Manipulating dopaminergic activity with bupropion: Effects on reward and emotional processing biases implicated in depression

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Abstract

Major depressive disorder is characterised by two core diagnostic symptoms, low mood and anhedonia, which have been suggested to be caused by negative biases in emotional processing and aberrant reward processing, respectively. Previous research using antidepressants predominantly affecting serotonin or noradrenaline activity suggests that antidepressants may act to reduce the negative biases in emotional processing, which then influences low mood; however, such antidepressants do not fully correct the anhedonia. This lack of efficacy underlines the need for further research to unravel the roles of various neurotransmitter systems in the modulation of processing biases implicated in major depressive disorder. For example, it is still unclear how the initial antidepressant effects on biases in emotional processing relate to effects on aberrant reward processing in major depressive disorder and whether they are also targeted by antidepressants affecting dopaminergic activity, such as bupropion. The current thesis aimed to delineate the role of the dopaminergic reward system in the cognitive neurobiological processes underlying the two symptom clusters of low mood and anhedonia in major depressive disorder. In order to do this, two experimental studies manipulating dopaminergic activity with the antidepressant bupropion were completed and the effects on reward and emotional processing biases implicated in major depressive disorder were assessed.

The first study investigated the acute effects of bupropion on behavioural measures of reward and emotional processing in a non-clinical population. Compared to placebo, an acute dose of bupropion was found to reduce negative and increase positive emotional processing with little effect on reward processing.

The second study investigated the early and longer-term effects of bupropion on behavioural and neural measures of reward and emotional processing in individuals suffering with major depressive disorder. Compared to untreated healthy controls, bupropion was again found to reduce negative and increase positive emotional processing but was found to worsen aberrant reward processing at 2 weeks prior to normalisation after the full 6 week treatment. Higher right lateral orbitofrontal cortex activity in response to happy faces and decreased right amygdala activity in response to sad faces at 2 weeks was associated with decreased HAM-D score at 6 weeks. This suggests a causal role for the early neural effects of bupropion in later change in subjective symptoms and has clinical implications providing further evidence for the potential to predict clinical outcome from the early effects of antidepressants.

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Abbreviations

ACC	Anterior cingulate cortex
BFS	Befindlichkeits scale
BMI	Body mass index
BOLD	Blood oxygen level dependent
CBT	Cognitive behavioural therapy
DA	Dopamine
dl	Dorsolateral
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
ECAT	Emotional Categorisation Task
ECG	Electrocardiogram
EEfRT	Effort Expenditure for Rewards Task
EMEM	Emotional Recognition Memory Task
EPI	Echo planar image
EPQ	Adult Eysenck Personality Questionnaire
EREC	Emotional Recall Task
ETB	Emotional Test Battery
FDOT	Facial Dot-Probe Task
FERT	Facial Expression Recognition Task
fMRI	Functional magnetic resonance imaging
FMRIB	Functional Magnetic Resonance Imaging of the Brain
FSL	FMRIB Software Library
GLM	General linear model
HAM-D	Hamilton Rating Scale for Depression
HC(s)	Healthy control(s)
5-HT	Serotonin
ICSS	Intracranial self-stimulation
IM	Immediate release
L-DOPA	3,4-dihydroxy-L-pheylalanine
ll	Left lateral
lm	Left medial
MASQ	Mood and Anxiety Symptom Questionnaire
MDD	Major depressive disorder
MNI	Montreal Neurological Institute

NA	Noradrenaline
NAcc	Nucleus accumbens
NART	National Adult Reading Test
OFC	Orbitofrontal cortex
OQuESA	Oxford Questionnaire on the Emotional Side-effects of Antidepressants
PANAS	Positive and Negative Affect Schedule
PET	Positron emission tomography
PFC	Prefrontal cortex
rl	Right lateral
rm	Right medial
ROI	Region of interest
RPE	Reward prediction error
SCID	Structured Clinical Interview for DSM-IV
SHAPS	Snaith-Hamilton Pleasure Scale
SNRI	Serotonin-noradrenaline reuptake inhibitor
SR	Sustained release
SSRI	Selective serotonin reuptake inhibitors
SVC	Small volume corrected
TE	Echo time
TR	Repetition time
TRP	Tryptophan
VAS	Visual analogue scale
vm	Ventromedial
VS	Ventral striatum
VTA	Ventral tegmental area
XL	Extended release

CHAPTER 1

RESEARCH BACKGROUND

1.1 Major Depressive Disorder: Burden & Current Limitations in Treatment

Major depressive disorder (MDD) is a psychiatric disorder characterised by two core diagnostic symptoms, low mood and the inability to experience interest or pleasure in activities that were previously enjoyable, known as loss of reward or anhedonia (Diagnostic and Statistical Manual of Mental Disorders, 5th edition; DSM-V; American Psychiatric Association, 2013). Cognitive (excessive guilt, feelings of worthlessness and hopelessness), neurovegetative (disturbances in sleep and appetite), psychomotor (agitation or retardation) and social withdrawal symptoms may also be present (Fava and Kendler, 2000).

MDD is one of the most prevalent psychiatric disorders and is associated with substantial disability. In a summary of the World Health Organisation Surveys conducted across 60 countries, 1 year prevalence averaged 9.3 - 23.0% and 3.2% in those with or without comorbid conditions, respectively and MDD had the largest effect on worsening health compared to the other comorbid conditions (Moussavi et al., 2007). MDD accounted for 4.4% of total disability adjusted life years in 2000 and is predicted to become the second leading contributor to the global burden of disease across all age groups by 2020 (Murray and Lopez, 1996, Kessler and Bromet, 2013).

This burden is severely exacerbated by limitations in current understanding of and treatments for MDD. Two main treatment options currently exist following diagnosis of MDD. Individuals are either referred for psychological therapy, such as cognitive behavioural therapy (CBT), or are prescribed antidepressant medication. The acute neuropharmacological actions of first-line antidepressants at cellular and molecular level are now well characterised, with most acting as serotonin (5-HT) and/or noradrenaline (NA)

reuptake inhibitors (SSRIs / SNRIs). Whilst these actions are detectable immediately after the administration of the antidepressant, repeated administration over a number of weeks is required before a clinically important therapeutic effect is observed. MDD is also a wide diagnostic category with substantial heterogeneity between patients in terms of neurochemistry, biological correlates, resulting symptoms and response to treatment. Indeed, among outpatients starting first-line antidepressant treatment, approximately only half will respond and only one third will achieve remission (Rush et al., 2006, Trivedi et al., 2006). Whilst SSRIs tend to treat the low mood, they do not appear to fully correct the anhedonia (Nutt et al., 2007) and may actually exacerbate the loss of reward (McCabe et al., 2010), which some believe contributes to the phenomenon of emotional blunting (Price et al., 2009). On the other hand, there is evidence to suggest that SNRIs and antidepressants that enhance dopaminergic (DA) neurotransmission may better target the anhedonia (Nutt et al., 2007). It may be that the increased negative affect in low mood and the aberrant processing of reward or other positive stimuli in anhedonia are distinct dimensions independent of each other and are associated with disturbances in different neurotransmitter systems; the former the result of under-functioning in the 5-HT emotion system and the latter the result of under-functioning in NA or the DA reward system (Argyropoulos and Nutt, 2013, Pringle et al., 2013). Indeed, it has long been established that negative and positive affect are two relatively independent dimensions (Watson et al., 1988) and should therefore be treated that way in MDD. Focusing treatment on the predominant symptomatology for an individual patient could potentially be the key to improve rates of response and remission (Nutt et al., 2007).

1.2 Aberrant Reward Processing in Anhedonia in MDD

1.2.1 The Importance of Researching Anhedonia

Anhedonia has typically been regarded as a symptom of MDD; however, from epidemiological research it is becoming increasingly recognised as a state-independent marker of vulnerability to MDD and potential specific endophenotype for MDD (Hasler et al., 2004). Anhedonia often precedes the onset of MDD with a strong positive correlation between altered performance on reward tasks and the onset of MDD in the following year. For example, in a longitudinal study of young boys, low frequency of reward-seeking decisions was found to predict depressive symptoms and disorders one year later (Forbes et al., 2007). There is also evidence that anhedonia is a marker of resistance to treatment (Vrieze et al., 2014). Within a group of MDD patients, individuals with high anhedonic symptoms were found to show reduced response bias for high-probability reward compared to individuals with low anhedonic symptoms and such reduced reward responsiveness at baseline was a unique predictor of continued MDD diagnosis after 8 weeks of treatment (Vrieze et al., 2013). Reward-related brain activity in the ventral striatum (VS) and ventromedial prefrontal cortex (vmPFC) at baseline has also been associated with post-treatment symptom severity, with those who show the greatest increase in VS activity or decrease in vmPFC activity having a greater rate of symptom reduction (Forbes et al., 2010).

1.2.2 The Neurobiology of Reward Processing and the Role of Dopamine

The neural circuitry supporting reward processing is now well characterised with a key role for the mesocorticolimbic DA pathway, projecting from the ventral tegmental area (VTA) to the VS (nucleus accumbens; NAcc) and the frontal cortex (orbitofrontal cortex; OFC), as well as the amygdala and the hippocampus (Nestler and Carlezon, 2006). Understanding of the importance of this neural circuitry in reward processing began with the discovery of intracranial self-stimulation (ICSS) in rats. It was observed that rats would

repeatedly self-stimulate following implantation of electrodes in certain regions of the brain, particularly the septal nuclei (Olds and Milner, 1954). The enhancing influence of psychotropic drugs acting on DA neurotransmission on ICSS provided evidence for a specific involvement of DA in reward processing (Stein, 1964, Crow, 1970). The development of functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) allowed observation of increased activity in the mesocorticolimbic DA pathway during the presentation of rewarding stimuli in humans (Knutson and Cooper, 2005, Martin-Soelch, 2009).

Whilst the mesocorticolimbic DA pathway is clearly important in reward processing, the specific function it plays remains controversial (Berridge and Robinson, 1998). So far here reward processing has been conceptualised as a single psychological process; however, it appears to be composite and can be parsed into several reward-related components. These are the motivation to obtain reward, carrying out of reward-seeking behaviours (appetitive) and the hedonic impact or pleasure of experiencing reward (consummatory) (Forbes and Dahl, 2005).

1.2.2.1 Anhedonia Hypothesis

In the anhedonia hypothesis developed by (Wise, 1982), it was suggested that DA mediates the hedonic impact or pleasure of experiencing reward. This hypothesis was centred around the selective reduction in the hedonic impact of rewarding stimuli by administration of DA antagonists in rats (Wise et al., 1978). More recent PET studies still find that DA may mediate hedonic impact, for example, amphetamine-induced DA release in the human VS was found to positively correlate with subjective experience of euphoria (Drevets et al., 2001). However, the anhedonia hypothesis comes into question when considering the timing and nature of activation of the DA reward system.

1.2.2.2 Reward Learning

The DA reward system is often activated prior to receiving a rewarding stimulus, indicating that DA may be more involved in the motivational or appetitive rather than consummatory aspects of reward. More specifically, a series of neurophysiological studies in awake primates (Schultz et al., 1997, Schultz, 2000) provided evidence for a role of DA in associative reward learning. A simple form of associative learning is classical conditioning, whereby a previously neutral (conditioned) stimulus acquires a response similar to the potent (unconditioned) stimulus, which may be rewarding, such as food, or aversive, such as an electric shock, after repeated pairings and becomes a predictive cue. The DA neurons of inexperienced primates that had not received classical conditioning did discharge only when they received food or liquid rewards (Apicella et al., 1991). However, following classical conditioning, the DA neurons stopped responding to the reward itself and instead responded robustly to the conditioned stimulus predictive of the reward, to a greater extent than when an inexperienced primate received a reward (Schultz et al., 1993). Such alterations in neuronal response are strongly diminished after extinction of the conditioned behaviour (Tremblay et al., 1998). In further studies, it was found that DA neurons display phasic activity that could encode the reward prediction error (RPE), that is, the difference between actual reward occurrence and predicted reward occurrence. VS and OFC DA neurons display a higher firing rate to unpredicted than to predicted rewards and fail to discharge if there is no reward at all (Tremblay and Schultz, 2000). This may result in the modulation of synaptic strength and such DA-dependent synaptic plasticity could provide the neural mechanism for a further associative learning process, known as instrumental conditioning, whereby reward or punishment can act to increase or decrease the probability that a certain behaviour will occur again in the future, respectively (Glimcher, 2011).

Similar results have also been reported in humans, for example, in monetary incentive delay tasks during which participants have to respond to either a cued target for monetary reward, cued target for no reward or no response to a cued target, the VS shows

reward-proportional increased activity in response to the anticipation of increasing monetary rewards, but not punishments, which instead recruit the medial caudate (Knutson et al., 2001a, Knutson et al., 2001b, Breiter et al., 2001). It is also becoming clearer that there is a dissociation between reward anticipation and outcome in both the primate (Schultz et al., 2000) and human (Knutson et al., 2001a, Knutson et al., 2001b) brain, with the VS activated more by anticipation of reward than no reward and the OFC activated more by outcome of reward than no reward.

Final evidence for a role of DA in reward learning comes from studies showing that administration of drugs enhancing or reducing DA activity can modulate both neural activity and behaviour during reward learning tasks. For example, during a probabilistic instrumental learning task, participants have to learn to choose a stimulus associated with high-probability win and avoid a stimulus associated with high-probability loss in order to maximise payoffs. Participants treated with a drug enhancing DA activity (3,4-dihydroxy-L-pheylalanine; L-DOPA; a metabolic precursor of DA) were more likely to choose the high-probability win, subsequently winning more money than participants treated with the DA receptor antagonist, haloperidol, although not placebo. In addition, L-DOPA significantly enhanced activity in the VS reflecting RPE-related responses to wins compared to haloperidol. Note that neither drug altered the amount lost or brain activity in response to losses. Therefore, enhancing DA activity was found to increase reward-seeking behaviour, but not avoidance of punishments (Pessiglione et al., 2006). Administration of L-DOPA has also been shown to restore RPE-related responses in groups vulnerable to aberrant reward learning, such as older adults (Chowdhury et al., 2013), suggesting that enhancing DA activity could have the same restorative effects on aberrant reward learning in MDD patients.

1.2.2.3 Incentive Salience Hypothesis

A further challenge to the anhedonia hypothesis came from Berridge and Robinson who discovered that hedonic and aversive taste reactivity remains unchanged in rats subjected to either intranigral 6-hydroxydopamine injections such that they could not swallow

(Berridge et al., 1989) or conversely, electrical stimulation of the lateral hypothalamus and subsequent activation of ascending DA projections that elicits robust eating (Berridge and Valenstein, 1991). This suggested that the motivated appetitive behaviour of eating or swallowing and the hedonic impact of the food are mediated by different neural systems. Berridge and Robinson describe the motivation for, or ‘’incentive salience’’ of a reward as ‘’wanting’’ and the hedonic impact of a reward as ‘’liking’’, with DA mediating the ‘’wanting’’ but not the ‘’liking’’, or associative learning (Berridge and Robinson, 2003, Romer Thomsen et al., 2015). Such DA-mediated incentive salience or wanting can interact with but is separate from the hedonic and associative learning aspects of reward processing.

According to the incentive salience hypothesis, the phasic activity of DA neurons acts to transform the neural representation of a stimulus, such that it becomes a wanted, salient incentive that can elicit an animal to work for its acquisition. Indeed, several studies have now shown that enhancement or reduction of DA activity can elicit an increase or decrease in the willingness of rats to exert effort for a food reward, respectively, whilst ‘’liking’’ remains preserved (Pecina et al., 2003, Salamone et al., 2007, Bardgett et al., 2009, Ward et al., 2012). Similarly, enhancing DA activity through d-amphetamine administration has also been shown to increase willingness to exert effort for rewards in humans (Wardle et al., 2011).

There is also evidence that it is possible to parse incentive salience apart from associative learning, particularly from the way microinjections of a DA enhancing drug affect the neural coding in the ventral pallidum neurons in rats. Amphetamine was found to increase the incentive salience but not the learned prediction firing signals to a conditioned stimulus (Tindell et al., 2005, Smith et al., 2011). Finally, a learned repulsion can be transformed into motivational ‘’wanting’’, for example, sodium-depletion in rats was found to transform a learned repulsion toward a conditioned stimulus predicting saltiness into a wanted, salient incentive (Robinson and Berridge, 2013).

It is clear that the mesocorticolimbic DA system is involved in reward processing, be it the hedonic impact, associative learning or motivational aspects of reward, and it is

hypothesised that hypofunction of this system may underlie the anhedonia observed in MDD (Nestler and Carlezon, 2006, Martin-Soelch, 2009, Argyropoulos and Nutt, 2013).

1.2.3 Dopamine Hypofunction & Aberrant Reward Processing in MDD

Support for the DA hypofunction hypothesis of anhedonia firstly comes from evidence of reduced central DA neurotransmission in MDD. For example, concentrations of DA metabolites, such as homovanillic acid, are found to be lower in the cerebrospinal fluid and vein blood plasma of MDD patients, particularly those who have attempted suicide (Asberg et al., 1984, Roy et al., 1986). Studies manipulating DA activity have shown that DA agonists exert antidepressant effects, whilst DA depletion can induce depression in susceptible individuals (Hasler et al., 2008). Finally, PET studies have shown evidence of reduced VS DA receptor binding in MDD (Meyer et al., 2001, Cannon et al., 2009), although findings are often inconsistent.

Further evidence of DA hypofunction comes from behavioural and neuroimaging studies of aberrant reward processing in MDD patients. However, the precise mechanism of how such DA hypofunction and subsequent aberrant reward processing leads to anhedonia remains poorly understood with potential impairments in some or all of the reward-related processes considered above. Given the very definition of anhedonia, it was originally thought that it may be associated with impairments in the consummatory, hedonic aspects of reward processing; however recent evidence indicates more motivational or appetitive involvement (Argyropoulos and Nutt, 2013, Treadway and Zald, 2013, Romer Thomsen et al., 2015).

1.2.3.1 Impairments in Responsiveness to Reward

There is evidence that at least MDD patients, but not specifically anhedonic individuals per se, display blunted responsiveness to reward. For example, emotional reactivity in response to both anticipated reward and actual reward outcome has been found to be significantly reduced in MDD patients compared to healthy controls (McFarland and

Klein, 2009). Many studies have also shown altered brain activity in response to both anticipated reward and reward outcomes, such as the taste of chocolate, in currently depressed, recovered depressed and individuals at risk of depression. These studies and brain regions involved are summarised in Table 1.1. Overall, the most consistent finding is of reduced VS activity in MDD patients in response to reward. Furthermore, these behavioural and neural abnormalities have been found to correlate specifically with severity of self-reported anhedonia, independently of overall depression severity. In particular, VS and vmPFC activity in response to reward have been found to negatively and positively correlate with anhedonia severity, respectively (Keedwell et al., 2005, Epstein et al., 2006).

Table 1.1 Alterations in Brain Activity in Response to Rewarding and Aversive Stimuli in MDD

Reference	MDD Population Studied	Type of Stimulus / Task	Affective Valence of Stimulus	Brain Region	Direction of Change
(Mitterschiffthaler et al., 2003)	Current Medicated	Pictures	Positive	Right precentral gyrus Right inferior parietal gyrus Bilateral lingual gyrus Left medial frontal gyrus CC – posterior, anterior Left insula Thalamus Left superior / middle temporal gyrus Right inferior frontal gyrus	↓ ↑
(Epstein et al., 2006)	Current Unmedicated	Words	Positive	Bilateral VS Left dorsomedial frontal gyrus Parahippocampal gyrus Thalamus Hypothalamus	↓
			Negative	Parahippocampal gyrus Right middle frontal gyrus Left insula CC - subgenual	↓ ↑
(Knutson et al., 2008)	Current Unmedicated	Monetary Incentive Delay Task	Gain Anticipation	Left ACC	↑
			Gain Outcome	Bilateral putamen Right mPFC Left insula	↓

(Pizzagalli et al., 2009)	Current Unmedicated	Monetary Incentive Delay Task	Gain Anticipation	Left putamen	↓
			Gain Outcome	Left VS / NAcc Bilateral caudate	↓
(McCabe et al., 2009)	Recovered Unmedicated	Picture and/or taste	Chocolate	VS CC – anterior, pregenual, subgenual vmPFC	↓
			Mouldy Strawberry	Bilateral caudate	↑
(McCabe et al., 2012)	At Risk (family history)	Picture and/or taste	Chocolate	ACC OFC	↓
			Mouldy Strawberry	ACC lOFC Insula	↓ ↑

1.2.3.2 Impairments in Reward Learning

There is evidence that such blunted responsiveness to anticipated reward and actual reward outcomes considered above may lead to a failure of instrumental conditioning, that is, failure to use positive or negative feedback to adjust future behaviour, resulting in subsequent abnormal reward-based decision making. This can be observed in studies measuring the trial-by-trial adjustment of reaction times following positive or negative feedback. Healthy controls typically show a decrease in reaction time following negative feedback and an increase in reaction time following positive feedback. In addition the extent to which reaction time decreases or increases correlates with ACC and VS activity, respectively. Reduced ACC and VS activity and a lack of alteration of reaction times following feedback is observed in MDD patients (Steele et al., 2007, Chase et al., 2010). Some have reported that the lack of post error slowing in MDD patients is associated with an increased risk of committing subsequent errors (Elliott et al., 1996, Elliott et al., 1997).

Further evidence comes from abnormal response selection in probabilistic instrumental learning tasks. In such tasks, healthy controls develop a reward response bias, learning to choose the stimulus associated with high-probability win and avoid the stimulus associated with high-probability loss in order to maximise payoffs. It is observed that MDD patients fail to develop a reward response bias resulting in reduced frequency of choosing the high-probability win (Henriques and Davidson, 2000, Pizzagalli et al., 2008, Vrieze et al., 2013). These studies are complemented by neuroimaging results that find significantly reduced RPE-related responses in the VS, ACC and hippocampus of MDD patients (Steele et al., 2004, Kumar et al., 2008, Robinson et al., 2012). Importantly, again, these impairments in instrumental conditioning have been found to correlate specifically with the severity of self-reported anhedonia, independently of overall depression severity (Steele et al., 2007, Chase et al., 2010, Vrieze et al., 2013, Steele et al., 2004) as well as predict persisting diagnosis of MDD after 8 weeks treatment (Vrieze et al., 2013).

1.2.3.3 Impairments in Motivation for Reward

The motivation to obtain reward can be assessed by measuring the willingness to exert effort for rewards. An example of this is the Effort Expenditure for Rewards Task (EEfRT) developed by Treadway and colleagues, during which participants are offered a choice of exerting greater effort for greater reward or less effort for less reward. Currently depressed and recovered depressed individuals were found to show less willingness to exert greater effort for greater reward (Treadway et al., 2012, Yang et al., 2014). A negative correlation between willingness to exert effort for rewards and self-reported anhedonia has been found in both healthy controls (Treadway et al., 2009) and MDD patients (Yang et al., 2014).

