Another study found that neuroticism modulated amygdala response to facial expressions only if the task immediately followed a stressful film clip showing

aggressive behaviour (Everaerd et al., 2015). The lack of any effect in our study may have been due to the lack of any control over individuals' stress levels. In future studies it would be worth investigating the effects of stress on BOLD response in high and low Ns, and in particular whether antidepressant treatment can modulate this response.

6.4.4 **Future directions**

Overall, then, our results suggest that high and low Ns show a dissociation in BOLD response to neutral and emotional faces, both at baseline and in terms of drug effects. This has several implications for future study design. Firstly, it suggests that neutral faces are not necessarily an adequate baseline. If we had not separated out responses to emotional and neutral faces in our emotion > neutral contrasts, we might have assumed our results represented modulation of response to the happy and fearful expressions, and would have come to quite different conclusions. This is not the first study to highlight the problems of using neutral faces as a baseline (Somerville et al., 2004), but it is clear that researchers must also investigate potential group differences in baseline measures in order to fully understand the meaning behind a contrast.

Secondly, our study shows that even amongst healthy controls, individual differences can have a profound effect upon results. For our purposes, these effects of personality are of central interest. They suggest that high Ns may be a more useful population for studying the effects of antidepressants than unselected healthy controls, as baseline biases in neuropsychological responses may render them more similar to depressed patients. Future research should directly compare neuropsychological effects of antidepressants between high Ns and patients with depression to further investigate this hypothesis.

However, for the researcher interested in healthy controls in general, it is clear that levels of neuroticism - and potentially other indicators of personality – should be carefully controlled for. Were high Ns to be overrepresented in one intervention compared to the other, then any effect of the intervention could be easily confounded by an effect of neuroticism.

Chapter 7

Neuroticism and resting state functional connectivity

7.1 Introduction

Task-based fMRI has inherent limitations. Group differences between, for example, a drug and placebo group could reflect a specific effect of the drug on that task rather than more general differences in underlying processes. On the other hand, the absence of an effect could simply mean that the choice of task is not tapping in to the particular processes affected by the drug.

A complementary approach is to examine patterns of neural activity at rest. In its resting state, the brain shows low frequency fluctuations in BOLD signal, which appear to be spatially diverse but functionally linked (Beckmann, DeLuca, Devlin, & Smith, 2005; Damoiseaux et al., 2006). Because these BOLD effects are measurable in the absence of a task, they could reveal information about group differences that are not detectable in a task paradigm.

There are two major approaches for investigating resting state activity. Independent components analysis (ICA) involves decomposing the resting state data into individual functionally connected resting state networks, while seed-based approaches examine the functional connectivity of a specific seed region with that of the rest of the brain. Studies utilising these two approaches to examine the effects of depression, neuroticism and antidepressants are briefly examined below.

7.1.1 Depression and resting state connectivity

Default Mode Network

The default mode network (DMN) has been of particular interest in depression. This network incorporates mPFC, medial parietal areas including PCC and precuneus, and the inferior parietal lobule (Smith et al., 2009). Medial temporal regions, particularly hippocampus and

parahippocampus, are also often included in the DMN (Greicius, Srivastava, Reiss, & Menon, 2004; Sheline et al., 2009).

The DMN was originally conceptualised as a network of areas which were more active in the "passive" conditions of task-based fMRI studies (Shulman et al., 1997). However, more recently the DMN has been found to be involved in introspective thinking, and especially thoughts about oneself (Buckner, Andrews-Hanna, & Schacter, 2008). The DMN has thus become a prime candidate for study in depression, a disorder which by definition involves negative self-referential thinking. Indeed, task-based fMRI studies have found dysfunction in DMN structures during emotional regulation, both in depressed patients (Sheline et al., 2009) and healthy controls who display high rumination (Ray et al., 2005).

A number of studies have found that resting DMN connectivity is also increased in patients with depression. In ICA analyses, depressed patients have been found to have increased functional connectivity within areas of the DMN, particularly anterior regions including medial PFC, subgenual ACC, pregenual ACC, and OFC (Greicius et al., 2007; Zhu et al., 2012). Posterior regions such as precuneus have shown both increases (Greicius et al., 2007; Li et al., 2013) and decreases (Zhu et al., 2012) in connectivity, leading to suggestions that depression might affect the anterior and posterior parts of the DMN differently (Mulders, van Eijndhoven, Schene, Beckmann, & Tendolkar, 2015).

Seed-based analyses have also highlighted abnormal connectivity of certain DMN structures in depression. Most of these analyses have found an increase in DMN connectivity: for example, increased functional connectivity has been found between a PCC seed and both anterior (subgenual ACC, vmPFC) and posterior (precuneus) DMN regions (Alexopoulos et al., 2012), and increased connectivity has been found between a subgenual ACC seed and insula (Connolly et al., 2013). However, reduced connectivity has been found between a precuneus/PCC seed and

caudate (Bluhm et al., 2009), again highlighting the potential dissociation between anterior and posterior DMN in depression.

Recently, the dmPFC has emerged as a particularly key area of the DMN that shows altered functional connectivity to a number of regions in depression. Sheline and colleagues found that seeds in the precuneus, subgenual ACC and dlPFC all had increased connectivity with an overlapping area of dmPFC they termed the "dorsal nexus" (Sheline, Price, Yan, & Mintun, 2010). A number of other studies have confirmed the importance of this particular part of the DMN in depression (see sections on neuroticism and antidepressants below).

Cortical-amygdala connectivity

Another consistent finding in the resting state literature amongst patients with depression is abnormal functional connectivity between cortical and limbic regions (Wang, Hermens, Hickie, & Lagopoulos, 2012). Particularly important may be connectivity with the amygdala (Córdova-Palomera et al., 2015). In healthy controls, amygdala activity is negatively correlated with higher-level cortical areas involved in emotional processing, including dmPFC and insula (Tahmasian et al., 2013). However, in depression reduced negative connectivity (or sometimes the presence of *positive* connectivity) between amygdala and a number of cortical areas has been found, including ACC, dmPFC, vPFC, precuneus and insula (Anand, Li, Wang, Lowe, & Dzemidzic, 2009; Cullen et al., 2014; Tahmasian et al., 2013; Tang et al., 2013; Veer et al., 2010). The overall pattern of results therefore suggests reduced top-down regulation over amygdala (and possibly other limbic areas too; Anand et al., 2005a) by cortical regions; DMN regions like mPFC and ACC may be particularly important (Wang et al., 2012).

7.1.2 **Neuroticism and resting state connectivity**

A few studies have related resting state functional connectivity to neuroticism, and have tended to show similar patterns to those with depressed patients. In particular, heightened functional connectivity of areas within the DMN has been associated with neuroticism. Higher levels of neuroticism are related to increased connectivity between the dmPFC and the precuneus, and the precuneus itself also shows increased internal functional connectivity (Adelstein et al., 2011).

Neuroticism is also associated with aberrant cortical-amygdala connectivity. Participants who score low on neuroticism show the normal negative connectivity between precuneus and amygdala (Aghajani et al., 2014); however, participants with higher scores actually show *positive* connectivity between the two areas, similar to depressed patients (Cullen et al., 2014). As in the literature on depression, the authors suggest that the reversal of normal negative connectivity relates to an increase in self-referential processing and rumination.

7.1.3 **Effects of antidepressants on resting state connectivity**

Antidepressant treatment may work to "correct" the dysfunctional connectivity associated with depression. A number of studies have examined the effects of antidepressants on functional connectivity, both in healthy controls and in patient groups.

Patient studies

There is some evidence that antidepressants can normalise DMN connectivity. Posner and colleagues (Posner et al., 2013) found that patients with dysthymia had increased DMN connectivity, which was reduced after a 10 week course of duloxetine. Similarly, after 12 weeks of antidepressant treatment, heightened activity in the posterior, but not anterior, DMN was normalised in a group of patients with depression (Li et al., 2013).

Studies have also found that antidepressant treatment remediates the reduced cortical-amygdala connectivity seen in patients. For example, six weeks of sertraline increased functional

connectivity between ACC and amygdala, as well as thalamus and pallidostriatum (Anand, Li, Wang, Gardner, & Lowe, 2007; Anand et al., 2005b). A recent review suggests that treatment responders show a greater increase in frontal-subcortical connectivity than non-responders (Dichter, Gibbs, & Smoski, 2015).

On the other hand, a number of studies have included patients who were taking antidepressant medications and have still found heightened DMN or reduced cortical-amygdala connectivity (e.g. Bluhm et al., 2009; Greicius et al., 2007; Tahmasian et al., 2013). This highlights one of the limitations in studying this population: it can often be hard to find a homogeneous sample in which the effects of the illness and the effects of the drug can be clearly disentangled.

Healthy volunteers

Healthy volunteer studies overcome the issues surrounding concurrent medication use. These studies have also tended to find that antidepressant treatment reduces functional connectivity in the DMN. A week of citalopram reduced connectivity between the left hippocampus and the left dorsal nexus (McCabe et al., 2011). An acute dose of ketamine also reduced connectivity between the subgenual ACC and dmPFC and between posterior areas and anterior DMN (PCC to medial PFC/pregenual ACC; Scheidegger et al., 2012).

In contrast to studies on depressed patients, McCabe and colleagues found that a week-long course of citalopram *reduced* functional connectivity between the right amygdala and left vmPFC, while reboxetine reduced connectivity between both the amygdala and the nucleus accumbens and the OFC (McCabe & Mishor, 2011). These findings may be more relevant to an understanding of how antidepressants affect the amygdala-OFC reward-processing network, which is somewhat distinct from the amygdala-DMN connections which have been examined in patient studies (Catani, Dell'Acqua, & Thiebaut de Schotten, 2013).