1.2.4 Could Dopaminergic Antidepressants Target Aberrant Reward Processing and Anhedonia?

It is becoming clear that SSRIs do not fully correct the anhedonia observed in MDD (Nutt et al., 2007) and may actually exacerbate the loss of reward. Indeed, several studies have shown that at least in healthy controls, SSRIs do reduce reward-related brain activity. For example, healthy controls given an acute dose of the SSRI citalopram were found to have blunted RPE signals (Kumar et al., 2008). Treatment for 7 days with the SSRI citalopram, but not the SNRI reboxetine, was found to reduce VS and vmOFC activity in response to a chocolate reward in healthy controls. By contrast, the reboxetine was found to increase medial OFC activity in response to a chocolate reward. Both drugs were found to reduce lateral OFC and insular activity in response to aversive stimuli, but reboxetine to a lesser extent (McCabe et al., 2010). Two weeks of treatment with the SNRI duloxetine has also been found to enhance reward-related VS activity in healthy controls (Ossewaarde et al., 2011). Therefore, it appears that whilst SNRIs can enhance reward processing, SSRIs actually diminish neural processing of both aversive and rewarding stimuli resulting in a general dampening of all negative and positive affective experiences, similar to the effect of

dopamine receptor antagonists (McCabe et al., 2011). This may provide a plausible neural mechanism for the emotional blunting phenomenon observed clinically in some MDD patients during SSRI treatment (Price et al., 2009).

It may be that NA and/or DA antidepressants could better target the aberrant reward processing and anhedonia in MDD (Pringle et al., 2013). As previously discussed, altering DA activity can alter reward learning in healthy controls (Pessiglione et al., 2006) and enhancing DA activity may have the potential to restore blunted RPE-related responses, at least in old age (Chowdhury et al., 2013); however, few studies specifically assessing the effects of DA antidepressants, such as bupropion, on reward processing in MDD have been completed.

Bupropion acts as a dual DA and NA re-uptake inhibitor (Figure 1.1). Despite not having any 5-HT activity, it demonstrates efficacy in treating MDD comparable to that of SSRIs or tricyclic antidepressants (Horst and Preskorn, 1998, Stahl et al., 2004, Fava et al., 2005). It is licensed and very commonly used as an antidepressant in the USA and some parts of Europe; however, it is not licensed as an antidepressant in the UK, where it is instead used as an aid for the cessation of smoking. Bupropion is typically used on its own as a first-line antidepressant; however, some have considered its use as an adjunctive treatment either to alleviate side-effects associated with SSRIs or augment antidepressant efficacy in non-responders to other antidepressants (Rush et al., 2011). For either usage, bupropion is available in 3 oral formulations: immediate release (IR) given three times a day, sustained release (SR) given two times a day and extended release (XL) given once daily (Fava et al., 2005, Jefferson et al., 2005).

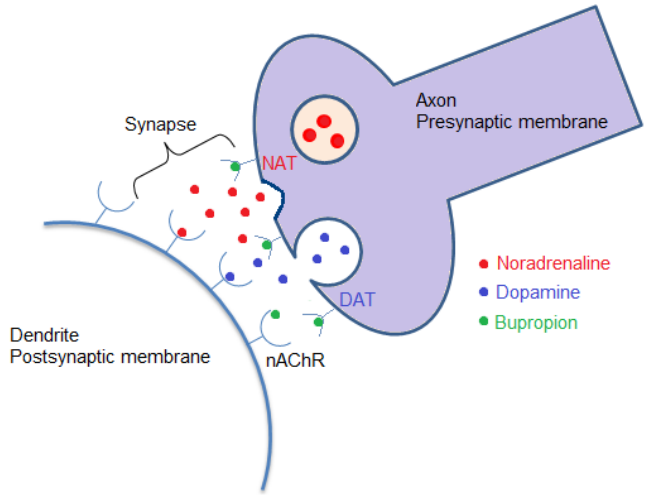


Figure 1.1 – Bupropion Mechanism of Action: bupropion acts at the noradrenaline (NAT) and dopamine (DAT) transporters to block the re-uptake of noradrenaline and dopamine, respectively. Bupropion also acts as a non-competitive antagonist at nicotinic acetylcholine receptors, which is thought to mediate its role as a smoking cessation aid.

Whilst bupropion has been shown to have a more robust effect on anhedonia than placebo (Tomarken et al., 2004), its effects on reward processing remain unclear. In a recently published study (Dean et al., 2016), healthy controls completed an fMRI reward task following 7 day treatment with bupropion or placebo. The task covered all aspects of reward processing with a cue for the anticipation of reward, effort exerted to receive the reward and actual receipt of the reward. Compared to placebo, bupropion was found to increase activity during anticipation of the reward in the vmPFC and caudate, exertion of effort for the reward in the vmPFC, VS and ACC and finally receipt of the reward in the medial OFC. Bupropion was also found to increase activity to an aversive stimulus in the medial OFC, VS and amygdala. However, no behavioural effects of bupropion were found. This provides some preliminary evidence for an effect of bupropion on reward processing but it remains to be seen whether it can remediate aberrant reward processing in MDD patients, as well as the negative affective biases also observed in MDD, discussed below.

1.3 Increased Negative Affect & Low Mood in MDD

Negative biases in emotional processing refer to the tendency to extract negative rather than positive affective information from a variety of social and affectively valenced stimuli. The importance of such negative affective biases in the aetiology and maintenance of MDD has long been known and they are believed to critically fuel the low mood and negative views about oneself, the world and the future observed in MDD (Beck et al., 1979).

Numerous studies implementing various behavioural paradigms have shown that MDD patients display negative affective biases across a range of cognitive domains, including perception, attention and memory. MDD patients have an inclination to perceive ambiguous information as negative, preferentially attend to negative affective stimuli and better recall negative information (Mathews and MacLeod, 2005, Leppanen, 2006, Elliott et al., 2011, Roiser et al., 2012). For example, in a facial expression recognition task, MDD patients are more sensitive to lower sad intensities and are more likely to interpret ambiguous or neutral facial expressions as negative, whilst their sensitivity to happy facial expressions is reduced (Gur et al., 1992, Bouhuys et al., 1999, Gilboa-Schechtman et al., 2002). In a dot-probe task where the attention to one word in a pair can be determined from the response latency to the appearance of a dot probe in the place of one of the words, MDD patients display an attentional bias towards negatively valenced words (Mogg et al., 1995), which may represent a difficulty in disengaging attention from negative affective information (Gotlib and Joormann, 2010). Finally, MDD patients also recall more negative rather than positive self-referent personality descriptive words (Matt et al., 1992).

Neuroimaging studies have revealed that such negative affective biases may be associated with aberrant activity in limbic and striatal circuitry involved in the initial appraisal and memory of affective stimuli (Leppanen, 2006, Fitzgerald et al., 2008). For example, MDD patients exhibit enhanced activity in the amygdala, VS and insula in response to negative affective stimuli (Sheline et al., 2001, Fu et al., 2004, Surguladze et al., 2005, Fu et al., 2008, Victor et al., 2010) and reduced activity within the amygdala, thalamus, putamen

and hippocampus to positive affective stimuli (Lawrence et al., 2004, Fu et al., 2007), such as facial expressions in particular. A similar pattern has also been observed in the extrastriate cortex, fusiform gyrus and frontal regions, involved in visual processing, recognition and higher-order control of emotional processing, respectively (Lawrence et al., 2004, Surguladze et al., 2005). Such aberrant neural activity has been found to be associated with response to treatment with the higher the pre-treatment activity in the midbrain, dlPFC, ACC, thalamus and caudate in response to negative self-referent words, the worse the response to escitalopram treatment (Miller et al., 2013).

These negative affective biases are also exhibited by individuals who have never been depressed but are at high risk of the disorder by virtue of high neuroticism (Chan et al., 2007, Haas et al., 2007, Robinson et al., 2010) or family history of MDD (Joormann et al., 2007). They have also been found to correlate with later depression severity (Hale, 1998), predict later relapse (Bouhuys et al., 1999) and persist following remission (Joormann and Gotlib, 2007, Bhagwagar and Cowen, 2008, Kerestes et al., 2012). Therefore, negative affective biases certainly play a role in the vulnerability to and aetiology of MDD as well as the reinforcement and maintenance of low mood (Teasdale and Russell, 1983) rather than simply being a secondary consequence of low mood. Table 1.2 summarises the effects of MDD on emotional processing and associated brain activity.

Table 1.2 Summary of the Effects of MDD on Emotional Processing and Associated Brain Activity

Reference	MDD Population Studied	Type of Stimulus / Task	Affective Valence of Stimulus	Direction of Change of Emotional Processing	Direction of Change of Associated Brain Activity
(Gur et al., 1992)	Current Medicated	Facial Expression Recognition	Happy	↓	N/A
			Sad	↑	N/A
(Matt et al., 1992)	Current & Induced Depressed Mood	Self-referent Words	Positive	↓	N/A
			Negative	↑	N/A
(Mogg et al., 1995)	Current	Word Dot-probe	Negative	↑	N/A
(Bouhuys et al., 1999)	Current Medicated	Facial Expression Recognition	Positive	↓	N/A
			Negative	↑	N/A
(Sheline et al., 2001)	Current Unmedicated	Faces	Happy	N/A	Left amygdala ↑
			Fear	N/A	Left amygdala ↑
(Fu et al., 2004)	Current Unmedicated	Faces	Sad	N/A	CC - anterior, middle, posterior ↑ Left inferior parietal cortex Left superior temporal cortex

					Left inferior temporal cortex Left precuneus Left premotor cortex Right precentral gyrus Left insula Left amygdala Left parahippocampal gyrus Left putamen Left caudate Left thalamus
(Lawrence et al., 2004)	Current Medicated	Faces	Happy	No difference in accuracy of gender discrimination or memory for facial expressions	Left amygdala ↓ Left putamen Left caudate Right globus pallidus / anterior thalamus Right parahippocampal gyrus Right vm and dl PFC
			Sad		OFC ↓ Right dorsal cingulate gyrus Bilateral dlPFC
			Fear		Right amygdala ↓ Right parahippocampus gyrus Left vm and right dl PFC Right globus pallidus / anterior thalamus
(Surguladze et al., 2005)	Current Medicated	Faces	Happy	No difference in accuracy of gender discrimination	Bilateral fusiform gyrus ↓ Right putamen
			Sad		Right fusiform gyrus ↑ Left putamen Left parahippocampal gyrus Left amygdala
(Fu et al., 2007)	Current Unmedicated	Faces	Happy	↓ Patients slower and less accurate at gender discrimination	Left posterior cingulate gyrus ↓ Right precuneus Left lingual gyrus Left hippocampal gyrus / hippocampus

					Right thalamus Right putamen Right cerebellum Right brain stem
(Fu et al., 2008)	Current Unmedicated	Faces	Sad	N/A	Right amygdala ↓ Right hippocampus CC – posterior, anterior ↑ Superior frontal gyrus Parietal cortex Precuneus
(Victor et al., 2010)	Current & Remitted Unmedicated	Backward Masked Face	Happy	No difference in behavioural performance	Bilateral amygdala ↓
			Sad		Bilateral amygdala ↑
(Chan et al., 2007)	Never-depressed At risk – neurotic	Facial Expression Recognition Self-referent Word Categorisation & Memory	Positive	↓	N/A
			Negative	↑	N/A
(Joormann et al., 2007)	Never-depressed At risk – family history Induced Depressed Mood	Faces Dot-probe	Positive	↓	N/A
			Negative	↑	N/A

(Joormann and Gotlib, 2007)	Current & Remitted Depressed	Faces Dot-probe	Happy	↓	N/A
			Sad	↑	N/A
(Kerestes et al., 2012)	Remitted Depressed	Facial Expression n-back	Happy	No difference in behavioural performance	VS ↓ Right dlPFC vlPFC
			Fear		Left OFC ↑ Left amygdala Left dl and right vlPFC

1.4 Theories of Antidepressant Action

A significant unresolved question in psychiatry is the delay between the immediate neuropharmacological actions of antidepressants and their therapeutic effect. It was thought that this delayed onset of therapeutic effect may be the result of an antidepressant-induced cascade of downstream neuroadaptive changes. For example, changes in receptor expression, such as the downregulation of 5-HT or NA transporters or desensitisation of 5-HT or NA autoreceptors (Blier and de Montigny, 1994). There is also evidence for antidepressant-induced activation of second messengers and subsequent changes in gene expression leading to increased production of neurotropic factors, such as brain-derived neurotrophic factor, with roles in synaptic plasticity and neurogenesis, particularly in the hippocampus (Malberg et al., 2000, Santarelli et al., 2003, Duman and Monteggia, 2006, Castren and Rantamaki, 2010, Bhagya et al., 2015). However, these purely neurobiological models of antidepressant action are limited in that they cannot explain how these actions relate to an improvement in mood and overall symptomatic relief (Groves, 2007, Miller and Hen, 2015). Indeed, antidepressants do not immediately elevate mood in healthy individuals (Harmer et al., 2003a, Harmer et al., 2004) and psychostimulant drugs that act to immediately elevate mood have no advantage over placebo in MDD patients (Satel and Nelson, 1989), suggesting that antidepressants may not be acting as direct mood enhancers. The focus has now moved to the neuropsychological level and a recent series of studies have suggested that antidepressants may instead act upon the negative affective biases discussed above.

1.4.1 Cognitive Neuropsychological Theory of Antidepressant Action

It has been hypothesised that the direct action of antidepressants is to alter the balance between positive and negative emotional processing. Importantly, these actions occur early in treatment before any clinically therapeutic effects are seen but are not

consciously accessible, hence a subjective delay in the action of antidepressants. As such, this theory provides the missing link between the acute neuropharmacological actions of antidepressants and the delay in mood improvement and symptom resolution (Harmer et al., 2009a, Pringle et al., 2011, Roiser et al., 2012, Harmer and Cowen, 2013). This theory of antidepressant action is supported by extensive evidence from studies investigating the effect of tryptophan (TRP) depletion and repeated, subchronic (7 day) or acute antidepressant treatment on emotional processing in healthy controls and MDD patients.

TRP is the precursor amino acid in the synthesis of 5-HT, therefore, its depletion results in low 5-HT neurotransmission and a biological induction of depressed mood. TRP depletion has been found to reduce emotional processing of positive information, such as attention directed towards happy faces (Murphy et al., 2002) and the recall of positive self-referent words (Klaassen et al., 2002).

Conversely, subchronic antidepressant treatment has been found to increase emotional processing of positive information in healthy volunteers. For example, 7 days treatment with the SSRI citalopram or the SNRI reboxetine increased recall of positive self-referent words and the inclination to perceive ambiguous facial expressions as happy (Harmer et al., 2004). Even an acute dose of citalopram (Harmer et al., 2003a) or reboxetine (Harmer et al., 2003b) has been found to be sufficient to increase the recognition of happy facial expressions. Subchronic or acute treatment with either citalopram or reboxetine also results in associated neural alterations, including attenuated amygdala responses to negative affective stimuli (Harmer et al., 2006, Anderson et al., 2007, Norbury et al., 2007, Murphy et al., 2009), increased fusiform responses to happy versus neutral facial expressions (Norbury et al., 2007) and increased or decreased frontoparietal activity during classification and recognition of positive versus negative self-referent words, respectively (Norbury et al., 2008, Di Simplicio et al., 2012). Importantly, all of these effects of antidepressants on emotional processing occur prior to any significant changes in subjective mood.

An acute dose of reboxetine has also been shown to alter the balance of positive to negative emotional processing in facial expression recognition and the recall of self-referent words in MDD patients in the absence of any changes in subjective mood (Harmer et al., 2009b). The majority of neuroimaging studies conducted in MDD patients have investigated the longer-term effects of antidepressant treatment. For example, 8 weeks treatment with SSRIs in MDD patients normalises hyperactive amygdala, VS and frontoparietal cortical responses to negative affective stimuli (Sheline et al., 2001, Fu et al., 2004). Treatment with the SNRI venlafaxine for 8 weeks also normalises hypoactive ACC responses to negative affective stimuli (Davidson et al., 2003). Repeated SSRI or SNRI treatment can also normalise hypoactive responses to positive affective stimuli (Schaefer et al., 2006, Fu et al., 2007). Due to the long-term nature of these studies, it has not been clear whether these effects of antidepressants on neural activity are the result of, or occur prior to, mood improvement in MDD patients. However, it has since been demonstrated that whilst both placebo- and escitalopram-treated MDD patients display similar symptom severity at 7 days, such short-term escitalopram treatment is sufficient to normalise amygdala hyperactivity in response to negative facial expressions (Godlewska et al., 2012).

Preliminary evidence suggests that bupropion can also influence cognitive function in MDD patients, such as memory (Soczynska et al., 2014) and attenuate amygdala hyperactivity, which was also found to correlate with improvement in symptom severity (Robertson et al., 2007). Table 1.3 summaries the effects of pharmacological manipulation on emotional processing and associated brain activity.

Table 1.3 Summary of the Effects of Pharmacological Manipulation on Emotional Processing and Associated Brain Activity

Reference	Population Studied	Type of Stimulus / Task	Pharmacological Manipulation	Affective Valence of Stimulus	Direction of Change of Emotional Processing	Direction of Change of Associated Brain Activity
(Murphy et al., 2002)	Healthy Controls	Self-referent Word Memory	TRP Depletion	Positive	↓	N/A
				Negative	No Effect	N/A
(Klaassen et al., 2002)	Healthy Controls	Faces Dot-probe	TRP Depletion	Happy	↓	N/A
				Sad	No Effect	N/A
(Harmer et al., 2004)	Healthy Controls	Facial Expression Recognition Task Self-referent Word Memory	SSRI Citalopram 7 day 20 mg / day	Positive	↑	N/A
				Negative	↓	N/A
			SNRI Reboxetine 7 day 8 mg / day	Positive	↑	N/A
				Negative	↓	N/A
(Harmer et al., 2006)	Healthy Controls	Facial Expression Recognition	SSRI Citalopram 7 day 20 mg / day	Happy	No Effect	No Effect
				Fear	↓	Right parahippocampal gyrus ↓ Right amygdala mPFC
(Di Simplicio et al., 2012)	Healthy Controls	Self-referent Word Categorisation	SSRI Citalopram 7 day 20 mg / day	Positive	No Effect	No Effect
				Negative	No Effect	vmPFC ↓ Rostral ACC OFC Frontal pole

(Norbury et al., 2007)	Healthy Controls	Faces	SNRI Reboxetine 7 day 8 mg / day	Happy	No difference in accuracy of gender discrimination	Right fusiform gyrus ↑
				Fear		Right amygdala ↓
(Norbury et al., 2008)	Healthy Controls	Self-referent Word Categorisation & Memory	SNRI Reboxetine 7 day 8 mg / day	Positive	No Effect	Precuneus ↑ Inferior frontal gyrus
				Negative	No Effect	Medial frontal gyrus ↑ CC - middle Precuneus
(Harmer et al., 2003a)	Healthy Controls	Facial Expression Recognition	SSRI Citalopram Acute 10 mg infusion	Happy	↑	N/A
				Fear	↑	N/A
(Anderson et al., 2007)	Healthy Controls	Faces	SSRI Citalopram Acute 7.5 mg infusion	Fear	No difference in accuracy of gender discrimination	Right amygdala ↓
				Disgust		Left amygdala ↓ Right temporal cortex Bilateral posterior insula ↑ Left dlPFC Bilateral occipital cortex Thalamus
(Murphy et al., 2009)	Healthy Controls	Facial Expression Recognition	SSRI Citalopram Acute 20 mg	Happy	↑	No Effect
				Fear	No Effect	Right amygdala ↓

(Harmer et al., 2003b)	Healthy Controls	Facial Expression Recognition Self-referent Word Categorisation & Memory	SNRI Reboxetine Acute 4 mg	Positive	↑	N/A
				Negative	↓	N/A
(Sheline et al., 2001)	Current MDD	Faces	SSRI Sertraline 8 weeks, ~100 mg / day	All Faces, particularly fear	No difference in accuracy of gender discrimination	Left amygdala ↓
(Fu et al., 2004)	Current MDD	Faces	SSRI Fluoxetine 8 weeks, 20 mg / day	Sad	No difference in accuracy of gender discrimination	Left CC – anterior, middle, posterior ↓ Left medial premotor cortex Right inferior parietal cortex Left precuneus Bilateral precentral gyrus Bilateral postcentral gyrus Left insula Left amygdala Bilateral putamen Left caudate Bilateral thalamus
(Fu et al., 2007)	Current MDD	Faces	SSRI Fluoxetine 8 weeks, 20 mg / day	Happy	No difference in accuracy of gender discrimination	Bilateral posterior CC ↑ Left precuneous Bilateral lingual gyrus Right cerebellum
(Davidson et al., 2003)	Current MDD	Affective Pictures	SNRI Venlafaxine 6 weeks, ↑ dose	Positive	N/A	No Effect
				Negative	N/A	Left insula ↑ Left ACC
(Schaefer et al., 2006)	Current MDD	Faces	SNRI Venlafaxine 7 day, 37.5-75 mg / day	All Faces	N/A	Left medial gyrus ↑ Left inferior gyrus Left superior gyrus

						Bilateral insula Right thalamus Right fusiform gyrus
(Harmer et al., 2009b)	Current MDD	Facial Expression Recognition Self-referent Word Categorisation & Memory	SNRI Reboxetine Acute 4 mg	Positive	↑	N/A
				Negative	↓	N/A
(Godlewska et al., 2012)	Current MDD	Faces	SSRI Escitalopram 7 day 10 mg / day	Happy	No difference in accuracy of gender discrimination	No Effect
				Fear		Right amygdala ↓
(Robertson et al., 2007)	Current MDD	Emotional Oddball	Bupropion 8 weeks, 150-450 mg / day	Emotionally Unpleasant Distractor	N/A	Right OFC ↓ Right vmPFC Right ACC Left PCC Right inferior frontal cortex Right amygdala Right parahippocampus Right caudate Right fusiform gyrus Left dmPFC Left inferior frontal cortex ↑ Left fusiform gyrus

1.4.1.1 Can this Theory Explain the Delayed Onset of Clinical Effect and Symptomatic Relief?

It certainly appears that acute antidepressant treatment can produce early changes in emotional processing prior to an improvement in mood. It is argued that such changes are not consciously accessible and require interaction with the social environment to provide the improvement in mood. Indeed, it has been demonstrated that experimentally inducing a negative affective bias in healthy volunteers does not affect mood until exposure to a stressor (MacLeod et al., 2002). A positive correlation exists between negative affective bias and impairments in mood recovery following a negative mood induction (Clasen et al., 2013). Furthermore, the magnitude of the initial effect of the SSRI citalopram on negative affective biases in memory predicts later resilience to negative mood induction (Browning et al., 2011). Such a process might involve re-learning a range of emotional associations, which fits well with the observation that antidepressants also promote synaptic plasticity, hippocampal neurogenesis and learning in animal models (Bhagya et al., 2015).

The requirement for changes in emotional processing and interaction with the social environment may also help to explain some of the variance in clinical response to antidepressant treatment. Treatment resistant MDD patients may have highly entrenched, long-standing negative affective biases that are resistant to change or highly adverse social environments that cannot support an improvement in mood even with remediation of the negative affective biases. Indeed, improved accuracy of happy facial expression recognition by perceived level of social support was found to be a significant predictor of change in symptom severity (Shiroma et al., 2014).

It is important to note that MDD is not only characterised by low mood but also by vegetative, cognitive and social symptoms. However, changes in emotional processing could potentially still account for the global resolution of the multiple symptom domains in MDD. A reduction in the preoccupation with negative information would certainly improve mood, reduce social withdrawal and increase the availability of information processing resources for

other cognitive tasks, such as episodic memory that is often impaired in MDD (Harmer et al., 2009a, Harmer and Cowen, 2013). Indeed, a range of antidepressants have been shown to increase affiliative problem solving behaviour and decrease submissive behaviour in healthy volunteers (Knutson et al., 1998, Tse and Bond, 2002, Tse and Bond, 2003), demonstrating that reduced negative affective biases are translated into improved social interactions and behaviour.

1.4.1.2 Clinical Applications

The cognitive neuropsychological theory of antidepressant action suggests that the early effects of antidepressants on emotional processing are critical for the later therapeutic effects. If this hypothesis is correct, it may be possible to predict how effective a particular antidepressant will be for a particular individual in the long-term from the magnitude of the initial effects of the antidepressant on behavioural or neural measures of emotional processing. Initial results are certainly promising, for example, the increase in the recognition of happy facial expressions produced by 2 weeks treatment of the SSRI citalopram or the SNRI reboxetine was found to be associated with the clinical improvement seen after 6 weeks of continued treatment (Tranter et al., 2009). Improved accuracy of happy facial expression recognition following 7 days citalopram treatment has also been found to be a marginally significant predictor of antidepressant response after 56 days in later-life MDD (Shiroma et al., 2014). It is also promising that the effects of antidepressants on emotional processing correspond with clinical observations. For example, whilst repeated treatment with either the SSRI citalopram or the SNRI reboxetine reduces the perception of negative facial expressions of fear, anger and disgust, indicating reduced threat-relevant processing (Harmer et al., 2004), acute citalopram treatment appears to increase threat-relevant processing as evidenced by increased fearful facial expression recognition and startle response (Harmer et al., 2003a, Browning et al., 2007, Grillon et al., 2007). This is consistent with the clinical observation of an initial exacerbation of anxiety early in SSRI treatment prior to the production of long-term anxiolytic effects. This also provides further evidence that

different neurotransmitters play different roles in emotional processing and has implications for stratified medicine, where treatment is tailored towards the predominant symptomatology for an individual MDD patient (Pringle et al., 2013, Gerra et al., 2014).

Since emotional processing appears to be altered by a wide range of antidepressants with different neuropharmacological actions in healthy volunteers, individuals at high risk from MDD and MDD patients, behavioural and neural measures of emotional processing could also be a useful predictor of the therapeutic potential of novel, candidate antidepressants. Indeed, the novel candidate antidepressants aprepitant, a antiemetic neurokinin 1 receptor antagonist and memantine, a glutamate receptor antagonist, that have since failed clinical trials (Keller et al., 2006, Zarate et al., 2006) also failed to modify emotional processing (Chandra et al., 2010, Pringle et al., 2012), whilst rimonabant, a anorectic cannabinoid 1 receptor antagonist known to cause psychiatric side-effects including MDD, produced negative affective biases (Horder et al., 2012).

Future research must focus on replicating these predictive effects in larger MDD patient samples with antidepressants influencing different neurotransmitter systems such that emotional processing measures can be used in the development and efficacy screening of much needed novel antidepressants and the tailoring of treatment to individuals.

1.5 Summary and Overall Project Aims

The majority of research on the effects of antidepressants has been conducted using antidepressants predominately affecting 5-HT or NA activity; however, SSRI or SNRI antidepressant treatment alone is clearly not sufficiently effective in all MDD patients. It is becoming clearer that SSRIs better target negative affective biases and low mood, whilst SNRIs better target reduced positive affect. However, questions remain as to the specific role of the various neurotransmitters in the manifestation of negative affective biases and aberrant reward processing in MDD. For example, it is still unclear how the initial antidepressant effects on biases in emotional processing relate to effects on aberrant reward

processing in MDD patients and whether they are also targeted by antidepressants with DA activity, such as bupropion.

This project comprises two studies: a double-blind randomised trial in which non-clinical participants receive acute bupropion treatment, placebo or no treatment prior to behavioural assessment of reward and emotional processing and a large, repeated measures, open-label, experimental medicine study in which a cohort of MDD patients receive bupropion and their reward and emotional processing is assessed at baseline, 2 weeks and 6 weeks via behavioural and fMRI methods. To summarise, in a series of studies manipulating DA activity with bupropion in either healthy controls or MDD patients we aim to delineate the role of the DA reward system in the cognitive neurobiological processes underlying different symptom clusters in MDD.

CHAPTER 2

THE ACUTE EFFECTS OF BUPROPION AND PLACEBO ON BEHAVIOURAL MEASURES OF REWARD AND EMOTIONAL PROCESSING IN HEALTHY VOLUNTEERS

2.1 Introduction & Study Aims

As discussed in Chapter 1, the neuropsychological theory of antidepressant action is supported by evidence from various behavioural paradigms and functional neuroimaging investigations following TRP depletion or repeated and acute antidepressant treatment in healthy volunteers, individuals at high risk for MDD and MDD patients (Harmer et al., 2009a, Pringle et al., 2011, Roiser et al., 2012, Harmer and Cowen, 2013). The majority of research has been conducted using SSRIs or SNRIs; however, whilst SSRIs treat the negative biases in emotional processing underlying low mood, it is becoming clear that they do not fully correct the anhedonia observed in MDD (Nutt et al., 2007) and may actually exacerbate it (Kumar et al., 2008, McCabe et al., 2010). The anhedonia observed in MDD is associated with resistance to treatment and poor clinical outcome. MDD patients with high anhedonic symptoms show a greater reduction in reward response bias and reward-related brain activity in the VS and this is a unique predictor of continued MDD diagnosis after treatment (Forbes et al., 2010, Vrieze et al., 2013, Vrieze et al., 2014). It may be that drugs enhancing reward-related DA activity, such as bupropion, may better target the aberrant reward processing and anhedonia in MDD.

Therefore, the current Chapter aims to define the effects of an acute dose of bupropion compared to placebo on emotional and reward processing in healthy volunteers. Specifically, we sought to investigate whether bupropion had similar effects on emotional processing to SSRIs, acting to reduce negative affective biases, or had more specific effects on processing of positive stimuli and reward. A double-blind randomised study with three

parallel groups was conducted, in which healthy participants received an acute dose of bupropion, acute dose of placebo or no treatment prior to behavioural assessment of reward and emotional processing. The primary hypothesis was that, with its DA and NA enhancing effects, an acute dose of bupropion would improve performance in a reward task and increase positive emotional processing. A secondary aim was to explore whether the ETB tasks were influenced by the placebo effect or whether performance was similar between the no treatment and placebo groups.

2.2 Methods

This study was approved by the University of Oxford research ethics committee (MSD-IDREC-C3-2014-004) and was carried out in accordance with the protocol and provisions of the World Medical Association Declaration of Helsinki. All participants provided written informed consent.

2.2.1 Participant Recruitment, Screening & Randomisation

A total of 20 x 3 healthy participants were recruited from the general population via posters displayed in University colleges, departments and email newsletters and via advertisement on the internet. Participants first attended a screening visit, which involved the Structured Clinical Interview (SCID) for DSM-IV (First, 1997), the National Adult Reading Test (NART) (Nelson and Willison, 1991) to provide an estimate of verbal IQ, collection of information on date of birth, body mass index (BMI; height and weight) and medical history, assessment of the participant's cardiovascular system, including electrocardiogram (ECG), pulse rate and blood pressure checked by the study medic and finally a urine drug and pregnancy test. Participants were required to be between the ages of 18 and 45 years and sufficiently fluent in English to complete the behavioural tasks. Participants were excluded if they had current or past history of any Axis 1 DSM-IV psychiatric illness, had any medical

condition or had taken any medications or drugs that could impact upon the safety or effect of bupropion, or had previous experience of the behavioural tasks.

Eligible participants were randomised into the three parallel groups, with 20 participants per group, stratified for gender. Participants were also matched for age and verbal IQ across the three treatment groups. Participants received an acute dose of SR bupropion, acute dose of placebo or no treatment. Those in the bupropion and placebo groups were blinded to the treatment they received and the treatment was administered by an independent member of staff such that the investigator also remained blind to the treatment group. A 3 hour wait period followed treatment administration since this is the t_{max} of the SR formulation of bupropion and allowed for testing at maximum plasma concentration (Jefferson et al., 2005). Participants then completed a reward task to assess reward processing (Section 2.2.3) and the Emotional Test Battery (ETB) to assess emotional processing (Section 2.2.4). Participants also completed a variety of questionnaires (Section 2.2.2) and provided salivary cortisol samples before and after behavioural assessment of reward and emotional processing. Salivary cortisol can act as a marker of bupropion absorption of activity as the enhanced DA activity increases stimulation of the paraventricular nucleus of the hypothalamus promoting synthesis and release of corticotrophin-releasing hormone and subsequent release of cortisol (Rao et al., 2005). Figure 2.1 summarises participant recruitment and the study design.

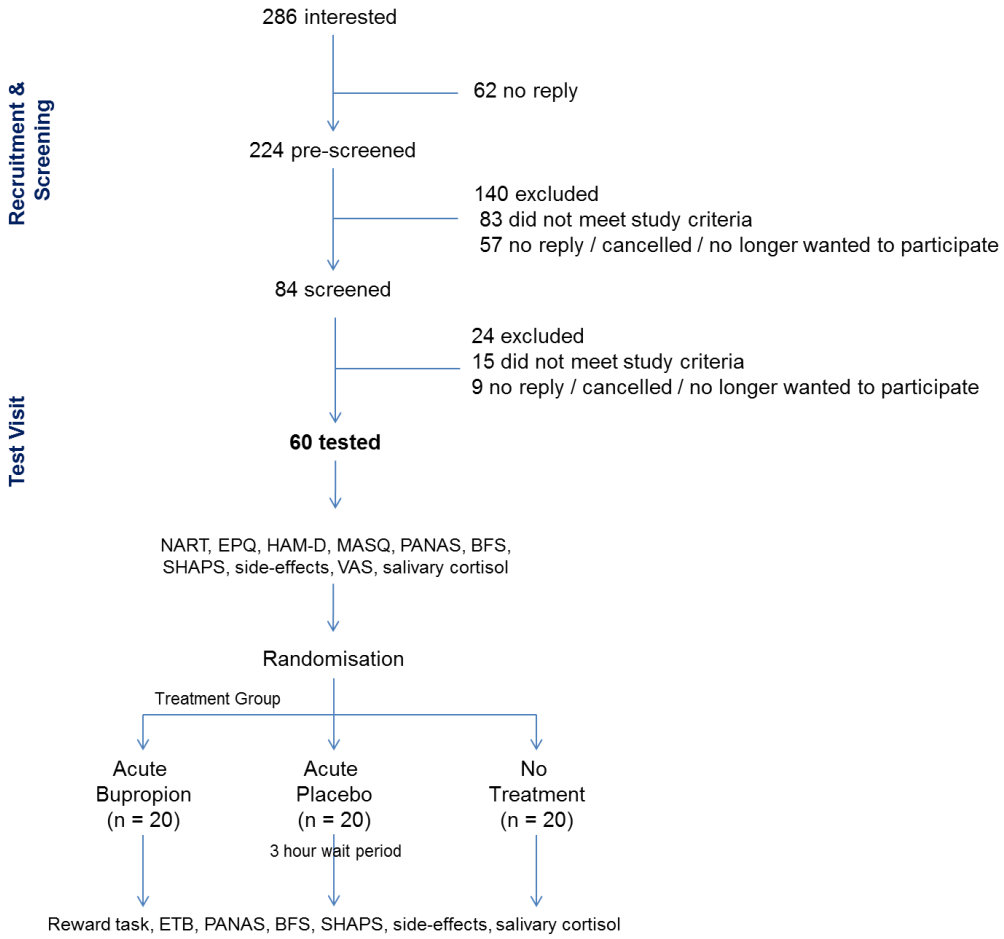


Figure 2.1 – Summary of Participant Recruitment and Study Design: displays the number of participants recruited, screened and tested. The study was a double-blind, randomised design with three parallel groups. Eligible participants completed a variety of questionnaires prior to randomisation to their treatment group and behavioural assessment of reward and emotional processing.

2.2.2 Questionnaire Measures

Prior to treatment administration and behavioural assessment, participants completed a number of questionnaires. Firstly, the Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960) was administered via a semi-structured interview with a trained experimenter. This consists of 17 items and measures the severity of depressive symptoms ranging from normal (below 7) to very serve depression (greater than 23).

The rest of the questionnaires were self-report questionnaires completed on a computer. The Adult Eysenck Personality Questionnaire (EPQ) (Eysenck, 1975) comprises 90 items and provides measures of four personality characteristics: neuroticism, psychoticism, extraversion and susceptibility to lie on questionnaires. The Full Mood and Anxiety Symptom Questionnaire (MASQ) (Clark and Watson, 1991) comprises 90 items and provides measures of general distress (depressive, anxious and mixed), anxious arousal and anhedonic depression experienced in the past week. The Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) is a list of 20 affective words covering two primary dimensions of mood: positive and negative affect. Participants were required to rate the extent to which they felt a certain mood on the day of testing. The Befindlichkeits Scale (BFS) (Von Zerssen and Petermann, 2011) comprises 22 affective words and their antonyms, which participants were required to choose between depending on how they felt on the day of testing, to give measures of mood and energy levels. The Snaith-Hamilton Pleasure Scale (SHAPS) (Snaith et al., 1995) comprises 14 items to provide measures of anhedonia. Each item describes a pleasurable situation covering one of four domains of pleasure: interests / pastimes, social interaction, sensory experience and food / drink and participants are asked to agree or disagree with each statement based on the past few days (Romer Thomsen et al., 2015). In this study, the ratings of strongly agree, agree, disagree and strongly disagree were converted into a 4-point scale, with higher scores indicating higher anhedonia. A typical side-effects questionnaire asks participants to rate whether certain side-effects were absent, mild, moderate or severe. For this study, the side-effects listed were the ones most common for bupropion, including headache, nausea, dizziness, dry mouth, agitation, itching and sweating. Finally, participants were also asked to provide a rating for expectation of change in their emotional processing resulting from treatment on a visual analogue scale (VAS). After treatment administration and behavioural assessment, participants repeated the PANAS, BFS, SHAPS and side-effects questionnaires.

2.2.3 Salivary Cortisol

Salivary cortisol samples were provided before and after treatment administration and behavioural assessment. These samples were collected using Salivette cotton swabs that were centrifuged to extract the saliva, which was then frozen ready for analysis. Absorption spectroscopy with area under the curve (AUC) correction was used to determine the concentration of cortisol in each sample and the mean for each treatment group was calculated.

2.2.4 Reward Task

The reward task was a probabilistic instrumental learning task involving monetary wins or losses based on the task described in (Pessiglione et al., 2006). It was developed in Presentation (Neurobehavioural Systems, Inc., Berkeley, USA) and piloted on 15 individuals prior to its use in any studies. The task stimuli consisted of two pairs of symbols, based on letters taken from the Agathodaimon font. Each pair of symbols had an associated pair of outcomes, with each symbol in the pair corresponding to reciprocal probabilities of the associated outcome occurring. One pair of symbols was associated with wins, with the correct symbol of choice having a probability of winning £1 70% of the time over winning nothing 30% of the time and the incorrect symbol of choice having a probability of winning nothing 70% of the time over winning £1 just 30% of the time. The other pair of symbols was associated with losses, with the correct symbol of choice having a probability of losing nothing 70% of the time over losing £1 just 30% of the time and the incorrect symbol of choice having a probability of losing £1 70% of the time over losing nothing 30% of the time.

Participants first performed a shortened, 10 trial familiarization version of the reward task. Participants then performed two, 60 trial runs with each run containing a different set of 4 symbols. Participants began the task with £5. On each trial, participants were randomly presented with one of the two pairs of symbols on a display screen for 4000 ms, with each symbol randomly positioned either to the left or the right of a central fixation cross. Win a

loss trials were interspersed and whilst the contingencies were randomised within a run, they were fixed across runs and visits. Participants were instructed to choose between the two symbols in order to maximise payoffs. Once a choice was made, the associated outcome was presented on the display screen (Figure 2.2). In order to maximise payoffs, participants should use the outcome feedback to gradually learn the symbol-outcome associations by trial and error over time, such that they consistently choose the symbol with the high-probability win and avoid the symbol with the high-probability loss. Outcome measures included end total, total won and lost, choice frequency, percentage of choices identical to the preceding one (percentage consistency) and reaction time.

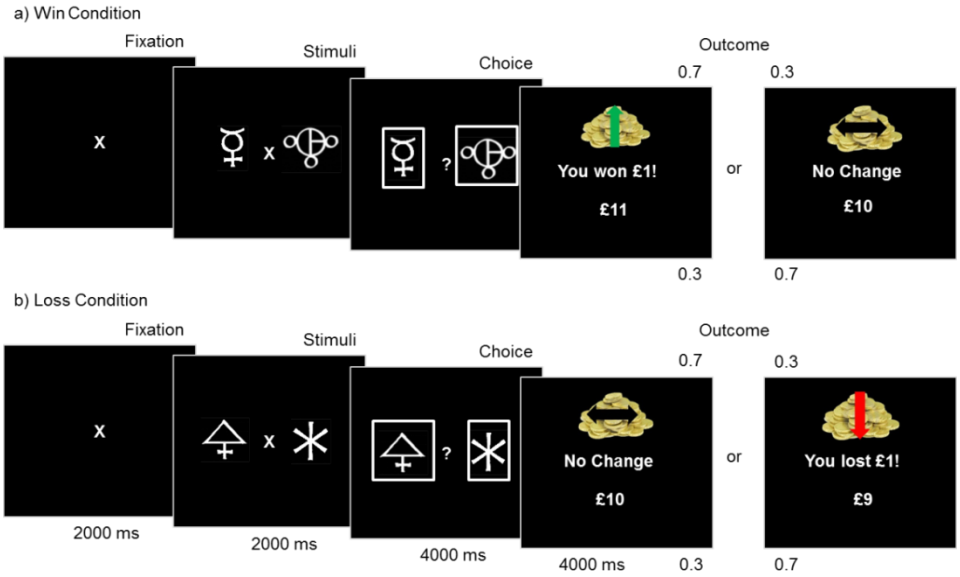


Figure 2.2 - Probabilistic Instrumental Learning Task with Monetary Outcomes: task stimuli consisted of two pairs of symbols, with one pair associated with **(a)** win outcomes and the other associated with **(b)** loss outcomes. Each symbol in the pair corresponded to reciprocal probabilities (0.7 or 0.3) of the associated outcome occurring. On each trial, participants were randomly presented with one of the two pairs of symbols, with each symbol randomly positioned either to the left or the right of a central fixation cross. Participants were required to choose between the two symbols in order to maximise payoffs. Once a choice was made, outcome feedback was provided.

2.2.5 Emotional Test Battery

The Emotional Test Battery (ETB; P1vital, Oxford, UK) comprises five validated, computerised cognitive tasks designed to assess the processing of a variety of emotionally valenced stimuli. It has found to be sensitive to the negative biases in emotional processing observed in depression and to the early effects of antidepressants on emotional processing (Harmer et al., 2009a). Given that two of the tasks are memory tasks that have to appear near the end and no effects of task order have been found (Adams et al., 2016), the order of administration was fixed and was as follows:

2.2.5.1 Facial Expression Recognition Task (FERT)

Task stimuli consisted of a series of facial expressions associated with six basic emotions: anger, disgust, fear, happy, sad, surprise and neutral. Each emotion also had a range of different intensity levels by using faces morphed between neutral (0%) and full intensity (100%) in 10% steps (Young et al., 1997). For each emotion, there were 4 faces for each intensity level, totalling 280 emotional facial expressions randomly presented in the centre of a display screen, each for 500 ms, divided across 4 blocks. Participants were instructed to indicate the emotional expression on the face by pressing a correspondingly labelled button. Outcome measures were percentage accuracy for facial expression recognition, percentage misclassifications and average reaction time for correct responses for each emotion. Signal detection theory was also used to provide estimates of target sensitivity (d') and beta. Increased beta indicates decrease response bias for a particular emotional facial expression. To calculate d' and beta, percentage accuracy and percentage misclassification were first divided by 100 then the formulae used were as follows, where x is the percentage misclassification/100 and y is the percentage accuracy/100:

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

$$beta = (y*(1-y)-x*(1-x))/(y*(1-y)+x*(1-x))$$

2.2.5.2 Emotional Categorisation Task (ECAT)

A series of 60 words, selected to be either positive or negative descriptors of personality, were randomly presented in the centre of a display screen, each for 500 ms. The personality descriptive words were matched for word length and ratings of frequency and meaningfulness. Participants were instructed to indicate whether they would like or dislike to be referred to as each personality descriptive word by pressing a correspondingly labelled button. Outcome measures were percentage accuracy for categorisation and average reaction time for correct responses for positive versus negative self-referent personality descriptive words.

2.2.5.3 Facial Dot-Probe Task (FDOT)

During each trial of the FDOT, a pair of faces was presented vertically on a display screen prior to the appearance of a dot-probe. There were 96 trials in total, with 48 trials showing a positive facial expression (happy) and 48 trials showing a negative facial expression (fearful), each time paired with a neutral facial expression. Additionally, the pairs of faces could either be unmasked and presented for 500 ms or briefly presented for 14 ms and then replaced by a jumbled face mask. The pairs of faces were presented in a random order, divided across 4 blocks. The dot-probe appeared either in the same position as the emotional facial expression (congruent trials) or in the opposite position to the emotional facial expression (incongruent trials). The dot-probe comprised a pair of dots that could either be vertically or horizontally aligned and participants were instructed to indicate the alignment of the dot-probe by pressing a correspondingly labelled button as quickly as possible. Reaction time was recorded and used to calculate attentional vigilance scores for each face emotion.

2.2.5.4 Emotional Recall Task (EREC)

Participants were given a surprise free recall task where they were asked to write down as many of the self-referent words that they could remember from the ECAT within a 2

minute time limit. The totals for positive versus negative self-referent words correctly and falsely recalled were calculated.

2.2.5.5 Emotional Recognition Memory Task (EMEM)

A series of 120 words, comprising the positive and negative self-referent words included in the ECAT and previously unseen distractor self-referent words were randomly presented in the centre of a display screen, each for 500 ms. Participants were instructed to indicate whether they had previously been presented with the self-referent word or not. Outcome measures were percentage accuracy, percentage misclassification of familiar words as novel and vice versa and average reaction time for correct responses for positive versus negative self-referent words. Signal detection theory was also used to provide estimates of target sensitivity (d') and beta / response bias for positive versus negative self-referent words.

2.2.6 Statistical Analysis

2.2.6.1 Data Cleaning

Prior to statistical analysis, raw behavioural data was visually inspected for anomalous values through the use of scatter graphs. Scatter graphs of overall totals in the reward task revealed a distinct group of participants for each treatment group with very low overall totals, including some participants that performed worse that chance and totalled negative monetary amounts. It was assumed that these participants did not understand the task and any participant totalling an average across the two runs below the starting £5 was excluded. A total of 10 participants were excluded from all reward task analyses for this reason: 4 from the no treatment group, 3 from the placebo group and 3 from the bupropion group. One participant in the placebo group was excluded from the analysis of reaction times for the FERT since they scored 0% accuracy for recognising fearful faces meaning the average reaction time for correct responses could not be calculated. One participant in the

no treatment group was excluded from the FDOT analysis due to a large proportion of no responses (64 out of 192) and an outlying average reaction time.