7.1.4 **Current study**

There is thus strong evidence for aberrant functional connectivity in depression, both within the DMN and between the amygdala and a number of cortical areas. Similar patterns of connectivity are seen in high neurotics, who, similar to patients with depression, often display negative self-referential thinking. Antidepressant treatment appears to remediate dysfunctional connectivity in at least some studies; however, the limitations inherent in studying either patients who might already be on treatment or healthy volunteers who do not display baseline altered connectivity mean that the effects of treatment are not always clear cut. In addition, studies in healthy volunteers have been restricted to short, week-long treatment courses, and so the effects of longer-term doses in non-depressed populations remain to be investigated.

In the current study we sought to overcome these limitations by examining functional connectivity in patients with high neuroticism after a four week treatment with citalopram or placebo. We also compared this group to a group of low neurotics who have received similar treatment. On the basis of the above research, we made a number of predictions about the pattern of resting state activity we would see in our study:

1. There would be increased functional connectivity within the DMN and from a dmPFC seed to other DMN structures for high neurotics given placebo compared to low neurotics given placebo.
2. There would be reduced functional connectivity between the amygdala and cortical regions, particularly medial frontal areas, for high neurotics given placebo compared to low neurotics given placebo.
3. Four weeks of citalopram would reduce DMN connectivity and connectivity from dmPFC to other DMN structures, and increase functional connectivity between amygdala and cortical regions, for both high and low neurotics.

7.2 Methods

7.2.1 Participants

Resting state scans were taken after four weeks of citalopram or placebo treatment in the same study as in the previous two chapters. Resting state imaging data was not available for five participants: time constraints meant the resting scan was not completed for three participants, a structural image was not obtained for one participant, and one participant was not able to remove jewellery and so could not be scanned. A further participant was excluded from analysis due to incidental findings during the MRI.

Demographic data for the 63 participants for whom resting state data is available is displayed in Table 7.1. As in the previous chapter, a chi-square test found no evidence of association between neuroticism group and treatment group, $\chi^2(1) = 0.51$, $p = .52$. A chi-square test across all four combined neuroticism/treatment groups also found that gender distribution did not differ between groups, $\chi^2(3) = 0.27$, $p = .97$. To compare age and years of education, 2 x 2 ANOVAs were conducted. There were no main effects of neuroticism or treatment, and no interaction, suggesting that the groups did not differ on these variables (ps > .05).

Table 7.1 Demographic data for participants with resting state data

	High Neurotics				Low Neurotics			
	Citalopram (*n*=13)		Placebo (*n*=17)		Citalopram (*n*=17)		Placebo (*n*=16)	
Gender	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Male	5	38.46	7	41.18	6	35.29	7	43.75
Female	8	61.54	10	58.82	11	64.71	9	56.25
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Age	21.15	3.16	22.35	3.24	23.47	4.19	22.56	3.86
Years of education[1]	15.33	2.10	15.82	1.98	16.47	2.79	16.13	1.89

[1] Not available for one participant in the high N citalopram group

7.2.2 **Scanning Protocol and Analysis**

The scan took place on the 28th day of treatment; details of the scanner and T1 image acquisition can be found in the previous chapter. During acquisition of the resting state scan, participants were told to lie still with their eyes open. $T2^*$-weighted gradient-echo EPI slices were acquired with a TR of 2410ms and TE of 30ms. Voxel dimension was $3mm^3$. Images were acquired with a tilt angle of 30° in order to reduce susceptibility-induced signal losses in the frontal cortex (Deichmann et al., 2003). A total of 128 volumes were acquired and acquisition took 5m 16s.

ICA Analysis

Analysis was conducted using FSL v5.08 (Smith, Jenkinson, Woolrich, Beckmann, Behrens, Johansen-Berg, Bannister, De Luca, Drobnjak, & Flitney, 2004). Pre-processing of the functional data involved brain extraction using the Brain Extraction Tool (BET; Smith, 2002), motion correction using MCFLIRT (Jenkinson et al., 2002), spatial smoothing using a Gaussian kernel of 6mm FWHM, grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor, and highpass temporal filtering with a cutoff of 150s. Functional data was registered to each participant's structural image using FMRIB's Linear Image Registration Tool (FLIRT; Jenkinson et al., 2002) and optimised using the Boundary-Based Registration (BBR) technique (Greve & Fischl, 2009). Images were then registered to standard space (MNI-152 template) using FMRIB's Nonlinear Registration Tool (FNIRT; Andersson et al., 2007).

Independent components analysis (ICA) was conducted using the MELODIC tool. A single-session ICA design was used to decompose each individual's functional data into components. FMRIB's ICA-based Xnoiseifier (FIX; Salimi-Khorshidi et al., 2014), was used to auto-classify components as signal or noise, based on hand-classified training data from a separate dataset. Components defined as noise were removed, and the subsequent denoised data was then registered to standard space as above.

The next stage in ICA is to run a group-level analysis, in order to concatenate components across all subjects, identifying group-average components that can then be modelled for each individual subject. Because we acquired images from two different scanners, we chose to run a group ICA on a separate group of 26 healthy controls who had undergone a resting state scan on a 3T scanner for another project in the lab, in order to avoid any confounding effects of scanner on the analysis. The functional data for these subjects had been preprocessed as above. A total of 50 components were identified in the group ICA.

The set of 50 spatial maps from the group analysis was then used to create subject-specific spatial maps and associated time series using dual regression (Beckmann, Mackay, Filippini, & Smith, 2009; Filippini et al., 2009). For each participant the group spatial maps were regressed into the participant's pre-processed functional data (spatial regression), creating a subject-specific timeseries for each of the 50 group spatial maps. These timeseries were then regressed into each individual's data (temporal regression) to create subject-specific spatial maps.

The eight resting state networks found by Beckmann and colleagues (Beckmann et al., 2005), as well as an additional salience network, were identified from within these 50 spatial maps. In order to examine group differences, nonparametric permutation inference was employed using the FSL tool randomise (Winkler, Ridgway, Webster, Smith, & Nichols, 2014), with 5000 permutations. Contrasts examined the main effects of neuroticism and treatment, as well as the interaction between the two variables. In order to control for confounding effects of scanner, this was entered as a demeaned covariate of no interest. Grey matter maps, prepared using the FSL feat_gm_prepare script, were entered as voxelwise covariates of no interest to ensure that any effects were not produced as the result of structural differences.

The resulting statistical maps were thresholded using cluster-mass based thresholding, at $Z > 2.3$ and $p < .05$. Where significant effects were found, the resulting clusters were binarised and

registered back into each individual's functional space. These masks were used to extract the mean time series from each participant in order to further interrogate the results.

Seed Analysis

The denoised functional data from the ICA analysis was used in the seed analysis. Masks of the left and right amygdala were created from the probabilistic maps provided by the Harvard-Oxford Subcortical Structural Atlas within FSL. These were thresholded to 90% and binarised. Masks of the left and right dorsal nexus were made by creating a 10mm radius sphere centred on the co-ordinates provided by Sheline et al. (2010). Masks of an area of white matter (WM) and cerebrospinal fluid (CSF) were also created. Inverse registration maps were used to convert all masks from standard space into each individual's functional space. The mean time series for voxels within the masks were calculated for each individual.

Seed-based correlations were carried out for each participant using FMRI Expert Analysis Tool (FEAT) v6.0. The time series derived from the mask corresponding to the relevant seed (i.e. left dorsal nexus, right dorsal nexus, left amygdala or right amygdala) was included as an explanatory variable (EV) to determine which other brain areas significantly correlated with this time course. Time series derived from WM and CSF masks were included as confound regressors.

Group FEAT analyses were then run for each seed, to determine whether group membership affected functional connectivity with the seed. This analysis utilised FMRIB's Local Analysis of Mixed Effects (FLAME) with automatic outlier detection (Beckmann et al., 2003; Woolrich, 2008; Woolrich et al., 2001). Contrasts examined the main effects of neuroticism and treatment, as well as the interaction between the two variables. Grey matter maps and scanner were included as covariates as above.

Statistical maps were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significance threshold of $p < .05$. As above, where significant effects were found, mean timeseries for each participant were extracted to investigate the results in more detail.

7.3 Results

7.3.1 Measures of mood, anxiety and personality

Baseline measures

As in the Chapter 5, screening questionnaire data were analysed in 2 x 2 ANOVAs to investigate any baseline between-group differences. The pattern in this sample was exactly the same as that in the larger sample; details are given in Table 7.2.

Table 7.2 Baseline mean (SD) scores on measures of mood and anxiety for participants included in resting fMRI analysis

	High Neurotics		Low Neurotics		
	Citalopram	Placebo	Citalopram	Placebo	Sig effects
BDI[1]	7.00 (4.81)	7.44 (6.77)	1.00 (1.37)	1.00 (1.07)	High > Low
MDQ[1]	4.38 (3.12)	3.00 (2.94)	3.06 (3.15)	2.86 (3.66)	-
SHAPS[2]	0.32 (0.67)	0.59 (1.12)	0.12 (0.33)	0.13 (0.35)	-
EPQ					
P	7.69 (3.57)	6.12 (2.57)	8.88 (3.33)	7.01 (4.50)	-
E	13.78 (5.81)	11.71 (5.45)	17.63 (3.44)	18.06 (3.17)	Low > High
N	18.77 (2.31)	18.71 (2.49)	3.00 (1.73)	2.94 (1.57)	High > Low
L	5.69 (3.79)	5.48 (3.00)	7.00 (2.76)	7.20 (2.89)	-
Trait anxiety[2]	41.77 (10.52)	43.53 (9.49)	30.66 (5.36)	30.83 (5.71)	High > Low
State anxiety[2]	39.20 (6.21)	42.66 (7.58)	36.99 (3.69)	36.33 (2.87)	High > Low
LSAS[2]					
Social Fear	7.92 (4.94)	8.35 (4.91)	3.94 (3.78)	4.75 (4.04)	High > Low
Social Avoid.	8.25 (5.96)	7.47 (4.52)	2.82 (3.24)	4.25 (3.59)	High > Low
Perform. Fear	10.50 (4.08)	9.06 (5.04)	6.59 (4.72)	5.56 (5.10)	High > Low
Perform. Avoid.	7.25 (5.41)	7.06 (4.85)	3.94 (3.83)	4.06 (4.11)	High > Low

[1] Data missing from two participants
[2] Data missing from one participant

Effect of four weeks' citalopram treatment

We again examined whether four weeks' citalopram treatment affected scores on BDI, state anxiety, neuroticism, SHAPS, and VAS scales in this subsample of participants. The pattern of results was exactly the same as in Chapter 5.

7.3.2 **ICA analysis**

The ICA analysis identified the eight networks reported by Beckmann and colleagues (Beckmann et al., 2005), with the sensorimotor network split into two components. We also identified a ninth network incorporating insular cortex and subcortical structures which appears similar to the salience network, reported by Seeley (Seeley et al., 2007; though in our case the network did not include ACC). Figure 7.1 displays the resting state networks identified by the ICA.

None of the ten networks showed any main effect of treatment or neuroticism. However, for both the lateral visual network (LVN) and sensorimotor network I (SMN I), there was an interaction between treatment and neuroticism. This interaction did not occur in clusters within the networks themselves; rather in both cases it represented altered functional connectivity between the network and a cluster in the precuneus/posterior cingulate. Details are provided in Table 7.3.

Table 7.3 Networks displaying a significant interaction between neuroticism and treatment

Network	Anatomical location	MNI (x y z)			p-value
LVN	Bilateral Precuneus, posterior cingulate	45	53	52	.009
SMN I	Left precuneus, posterior cingulate	49	49	50	.014

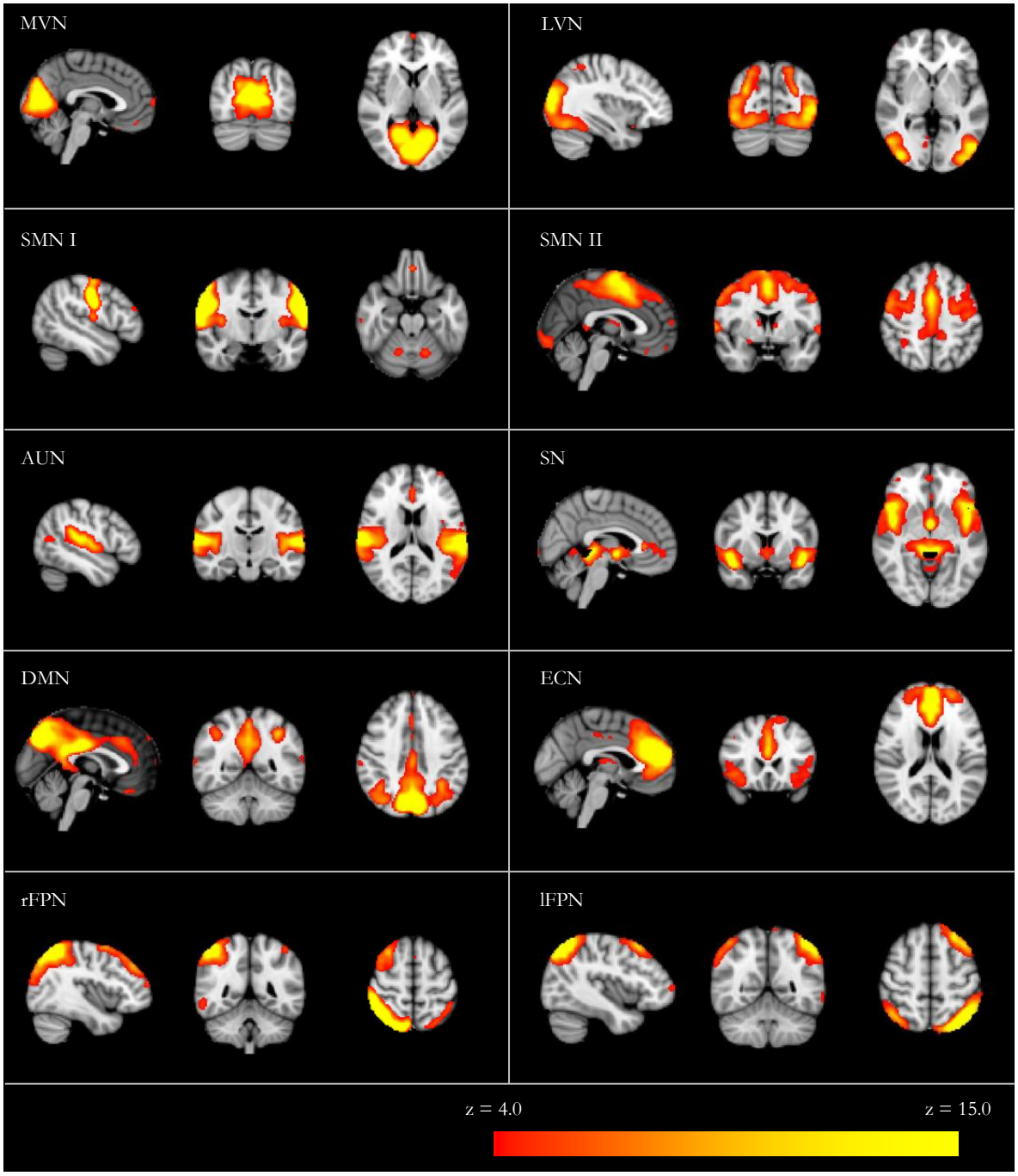

Figure 7.1 Resting state networks identified by the independent components analysis
Spatial maps are thresholded at z=4.0 and overlayed on the MNI-152 2mm template. *MVN* = medial visual network; *LVN* = lateral visual network, *SMN* = sensorimotor network; *AUN* = auditory network; *SN* = salience network; *DMN* = default mode network; *ECN* = executive control network; *rFPN* = right frontoparietal network; *lFPN* = left frontoparietal network

Figure 7.2 displays the clusters showing altered functional connectivity for each network. In order to examine group differences within this area in more detail, a mask of the cluster was used to extract the mean time series for each individual in standard space. Group averages of these are displayed to the right in Figure 7.2.

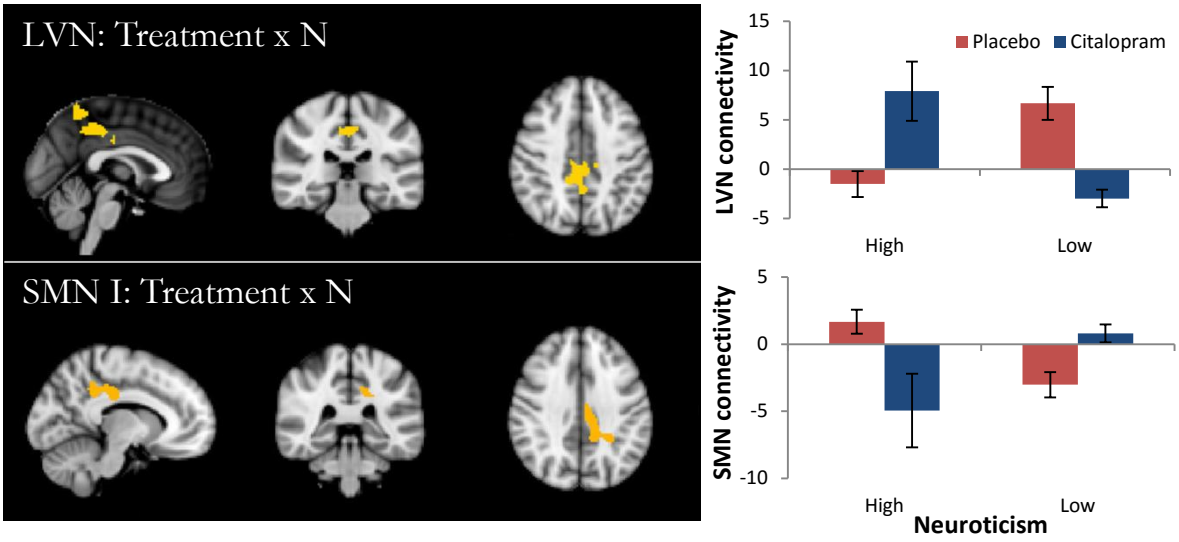

Figure 7.2 Altered functional connectivity in ICA analysis
Left: Clusters showing a significant interaction between treatment and neuroticism for functional connectivity with the LVN (*top*) and SMN (*bottom*) resting state networks.
Right: Mean timecourse extracted from each cluster.

A 2 (treatment) x 2 (neuroticism) ANOVA was run on the extracted LVN data. There was a significant interaction between treatment and neuroticism, $F(1,59) = 30.21$, $p < .001$. There were no main effects of treatment or neuroticism (ps > .05).