The mean is particularly sensitive with regards to reaction times since anomalously high reaction times as a result of distractions can produce a positive bias in the mean; therefore, with the exception of the FDOT, reaction times were trimmed at the participant level: reaction times above 3 standard deviations from the mean or below 200 ms were excluded prior to calculating the mean. For the FDOT, previous papers have calculated median reaction times at the participant level prior to calculating the mean to reduce the size of the standard error of the mean (Price et al., 2015); therefore, the same method was implemented in this study.

2.2.6.2 Reward Task Analysis

Statistical analyses used in the reward task are summarised in Table 2.1. Unless otherwise stated, post hoc planned comparisons were independent samples t-tests between the treatment groups of interest.

Table 2.1 Summary of Statistical Analyses used for the Reward Task

Outcome Measures		Statistical Analysis Used	Factors		Critical Effects
			Between	Within	
Monetary Totals	Overall total	Repeated measures ANOVA	Treatment group	Run (2 levels – run 1, run 2)	If no main effect of run, not included as a within subjects factor and average across 2 runs used in ANOVA
		ANOVA	Treatment group		Treatment group
	Amount won	Repeated measures ANOVA	Treatment group	Run (2 levels – run 1, run 2)	If no main effect of run, not included as a within subjects factor and average across 2 runs used in ANOVA
		ANOVA	Treatment group		Treatment group
	Amount lost	Repeated measures ANOVA	Treatment group	Run (2 levels – run 1, run 2)	If no main effect of run, not included as a within subjects factor and average across 2 runs used in ANOVA
		ANOVA	Treatment group		Treatment group
Response	Reaction time	Repeated measures ANOVA	Treatment group	Run (2 levels – run 1, run 2) Condition (2 levels – win, loss)	If no main effect of run, not included as a within subjects factor and average across 2 runs used Treatment group Condition x treatment group
Learning	Average of last 50% of trials in which correct symbol chosen (Harrison et al., 2016)	Repeated measures ANOVA	Treatment group	Condition (2 levels – win, loss)	Treatment group Condition x treatment group
	Consistency	Repeated measures ANOVA	Treatment group	Condition (2 levels – win, loss)	Treatment group Condition x treatment group

2.2.6.3 ETB Analysis

Data from each task of the ETB was analysed using repeated measure ANOVAs with different within subject factors depending on the task. These are summarised in Table 2.2. Unless otherwise stated, post hoc planned comparisons were independent samples t-tests between the treatment groups of interest. Note that the FDOT can be analysed either using the reaction time data in a 2x2x2 ANOVA including congruency as a within subjects factor or using the attentional vigilance data derived from reaction times in a 2x2 ANOVA not including congruency. Congruency refers to whether the dot-probe appeared in the place of a neutral (incongruent) or emotionally valenced (congruent) face and allows assessment of whether participants displayed quicker reaction times and therefore a response bias to emotionally valenced faces. The former allows investigation of the main effects for reaction time but the latter is simpler to understand so both methods were used.

Table 2.2 Summary of Repeated Measure ANOVAs for the ETB Tasks

ETB Task	Within Subject Factors	Number of Levels	Outcome Measures	Critical Contrasts of Interest
FERT	Face emotion	6 (anger, disgust, fear, happy, surprise – neutral excluded) OR 3 (happy, sad, external – average of anger, disgust, fear, surprise)	% accuracy % misclassifications Average reaction time for correct responses Target sensitivity / d' Beta / response bias	Face emotion x treatment group
	Emotion intensity	10 (10-100%)	% accuracy	Emotion intensity x treatment group
ECAT	Word valence	2 (like, dislike)	Average reaction time for correct responses % accuracy	Word valence x treatment group
EREC	Word valence	2 (positive, negative)	Number of words recalled Number of commission errors	Word valence x treatment group
EMEM	Word valence	2 (positive, negative)	% accuracy % misclassifications Average reaction time for correct responses Target sensitivity / d' Beta / response bias	Word valence x treatment group
	Misclassification type (only used for % misclassifications)	2 (novel as familiar, familiar as novel)		Word valence x treatment group Word valence x misclassification type x treatment group
FDOT	Face emotion Probe duration / masking	2 (fear, happy) 2 (masked, unmasked) First 2X2 then split into 2 separate repeated measure ANOVAs	Attentional vigilance (derived from reaction times) Average reaction time for correct responses	Face emotion x treatment group Face emotion x probe duration x treatment group
	Probe location / congruency (only used for reaction time)	2 (congruent with emotional face, incongruent with emotional face)		

2.3 Results

2.3.1 Participant Demographics & Characterisation

As can be seen from Table 2.3 there were no significant differences between treatment groups with regards to gender, age, NART-derived verbal IQ and baseline scores on the HAM-D and self-report questionnaires.

Table 2.3 Participant Demographics & Baseline Questionnaire Scores

	No Treatment	Placebo	Bupropion	ANOVA p
n	20	20	20	
Gender (Male:Female)	10:10	10:10	10:10	
Age	22.55 ± 4.61	24.45 ± 3.49	23.20 ± 4.66	0.37
NART	116.09 ± 4.46	115.31 ± 6.15	114.85 ± 5.91	0.78
HAM-D	0.75 ± 0.79	0.60 ± 0.99	0.95 ± 1.70	0.67
SHAPS*	19.75 ± 4.15	22.85 ± 5.92	21.15 ± 4.49	0.15
VAS Expectation	34.25 ± 18.85	31.10 ± 23.14	43.20 ± 26.47	0.24
EPQ				
Neuroticism	5.05 ± 3.66	5.10 ± 4.18	5.20 ± 4.05	0.99
Psychoticism	2.95 ± 2.56	3.00 ± 2.05	2.70 ± 1.87	0.90
Extroversion	14.15 ± 4.13	13.85 ± 4.59	14.90 ± 4.94	0.76
Lie	8.7 ± 4.60	8.30 ± 3.56	9.00 ± 2.99	0.84
PANAS*				
Positive Affect	30.45 ± 4.41	30.85 ± 5.95	31.75 ± 5.79	0.74
Negative Affect	11.85 ± 2.21	13.00 ± 4.45	12.85 ± 3.00	0.50
MASQ				
General Distress: Mixed	21.00 ± 4.09	23.80 ± 6.69	22.65 ± 4.53	0.24
General Distress: Anxious	15.65 ± 2.46	16.25 ± 3.52	16.15 ± 3.15	0.80
Anxious Arousal	18.35 ± 1.98	19.00 ± 1.49	18.95 ± 2.37	0.52
General Distress: Depressive	16.40 ± 3.17	16.50 ± 4.07	15.30 ± 2.99	0.48
Anhedonic Depression	49.45 ± 7.31	50.80 ± 9.66	50.05 ± 8.71	0.88
BFS*				
Energy	2.95 ± 2.95	4.50 ± 4.74	3.05 ± 3.19	0.34
Mood	9.50 ± 7.22	14.50 ± 10.71	12.25 ± 9.88	0.25
Overall	12.45 ± 8.92	19.00 ± 13.56	15.30 ± 12.44	0.22
Side-effects*				
Nausea	1.00 ± 0.00	1.05 ± 0.22	1.15 ± 0.49	0.31
Dizziness	1.00 ± 0.00	1.05 ± 0.22	1.00 ± 0.00	0.37
Dry Mouth	1.00 ± 0.00	1.15 ± 0.37	1.10 ± 0.45	0.36
Headache	1.20 ± 0.41	1.15 ± 0.37	1.15 ± 0.37	0.89
Agitation	1.00 ± 0.00	1.00 ± 0.00	1.20 ± 0.52	0.06
Itching	1.05 ± 0.22	1.00 ± 0.00	1.15 ± 0.67	0.50
Sweating	1.10 ± 0.31	1.10 ± 0.31	1.15 ± 0.49	0.89

Values are reported as means ± standard deviation
* measured before and after treatment administration; only baseline is shown here

2.3.2 Changes in Mood

Table 2.4 shows the scores for those questionnaires completed again after treatment administration and behavioural assessment. Repeated measure ANOVAs revealed a significant main effect of time for all of the questionnaires, with a general reduction in positive mood over time. With the exception of the SHAPS, this was seen across all treatment groups. A significant time by treatment group interaction was observed for the SHAPS ($F_{2, 57} = 4.58$, $p < 0.05$) and post hoc paired t-tests indicated that SHAPS scores increased over time in the no treatment (+0.85 ± 1.42; $t_{19} = -2.67$, $p < 0.05$) and bupropion (+1.40 ± 3.17; $t_{19} = -2.04$, $p = 0.06$) groups but not in the placebo group (-1.25 ± 3.77; $t_{19} = 1.48$, $p = 0.15$). However, overall, the reduction in mood was not specific to any particular treatment group and was therefore most likely to be the result of the relatively long 5 hour study period, including a 3 hour wait period, rather than any drug effects. Side-effect ratings were very low with the majority of participants rating that side-effects were absent (1.00) pre- and post-treatment.

Table 2.4 Questionnaire Scores Post Treatment & Changes Over Time

	No Treatment	Placebo	Bupropion	Over Time	p
SHAPS 2	20.6 ± 4.49	21.60 ± 4.83	22.55 ± 5.61	Increases in no treatment & bupropion	p < 0.05
PANAS 2					
Positive Affect	27.20 ± 5.12	28.80 ± 5.36	28.95 ± 7.79	Decreases	p < 0.001
Negative Affect	11.25 ± 1.92	12.60 ± 3.50	11.70 ± 2.94	Decreases	p < 0.05
BFS 2					
Energy	5.90 ± 5.37	6.60 ± 5.47	5.55 ± 4.81	Increases	p < 0.001
Mood	13.80 ± 11.46	15.55 ± 12.61	12.85 ± 11.38	Increases	p < 0.05
Overall	19.70 ± 15.57	22.15 ± 16.64	18.40 ± 15.15	Increases	p < 0.001
Side-effects 2					
Nausea	1.05 ± 0.22	1.00 ± 0.00	1.10 ± 0.45	No change	
Dizziness	1.10 ± 0.31	1.00 ± 0.00	1.15 ± 0.37	No change	
Dry Mouth	1.00 ± 0.00	1.20 ± 0.41	1.10 ± 0.31	No change	
Headache	1.15 ± 0.49	1.05 ± 0.22	1.30 ± 0.57	No change	
Agitation	1.05 ± 0.22	1.00 ± 0.00	1.20 ± 0.52	No change	
Itching	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	No change	
Sweating	1.05 ± 0.22	1.10 ± 0.31	1.05 ± 0.22	No change	

Values are reported as means ± standard deviation

2.3.3 Salivary Cortisol

Salivary cortisol samples were also taken before and after treatment administration and behavioural assessment to act as a marker of bupropion absorption and activity. Whilst the no treatment group seemed to have a slightly higher baseline level of salivary cortisol from Figure 2.3, an ANOVA did not find a significant difference between treatment groups at baseline ($F_{2, 57} = 0.42$, $p = 0.66$). The diurnal cycle of cortisol can clearly be seen in Figure 2.3, with the highest levels of cortisol observed in the morning, decreasing throughout the day. This was reflected in a repeated measures ANOVA as a significant main effect of time ($F = 34.86_{1, 57}$, $p < 0.001$). Despite administration of bupropion to one group, there was no time by treatment group interaction ($F_{2, 57} = 0.47$, $p = 0.63$) for salivary cortisol.

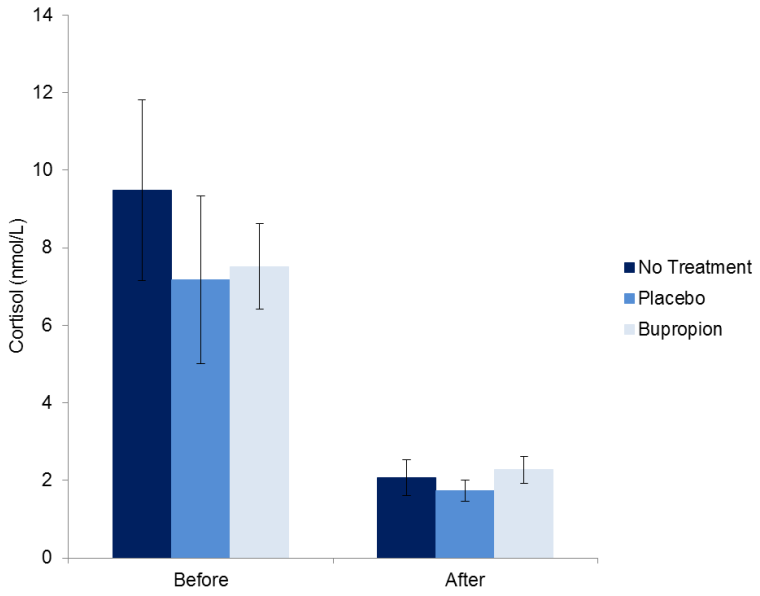


Figure 2.3 – Salivary Cortisol: measured before and after treatment administration and behavioural assessment. Values are reported as means ± standard error of the mean.

2.3.4 Acute Effects of Bupropion on Reward Processing

Participants performed a reward task, during which they were presented with two symbols per trial, where each symbol was associated with a certain win, loss or no change

outcome probability. Participants were required to choose between the two symbols in order to maximise payoffs.

Figure 2.4 shows the average overall total at the end of the reward task, amount won and amount lost per run as well as averaged across the two runs for each treatment group. Analysis of totals was first carried out using repeated measures ANOVAs with run as a within subjects factor; however, no significant main effect of run was found for each outcome condition. Therefore, run was excluded from further analyses and the averages across runs were used in separate ANOVAs for each outcome condition. There was no main effect of treatment group for overall total ($F_{2, 49} = 0.15$, $p = 0.86$), amount won ($F_{2, 58} = 0.15$, $p = 0.86$) or amount lost ($F_{2, 58} = 0.78$, $p = 0.46$).

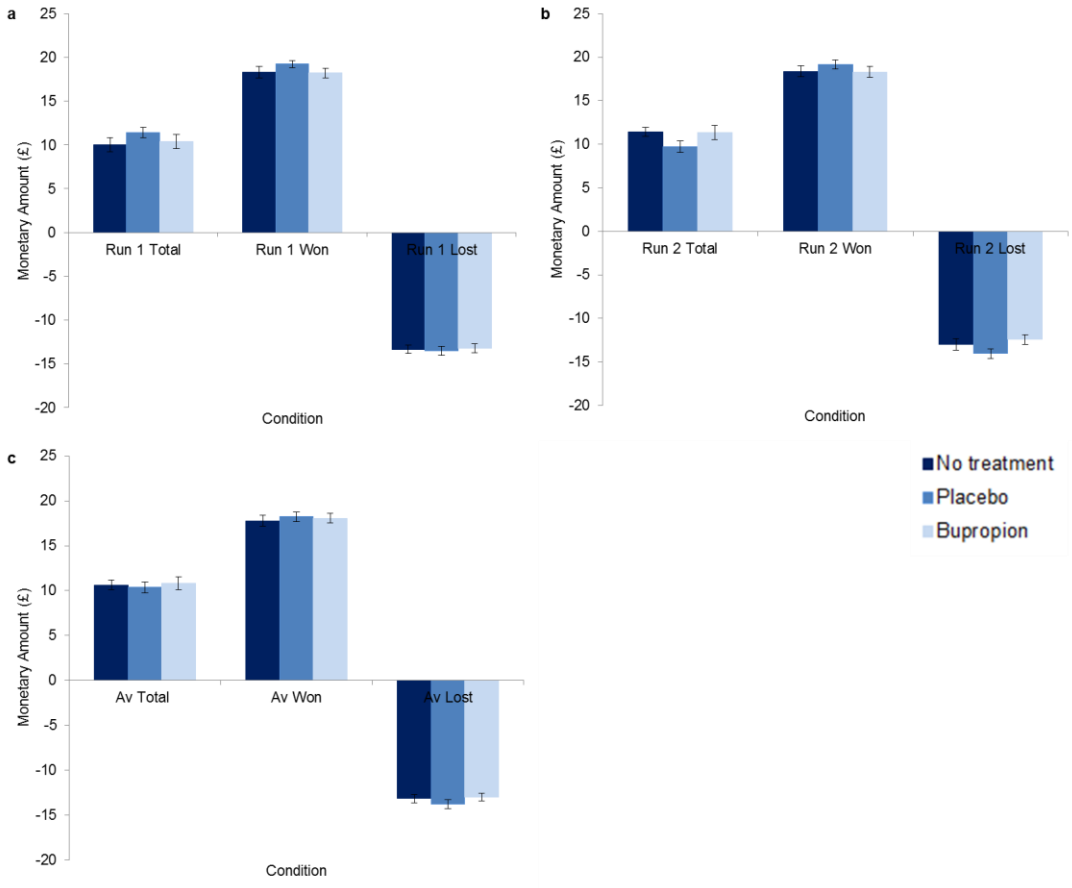


Figure 2.4 - Reward Task Monetary Totals: the average total at the end, amount won and amount lost for each treatment group for **(a)** run 1**, (b)** run 2 and **(c)** averaged across the two runs of the reward task. Values are reported as means ± standard error of the mean.

Figure 2.5a shows learning curves for each treatment group depicting trial-by-trial the proportion of participants that chose the correct symbol in the win condition, associated with high-probability win and the incorrect symbol in the loss condition, associated with high-probability loss. With the exception of those who were excluded (Section 2.2.5.1), participants generally learnt within the 30-trial run for each condition to choose the high-probability win and avoid the high-probability loss. Through visual inspection of Figure 2.5a, there does not appear to be any difference in learning about wins or losses between the three treatment groups, consistent with the monetary total results.

Whilst the learning curve can provide more temporal information about reward learning differences between treatment groups than monetary totals, it is less quantifiable. However, making the assumption that the participants have learnt to choose the high-probability win and avoid the high-probability loss by the last 50% of the trials, the average of the last 50% of the trials (last 15 out of 30) in which participants chose the correct symbol was calculated for both the win and loss conditions for each treatment group (Figure 2.5b) (Harrison et al., 2016). A repeated measures ANOVA to statistically analyse reward learning differences could now be performed. A significant main effect of condition was found ($F_{1, 42}$ = 79.51, $p < 0.001$) with participants more likely to choose the correct symbol in the win condition across all treatment groups despite the same reciprocal outcome probabilities in both the win and loss conditions. However, no main effect of treatment group ($F_{2, 42}$ = 0.52, p = 0.60) or condition by treatment group interaction ($F_{2, 42}$ = 1.73, p = 0.19) was found. A post hoc independent samples t-test did find a significant difference in percentage correct symbol choice for the win condition between the placebo and bupropion groups, with the bupropion group less likely to choose the correct symbol in the win condition compared to the placebo group (t_{28} = 2.14, $p < 0.05$); however, the bupropion group was still very similar to the no treatment group (t_{28} = -0.49, p = 0.63). Visual inspection of Figure 2.4b suggests that the bupropion group was also more likely to choose the correct symbol in the loss condition, indicating increased aversion to losses; however, this was not found to be significant (t_{28} = -1.17, p = 0.25).

Consistency, defined as the percentage of choices identical to the preceding one (Harrison et al., 2016) was also calculated and analysed (Figure 2.5c). Again, run was initially included as an additional within subjects factor in a repeated measures ANOVA; however, no significant main effect of run was found so it was excluded from the analysis and the averages across runs were used. Whilst a significant main effect of condition was found ($F_{1, 57} = 74.99$, $p < 0.001$), with participants displaying increased consistency in the win compared to the loss condition across all treatment groups, again no treatment group effects were found (main effect, $F_{2, 57} = 0.68$, $p = 0.51$; interaction, $F_{2, 57} = 1.29$, $p = 0.28$).

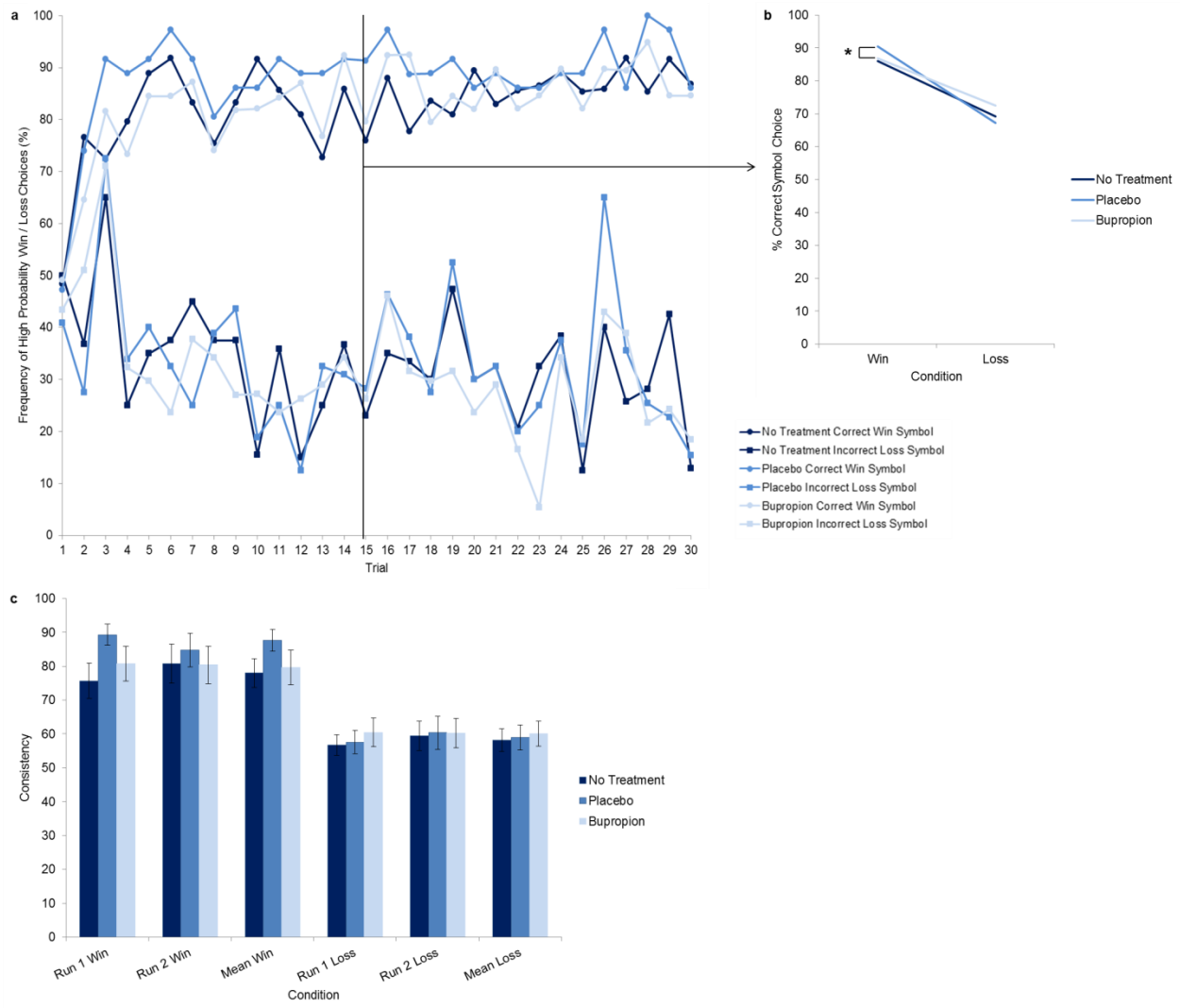


Figure 2.5 – Reward Task Learning: (a) learning curves for each treatment group depicting trial-by-trial the proportion of participants that chose the correct symbol in the win condition, associated with high-probability win (top lines) and the incorrect symbol in the loss condition, associated with high-probability loss (bottom lines). Learning curves were very similar for run 1 and run 2 of the reward task; therefore, only the average across the two runs are displayed here. **(b)** Average of the last 50% of trials in which participants chose the correct symbol for both the win and loss conditions for each treatment group. **(c)** Average consistency for the win and loss conditions for each treatment group for run 1, run 2 and averaged across the two runs of the reward task. Values are reported means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$).