In order to examine the interaction in more detail, individual t-tests were conducted between the different groups. There were significant differences between the high N citalopram and high N placebo groups ($t(28) = 3.12$, $p < .01$), low N citalopram and low N placebo groups ($t(28) = 5.19$, $p < .001$), high N placebo and low N placebo groups ($t(31) = 3.88$, $p < .01$) and high N citalopram and low N citalopram groups ($t(28) = 3.88$, $p < .01$). That is, functional connectivity between the LVN and this precuneus/PCC cluster differed at baseline, with high Ns on placebo

showing reduced connectivity compared to low Ns on placebo. Citalopram significantly increased functional connectivity with this cluster for high Ns, but reduced connectivity for low Ns.

A 2 (treatment) x 2 (neuroticism) ANOVA was also run on the extracted SMN data. There was a significant interaction between treatment and neuroticism, $F(1,59) = 14.42$, $p < .001$. There were no main effects of treatment or neuroticism (ps > .05).

Individual t-tests were again conducted between the different groups. There were significant differences between high N citalopram and high N placebo groups ($t(28) = 2.54$, $p < .05$), low N citalopram and low N placebo groups ($t(28) = 3.35$, $p < .01$), high N placebo and low N placebo groups ($t(31) = 3.62$, $p < .01$) and high N citalopram and low N citalopram groups ($t(28) = 2.29$, $p < .05$). That is, the pattern of results was the opposite of that found within the LVN, with high Ns on placebo showing greater connectivity between the SMN and precuneus/PCC than low Ns on placebo. Citalopram significantly reduced functional connectivity with this cluster for high neurotics, but increased connectivity for low neurotics.

7.3.3 **Seed-based analysis**

Table 7.4 provides a summary of clusters where functional connectivity with the seeds was influenced by neuroticism, treatment, or an interaction between the two. The findings for each seed are examined in more detail below.

Left dorsal nexus

Figure 7.3 displays the cluster showing altered functional connectivity with the left dorsal nexus seed for the treatment x neuroticism interaction. This cluster was located in the left hippocampus and parahippocampal gyrus. In order to examine group differences within this area in more detail, a mask of the cluster was warped to the functional space of each individual. The mean

time series within this mask was then extracted from each individual. Group averages of these are displayed to the right in Figure 7.3.

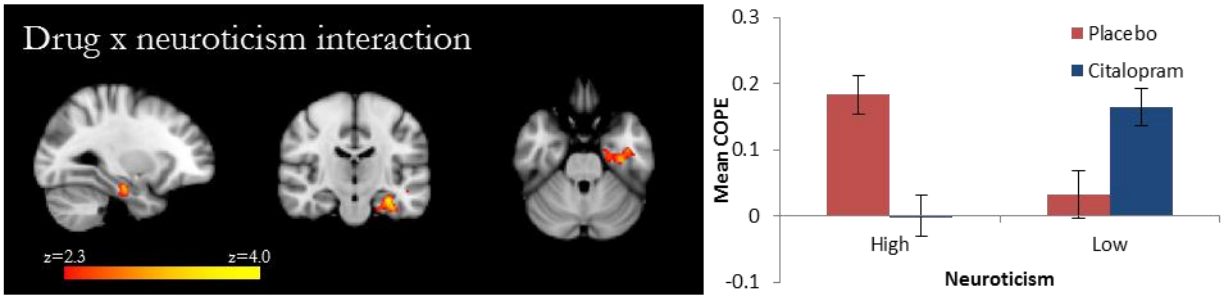

Figure 7.3 Altered functional connectivity with left dorsal nexus seed
Left: Cluster showing a significant interaction between treatment and neuroticism for functional connectivity with the left dorsal nexus seed.
Right: Group mean timecourse extracted from this cluster. Error bars represent SEM.

A 2 (treatment) x 2 (neuroticism) ANOVA was run on the extracted data. There was a significant interaction between treatment and neuroticism, $F(1,59) = 25.40$, $p < .001$. There were no main effects of treatment or neuroticism (ps >.05).

Individual t-tests were conducted between the different groups. There were significant differences between the high N citalopram and high N placebo groups ($t(28) = 4.29$, $p < .001$), low N citalopram and low N placebo groups ($t(28) = 2.93$, $p < .01$), high N placebo and low N placebo groups ($t(31) = 3.28$, $p < .01$) and high N citalopram and low N citalopram groups ($t(28) = 3.95$, $p < .001$). That is, functional connectivity differed at baseline, with high Ns on placebo showing greater connectivity between left dorsal nexus and the hippocampal/parahippocampal cluster than low Ns on placebo. Citalopram significantly reduced functional connectivity with this cluster for high neurotics, but increased connectivity for low neurotics.

Table 7.4 Clusters showing significant group differences in connectivity with left/right dorsal nexus and left/right amygdala

Seed/contrast	Anatomical location	MNI (x y z)			p-value
Left DN					
T x N	L hippocampus, parahippocampal gyrus	-30	-18	-20	0.043
Right DN					
High > Low	R angular gyrus, superior lateral occipital cortex, posterior supramarginal gyrus	46	-48	52	0.002
T x N	L superior lateral occipital cortex, angular gyrus	-48	-70	24	<0.001
	R middle frontal gyrus, superior frontal gyrus	42	20	52	0.002
	L middle frontal gyrus	-32	0	54	0.003
	L hippocampus, parahippocampal gyrus, posterior temporal fusiform, inferior temporal gyrus	-30	-18	-20	0.005
	L superior parietal lobule, supramarginal gyrus, angular gyrus	-34	-52	42	0.008
	L inferior frontal gyrus, OFC, frontal operculum	-38	38	12	0.014
	L frontal pole	-22	58	30	0.018
	L middle temporal gyrus, superior temporal gyrus	-44	-6	-20	0.028
Left Amyg					
Plac. >Cital.	R frontal pole, OFC, insular, inferior frontal gyrus, frontal operculum	30	46	0	<0.001
	Bilateral cuneal cortex, supracalcarine cortex, intracalcerine, occipital cortex	-10	-82	20	0.008
T x N	L frontal pole, middle frontal gyrus, precentral gyrus, postcentral gyrus, superior parietal lobule, supramarginal gyrus, angular gyrus, superior lateral occipital cortex	-34	-10	44	<.001
	Bilateral superior frontal gyrus, SMC, PCC; L middle frontal gyrus	22	20	64	<.001
	R putamen, caudate; bilateral thalamus	26	-8	10	<.001
	R middle frontal gyrus, precentral gyrus	34	22	36	0.001
	L putamen, amygdala, thalamus, caudate, pallidum	-20	0	10	0.002
	Bilateral PCC, ACC	-2	-28	32	0.022
Right Amyg					
T X N	Left postcentral gyrus, precentral gyrus	-36	-26	42	0.039

PCC = posterior cingulate cortex; ACC = anterior cingulate cortex; OFC = orbitofrontal cortex; SMC = supplementary motor cortex; DN = dorsal nexus; T = treatment

Right Dorsal Nexus

Figure 7.4 displays the clusters showing altered connectivity with the right dorsal nexus seed for the high vs low contrast and treatment x neuroticism interaction. Mean time series were extracted from individuals as above and are displayed to the right of the corresponding contrast.

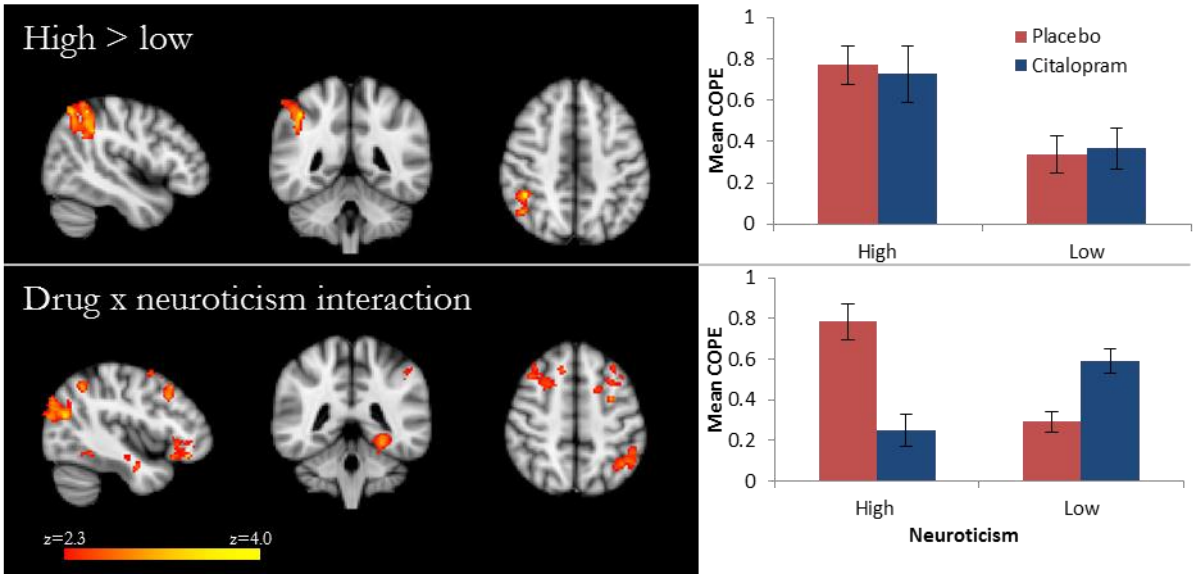

Figure 7.4 Altered functional connectivity with right dorsal nexus seed
Left: Cluster showing main effect of neuroticism (top) and interaction between treatment and neuroticism (bottom) for functional connectivity with the right dorsal nexus seed.
Right: Group mean timecourse extracted from corresponding clusters. Error bars represent SEM.

For the high vs low contrast, there was a cluster incorporating right angular gyrus and superior lateral occipital cortex. A 2 x 2 ANOVA was conducted on the extracted data. There was a significant effect of neuroticism, $F(1,59) = 14.82$, $p < .001$. There was no main effect of treatment and no treatment x neuroticism interaction (ps > .05). That is, high neurotics had greater functional connectivity between the right dorsal nexus and this lateral patietal-occipital area than low neurotics, and connectivity did not appear to be influenced by citalopram treatment.

For the treatment x neuroticism interaction, a number of clusters showed significant differences in functional connectivity, with areas including left hippocampus and fusiform, left lateral superior parietal and occipital areas, bilateral frontal gyri, left OFC, and left middle and superior temporal gyri. A 2 x 2 ANOVA was conducted on extracted data (Figure 7.4, bottom). There was a significant interaction between treatment and neuroticism, $F(1,59) = 33.90$, $p < .001$. There were no main effects of treatment or neuroticism (ps > .05).

In order to examine the interaction in more detail, individual t-tests were conducted between the different groups. There were significant differences between the high N citalopram and high N placebo groups ($t(28) = 4.36$, $p < .001$), low N citalopram and low N placebo groups ($t(31) = 3.78$, $p < .01$), low N placebo and high N placebo groups ($t(31) = 4.71$, $p < .001$) and low N citalopram and high N citalopram groups ($t(28) = 3.54$, $p < .01$). That is, functional connectivity differed at baseline, with high Ns on placebo showing greater connectivity than low Ns between the right dorsal nexus and these clusters. Citalopram significantly reduced functional connectivity with these clusters for high neurotics, but increased connectivity for low neurotics.

Left amygdala

Figure 7.5 displays the clusters showing altered connectivity with the right dorsal nexus seed for the placebo vs citalopram contrast and treatment x neuroticism interaction. Mean time series were extracted from individuals as above and displayed to the right of the corresponding contrast.

For the placebo vs citalopram contrast, the cluster included inferior frontal gyrus, operculum, OFC and a small portion of insula, as well as a posterior area within the cuneal cortex. A 2 x 2 ANOVA was conducted on the extracted data. There was a significant effect of treatment, $F(1,59) = 19.40$, $p < .001$. There was no main effect of neuroticism and no treatment x neuroticism interaction (ps > .05). That is, compared to participants on placebo, those on

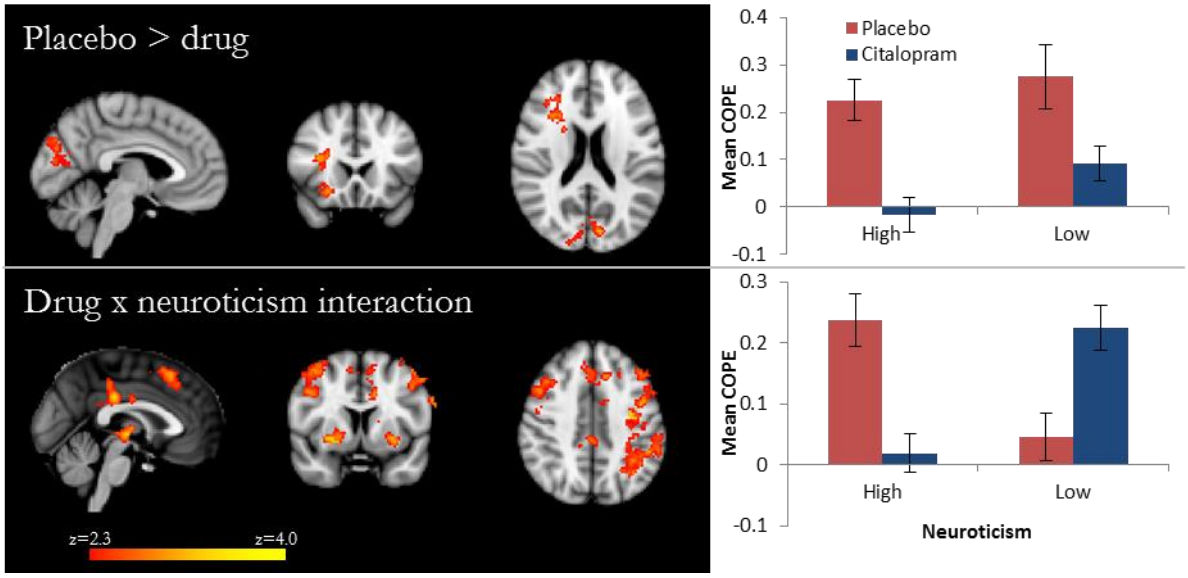

Figure 7.5 Altered functional connectivity with left amygdala seed
Left: Cluster showing main effect of treatment (top) and interaction between treatment and neuroticism (bottom) for functional connectivity with the left amygdala seed.
Right: Group mean timecourse extracted from corresponding clusters. Error bars represent SEM.

citalopram had reduced functional connectivity between the left amygdala and this area, and connectivity did not appear to be influenced by neuroticism.

For the treatment x neuroticism interaction, a number of clusters showed altered functional connectivity, incorporating regions including left middle frontal gyrus and superior parietal and occipital areas, bilateral superior frontal gyrus and paracingulate, bilateral caudate, putamen and cingulate, and bilateral posterior and anterior cingulate. A 2 x 2 ANOVA was conducted on extracted data. There was a significant interaction between treatment and neuroticism, $F(1,59) = 26.28$, $p < .001$. There were no main effects of treatment or neuroticism (ps > .05).

Individual t-tests were conducted between the different groups. There were significant differences between the high N citalopram and high N placebo groups ($t(28) = 3.88$, $p < .01$), low N citalopram and low N placebo groups ($t(31) = 3.37$, $p < .01$), high N placebo and low N placebo groups ($t(31) = 3.30$, $p < .01$) and high N citalopram and low N citalopram groups ($t(28)$

= 4.10, $p < .001$). That is, functional connectivity differed at baseline, with high Ns on placebo showing greater connectivity than low Ns between left amygdala and these clusters. Citalopram significantly reduced functional connectivity with these clusters for high neurotics, but increased connectivity for low neurotics.

Right amygdala

Figure 7.6 displays the cluster showing altered connectivity with the right dorsal nexus seed for the treatment x neuroticism interaction. Mean time series were extracted from individuals as above and are displayed to the right of the figure.

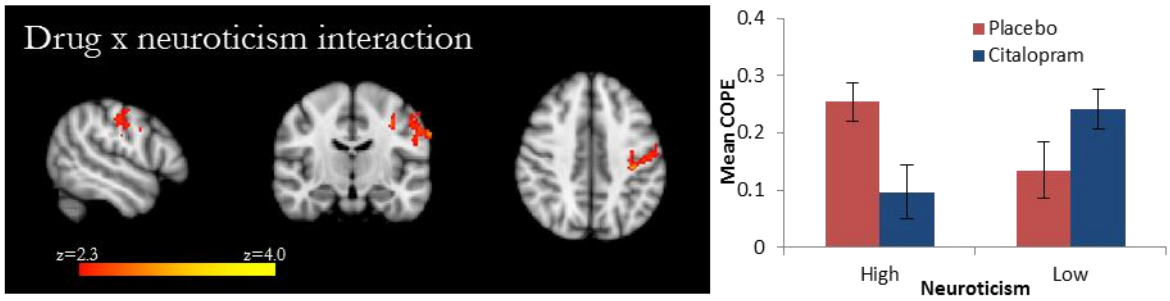

Figure 7.6 Altered functional connectivity with right amygdala seed
Left: Cluster showing a significant interaction between treatment and neuroticism for functional connectivity with the right amygdala seed.
Right: Group mean timecourse extracted from this cluster (right). Error bars represent SEM.

The cluster showing altered functional connectivity included left post- and pre-central gyrus. A 2 x 2 ANOVA was conducted on extracted data. There was a significant interaction between treatment and neuroticism, $F(1,59) = 10.06$, $p < .01$. There were no main effects of treatment or neuroticism (ps > .05). In order to examine the interaction in more detail, individual t-tests were conducted between the different groups. There were significant differences between the high N citalopram and high N placebo groups ($t(28) = 2.76$, $p < .05$) and high N citalopram and low N citalopram groups ($t(28) = 2.49$, $p < .05$). The difference between low N citalopram and low N placebo groups did not reach significance ($t(31) = 1.77$, $p = .09$), and the difference between the

high N placebo and low N placebo groups approached significance ($t(31) = 2.02$, $p = .05$). That is, there was a trend for high and low neurotics to differ in baseline functional connectivity between right amygdala and this post-/precentral gyrus region, and citalopram significantly decreased connectivity for high neurotics but had no effect for low neurotics.

7.4 Discussion

7.4.1 Dorsal Nexus seeds

As predicted, we found that high neuroticism was associated with increased connectivity between dmPFC and structures belonging to the DMN. Seeds placed in both the left and right dmPFC (the "dorsal nexus") showed increased functional connectivity with the left hippocampus amongst high neurotics on placebo compared to low neurotics on placebo. The right dorsal nexus seed also showed increased functional connectivity to a number of other cortical areas, most prominently left lateral occipital and superior parietal areas that form part of the DMN, but also bilateral middle frontal gyrus and left inferior frontal gyrus and OFC.

Relatively few studies have investigated DMN connectivity in neuroticism, but our results appear at least partly consistent with one previous study. Adelstein and colleagues also found that participants with higher levels of neuroticism showed increased connectivity between dmPFC and DMN structures – although in that case effects were found specifically between dmPFC and the precuneus (Adelstein et al., 2011). Our results suggest that neuroticism is associated with a broader increase in connectivity amongst a number of DMN structures and the dmPFC.