Figure 2.6 shows the average reaction time for the win and loss conditions of each run as well as averaged across the two runs for each treatment group. Again, a repeated measures ANOVA with run as a within subjects factor was first performed. A significant main effect of run was found this time ($F_{1, 57} = 9.20$, $p < 0.05$), with participants responding quicker in the second run across all treatment groups, likely as a result of practice effects; therefore run was kept in the analysis. A significant main effect of condition was also found ($F_{1, 57} = 41.87$, $p < 0.001$) with participants responding quicker in the win condition compared to the loss condition across all treatment groups. From Figure 2.6, it appears that the placebo group responded even quicker in the win condition compared to the other treatment groups. However, no main effect of treatment group ($F_{2, 57} = 1.28$, $p = 0.29$) or condition by treatment group interaction ($F_{2, 57} = 2.40$, $p = 1.00$) was found. Similar results were found if the average reaction time across the two runs was used.

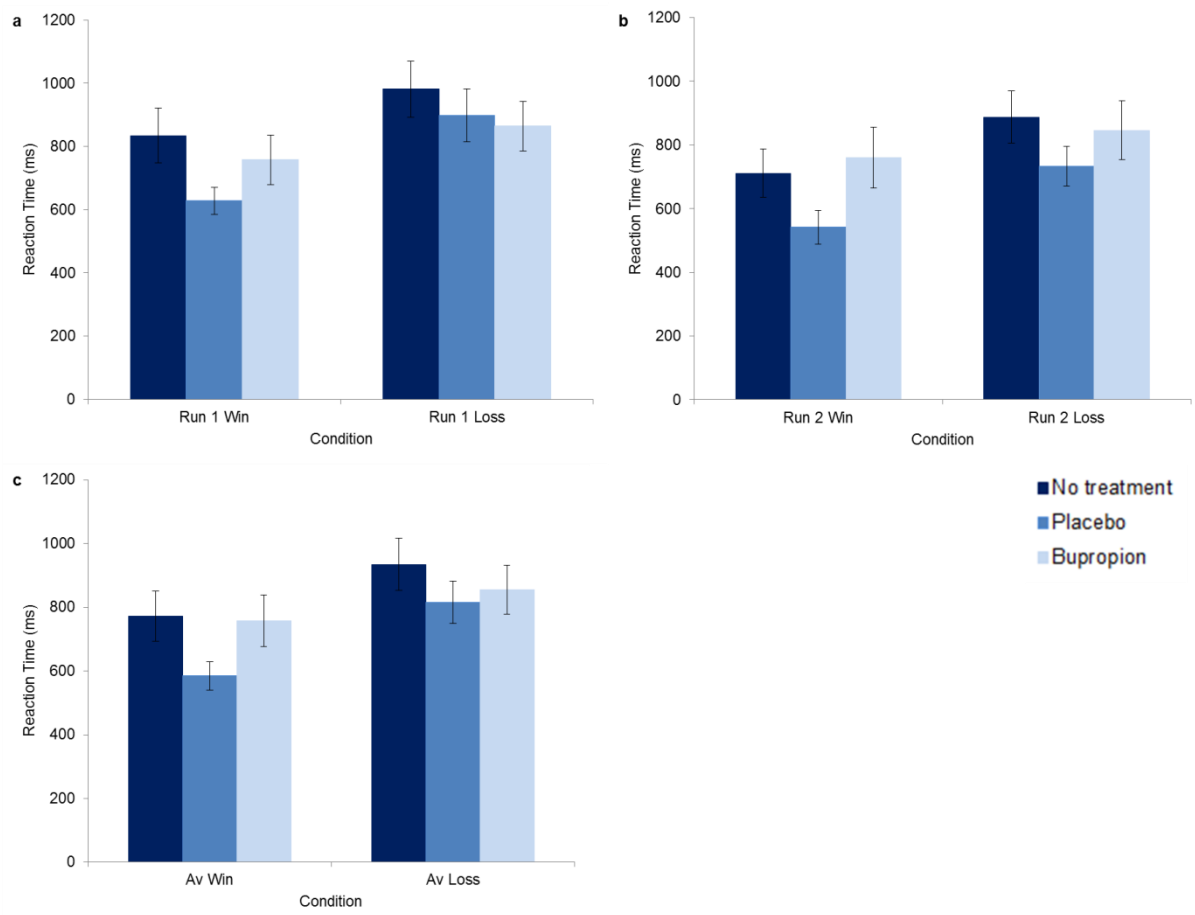


Figure 2.6 - Reward Task Reaction Times: the average reaction time for the win and loss conditions for each treatment group for **(a)** run 1, **(b)** run 2 and **(c)** averaged across the two runs of the reward task. Values are reported means ± standard error of the mean.

2.3.5 Acute Effects of Bupropion on Emotional Processing

2.3.5.1 FERT

The FERT requires participants to indicate whether an emotional facial expression is one of anger, disgust, fear, happy, sad, surprise or neutral (neutral was not included in the following statistical analyses as due to response bias the accuracy for neutral was near 100% for all treatment groups). A repeated measures ANOVA found a significant main effect of emotion ($F_{5, 285} = 47.55$, $p < 0.001$) for percentage accuracy, with participants most accurate at recognising happy faces. No main effect of treatment group ($F_{2, 57} = 0.50$, $p = 0.61$) or face emotion by treatment group interaction ($F_{10, 285} = 1.00$, $p = 0.48$) was found (Figure 2.7a). However, there was a trend towards significance for a face emotion by treatment group interaction ($F_{4, 114} = 2.32$, $p = 0.06$) if face emotions were combined into just 3 categories: happy faces, faces resulting from external environmental stimuli (anger, disgust, fear) and negative (sad) faces (Figure 2.6b). Indeed, ANOVAs considering each face emotion separately revealed a significant difference in percentage accuracy for recognising happy faces between treatment groups ($F_{2, 59} = 3.57$, $p < 0.05$). A post hoc independent samples t-test found the bupropion group to be significantly more accurate at recognising happy faces compared to the placebo group ($t_{38} = -2.33$, $p < 0.05$), but not compared to the no treatment group ($t_{38} = -0.31$, $p = 0.76$). A similar difference between bupropion and placebo groups was found for the d' for happy faces ($t_{38} = -2.18$, $p < 0.05$). When considering the different intensity levels of happy faces, a significant intensity by treatment group interaction was found ($F_{18, 513} = 2.21$, $p < 0.05$), with the bupropion group displaying higher recognition of happy faces at lower intensities than both the no treatment and placebo groups; however, post hoc independent samples t-tests again found this to be significant only when comparing against placebo (30% $t_{38} = -2.45$, $p < 0.05$; 40% $t_{38} = -2.73$, $p < 0.01$) (Figure 2.7c).

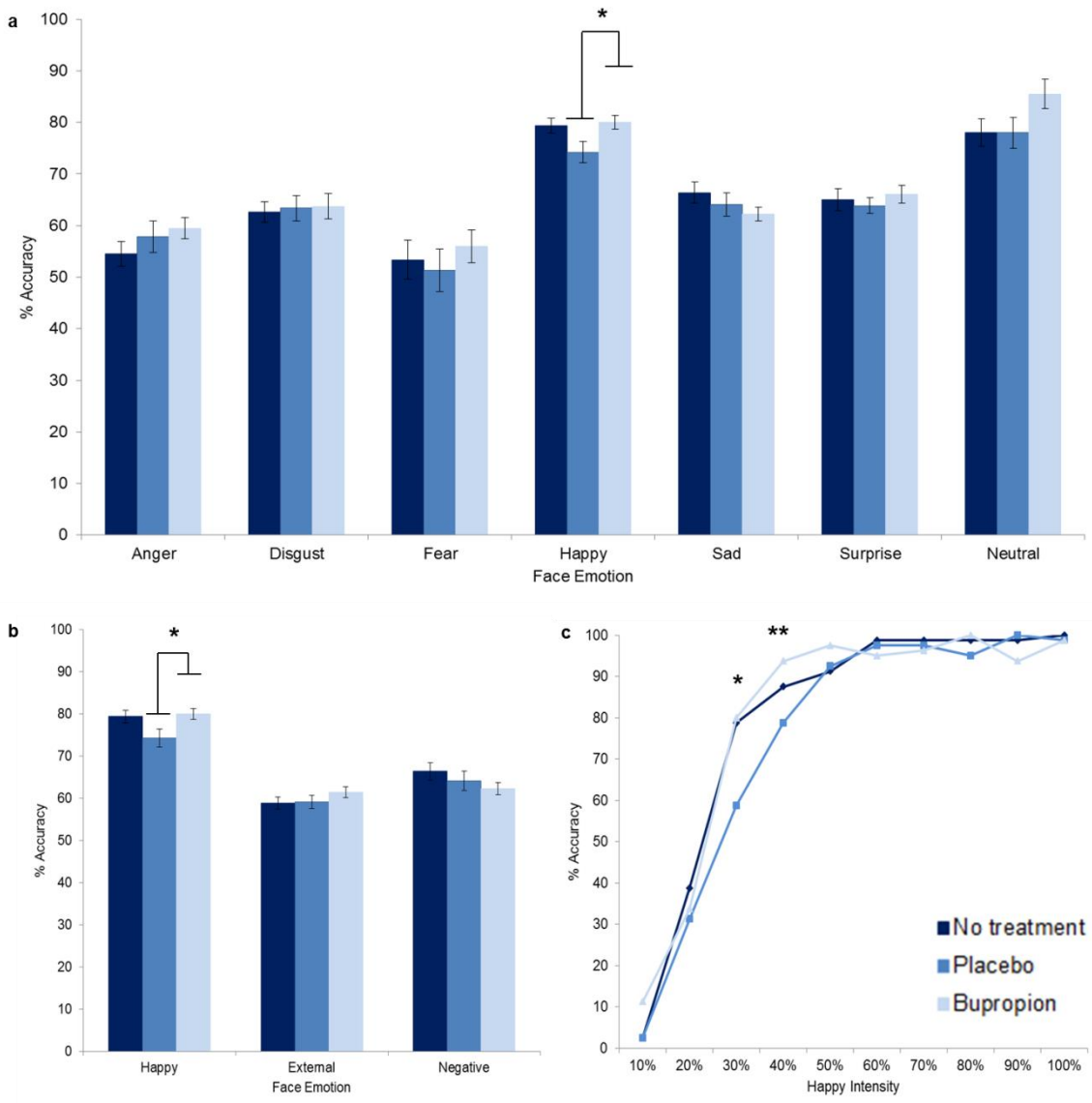


Figure 2.7 - FERT % Accuracy: (a) % accuracy for each face emotion for each treatment group. **(b)** % accuracy combined into happy faces, faces resulting from external environmental stimuli and negative faces for each treatment group. **(c)** % accuracy for each intensity of happy faces. Values are reported means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$, **$p < 0.01$).

The percentage misclassification of a particular face emotion as one of the other 6 face emotions was also calculated. A repeated measures ANOVA found a significant main effect of face emotion ($F_{5, 285} = 12.30$, $p < 0.001$) but no main effect of treatment group ($F_{2, 57} = 1.51$, $p = 0.23$) or face emotion by treatment group interaction ($F_{10, 285} = 1.54$, $p = 0.12$). However, visual inspection of Figure 2.8a does suggest some differences in percentage misclassifications between treatment groups, particularly for the percentage misclassification of other faces as sad. Indeed, an ANOVA considering just the percentage misclassification of other faces as sad found a main effect of treatment group ($F_{2, 57} = 4.64$, $p < 0.05$), with the bupropion group misclassifying significantly fewer faces as sad compared to both the no treatment ($t_{38} = 3.44$, $p < 0.01$) and placebo ($t_{38} = 2.36$, $p < 0.05$) groups. A significant face emotion by treatment group interaction was found for beta ($F_{10, 280} = 2.15$, $p < 0.05$) and results from planned post hoc comparisons reflected those found above. The bupropion group had a significantly higher beta value for sad faces compared to the no treatment ($t_{38} = -3.42$, $p < 0.01$) and placebo ($t_{38} = 2.32$, $p < 0.05$) groups, indicating a response bias away from sad faces (Figure 2.8b). Regarding reaction time, a significant main effect of face emotion was found ($F_{5, 280} = 49.12$, $p < 0.001$), with participants responding the quickest to fearful faces across all treatment groups (Figure 2.8c).

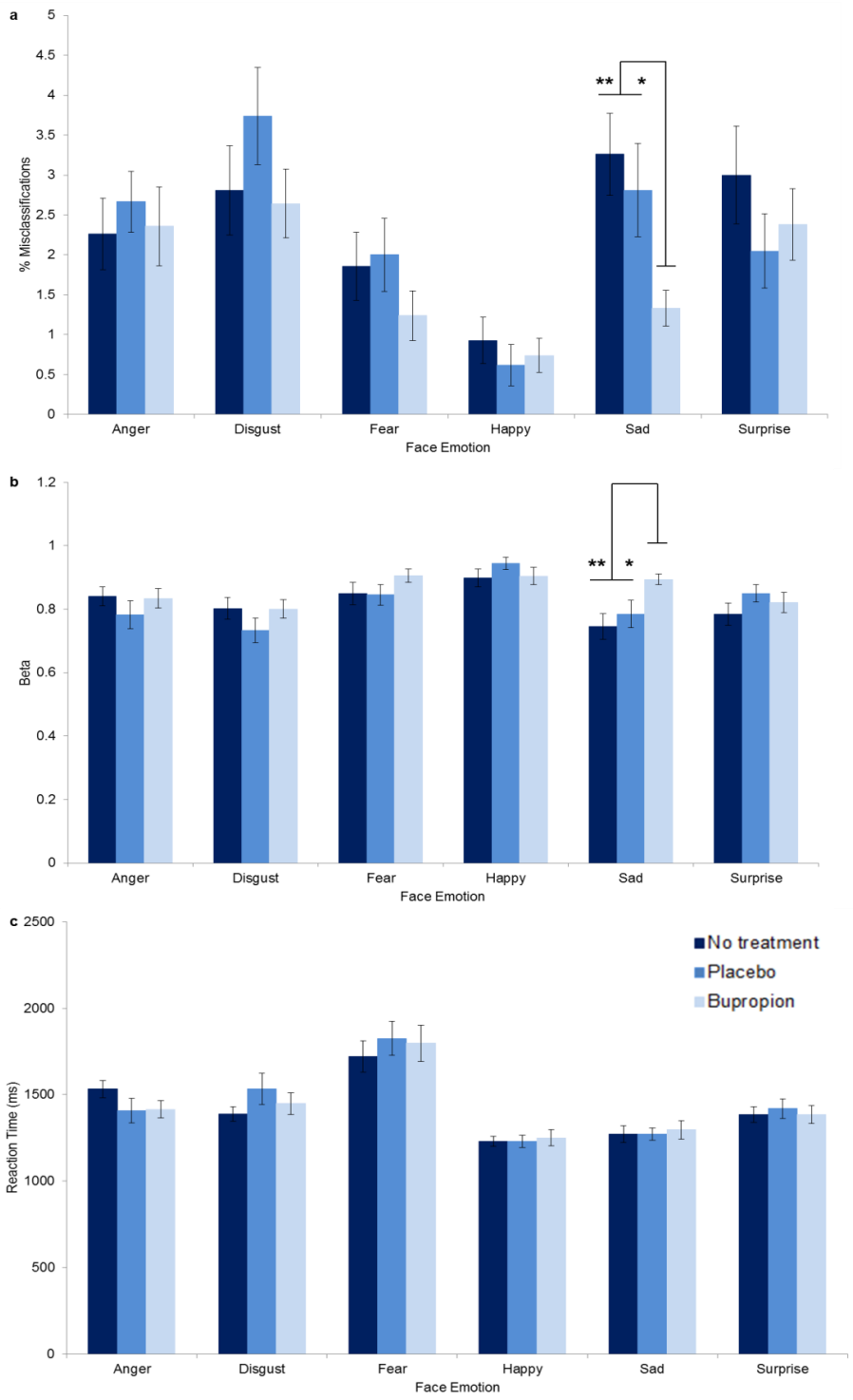


Figure 2.8 - FERT % Misclassifications, Beta / Response Bias and Reaction Time: (a) % misclassifications, **(b)** beta and **(c)** average reaction time for correct responses for each face emotion for each treatment group. Values are reported as means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*p < 0.05, **p < 0.01).

2.3.5.2 ECAT

The ECAT requires participants to indicate whether they would like or dislike to be described as certain negative and positive self-referent words. From Figure 2.9a and b, it seemed that the placebo group was less accurate for negative self-referent words and overall slower to respond compared to the no treatment and bupropion groups; however, this was not found to be significant (% accuracy interaction, $F_{2, 57} = 1.35$, $p = 0.27$; reaction time main effect of treatment group, $F_{2, 57} = 1.94$, $p = 0.15$).

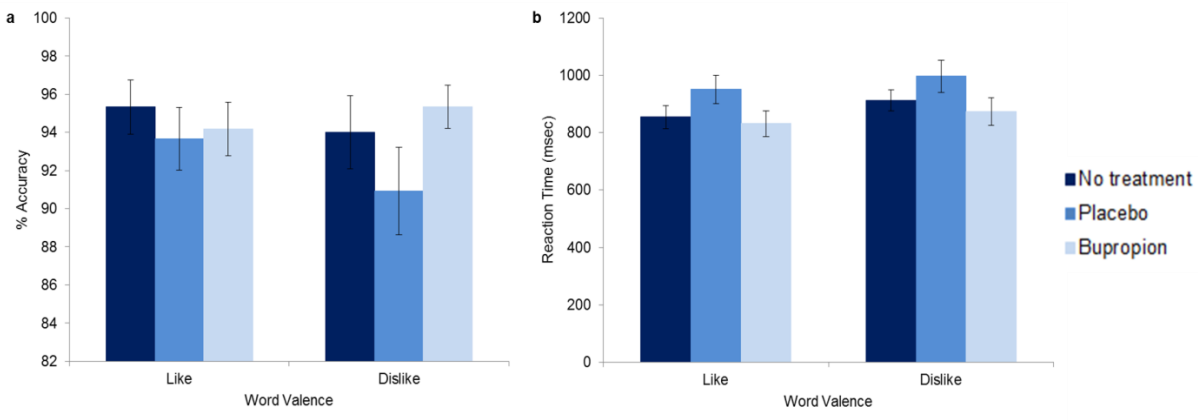


Figure 2.9 - ECAT % Accuracy and Reaction Time: (a) % accuracy and **(b)** average reaction time for correct responses for each word valence for each treatment group. Values are reported means ± standard error of the mean.

2.3.5.3 FDOT

The FDOT requires participants to respond to the alignment of a dot-probe appearing in the place of either a neutral, positively or negatively valenced face. Median reaction time to respond to the dot-probe was used to calculate attentional vigilance towards the positively or negatively valenced faces. Figure 2.10a suggests a potential reduction in reaction times in the placebo group compared to both the no treatment and bupropion groups that was non-valence specific but task-specific since this was not observed for any of the other ETB tasks. However, a repeated measures ANOVA failed to find a significant main effect of treatment group ($F_{2, 56} = 0.65$, $p = 0.53$), most likely as a result of the high intra-group variability

indicated by the large standard error bars. There was also no face emotion by probe duration by treatment group interaction ($F_{2, 56} = 0.54$, $p = 0.58$).

Large, overlapping error bars were also observed for attentional vigilance making discrimination between noise and actual treatment group differences difficult; although, it appears that the bupropion group displayed increased vigilance towards unmasked happy faces compared to the no treatment and placebo groups (Figure 2.10b and c). However, a 2x2x3 repeated measures ANOVA (emotion x probe duration x treatment group, $F_{2, 56} = 1.40$, $p = 0.25$), a repeated measures ANOVA with just one within subjects factor (face emotion: fear unmasked, happy unmasked; face emotion x treatment group, $F_{2, 56} = 1.50$, $p = 0.24$) and an independent samples t-test between the placebo and bupropion groups ($t_{37} = -1.16$, $p = 0.25$) all failed to find significance.

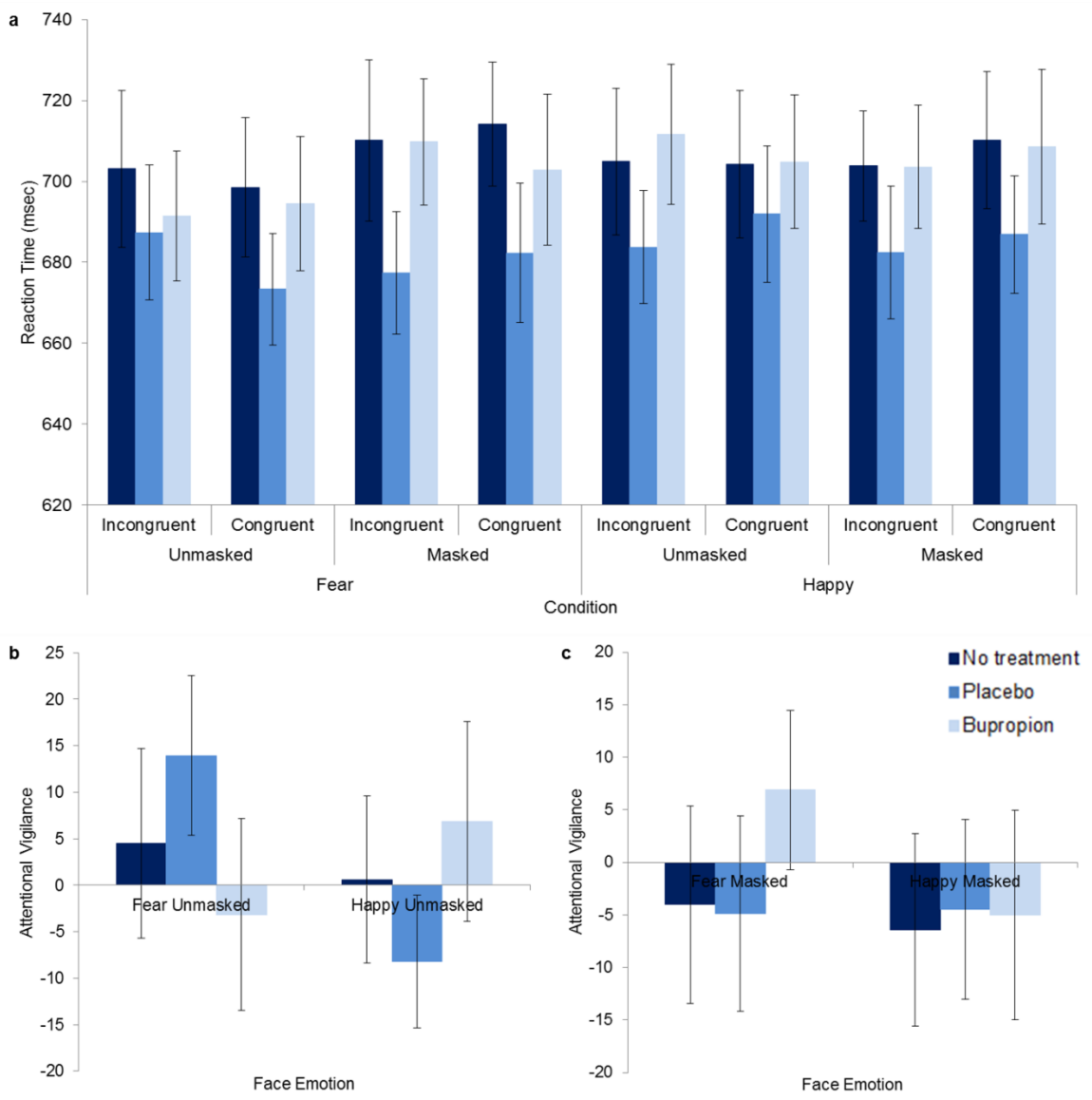


Figure 2.10 - FDOT Reaction Time and Attentional Vigilance: (a) average reaction time for correct responses for each condition of the FDOT for each treatment group. Attentional vigilance for each treatment group for each face emotion, which was either **(b)** unmasked or **(c)** masked. At the participant level, medians were used then values displayed here are means ± standard error of the mean.