This pattern of connectivity is also similar to that seen in depression, in which altered connectivity within the DMN network and especially with dmPFC has been found (Greicius et al., 2007; Sheline et al., 2010; Zhu et al., 2012). The increased connectivity we found between right dorsal nexus and other, non-DMN areas also appears consistent with the literature on

depression. In particular, Sheline and colleagues found increased connectivity in patients with depression between dorsal nexus and bilateral dlPFC (Sheline et al., 2010), very similar to the cluster in the middle frontal gyri in the present study.

In a parallel to the task-based fMRI findings reported in the previous chapter, the effects of treatment on functional connectivity with the dorsal nexus seeds were dependent on neuroticism group. For the high neurotic group, connectivity from both dorsal nexus seeds was reduced with citalopram. This is similar to effects seen in depressed patients (Posner et al., 2013) and suggests that citalopram normalises the heightened functional connectivity seen in this group. Heightened DMN connectivity appears likely to reflect the increase in rumination and negative thoughts about the self that is a defining feature of high neuroticism. Antidepressant drugs may therefore work to dampen DMN activity, thereby reducing this negative bias in processing emotional information concerning the self (Mulders et al., 2015).

Interestingly, the pattern of effects for the low neurotics was again in the opposite direction to that for high Ns: for low Ns, functional connectivity actually increased with treatment, although the effects were not as strong as for the high Ns. The reason behind these differences is unclear. It may be that citalopram does not have the same neural action in the absence of heightened connectivity; indeed, most studies investigating treatment effects have been conducted in depressed patients who do show a baseline increase in connectivity (Mulders et al., 2015). On the other hand, some healthy volunteer studies have also shown reduction in DMN connectivity in response to treatment (McCabe et al., 2011; Scheidegger et al., 2012). However, these have all used unselected healthy controls, while our sample of low Ns may not be representative of a population with a range of neuroticism scores. Whatever the case, our study again demonstrates that baseline characteristics of a sample can have a large impact on treatment effects.

Finally, high neurotics also showed an increase in functional connectivity between right dorsal nexus and a right superior lateral parietal-occipital cluster that forms part of the posterior DMN. Interestingly, this cluster was unaffected by treatment: high Ns on both drug and placebo showed greater connectivity than low Ns. Nevertheless, a very similar cluster in the left hemisphere was reduced by treatment in the high Ns. Previous studies have suggested that antidepressant treatment might have differential effects on posterior and anterior parts of the DMN (Li et al., 2013); however to our knowledge no-one has investigated potential hemispheric asymmetries in resting state connectivity in depression or with antidepressant treatment. There is some evidence of asymmetric distribution of the DMN in healthy controls (Saenger, Barrios, Martínez-Gudiño, & Alcauter, 2012), and it would be interesting to explore hemispheric differences in more detail.

7.4.2 **Amygdala seeds**

Contrary to predictions, we found that high neuroticism was associated with increased connectivity between the amygdala and a number of cortical and subcortical regions. For high Ns on placebo compared to low Ns on placebo, both left and right amygdala seeds showed increased connectivity to left post-/pre-central gyrus, and for the left amygdala there was additionally connectivity with a large cluster going from the left frontal pole through the frontal gyri to angular gyrus and superior lateral occipital cortex, as well as bilateral superior frontal cortex, PCC, and basal ganglia and thalamus.

These results are surprising, given past research showing that in depression the amygdala is "dissociated" from other brain areas involved in top-down emotional control, particularly ACC, mPFC and insula (Anand et al., 2009; Cullen et al., 2014; Tahmasian et al., 2013; Tang et al., 2013; Veer et al., 2010). However, in our study despite widespread heighted connectivity in the high Ns, the regions with significant clusters were largely different from those of previous studies: most cortical connectivity was situated in superior lateral cortex, with only a small cluster encroaching

into the ACC, no insula connectivity and little in the medial PFC. Our results may thus be seen more as a failure to replicate rather than being in direct conflict with those studies.

The clusters that we did find are not entirely inconsistent with previous research. One study found that depressed adolescents had increased amygdala connectivity with middle and inferior frontal gyrus and pre- and post-central gyrus, similar to our study (Pannekoek, 2014). The researchers suggest that in their patient population, the amygdala may be playing a greater part in directing cognitively controlled emotion processing in middle frontal gyrus and pre-/post-central gyrus. This raises the intriguing possibility of a dissociation between decreased top-down input to the amygdala from areas such as the insula and ACC, and increased bottom-up output to superior frontal and parietal areas. Both patterns of connectivity, or a combination of them, could explain how heightened limbic response is able to produce negative biases in depression, and in high neurotics.

As was the case for the dorsal nexus seeds, citalopram reversed the heightened connectivity for the high neurotic groups. If this connectivity reflects greater input from the amygdala to emotional processing areas, then this again suggests that the drug may be dampening down connectivity related to the production of negative emotional responses. Again, the pattern of results was opposite for the low neurotics, and the same ideas explored in the previous section are relevant here.

Finally, the drug also decreased connectivity for both high and low Ns between the left amygdala and a cluster in the frontal pole/OFC, stretching into the frontal tip of the insula, as well as an area of occipital cortex. Similar results have been found in healthy volunteers: McCabe and Mishor (2011) found that reboxetine reduced connectivity between amygdala and OFC in healthy controls. These results could reflect reduction of connectivity in a network involved in reward processing that is somewhat distinct from the network connecting amygdala to frontal

DMN areas (Catani et al., 2013). However, the lack of any baseline differences between high and low Ns limits the conclusions that can be drawn about the role of this network in neuroticism.

7.4.3 **ICA analysis**

In contrast to our seed-based analysis, our ICA analysis failed to find any effects within the DMN. However, we did find that two other networks displayed altered functional connectivity specifically with an area of DMN. For both the LVN and one of the SMN networks, an interaction was found in a cluster in the precuneus/PCC. For the LVN, there appeared to be reduced connectivity at baseline with this cluster for high Ns compared to low Ns. Treatment increased connectivity for high Ns but decreased it for low Ns. The cluster for the SMN was slightly inferior to that for the LVN, and the pattern of results was the exact opposite.

The precuneus/PCC clusters have previously been implicated in inter-network changes in depression. One patient study found that both superior posterior DMN (similar to the cluster found for the LVN in our study) and inferior posterior DMN (similar to the cluster found for the SMN in our study) showed reduced connectivity with a dorsal central executive network incorporating supramarginal gyrus and frontal gyri (Manoliu et al., 2014). The authors suggest that reduced connectivity between DMN and other networks results in a reduced ability to disengage the DMN. This could also be true in the current study, especially for the LVN which showed similarly reduced connectivity with the PCC in high Ns.

It is harder to explain the results for the SMN, with its *increased* connectivity with the PCC in high Ns. However, a clue might come from the Alzheimer's disease literature, in which the PCC has also been implicated. In fact, one study found a similar pattern, with decreased connectivity between PCC and visual areas in patients with mild AD, but increased connectivity with frontal and motor areas (Zhang et al., 2009). The increased connectivity is suggested to be a

compensatory response to the dissociation of the PCC with other networks, and it may be that the same is true here.

The implication of the above is that effects may not always be apparent by conducting an ICA analysis which includes whole resting state networks; rather, effects may be restricted to certain parts of the network, such as the PCC. Future research should conduct seed-based analysis from the PCC to further examine intra- or inter-network effects that could be particularly associated with this subregion.

Chapter 8

General discussion

This thesis has followed two lines of enquiry, both with a common goal: to evaluate the neurocognitive model of antidepressant treatment and provide further characterisation where necessary. In the introduction we set out the questions guiding these two lines of research. Below we discuss how this thesis has helped to answer these questions.

8.1 Do early neurocognitive changes generalise to other antidepressant treatments?

This thesis presents the first evidence that seven days of St John's wort produces a positive shift in emotional processing in healthy volunteers. It does so in the absence of changes in mood, general working memory or reward learning, strongly suggesting that these effects are not simply secondary to mood changes, nor the product of wider cognitive effects. Rather, they are direct effects of the drug which are specific to emotional processing tasks.

These findings are important because St John's wort is quite unlike any drug studied so far in the context of the neuropsychological model of treatment action in depression. It works through a novel mechanism – activation of sodium channels (Leuner et al., 2007) - and modulates levels of a number of neurotransmitters (Chatterjee et al., 1998). Nevertheless, the psychological effects are very similar to those seen in the case of SSRIs or SNRIs (Harmer et al., 2006), adding to a growing body of evidence that a fundamental action of antidepressant treatments is to produce early positive neuropsychological shifts in emotional processing.

8.1.1 St John's wort as a tool for future research

Further research is now required to fully characterise the effects of St John's wort. It would be interesting to see whether SJW also produces changes in neural activation consistent with other antidepressants (Harmer et al., 2006; Norbury et al., 2007). Future research should also extend

psychopharmacological research in SJW to depressed populations: if similar results were found, then this would further support the universal importance of these psychological changes.

If these effects are found, then SJW could also be used to test the cornerstone of the neuropsychological model: that early effects of antidepressants are directly responsible for producing improvement in mood several weeks later. By giving patients a long-term course of SJW, researchers could examine early changes in emotional processing and relate these to ultimate clinical improvement. Indeed, there is preliminary evidence that early changes do predict later mood changes (Tranter et al., 2009); however, further research is clearly needed to confirm these findings.

SJW may be a particularly useful substance with which to conduct this kind of longer-term research. SJW is a drug that is often classified as a "herbal remedy" and which many people may be more willing to take for long time periods: indeed, anecdotally, despite our clear description of SJW as a drug and discussion with participants of its associated risks and side-effects, in the current study some participants indicated that the fact that SJW was a "herbal" or "alternative" medicine influenced their willingness to participate. In addition, the reduced side-effects compared with, for example, the SSRIs, could reduce drop-out rates which are particularly problematic in long-term studies (such as in our high neurotic study). Future research should therefore consider the use of SJW in order to improve recruitment and retention rates.

8.1.2 **Limitations**

It is important to note that our findings cannot be used to imply anything about the efficacy of St John's wort in treating depression. It might be tempting to claim that they support the use of the drug in depression: if the kind of emotional processing changes that we saw in the study are fundamental to the clinical effect of antidepressant drugs, then does their presence not indicate

that the drug is an effective antidepressant? However, in the context of this study, that logic would be circular: we chose to investigate St John's wort precisely *because* there is strong evidence that the drug is an effective antidepressant, and so we wanted to know whether, given that fact, we would see alterations in emotional processing as we do in the case of other antidepressants. It would be fallacious to then claim that the presence of these changes supports the efficacy of the drug.

8.2 Are the same neurocognitive effects present in clinically-relevant populations?

The overarching theme to come out of the high N chapters is that changes produced by citalopram in this population are more complex than those reported in a general healthy population or in depressed patients. On the one hand, citalopram produced some changes in high Ns similar to those seen in previous literature in healthy controls: for example, increasing memory for positive words. When it came to facial expressions, the results were more nuanced. Citalopram decreased recognition of negative faces in low Ns, while for high Ns the drug *increased* recognition. However, at a neural level, there were no group effects concerning negative (or positive) faces – rather, high Ns showed a baseline increase in neural response to neutral faces, which was reduced with citalopram treatment.

These results are not necessarily contradictory. It may be that citalopram has different effects depending on the social relevance and/or threat level of the stimulus. That is, for general, non-threatening negative emotional information with low social relevance, citalopram may have similar effects in high Ns as in other populations, producing a positive shift in processing. Such emotional stimuli could be positive or negative words, or indeed neutral faces, which may not themselves carry much meaning or emotional value, but by nature of their ambiguity may be assigned an element of emotionality by high Ns. On the other hand, when it comes to more

socially-relevant stimuli, such as clearly negative facial expressions, then citalopram treatment reduces the inherent avoidance present in this group (i.e. increasing recognition).

Compared to the behavioural and task-based fMRI results, the resting state connectivity results appear relatively straightforward. Here high Ns showed very similar patterns to depressed populations: there was high baseline connectivity between dmPFC and DMN areas, which was reduced with citalopram, potentially reflecting high levels of rumination and negative self-thinking which, like the above forms of negative bias, are remediated by treatment. Similarly, increased bottom-up connectivity from amygdala to cortical areas in this group may relate to overactive resting input from the limbic system, again dampened by treatment. However, as in the previous chapters, the pattern of effects amongst low Ns was the opposite of that in high Ns.

8.2.1 **Reversal of avoidance may relate to early anxiogenic effects**

Interestingly, SSRIs have been reported to produce anxiety early on in treatment in some cases, which resolves with time (Griebel, Moreau, Jenck, Misslin, & Martin, 1994). Our study suggests a potential mechanism by which this may take place. If citalopram produces an increase in processing of threatening faces in depressed patients, as it appears to in our high N group, then this could produce anxiety as these stimuli – previously avoided – are suddenly in the centre of attention.

Future research should investigate the presence of early heightened threat processing in depressed patients given SSRIs, particularly after acute doses or doses of only a few days. If similar effects are found, then they could be correlated with any changes in anxiety between baseline and testing, to examine whether the two are indeed related. To date, these changes have only been examined in healthy controls, and while increased processing of fearful faces has been reported (Browning et al., 2007; Harmer, Bhagwagar, et al., 2003), in a non-depressed sample this

may not translate into anxiety. It would also be interesting to directly compare the early effects of SSRIs with those of antidepressants such as reboxetine, which are not known to cause anxiety: we would predict there would be no corresponding increase in threat-related processing.

Unexpectedly, in our high N group the increase in threatening face processing was apparent even after four weeks, long after early anxiogenic effects of antidepressants disappear. This persistent increase after longer-term doses in depressed patients has not been reported; rather, long-term treatment produces a reduction in neurocognitive response to threatening faces (Fu et al., 2004; Sheline et al., 2001). One possible explanation is that in a depressed sample, the gradual emergence of a clinical response over time results in top-down feedback that dampens the initial heightened threat processing. In contrast, because a healthy high-N sample does not actually benefit clinically from the drug, there are no mechanisms to reverse the initial increase in processing, and so even after several weeks those on the drug continue to show heightened threat processing. Research directly comparing high Ns and depressed patients would be needed to determine whether this theory might be true; whatever the case, these discrepant findings do highlight the fact that while high Ns may be more like depressed patients than unselected healthy controls, they are still not a perfect model.

8.2.2 **High N samples: beneficial for future research?**

It is clear from our study that baseline group characteristics can have a profound effect on how antidepressant treatments affect emotional processing. In many ways, our high N group appeared to more consistently show positive neurocognitive changes in response to citalopram treatment than our low Ns. This is consistent with the premise that this group may be more sensitive than unselected healthy controls to treatment effects, and so may be a more ideal population in which to conduct psychopharmacological studies. Future research should consider utilising high N

groups: in particular, if changes in emotional processing are to be used to screen possible novel interventions, then this sensitivity could be invaluable.

On the other hand, treatment effects such as increased recognition of negative faces hint at there being specific effects to threatening and/or socially-relevant stimuli in this group, which require validation and further characterisation. As above, previous research had suggested that these effects might only be an issue at early stages of treatment; however, our studies suggest that they persist even after several weeks. Such "paradoxical" changes could actually mask the presence of positive emotional processing changes in some instances, and so a complete understanding of the circumstances in which such effects occur will be necessary.

8.2.3 **Limitations**

One of the biggest limitations of our study was the lack of any baseline differences between high and low neurotics on behavioural measures of emotional processing. This is clearly a problem for the chapter examining how citalopram modulated performance on these tasks: it is unclear, for example, why citalopram should increase recognition of negative faces for high Ns and decrease recognition for low Ns, when the two placebo groups do not differ on recognition in the first place. It is hard to argue with certainty that these drug effects reflect a reversal of avoidance of threatening information in the high Ns, if that avoidance is not apparent in the placebo group.

The lack of baseline behavioural differences also affects interpretation of the two imaging chapters. In these chapters, there *were* differences in the two placebo groups. The unanswered question is how – or indeed whether – these neural changes translate into behavioural effects. This is particularly relevant to the task fMRI chapter, as here the high Ns compared to low Ns on placebo showed increased processing in neural areas related to emotional processing when viewing neutral faces, despite there being no behavioural differences in the earlier facial

expression recognition task. It may be that neuroimaging methods are better at picking up on subtle biases which are not strong enough to manifest behaviourally; conversely, our behavioural tasks may not be sensitive enough to tap into biases which become apparent mainly in relation to ambiguous emotional information, rather than to stimuli which are clearly positive or negative. Future behavioural studies on high Ns would do well to utilise tasks which are able to examine the way in which subjects assign emotionality to neutral stimuli.

Another limitation stems from the inclusion of the low N group. This group was included as a control against which to compare the high Ns. However, just as the high N group represents people at the very top of the neuroticism scale, the low N group represents those at the very bottom. That is, this group is not necessarily representative of the general population, and so by comparing our high Ns against this group we may be comparing against a group who also behave differently from the population more generally. This could explain some of the unexpected results we found for the low N group, for example, the *increased* dmPFC-DMN resting state connectivity with citalopram treatment. Researchers interested in comparing participants with different levels of neuroticism may do better to correlate behavioural and neural measures with neuroticism scores across the general population.

8.3 Future directions

The field is in an exciting place. Over the past decade a wealth of literature has provided support for and refined the neurocognitive model of antidepressant action, and this thesis contributes to that body of evidence. Now we are on the verge of seeing this research being translated into practical applications. This final section discusses some of the possible future directions of the field.

8.3.1 **Demonstrating the specificity of early neurocognitive changes**

Our study demonstrated that early positive shifts in emotional processing are common to a number of different antidepressants. However, just as important will be work demonstrating that these changes *do not* exist for drugs which are not effective antidepressants. That is, these early neuropsychological effects can only be useful in a practical setting if they are *specific to* antidepressant medications. There is some evidence for this specificity: for example, the neurokin 1 receptor antagonist aprepitant, for which clinical trials have not been hugely successful (Keller et al., 2006; Kramer et al., 2004), only produced very minimal behavioural or neural effects in terms of emotional processing (Chandra et al., 2010; McCabe, Cowen, & Harmer, 2009). Similarly, the failed antidepressant memantine had very limited effects on emotional tasks when compared to other antidepressants (Pringle et al., 2012).

On the other hand, in both cases there were some minor changes in emotional processing, prompting an important question that future research will need to answer: what is the extent of change in emotional processing that is needed to produce clinical effects? It will be vital to quantify the necessary changes in order to use the neurocognitive model as a tool for measuring antidepressant efficacy. Just as we examined St John's wort, a hitherto unstudied antidepressant, future research should examine other non-antidepressants and compare these to existing antidepressants, in order to answer this question.

8.3.2 **Reconciling cellular and neurocognitive accounts of antidepressants**

It will also be necessary to establish the underlying cellular and molecular mechanisms of the early cognitive and neural changes produced by antidepressant treatment. Cellular and molecular accounts of antidepressant action have tended to focus on relatively slow acting effects such as desensitisation of presynaptic autoreceptors (Stahl, 1998); however, it is clear from the neurocognitive model that more immediate effects on brain chemistry or structure must also be important.