2.3.5.4 EREC

The EREC requires participants to write down as many positive and negative self-referent words from the ECAT that they could remember. Figure 2.11 shows the average number of self-referent words correctly and falsely recalled. It is clear that more positive self-referent words were recalled across all treatment groups with a significant main effect of word valence for both correctly ($F_{1, 57} = 9.75$, $p < 0.01$) and falsely recalled ($F_{1, 57} = 16.06$, $p < 0.001$) self-referent words. For correctly recalled self-referent words, it appears that the bupropion group remembered more self-referent words overall, with no word valence specificity; however, no main effect of treatment group was found ($F_{2, 57} = 1.17$, $p = 0.32$).

With regards to commission errors, it appears that the bupropion group falsely recalled fewer negative and more positive self-referent words compared to the no treatment and placebo groups; however, this was also not found to be significant (word valence x treatment group, $F_{2, 57} = 0.34$, $p = 0.71$).

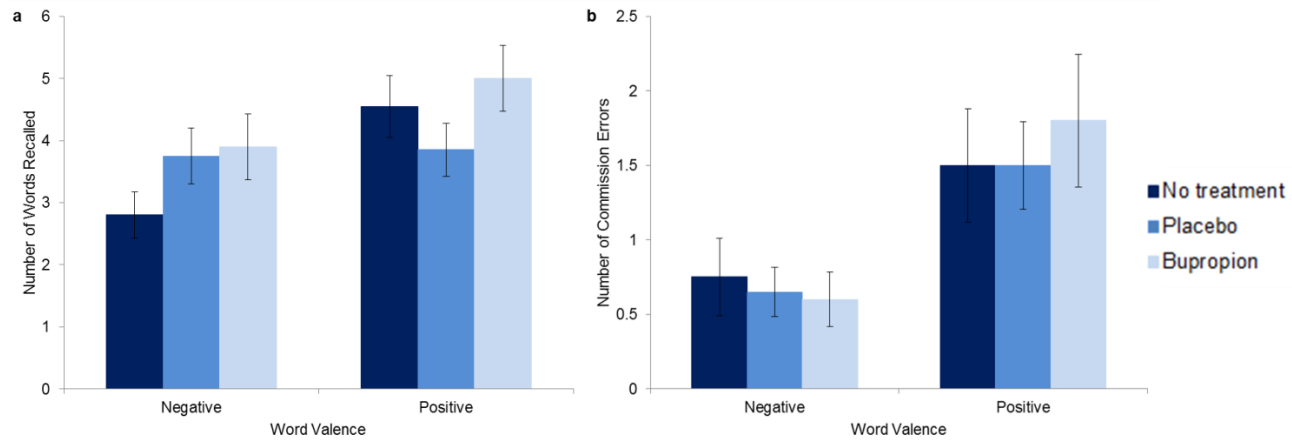


Figure 2.11 - EREC Self-Referent Words Correctly and Falsely Recalled: number of negative and positive **(a)** self-referent words correctly recalled and **(b)** commission errors made. Values are reported as means ± standard error of the mean.

2.3.5.5 EMEM

Memory for positive and negative self-referent words was also tested in the EMEM, during which participants were required to indicate whether a self-referent word was familiar to them from the ECAT or novel. As can clearly be seen in Figure 2.12, there was no main effect of word valence on percentage accuracy ($F_{1, 57} = 0.67$, $p = 0.42$) or reaction time ($F_{1, 57} = 1.51$, $p = 0.22$). There was also no word valence by treatment group interaction with regards to percentage accuracy ($F_{2, 57} = 0.21$, $p = 0.81$), d' ($F_{2, 57} = 0.02$, $p = 0.98$) or reaction time ($F_{2, 57} = 0.54$, $p = 0.59$). However, when considering percentage misclassifications, a repeated measures ANOVA did find a significant word valence by type of misclassification by treatment group interaction ($F_{2, 57} = 8.66$, $p < 0.01$). From Figure 2.13a, this was most likely the result of the bupropion group reporting more positive and fewer negative self-referent words as familiar or fewer positive and more negative self-referent words as novel; although this failed to reach significance in post hoc independent samples t-tests comparing against placebo (% misclassification of familiar positive self-referent words as novel, $t_{38} = 1.83$, $p = 0.08$; % misclassification of novel negative self-referent words as familiar, $t_{38} = 1.57$, $p = 0.13$).

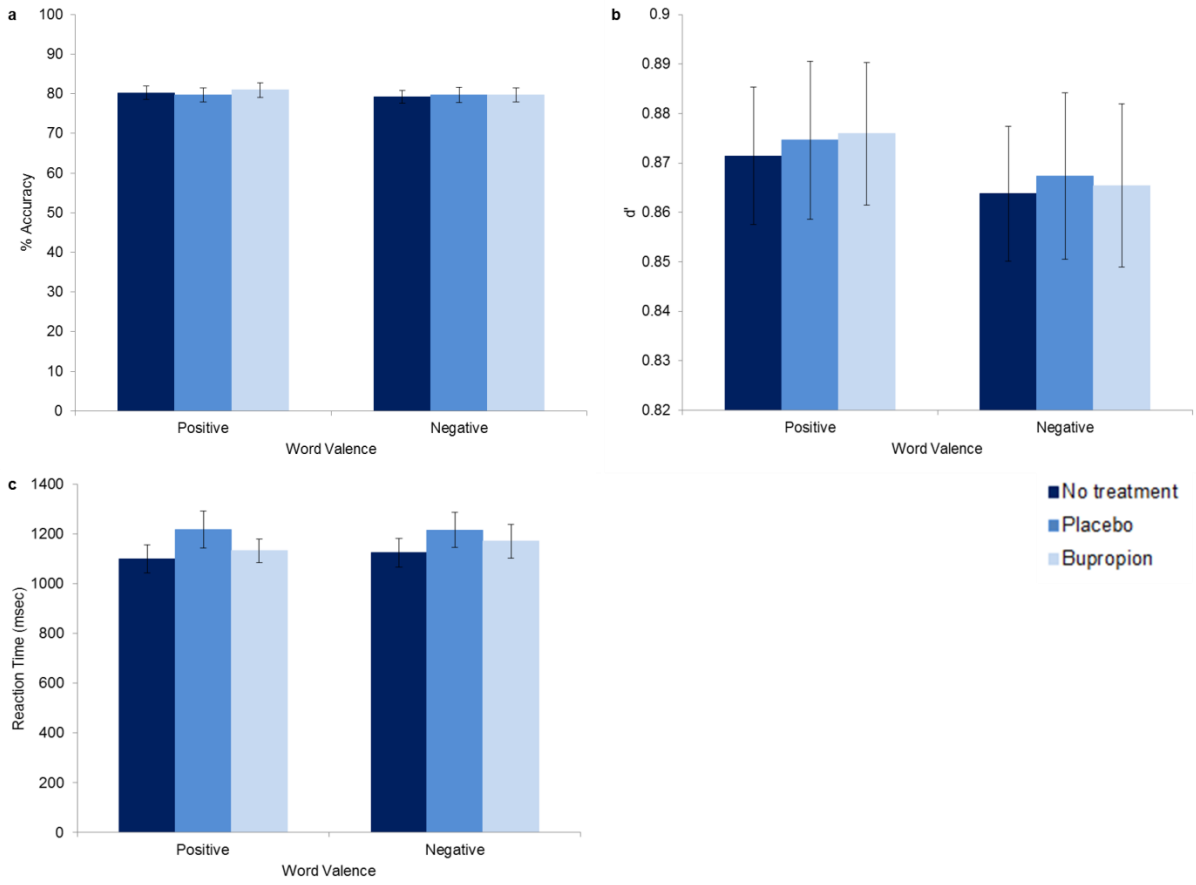


Figure 2.12 - EMEM % Accuracy, d' and Reaction Time: (a) % accuracy, **(b)** d' and **(c)** average reaction time for correct responses for each word valence for each treatment group. Values are reported means ± standard error of the mean.

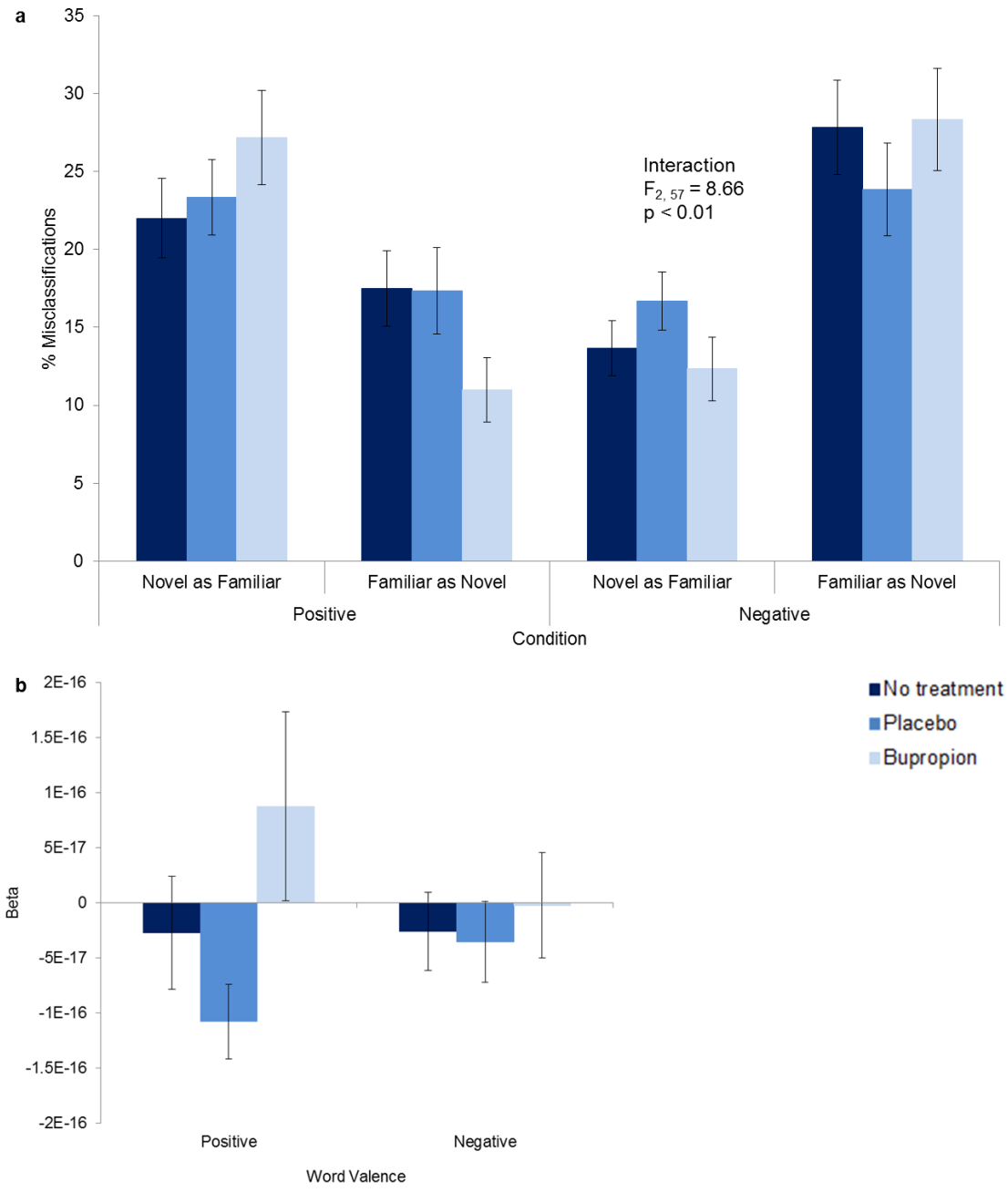


Figure 2.13 - EMEM % Misclassifications and Beta / Response Bias: (a) % misclassifications for each word valence for each treatment group, divided into whether the participant misclassified a novel self-referent word as familiar and vice versa. **(b)** Beta values for each word valence for each treatment group. Values are reported means ± standard error of the mean.

2.4 Summary

The current Chapter aimed to define the effects of an acute dose of bupropion compared to placebo on emotional and reward processing in healthy volunteers. Specifically, we sought to investigate whether bupropion had similar effects on emotional processing to SSRIs, acting to reduce negative affective biases, or had more specific effects on processing of positive stimuli and reward. The primary hypothesis was that, with its DA and NA enhancing effects, an acute dose of bupropion would improve performance in a reward task and increase positive emotional processing. A secondary aim was to explore whether the ETB tasks were influenced by the placebo effect or whether performance was similar between the no treatment and placebo groups.

With regards to emotional processing, it was found that an acute dose of bupropion did produce the expected increase in positive emotional processing, with a significant increase in the recognition of happy faces at lower happy intensities and familiarity of positive compared to negative self-referent words compared to placebo. However, an acute dose of bupropion was also found to decrease negative emotional processing, with a significant decrease in the misclassification of other faces as sad or response bias towards sad faces. These effects were not associated with a change in mood. Those ETB tasks that failed to show significance, still showed the hypothesised directions towards increased positive and decreased negative emotional processing with an acute dose of bupropion, such as increased vigilance for unmasked happy faces, increased number of positive self-referent words recalled and decreased number of negative self-referent words falsely recalled. These early effects of bupropion on emotional processing are similar to those produced by SSRIs and SNRIs and may be instrumental in its therapeutic effect as an antidepressant.

This particular study design and sample size did not detect any significant effect of an acute dose of bupropion on reward processing. Nor were there any significant differences between the no treatment and placebo groups for any behavioural outcome measure.

2.5 Discussion

2.5.1 Acute Effects of Bupropion on Emotional Processing

An acute dose of bupropion was found to increase positive emotional processing, with a significant increase in the recognition of happy faces and familiarity of positive self-referent words and decreased negative emotional processing, with a significant decrease in the misclassification of other faces as sad and response bias towards sad faces, compared to placebo, with no effect on mood. This provides evidence that a DA antidepressant also has early effects on emotional processing prior to a change in mood further supporting the neuropsychological theory of antidepressant action.

When comparing these acute effects of bupropion with the effects of other antidepressants on tasks assessing emotional processing, it seems that bupropion has a similar profile of perceptual and memory effects to the SNRI reboxetine. With reference back to Table 1.3 in Chapter 1, it can be seen that reboxetine increases the recognition of happy faces in the facial expression recognition task and alters the balance for memory of self-referent words, causing an increase in recall of positive words or decrease in the recall of negative words (Harmer et al., 2003b, Harmer et al., 2004, Harmer et al., 2009b). It is interesting to note that whilst reboxetine acts primarily as an SNRI, some have reported that it also increases DA activity in the frontal cortex (Linner et al., 2001, Page and Lucki, 2002). Likewise, although DA reuptake inhibition is the mechanism of action most commonly attributed to bupropion, the exact neuropharmacological actions of bupropion remain elusive, due to different actions in vitro versus in vivo (Horst and Preskorn, 1998). In vitro, bupropion is more potent at inhibiting DA rather than NA reuptake (IC_{50} of 2.0 and 5.0, respectively) (Horst and Preskorn, 1998) but the inhibition of DA reuptake itself is not particularly robust and wasn't thought to have pharmacological relevance (Ferris et al., 1983). In contrast, in vivo, an acute dose of bupropion has been found to affect the firing rate of NA neurons in the locus coeruleus of the rat at doses more similar to those required for antidepressant-like

activity in animal models (Cooper et al., 1994, Ascher et al., 1995). Therefore, the emotional processing and antidepressant effects of bupropion may be mediated by the DA and/or NA enhancing effect of bupropion and further research is required to unravel the roles of the two neurotransmitters in emotional processing.

2.5.2 Acute Effects of Bupropion on Reward Processing

Since the mesocorticolimbic DA pathway plays a crucial role in reward, it was hypothesised that an acute dose of bupropion acting to increase DA activity would alter reward processing and improve performance on a probabilistic instrumental reward task. Indeed, it has been found that participants treated with L-DOPA to enhance DA activity were more likely to choose a high-probability win, subsequently winning more money than participants treated with the DA receptor antagonist, haloperidol, although not placebo (Pessiglione et al., 2006). However, this study did not detect any significant effect of an acute dose of bupropion on reward processing during a probabilistic instrumental reward task and there could be a number of reasons for this.

Firstly, the sample size could have been too small to detect any significant effects of bupropion on reward processing. A reverse power calculation using the effect sizes observed in preceding studies of other antidepressants indicated a sample size of 20 participants per group would be sufficient to detect a significant difference between treatment groups with a power of 0.95. However, whilst there was a shortened, 10 trial familiarization version of the task, quite a few participants had very low overall totals and were assumed not to understand the task. As a result, 10 participants were excluded from the analysis (3/4 per treatment group) of the reward task and this likely had an impact on power. There is also the possibility that our adapted version of the probabilistic instrumental reward task isn't as sensitive to drug effects on reward processing.

Secondly, if the acute effects of bupropion are mediated primarily by enhancing NA activity as discussed above, then any alterations in reward processing wouldn't be expected.

In addition, as also discussed above, there is evidence that inhibition of DA reuptake may require higher doses than inhibition of NA reuptake. This reveals a potential problem with the dose of bupropion used in this study. For an antidepressant effect, the typical dose of SR bupropion is 150 mg twice a day 8 hours apart, totaling 300 mg per day. Since bupropion produces a dose-dependent lowering of the seizure threshold, for ethical reasons, the participants could only be provided with a single 150 mg dose during the 5 hour testing session. As a result the maximal inhibition of DA reuptake may not have been achieved in this study. Indeed, no group differences were found in the levels of salivary cortisol.

Finally, assuming bupropion was acting primarily though inhibition of DA reuptake, since the participants in this study were healthy controls, they may have had a roof level of reward processing that could not be further improved by an acute dose of bupropion. Indeed, in the (Pessiglione et al., 2006) study, the significant difference in performance on the probabilistic instrumental learning task was found when comparing between the L-DOPA and haloperidol groups but not between the L-DOPA and placebo groups. As a result, it may be possible that the L-DOPA was simply acting to restore the haloperidol-induced decrement in performance and cannot further improve performance in healthy controls where DA levels are normal. Therefore, there may still be effects of bupropion on performance on the probabilistic instrumental learning task in groups with DA hypoactivity and aberrant reward processing, such as MDD patients, discussed in the following Chapters.

2.5.3 Acute Effects of Placebo on Emotional and Reward Processing

Understanding the placebo effect is important, particularly in MDD, since antidepressant trials often fail to demonstrate superiority over placebo, with the placebo effect reported to account for up to 68 – 80 % of the benefit on an antidepressant (Kirsch, 2014). Furthermore, there is also evidence that the placebo effect can influence emotional processing. For example, treatment with a placebo anxiolytic can reduce the unpleasantness of distressing pictures in healthy volunteers (Petrovic et al., 2005). Therefore, with the

inclusion of a no treatment group, a secondary aim of this study was to explore whether the ETB tasks were influenced by the placebo effect or whether performance was similar between the no treatment and placebo groups.

The tasks assessing emotional processing in the ETB have been designed such that they measure negative affective biases that the participant is unaware of, which may decrease or eliminate the placebo effect observed with traditional scales used to measure symptom improvement. At least in this study, there were no significant differences between the no treatment and placebo groups for any behavioural outcome measure, providing preliminary evidence that the ETB could indeed be used to detect antidepressant effects on emotional processing without influence of the placebo effect and therefore be useful in the early stages of drug development of novel antidepressant agents. Given the focus of this thesis, further analysis was not conducted; however, it is important to note that further analysis is required to determine whether this result was true or just underpowered.

CHAPTERS 3 – 7

THE EFFECTS OF BUPROPION ON NEURAL AND BEHAVIOURAL MEASURES OF REWARD AND EMOTIONAL PROCESSING IN DEPRESSED PATIENTS

CHAPTER 3

INTRODUCTION, STUDY AIMS, DESIGN AND METHODS

3.1 Introduction

In the previous Chapter, the acute effects of the DA antidepressant bupropion on behavioural measures of reward and emotional processing in healthy controls were defined. There was an indication that bupropion does have early effects on emotional processing, similar to studies of other antidepressants. However, it remains unclear how these early effects on emotional processing relate to effects on the aberrant reward processing specifically in MDD patients and whether they are predictive of clinical outcome. Therefore, the remaining Chapters describe a large, repeated measures, open-label, experimental medicine study in which a cohort of MDD patients received bupropion and their reward and emotional processing was assessed at baseline, 2 weeks and 6 weeks via behavioural and fMRI methods. The current Chapter presents the methodology of the study and Chapters 4 – 7 present the results of the study.

3.2 Study Aims and Hypotheses

This study had three main aims, which were to investigate whether:

1. Dissociable baseline abnormalities in behavioural and neural measures of reward and emotional processing relate to baseline subjective experience of anhedonia and negative affect in MDD. It was expected that MDD patients would display abnormalities in behavioural and neural measures of reward and emotional processing compared to healthy controls, such as reduced response bias for reward with striatal hypoactivity to reward and negative affective biases with amygdala hyperactivity to negative stimuli, and such baseline measures would correlate with the baseline scores on questionnaires measuring anhedonia and negative affect.

2. Manipulating dopaminergic activity with bupropion affects behavioural and neural measures of reward and emotional processing in MDD. It was hypothesised that bupropion would act to remediate the above abnormalities in behavioural performance and neural response during tasks assessing reward and emotional processing by 2 weeks. Given the results of the acute study, it was also more specifically hypothesised that both an increase in positive emotional processing and a decrease in negative emotional processing would be observed.

3. These behavioural and neural effects predict later change in subjective experiences of anhedonia and negative affect in MDD. It was expected that the initial change in neural and/or behavioural effects at 2 weeks would correlate with the change in scores on questionnaires measuring anhedonia and negative affect over 6 weeks.