One possible locus for early neurocognitive changes is the hippocampus. There is some evidence that short-term antidepressant treatment may increase expression of neurotrophins and produce synaptic remodelling in the hippocampus (Hajszan, MacLusky, & Leranth, 2005; Musazzi et al., 2009). It remains to be seen whether these or any other early molecular effects underlie changes in emotional processing: the challenge lies in finding a way to either investigate emotional processing effects in animal models, or molecular changes in humans.

The latter is clearly more realistic, and we are beginning to see some indirect evidence that upregulation of neurotrophins may positively influence emotional processing. In particular, a series of studies found that the drug Erythropoietin (Epo; see the following section for details), which upregulates brain-derived neurotrophic factor (BDNF), produces a positive shift in emotional processing (Miskowiak et al., 2009; Miskowiak et al., 2008). Similarly, participants with a polymorphism that diminishes BDNF production showed increased activation in ACC and insula to fearful faces and reduced recognition of fear (Mukherjee et al., 2011).

However, attempts to consolidate neurocognitive and molecular accounts of antidepressant action have been minimal, and this will be a key role of future research. With the advent of higher resolution neuroimaging and magnetic resonance spectroscopy this may become easier, although it is likely that research will continue to rely on indirect measures of molecular markers and clever designs that overcome the inherent limitations of studying humans. Nevertheless, it is vital to recognise that neuropsychiatric disorders can be examined at multiple different levels, and that these levels should not be viewed as completely independent, but rather can inform each other.

8.3.3 **Using the neurocognitive model in drug development**

One of the most exciting future applications of the field lies in the potential to utilise the presence of early neurocognitive changes as a marker for antidepressant efficacy in novel drugs.

Indeed, this has already been done in the case of the drug Epo. Epo plays a key role in regulating red blood cells, but also has neurotrophic effects (Alural et al., 2014), and has been shown to reduce recognition of fearful faces and dampen BOLD response to fearful faces and negative scenes (Miskowiak et al., 2009; Miskowiak, O'Sullivan, et al., 2007). Recently, the first phase II trial of the drug has shown that weekly infusions of Epo does indeed improve scores on a number of measures of depression as well as cognitive function up to 14 weeks (Miskowiak et al., 2014).

Our SJW study in particular supports the hypothesis that these kinds of early changes in neuropsychological bias are fundamental to the clinical effect of antidepressant drugs, providing further evidence that these effects could be identified in a similar way to the Epo studies, in order to determine whether a novel substance might be a useful antidepressant. The discovery of new antidepressants is important because a large proportion of patients fail to respond to current drugs, even after switching treatments (Rush et al., 2006). Moreover, drugs which show promise in animal studies often fail to prove effective in patient samples, and so a means of quickly identifying a potentially useful drug in a human population would be invaluable.

8.3.4 **Using the neurocognitive model to refine treatment options**

Finally, as well as examining whether a novel substance may be a useful antidepressant, the neurocognitive model could also be used to determine whether a particular antidepressant may be beneficial to a single individual. Because antidepressants can take a relatively long period of time to have a clinical effect, a patient may take a drug for several weeks only to discover that it is not benefitting them. However, if early neurocognitive changes could be reliably used to predict later treatment response, then these could be examined only days or even hours after commencing treatment to determine whether a patient will respond.

In this context, further studies examining how individual differences influence neurocognitive changes will be vital. We have seen how high and low neurotics can show starkly contrasting effects of antidepressants on emotional processing, and so it is clear that there will not be a "one fits all" approach when it comes to determining whether a given pattern of early neurocognitive effects will likely correspond to later clinical benefit. Rather, it seems that individual differences will need to be taken into account – whether that refers to dimensions of personality, gender, or even genetic factors.

8.4 Conclusion

The neurocognitive model of antidepressant treatment provides an exciting approach to understanding the mechanisms of antidepressant drugs, one which could have wide-reaching implications for drug development and treatment methods. This thesis provides support for the model, showing that interventions with diverse mechanisms of action produce common psychological changes. However, it also highlights the fact that there are still gaps in our knowledge. In particular, translation of psychopharmacological research into the clinic can only be accomplished once we have fully quantified the neurocognitive changes required to produce clinical effects, as well as developed a more complete understanding of how individual differences modulate neuropsychological treatment effects. The research outlined in this thesis represents just an early step in that direction.

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Appendix

Table 9.1 Summary table of effects of pharmacological interventions on emotional processing measure
Details given for studies examining healthy volunteers, depressed patients and high N volunteers. Blank cells indicate that a task was not conducted; non-significant findings are noted.

	Dose (oral unless stated)	Face recognition	Emotional word categorisation (reaction time)	Emotional word recall	Other	Reference
Short-term (7 days unless stated)						
Healthy volunteers						
Citalopram	20mg	↓ fear, anger, disgust, surprise	Trend towards ↓ positive vs negative	↑ positive vs negative	↓ startle response during negative pictures	(Harmer et al., 2004)
Citalopram	20mg	↓ fear	-	-	-	(Harmer et al., 2006)
Citalopram	20mg	-	-	-	↓ attentional vigilance to fearful faces	(Murphy, Yiend, et al., 2009)
Reboxetine	4mg	↓ fear, anger	↓ positive vs negative	↑ positive vs negative	No effect on startle response	(Harmer et al., 2004)
Reboxetine	4mg	-	-	-	No effect on attentional vigilance to fearful faces	(Murphy, Yiend, et al., 2009)
Tryptophan	3g (14 days)	↑ happy, ↓ disgust	No sig effects	No sig effects	↓ attentional vigilance to negative words; ↓ startle response	(Murphy et al., 2006)
Agomelatine	25mg	↓ sad	No sig effects	↑ positive vs negative	↓ startle response during negative pictures, ↑ during positive pictures	(Harmer et al., 2011)
Depressed patients						
Citalopram	10mg	↑ happy	-	-	-	(Shiroma et al., 2014)

Citalopram	20mg (14 days)	↑ happy, disgust, surprise	-	-	-	(Tranter et al., 2009)
Reboxetine	4mg (14 days)	↑ happy, disgust, surprise	-	-	-	(Tranter et al., 2009)
High Neurotics						
Citalopram	20mg	↑ positive vs negative	-	-	↑ gaze at facial expressions	(Di Simplicio, Doallo, et al., 2014)
Acute dose						
Healthy Volunteers						
Citalopram	10mg (i.v.)	↑ happy, fear	-	-	-	(Harmer, Bhagwagar, et al., 2003)
Citalopram	20mg	↑ happy	-	-	-	(Murphy, Norbury, et al., 2009)
Citalopram	20mg	↑ fear	No sig effects	No sig effects	↑ attentional bias to positive words; ↑ baseline startle response	(Browning et al., 2007)
Reboxetine	4mg	↑ happy	↓ positive vs negative	↓ negative	-	(Harmer, Hill, et al., 2003)
Reboxetine	4mg	↑ happy	↓ positive vs negative	No sig effects	-	(Harmer et al., 2009)
Reboxetine	4mg	-	No sig effects	-	↓ RT in positive word recognition	(Miskowiak, Papadatou-Pastou, et al., 2007)
Duloxetine	60mg	↑ happy, disgust	No sig effects	↑ false positives	No effects on startle	(Harmer et al., 2008)
Mirtazapine	15mg	↓ fear	↓ positive *and* negative	↑ positive vs negative	↓ startle response	(Arnone et al., 2009)
Depressed Patients						
Reboxetine	4mg	↑ happy	↓ positive vs negative	↑ positive vs negative	-	(Harmer et al., 2009)

Table 9.2 Effects of acute and short-term antidepressant administration on BOLD response to emotional facial expressions
Arrows indicated increases or decreases in BOLD response for participants given drug compared to placebo

	Dose	Amygdala	Other areas	Reference
Short-term dose (7-10 days)				
Healthy Volunteers				
Citalopram	20mg	↓ fear	Amygdala-hippocampal area, medial frontal gyrus: ↓ fear	(Harmer et al., 2006)
Citalopram	20mg	↑ happy		(Norbury et al., 2009)
Reboxetine	4mg	↓ fear	R fusiform gyrus: ↑ happy	(Norbury et al., 2007)
Depressed patients				
Escitalopram	10mg	↓ fear		(Godlewska et al., 2012)
High Neurotics				
Citalopram	20mg	↑ happy, fear, neutral	PFC: ↑ fear vs happy	(Di Simplicio, Norbury, et al., 2014)
Acute dose				
Healthy Volunteers				
Citalopram	20mg	↓ fear		(Murphy, Norbury, et al., 2009)
Citalopram	7.5mg (i.v.)	↓ fear	L ACC: ↑ happy R posterior insula, R lateral OFC: ↓sad	(Anderson et al., 2011)
Citalopram	50mg over ~3hr	↓ fear	Fusiform gyrus, posterior occipital cortex, R superior temporal sulcus, ventral striatum, medial PFC: ↓ fear faces	(Grady et al., 2013)
Citalopram	7.5mg (i.v.)	↓ disgust, fear	Posterior insula: ↑ disgust	(Anderson et al., 2007)
Citalopram	7.5mg (i.v.)	↓ aversive (anger/disgust/fear)	Lateral OFC: ↓aversive Fusiform gyrus, thamlus ↑ aversive	(Del-Ben et al., 2005)
Citalopram	20mg over 30 min(i.v.)	↑ general emotional (anger/fear/surprise)		(Bigos et al., 2008)
Mirtazapine	15mg	↓ fear; ↑ happy	Fusiform gyrus: ↓ fear	(Rawlings et al., 2010)