3.3 Study Design

The study was an experimental medicine study with a repeated measures, open-label design. It involved administration of open-label SR bupropion to MDD participants over a six week period (Section 3.2.2) with a screening visit, clinical review visit and 3 test visits. MDD participants completed a reward task (Section 3.2.4.1) and covert faces task (Section 3.2.4.2) in the fMRI scanner, as well as the ETB (Section 3.2.5) at week 0 (test visit 1 / baseline) and week 2 (test visit 2) of bupropion treatment. At six weeks (test visit 3), MDD participants completed all the same tasks but did not undergo an fMRI scan. Each study visit also involved completion of a number researcher-administered and self-report questionnaires measuring severity of depression and symptoms of anhedonia and negative affect (Section 3.2.3). Ideally the study would have employed a double-blind design involving a separate control group of MDD participants receiving placebo for six-weeks; however, ethical concerns over the length of time patients would be treated with a placebo instead of a true medication prevented this. Instead, a separate group of healthy control (HC) participants were recruited and did not receive bupropion, placebo or any other intervention to allow investigation of baseline differences between MDD and HC groups and to control for the practice effects of repeated testing.

An important aspect of the study design was that all the MDD patients received the same bupropion treatment, which introduces a confound in the study: all participants who received bupropion were depressed, whereas all participants who received no drug treatment were not depressed. This makes it challenging to determine whether group by visit interactions result from drug effects or from differences between the depressed and healthy groups. Correlations between drug effects and clinical outcome were also performed, which are not influenced by this confound. In addition, the supplementary study described in Chapter 2 that did include a placebo group found no influence of the placebo effect on the behavioural measures of emotional and reward processing to be used in this study. Figure 3.1 displays a summary of the study design and timeline.

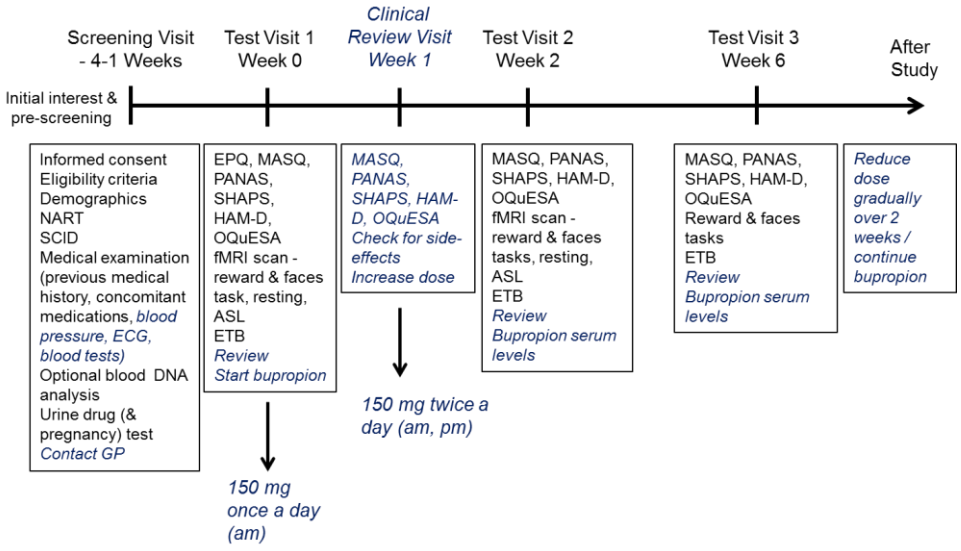


Figure 3.1 – Summary of Study Design and Timeline: HCs complete the same questionnaires and tasks as MDDs but do not receive bupropion. The additional procedures for the MDDs are shown in blue italics.

3.4 Methods

This study was approved by the University of Oxford Clinical Trials and Research Governance Team (CTRG) and a NHS Research Ethics Committee (NRES Committee South Central – Berkshire B) (13/SC/0569). It was carried out in accordance with the protocol and provisions of the World Medical Association Declaration of Helsinki. All participants provided written informed consent.

3.4.1 Participant Recruitment and Screening

The study required 40 MDD and 40 HC participants. During the course of the study 2 MDD participants could not undergo fMRI scanning (due to contraindications to fMRI scanning: tattoos and a copper coil) and a further 3 MDD and 2 HC participants withdrew from the study before the week 2 test visit. These participants were replaced and a total of 46 MDD and 42 HC participants were recruited and tested with complete data sets obtained for 41 MDD and 40 HC participants (Figure 3.2).

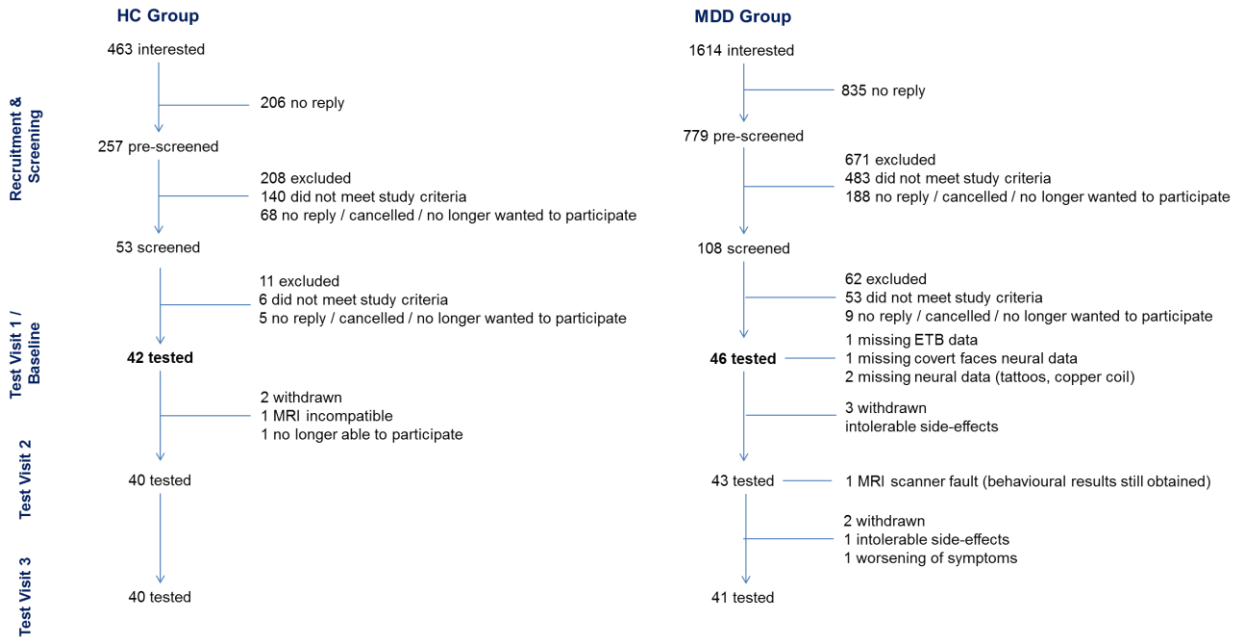


Figure 3.2 – Consort Diagram: displays the number of participants recruited, screened and tested at each study visit for each participant group.

All participants were recruited from the general population via a number of methods including targeting university colleges and departments (email newsletters and posters), radio advertisements (JACKfm, Oxford, UK), posters on the London Overground (ExterionMedia, London, UK) and advertisement on the internet (Dailyinfo, Gumtree and TrialSpark).

All participants first attended a screening visit, which involved the SCID for DSM-IV, the NART to provide an estimate of verbal IQ, collection of information on date of birth, BMI, medical history and any concomitant medications and finally a urine drug and pregnancy test. MDD participants also underwent a more in depth psychiatric assessment of their history of MDD and a physical, including pulse rate, blood pressure, an ECG and a blood sample (liver function tests, urea and electrolytes) by the study psychiatrist. Participants were both male and female and were required to be between the ages of 18 and 50 years, have a BMI of 18 to 36 kg/m^2 and be sufficiently fluent in English to complete the behavioural tasks. MDD participants had to satisfy a diagnosis of a current episode of MDD as determined by the SCID but were excluded if they had current or past history of any other

Axis 1 DSM-V psychiatric disorder, apart from co-morbid anxiety disorders. HC participants were excluded if they had current or past history of any Axis 1 DSM-IV psychiatric disorder. Other exclusion criteria for all participants included recent use of any psychotropic medication or drug within 3 weeks of test visit 1 or psychological treatment within 3 months of test visit 1, presence of any medical condition, allergy or concomitant medications that could impact upon the safety of the participant or scientific assessment, any contraindication to MRI scanning, heavy smoking (greater than 10 cigarettes a day) and finally participation in other studies involving the use of medication within the last 3 months or previous experience of any of the behavioural tasks. Suitable participants meeting all study criteria returned at least 1 but not more than 4 weeks later to complete the first test visit, allowing time to review results of the screening procedures above and contact the GPs of MDD participants.

3.4.2 Study Intervention / Medication

The study intervention was a 6 week treatment with 300 mg (150 mg twice daily) open-label SR bupropion given to the MDD group only. The MDD participants started the course of bupropion the morning after test visit 1 (week 0) and initially took 150 mg once daily in the morning for the first 7 – 10 days. MDD participants then attended a clinical review visit in week 1, after which the dose of bupropion was increased to the full 300 mg for the remaining 5 weeks, with one 150 mg tablet in the morning and another 150 mg tablet at least 8 hours after. If intolerable side-effects developed, the participant was offered the choice to either reduce the dose back down to 150 mg or withdraw from the study. All 4 of the participants who developed intolerable side-effects decided to withdraw from the study. At the end of the 6 week treatment, MDD participants were offered the choice to either discontinue the bupropion, in which case the dose was gradually reduced over the course of 2 weeks, or continue the bupropion under the supervision of the study psychiatrist or their own GP.

3.4.3 Questionnaire Measures

The questionnaires used in this study included some of those described for the acute study (Section 2.2.2). These were the HAM-D, EPQ, Full-MASQ, PANAS and SHAPS. All participants in this study also completed The Oxford Questionnaire on the Emotional Side-effects of Antidepressants (OQuESA) (Price et al., 2012), which consists of 3 sections designed to measure the symptoms of emotional blunting in MDD patients being treated with antidepressants. All questionnaires were completed at every study visit, apart from the EPQ, which was only completed at test visit 1.

3.4.4 ETB

The ETB has been described in full in Section 2.2.4, the only difference here being exclusion of the EMEM task due to insufficient validated self-referent words to cover the 3 test visits. In brief, participants completed the FERT, ECAT, FDOT and EREC at each test visit. All task stimuli were different for each test visit.

3.4.5 fMRI Tasks

All participants underwent an fMRI scan at test visit 1 and 2. Two tasks were completed during these fMRI scans; the covert faces task and a reward task. The same tasks were completed at test visit 3 but outside of the fMRI scanner on a computer.

3.4.5.1 Covert Faces Task

The covert faces task had been previously developed using E-Prime (Psychology Software Tools, Inc., Pittsburgh, USA) (Godlewska et al., 2012). Task stimuli consisted of a series of happy, sad or fearful facial expressions (Japanese and Caucasian Facial Expressions of Emotion) (Matsumoto and Ekman, 1989). A block-design was used, with four blocks of each face emotion and each block consisting of 10 (5 male, 5 female) facial expressions. Each facial expression was presented for 100 ms followed by a central fixation

cross for 2900 ms, during which participants were required to indicate the gender of the face via an appropriate button press. Each block was interspersed with a further central fixation cross lasting for 30 sec. Participants completed a single run of the covert faces task in the fMRI scanner at test visits 1 and 2 and outside the fMRI scanner at test visit 3. The same facial expressions were used for each visit; however, participants were randomised to receive different orders of the blocks, such that the starting emotion was varied for each participant at each visit. Outcome measures were percentage accuracy for gender and average reaction time for correct responses for each emotion.

3.4.5.2 Reward Task

This was the same reward task used in the acute study and has been described in full in Section 2.2.3. In brief, task stimuli consisted of two pairs of symbols with one pair associated with win outcomes (win £1 or no change) and the other associated with loss outcomes (lose £1 or no change). Each symbol in the pair corresponded to reciprocal probabilities (0.7 or 0.3) of the associated outcomes occurring. Participants were required to choose between the two symbols in order to maximise payoffs. Once a choice was made, outcome feedback was provided. In order to maximise payoffs, participants should use the outcome feedback to gradually learn the symbol-outcome associations by trial and error over time, such that they consistently choose the symbol with the high-probability win and avoid the symbol with the high-probability loss.

At test visit 1 only, participants performed a shortened, 10 trial familiarization version of the reward task outside of the fMRI scanner. Participants then performed two, 60 trial runs inside the fMRI scanner at test visits 1 and 2 and outside the fMRI scanner at test visit 3, with each run comprising a different set of 4 symbols to be learnt. The timings of the task were the same whether the task was performed inside or outside of the scanner and were as follows: central fixation cross with a 2-6 (mean of 4) second jitter, stimulus presentation for 2 seconds, choice presentation with a further 2-6 (mean of 4) second jitter and outcome presentation for 2 seconds.

3.4.6 Statistical Analysis

3.4.6.1 Data Cleaning

Prior to statistical analysis, raw behavioural data was visually inspected for anomalous values through the use of scatter graphs. This revealed a number of participants that performed worse than chance totalling an average across the two runs of the reward task below the starting £5. A further issue was found when looking at these participants reward task data on a trial-by-trial analysis where it was observed that they had also chosen the incorrect symbol all the way through the task. These participants were assumed to have misunderstood the task and were excluded from the reward task analysis (Table 3.1).

Number of responses made, reaction times and mean accuracy were also assessed. A large number of no responses, slow reaction times even after trimming or low accuracy were suggestive of inappropriate completion of the task resulting in exclusion of that data from subsequent analyses. With the exception of the FDOT, reaction times were trimmed at the participant level: reaction times above 3 standard deviations from the mean or below 200 ms were excluded prior to calculating the average. For the FDOT, previous papers have calculated median reaction times at the participant level prior to calculating the mean to reduce the size of the standard error of the mean (Price et al., 2015); therefore, the same method was implemented in this study.

Neural data was also visually inspected for artefacts, including excessive movement, incorrect registration or MRI scanner related problems.

As a result of data cleaning and participant attrition, the number of participants included may differ between the different analyses, for example, a participant who completed test visit 1 but not subsequent test visits would be included in baseline analyses but not repeated measures analyses. The number of participants excluded and the specific reason for exclusion for each task analysis is summarised in Table 3.1.

Table 3.1 Summary of the Number of Participants Excluded for each Task Analysis and Reasons for Exclusion

Task	Analysis	Number Excluded			Reason for Exclusion
		Visit 1	Visit 2	Visit 3	
FERT	% Accuracy Reaction Time	MDD - 1	HC – 1	HC - 1	0% accuracy for fear so could not calculate average reaction time for correct responses
ECAT	% Accuracy Reaction Time	0	0	HC – 1	Below 50% accuracy / performed below chance level
EREC	Words recalled Commission errors	0	0	0	
FDOT	Attentional Vigilance Reaction Time	0	0	0	
Covert Faces	% Accuracy Reaction Time	0	HC – 1	0	Fell asleep in the MRI scanner – 89 out of 120 no responses
Reward	Totals Trial-by-trial	HC - 5 MDD - 6	HC - 8 MDD - 7	HC - 4 MDD - 5	Totalled an average across the two runs of the reward task below the starting £5 and/or consistently chose the incorrect symbol all the way through the task
Neuroimaging	Cardiac physiological data not included in the analysis	0	HC – 2 MDD - 4	N/A	Failed to record cardiac data

3.4.6.2 Analysis of Behavioural Data

All analysis of behavioural data was performed in a statistical analysis software package (SPSS) (IBM, New York, USA).

3.4.6.2.1 Chapters 4 & 5 - Baseline Differences and Linking to Baseline Symptoms

In order to investigate baseline differences in behavioural measures of reward and emotional processing between the HC and MDD groups, a repeated measures ANOVA was performed for each outcome measure for each task with participant group as the between subjects factor. Each task had a number of different outcome measures and within subject factors, which are summarised in Table 3.2.

In order to investigate whether baseline subjective experiences of anhedonia and negative affect in MDD related to dissociable abnormalities in the behavioural measures of reward and emotional processing, the baseline data for the experimental measures was correlated with the baseline questionnaire scores.

Table 3.2 Summary of Within Subject Factors and Outcome Measures for Each Task

Task	Within Subject Factors	Number of Levels	Outcome Measures	Critical Contrasts of Interest
Reward	Run	2 (run 1, run 2)	Overall total Amount won Amount lost Reaction time Average of last 50% of trials in which correct symbol chosen (Harrison et al, 2016)	If no main effect of run, not included as a within subjects factor and average across 2 runs used in subsequent analyses
Covert Faces	Face emotion	3 (happy, sad, fear)	% accuracy Average reaction time for correct responses	Face emotion x group
FERT	Face emotion	6 (anger, disgust, fear, happy, surprise – neutral excluded) OR 3 (happy, sad, external – average of anger, disgust, fear, surprise)	% accuracy % misclassifications Average reaction time for correct responses Target sensitivity / d' Beta / response bias	Face emotion x group
	Emotion intensity	10 (10-100%)	% accuracy	Emotion intensity x group
ECAT	Word valence	2 (like, dislike)	Average reaction time for correct responses % accuracy	Word valence x group
EREC	Word valence	2 (positive, negative)	Number of words recalled Number of commission errors	Word valence x group
FDOT	Face emotion Probe duration / masking	2 (fear, happy) 2 (masked, unmasked) First 2X2 then split into 2 separate analyses	Attentional vigilance (derived from reaction times) Average reaction time for correct responses	Face emotion x group Face emotion x probe duration x group
	Probe location / congruency (only used for reaction time)	2 (congruent with emotional face, incongruent with emotional face)		

3.4.6.2.2 Chapter 6 - Effect of Bupropion

In order to investigate whether manipulating DA activity with bupropion affects behavioural measures of reward and emotional processing in MDD, repeated measures ANOVAs were performed on the behavioural data. Visit was now included as an additional within subjects factor with either 2 (baseline, test visit 2) or 3 levels (baseline, test visit 2, test visit 3) and participant group (HC, MDD) as the between subjects factor. Significant visit by group interactions indicated changes that could not solely be explained by practice effects suggestive of an effect of bupropion and were investigated using standard post hoc tests.

3.4.6.2.3 Chapter 7 - Predicting Clinical Outcome

In order to investigate whether the behavioural effects were associated with clinical outcome and later change in subjective experiences of anhedonia and negative affect in MDD, the relationship between the change in the behavioural measures at 2 weeks and the change in the questionnaire scores over 6 weeks for the MDD group was examined. This was achieved by calculating the differences scores for the behavioural measures and questionnaires, which were then used in partial correlations, with the baseline questionnaire score included as a covariate.

The MDD group was also split into two groups depending on their clinical outcome after the 6 week treatment with bupropion; responders and non-responders. Responders were classed as those MDD participants displaying a greater than 50% decrease between their baseline and final HAM-D scores and a final HAM-D score of less than 7 indicating remission. Non-responders were classed as those MDD participants displaying a less than 50% decrease between their baseline and final HAM-D scores. A repeated measures ANOVA with visit as the within subjects factor and clinical outcome group as the between-subject factor was then performed to determine if there was any difference in the change in the experimental measures between responders and non-responders. This additional responder versus non responder approach to analysis was included as it investigates associations at a more clinically meaningful level.

3.4.6.3 Image Acquisition and Analysis of Neural Data

T*1-weighted structural images and T*2-weighted echo planar images (EPIs) depicting blood oxygen level dependent (BOLD) contrast signal were acquired on a 3.0 Tesla TIM Trio scanner. T*1- weighted structural images were acquired using an MPRAGE sequence with the following parameters: voxel resolution 1x1x1 mm^3, repetition time (TR) = 2.4 s and echo time (TE) = 4.7 ms. EPIs were acquired using the following parameters: voxel resolution of 3x3x3 mm^3, TR = 2.71 s, TE = 35 ms, flip angle of 87^o, slice angle of 30^0 and whole brain coverage with a total of 45 slices.

All analysis of neural data was performed using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) (Analysis Group, FIMRIB, Oxford, UK) fMRI Expert Analysis Tool (FEAT) Version 6.00 and consisted of a number of stages. Firstly, neural data required pre-processing. This was followed by a first-level analysis, which investigated brain activity at an individual level. Two fixed effects analyses were then performed; one to average brain activity across the two runs of the reward task and the other to calculate the change in brain activity between test visit 1 and 2 for each individual. Finally, brain activity was compared across participants in a random effects second-level analysis. These stages are described in more detail in the following sections.

3.4.6.3.1 Pre-processing

Firstly, neural data was converted from Dicom to NIfTI format and non-brain tissue was removed from the T*1-weighted structural images using Brain Extraction Tool (BET) (Smith, 2002). EPIs were realigned with the middle image using a least squares approach and a 6 parameter rigid body motion correction using MCFLIRT (Jenkinson et al., 2002), unless a large motion artefact occurring in the middle was identified, in which case, a different image was used. The realigned data was then smoothed with a 5 mm full width half maximum Gaussian kernel. High-pass temporal filtering was applied to reduce the influence of low frequency drifts in the MRI scanner signal, with a cut off of 90 seconds for the reward task and 135 seconds for the covert faces task. Finally, the realigned images were

registered first to the participant's high resolution T*1-weighted structural image and then to a standard template (Montreal Neurological Institute; MNI) via a non-linear transformation.

3.4.6.3.2 First-level Analysis

Task specific regressors, describing the onset and duration of task relevant events were defined. The remaining fixation events were used as the implicit baseline. Pulse oximetry and respiratory bellows data collected during completion of the tasks were used to create 33 physiological noise nuisance regressors using the PNM tool.

For the reward task, two regressors representing the anticipation of win or loss and four regressors representing the outcome (win, loss, no win in win, no loss in loss) were defined. Linear contrasts were either relative to an implicit baseline or between these regressors to allow identification of brain regions which respond preferentially to the anticipation of win versus loss and to positive versus negative outcomes.

For the covert faces task, three regressors representing the blocks of happy, sad or fearful faces were defined. Linear contrasts were either relative to an implicit baseline or between these regressors to allow identification of brain regions where activity was greater for a particular face emotion compared to another.

For each task, the regressors were convolved with a standard haemodynamic response function, filtered using the same high-pass filters as above and regressed against the pre-processed EPIs. Linear contrasts of the regressors at the individual participant level produced contrast maps for each participant, run and visit.

3.4.6.3.3 Fixed Effects Analyses

Since the reward task consisted of 2 runs, contrast maps were averaged across the 2 runs using a fixed effects analysis. A separate fixed effects analysis was performed for each visit and participant. Separate fixed effects analyses were also used to calculate the difference between contrast images obtained at test visits 1 and 2 for each participant, such that any changes in brain activity induced by the bupropion could be investigated.

3.4.6.3.4 Second-level Analyses

Three different second second-level analyses were run; whole brain analyses looking at all voxels in the brain, small volume corrected (SVC) analyses limited to voxels in the pre-specified regions of interest listed in Section 3.4.6.3.6 and region of interest (ROI) analyses using mean percentage signal change extracted from the same pre-specified regions of interest.

For the image-based whole brain and SVC analyses, inputs to the second-level analyses were the contrast maps derived from the first-level or fixed effects analyses. Inputting contrast maps derived from the first-level analysis allowed comparison of brain activity between participant groups at baseline. Inputting the difference between contrast images derived from the fixed effects analyses allowed investigation of any bupropion-induced changes in brain activity between test visits 1 and 2. When investigating whether any of these bupropion-induced changes in brain activity correlated with clinical outcome, demeaned questionnaire difference scores and a covariate of demeaned baseline questionnaire scores were included in the analysis. Demeaning was carried out by subtracting the mean questionnaire score from each individual questionnaire score.

For the ROI analyses, summary estimates of mean percentage signal change within the pre-specified regions were obtained from the first-level analyses using Featquery and analysed at the second-level using SPSS, with a univariate ANOVA with percentage signal change as the dependent variable and the baseline contrast value included as a covariate when looking at changes across visits.

3.4.6.3.5 Correction for Multiple Comparisons

The above image-based statistical analyses were corrected for multiple comparisons, either across all voxels within the brain for the whole brain analyses, or across the voxels within the pre-specified region of interest mask used in the SVC analyses. In both cases,

correction for multiple comparisons was achieved using a cluster-based correction with a z threshold of 2.3 and a corrected *p* value of < 0.05.

It should be noted that a paper has recently been published questioning this approach, suggesting that either a higher z threshold or a non-parametric approach should be used (Eklund et al., 2016). However, there is still debate surrounding this issue and since the paper was published after analysis was complete, the above pre-specified approach was still used.

3.4.6.3.6 Pre-specified Regions of Interest

As described in Chapter 1, MDD individuals have been shown to display abnormalities in particular overlapping brain regions involved in emotional and reward processing. Therefore, masks of the following pre-specified regions of interest were created and used in the SVC and ROI analyses of both tasks:

- Left and right amygdala (as defined in the Harvard-Oxford Subcortical Atlas)
- Left and right VS (as defined in the Harvard-Oxford Subcortical Atlas)
- Rostral, dorsal and subgenual portions of the ACC (as defined in the Harvard-Oxford Subcortical Atlas, with the genu of the callosum defining the boundary between the rostral and dorsal portions)
- Left and right medial and lateral OFC (sphere with radius of 10 mm created from co-ordinates stated in (Kringelbach and Rolls, 2004) left medial OFC (x y z = -24 31 -14) left lateral OFC (x y z = -33 42 -14))

Table 3.3 provides a summary list of all the analyses for the neural data.

Table 3.3 Summary List of all the Analyses for the Neural Data

	Covert Faces Task	Reward Task
First-level Contrasts	Happy Sad Fear All Faces No Faces - Relative to an implicit baseline Happy – Sad Happy – Fear Sad – Happy Sad – Fear Fear – Happy Fear – Sad Positive (Happy) – Negative (Sad, Fear) Negative – Positive	Win Anticipation Loss Anticipation Win Outcome Neutral Outcome (No Win in Win No Loss in Loss) Loss Outcome - Relative to an implicit baseline Win Anticipation – Loss Anticipation Loss Anticipation – Win Anticipation Win Outcome – Neutral Outcome Win Outcome – Loss Outcome Loss Outcome – Neutral Outcome Loss Outcome – Win Outcome Positive (Win, No Loss in Loss) – Negative (Loss, No Win in Win) Negative – Positive
Fixed-effects	Mean across visits 1 and 2 Increase between visit 1 and 2 Decrease between visit 1 and 2	Mean across runs 1 and 2 Mean across visits 1 and 2 Increase between visit 1 and 2 Decrease between visit 1 and 2
Second-level Whole Brain, SVC, ROI		
Baseline Analyses		
Baseline Differences	All mean (main effect of task) HC mean MDD mean HC > MDD MDD > HC	All mean (main effect of task) HC mean MDD mean HC > MDD MDD > HC
Linking to Baseline Symptoms	Positive / negative correlation with: EPQ Neuroticism HAM-D SHAPS PANAS Positive Affect PANAS Negative Affect MASQ General Distress: Depressive MASQ Anhedonic Depression MASQ General Distress: Anxious MASQ Anxious Arousal	Positive / negative correlation with: EPQ Neuroticism HAM-D SHAPS PANAS Positive Affect PANAS Negative Affect MASQ General Distress: Depressive MASQ Anhedonic Depression
Change Over Time Analyses i.e. using contrast maps derived from the fixed-effects analysis		
Effect of Bupropion	Increase / decrease: HC mean MDD mean HC > MDD MDD > HC	Increase / decrease: HC mean MDD mean HC > MDD MDD > HC
Predicting Clinical Outcome	Increase / decrease correlation with: EPQ Neuroticism HAM-D SHAPS PANAS Positive Affect PANAS Negative Affect MASQ General Distress: Depressive MASQ Anhedonic Depression MASQ General Distress: Anxious MASQ Anxious Arousal OR Increase / decrease: Responder mean Non-responder mean Responder > Non-responder Non-responder > Responder	Positive / negative correlation with: EPQ Neuroticism HAM-D SHAPS PANAS Positive Affect PANAS Negative Affect MASQ General Distress: Depressive MASQ Anhedonic Depression OR Increase / decrease: Responder mean Non-responder mean Responder > Non-responder Non-responder > Responder

CHAPTER 4

PARTICIPANT CHARACTERISATION, CLINICAL OUTCOME AND BASELINE DIFFERENCES

The following Chapter discusses the characterisation and demographics of the MDD and HC participant groups, the clinical response of the MDD group to the 6 week bupropion treatment and the differences between the HC and MDD groups in behavioural and neural measures of emotional and reward processing at baseline.

4.1 Participant Characterisation

4.1.1 Participant Demographics and Baseline Symptom Severity

Tables 4.1 and 4.2 summarise the demographic and clinical data of the HC and MDD groups. There were no significant differences between the two participant groups with regards to gender, age or NART-derived verbal IQ. The MDD group had significantly higher scores for neuroticism and lower scores for extraversion on the EPQ than the HC group. The HAM-D is typically used to assess baseline symptom severity and the MDD group had significantly higher baseline HAM-D scores than the HC group. Figure 4.1 shows that the majority of MDD participants displayed mild to moderate depression at baseline. Note that inclusion into the study was based solely on the psychiatric assessment and no baseline criteria for the HAM-D were stated in this study; therefore, the single MDD patient with a baseline HAM-D score within the normal range was still included.

Table 4.1 Participant Demographics

	HC	MDD	Independent Samples t-test
n	42	46	
Gender (Male:Female)	13:29	13:33	
Age	30.21 ± 8.13	29.52 ± 9.01	t = 0.38, $p = 0.71$
NART	116.31 ± 4.82	115.81 ± 4.34	t = 0.71, $p = 0.48$
HAM-D	0.86 ± 0.95	13.09 ± 3.03	t = -25.03, $p < 0.001$
EPQ			
Neuroticism	4.86 ± 3.25	18.98 ± 3.32	t = -20.12, $p < 0.001$
Psychoticism	2.48 ± 2.32	4.72 ± 3.70	t = -3.37, $p < 0.01$
Lie	10.00 ± 3.84	8.41 ± 4.31	t = 1.82, $p = 0.07$
Extraversion	14.71 ± 4.27	7.54 ± 5.44	t = 6.84, $p < 0.001$

Values are reported as means ± standard deviation

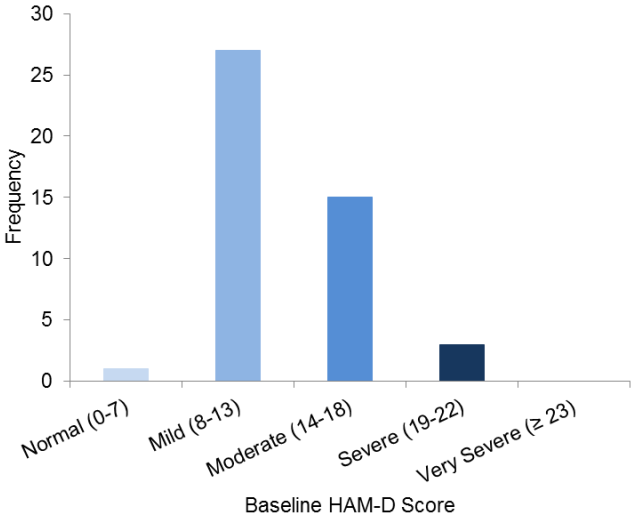


Table 4.2 Clinical History of MDD Group

	MDD
Age of Onset (years)	17.85 ± 7.91
Number of Previous Episodes	3.41 ± 6.58
Received Previous Treatment?	36 YES : 10 NO
Length of Current Episode (months)	45.65 ± 53.53

Values are reported as means ± standard deviation

Figure 4.1 – Baseline HAM-D of MDD Participants

The remaining questionnaire scores for each study visit are summarised in Table 4.3 and at baseline, the MDDs were found to be significantly different from HCs ($p < 0.001$) for every questionnaire measure below. Table 4.4 further summarises the change in questionnaire scores over time for the HC and MDD groups and displays the difference score between baseline and test visit 3.

Table 4.3 Baseline Symptom Scores & Change by Visit

	Baseline*		Review	Visit 2		Visit 3	
Questionnaire	**HC**	**MDD**	**MDD**	**HC**	**MDD**	**HC**	**MDD**
n	42	46	46	40	43	40	41
HAM-D	0.86 ± 0.95	**13.09 ± 3.03**	**10.48 ± 3.78**	0.83 ± 1.17	**10.37 ± 3.22**	0.88 ± 1.56	**6.05 ± 3.35**
SHAPS	20.02 ± 4.41	34.09 ± 5.30	32.35 ± 5.41	20.25 ± 4.24	31.60 ± 5.31	19.93 ± 5.08	26.56 ± 6.37
MASQ							
General Distress: Mixed	21.76 ± 5.25	46.48 ± 8.60	41.70 ± 10.12	21.18 ± 5.43	40.77 ± 9.44	21.33 ± 6.37	33.02 ± 8.52
General Distress: Anxious	14.21 ± 3.78	26.24 ± 6.43	23.30 ± 6.88	13.73 ± 3.15	22.23 ± 6.72	13.35 ± 2.82	19.34 ± 5.48
Anxious Arousal	19.29 ± 3.95	27.85 ± 8.00	26.37 ± 7.47	18.08 ± 1.42	26.63 ± 7.90	17.98 ± 1.29	25.00 ± 7.06
General Distress: Depressive	16.17 ± 5.23	41.00 ± 8.84	33.83 ± 9.97	15.45 ± 4.64	32.63 ± 10.53	16.15 ± 6.97	24.71 ± 8.44
Anhedonic Depression	45.10 ± 7.44	84.15 ± 10.27	77.02 ± 13.42	44.15 ± 8.89	74.42 ± 14.10	45.23 ± 13.54	61.83 ± 15.80
PANAS							
Positive Affect	33.55 ± 7.18	17.98 ± 5.49	19.26 ± 7.05	32.15 ± 7.24	20.70 ± 8.18	33.05 ± 8.63	25.17 ± 9.22
Negative Affect	11.48 ± 2.54	21.28 ± 6.77	19.43 ± 6.02	11.38 ± 2,25	20.28 ± 6.90	11.45 ± 2.49	16.17 ± 5.71
OQuESA							
Section 1	15.36 ± 3.97	42.41 ± 8.28	40.46 ± 9.82	15.53 ± 4.26	37.56 ± 10.18	14.88 ± 4.34	30.66 ± 11.53
Section 2	9.07 ± 3.54	32.33 ± 5.60	30.91 ± 5.90	8.63 ± 1.41	28.26 ± 7.53	8.63 ± 1.92	21.76 ± 9.01
Section 3			11.33 ± 5.34		10.56 ± 4.57		9.95 ± 5.63

Values are reported as means ± standard deviation
*Independent samples t-tests found MDDs to be significantly different from HCs ($p < 0.001$) for all baseline questionnaire scores
Bold red highlights scores for the HAM-D, typically used to assess baseline symptom severity and clinical outcome

Table 4.4 Difference Scores

	Difference Score (Visit 3 – Baseline)		Effect of Time	
Questionnaire	**HC**	**MDD**	**HC**	**MDD**
HAM-D	0.00 ± 1.63	-7.00 ± 3.91	No change, p = 0.89	Sig. decrease $p < 0.001$
SHAPS	0.10 ± 4.62	-7.24 ± 7.71	No change, p = 1.00	Sig. decrease $p < 0.001$
MASQ				
General Distress: Mixed	-0.58 ± 7.50	-12.66 ± 8.71	No change, p = 0.63	Sig. decrease $p < 0.001$
General Distress: Anxious	-0.75 ± 3.41	-6.51 ± 7.17	No change, p = 0.17	Sig. decrease $p < 0.001$
Anxious Arousal	-1.35 ± 3.66	-2.34 ± 8.17	Sig. decrease, p = 0.03	No change, p = 0.07
General Distress: Depressive	-0.13 ± 8.19	-15.34 ± 10.67	No change, p = 0.92	Sig. decrease $p < 0.001$
Anhedonic Depression	0.08 ± 12.72	-21.63 ± 18.24	No change, p = 0.97	Sig. decrease $p < 0.001$
PANAS				
Positive Affect	-1.03 ± 4.86	+6.71 ± 9.93	No change, p= 0.19	Sig. increase $p < 0.001$
Negative Affect	-0.05 ± 3.07	-4.37 ± 7.34	No change, p = 0.92	Sig. decrease $p < 0.001$
OQuESA				
Section 1	-0.55 ± 3.30	-11.83 ± 9.51	No change, p = 0.30	Sig. decrease $p < 0.001$
Section 2	-0.50 ± 3.95	-10.46 ± 8.59	No change, p = 0.43	Sig. decrease $p < 0.001$

Values are reported as means ± standard deviation

4.2 Clinical Outcome

4.2.1 Clinical Outcome based on the HAM-D

Change in HAM-D scores can also be used to assess clinical outcome, that is, whether the MDD participant has responded to treatment or not. A final HAM-D score of less than 7 is indicative of remission (Hamilton, 1960). However, since some individuals had relatively low baseline HAM-D scores, a final HAM-D score of less than 7 was achieved with only a modest decrease in HAM-D score. Therefore, responders were classed as those MDD participants displaying both remission and a greater than 50% decrease between their baseline and final HAM-D score. All remaining individuals were classed as non-responders. This did mean the inclusion of a single MDD participant who had a greater than 50% decrease in HAM-D score but no remission into the non-responder group. Table 4.5 displays clinical outcome based on percentage HAM-D decrease on an individual basis and this is then summarised in Table 4.6. Of the 46 MDD participants treated, 4 had to be withdrawn due to unmanageable side-effects and 1 could not attend the final test visit due to increased severity of symptoms and was therefore classed as a non-responder leaving 28 (66.7%) responders and 14 (33.3%) non-responders.

Table 4.5 Clinical Outcome based on % HAMD Decrease

Subject No.	Baseline HAM-D	6 week HAM-D	% Decrease	Clinical Outcome*	Decision**
2001	17	2	88.2	Responder, remission	Continue
2003	20	9	55.0	Responder	Continue
2004	14	3	78.6	Responder, remission	Continue
2005	11	1	90.9	Responder, remission	Continue
2007	20	4	80.0	Responder, remission	Continue
2008	18	9	50.0	Responder, remission	Continue
2010	15	Withdrawn			
2012	20	15	25.0	Non-responder	Discontinue
2013	11	10	9.1	Non-responder	Discontinue
2015	13	5	61.5	Responder, remission	Continue
2017	13	6	53.8	Responder, remission	Discontinue
2018	9	6	33.3	Non-responder but remission due to low baseline HAM-D	Continue
2019	7	3	57.1	Responder, remission	Continue
2020	12	3	75.0	Responder, remission	Continue
2023	15	Withdrawn			
2024	13	6	53.8	Responder, remission	Continue
2025	14	5	64.3	Responder, remission	Continue
2026	10	1	90.0	Responder, remission	Continue
2027	12	14	-16.7	Non-responder	Discontinue
2028	16	5	68.8	Responder, remission	Continue
2029	13	Withdrawn			
2031	17	7	58.8	Responder, remission	Continue
2032	15	7	53.3	Responder, remission	Continue
2033	15	14	6.7	Non-responder	Continue
2035	10	7	30.0	Non-responder but remission due to low baseline HAM-D	Discontinue
2036	12	4	66.7	Responder, remission	Continue
2038	16	4	75.0	Responder, remission	Continue
2039	14	4	71.4	Responder, remission	Continue
2040	9	7	22.2	Non-responder but remission due to low baseline HAM-D	Continue
2041	10	5	50.0	Responder, remission	Continue
2043	11	6	45.5	Non-responder but remission due to low baseline HAM-D	Continue
2045	12	4	66.7	Responder, remission	Continue
2051	14	4	71.4	Responder, remission	Continue
2053	9	4	55.6	Responder, remission	Continue
2055	10	9	10.0	Non-responder	Continue
2057	11	Withdrawn			
2059	12	2	83.3	Responder, remission	Continue
2061	13	Withdrawn		Couldn't attend last visit due to symptoms, classed as non-responder	
2065	15	9	40.0	Non-responder	Discontinue
2067	12	6	50.0	Responder, remission	Continue
2071	12	6	50.0	Responder, remission	Continue at half dose
2073	15	5	66.7	Responder, remission	Continue
2075	9	4	55.6	Responder, remission	Continue
2077	12	5	58.3	Responder, remission	Continue
2079	13	12	7.7	Non-responder	Continue
2081	11	6	45.5	Non-responder but remission due to low baseline HAM-D	Discontinue

*Responder – greater than 50% decrease between baseline and final HAM-D scores
Non-responder – less than 50% decrease between baseline and final HAM-D scores
Remission – final HAM-D score of 7 or less
**Decision indicates whether the participant decided to continue or discontinue the bupropion at the end of the 6 week study

Table 4.6 Clinical Response by HAM-D Summary Table

''Robust Clinical Response''	Frequency	% of Sample (excluding withdrawn)
Responded AND Remitted	28	66.7
Non-responders	14	33.3

4.3 Baseline Differences between the MDD and HC Groups

4.3.1 ETB

4.3.1.1 FERT

The FERT requires participants to indicate whether an emotional facial expression is one of anger, disgust, fear, happy, sad, surprise or neutral (neutral was not included in the following statistical analyses as due to response bias the accuracy for neutral was near 100% for both participant groups). A repeated measures ANOVA with face emotion as the within subjects factor and participant group as the between subjects factor was performed on the baseline data for each outcome measure of the FERT.

A significant main effect of face emotion was found for percentage accuracy ($F_{5, 425}$ = 90.19, $p < 0.001$) and d' ($F_{5, 425}$ = 75.73, $p < 0.001$), where participants were most accurate at recognising happy faces; however, no main effect of group ($F_{1, 85}$ = 0.43, $p = 0.51$) or face emotion by group interaction ($F_{5, 425}$ = 0.47, $p = 0.80$) was found (Figure 4.2 a and b). A significant main effect of face emotion was also found for reaction time ($F_{5, 415}$ = 103.37, $p < 0.001$), with participants responding quickest to fearful faces. There was a main effect of group for reaction time ($F_{1, 83}$ = 5.15, $p < 0.05$), with the MDD group responding significantly slower than the HC group. Whilst there was no face emotion by group interaction ($F_{5, 415}$ = 1.79, $p = 0.11$), post hoc independent samples t-tests found the MDD group to be significantly slower than the HC group specifically to fearful (t_{83} = -2.09, $p < 0.05$), happy (t_{85} = -3.14, $p < 0.01$) and surprised faces (t_{85} = -3.25, $p < 0.01$) (Figure 4.2c).

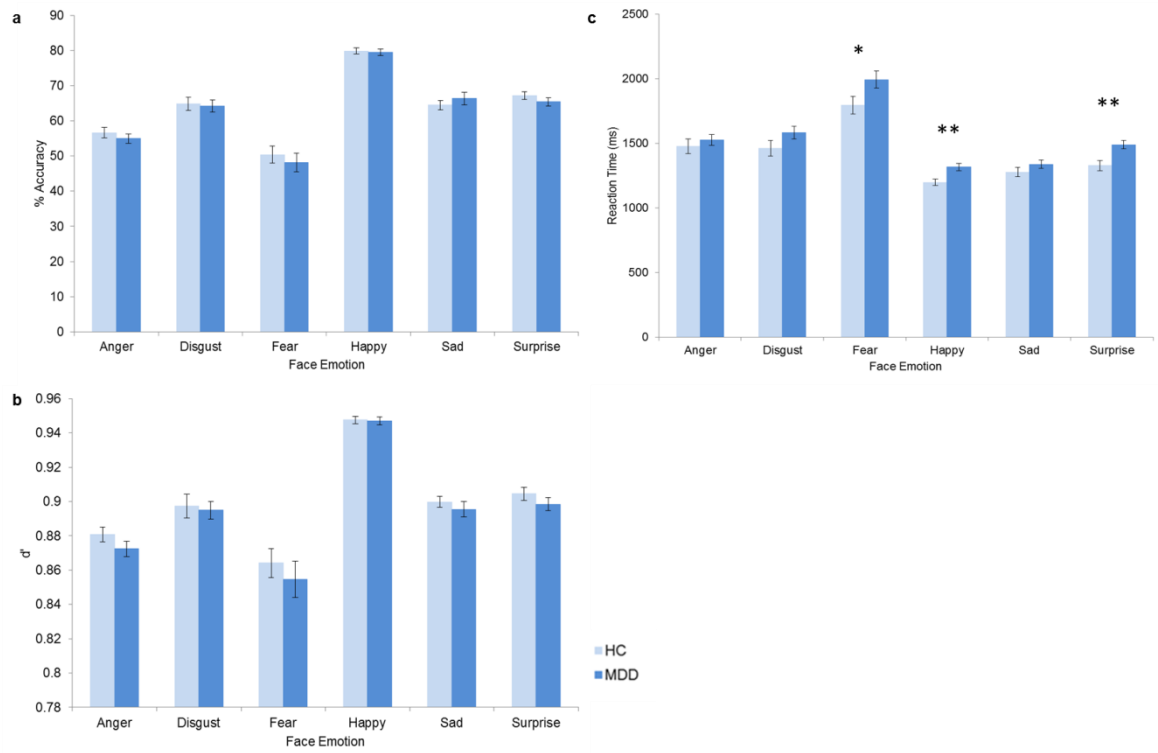


Figure 4.2 – FERT % Accuracy, d' and Reaction Time: (a) % accuracy, **(b)** d' and **(c)** average reaction time for correct responses for each face emotion for each participant group. Values are reported as means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$, **$p < 0.01$).

A significant main effect of face emotion was also found for percentage misclassifications ($F_{5, 425} = 20.58$, $p < 0.001$) and beta ($F_{5, 420} = 19.17$, $p < 0.001$). A significant main effect of group was found for percentage misclassifications ($F_{1, 85} = 4.94$, $p < 0.05$) but not for beta ($F_{1, 84} = 2.37$, $p = 0.13$). There was a face emotion by group interaction for both percentage misclassifications ($F_{5, 425} = 3.16$, $p < 0.01$) and beta ($F_{5, 420} = 3.23$, $p < 0.01$). Post hoc independent-samples t-tests found that the MDD group misclassified significantly more faces as sad compared to the HC group ($t_{85} = 2.32$, $p < 0.05$), which can clearly be seen in Figure 4.3a. This was reflected in significantly reduced beta ($t_{85} = 3.32$, $p < 0.05$) and therefore increased response bias towards sad faces in the MDD group compared to the HC group (Figure 4.3b). It is worth noting here that an acute dose of bupropion was found to reduce a response bias towards sad faces in Chapter 2.

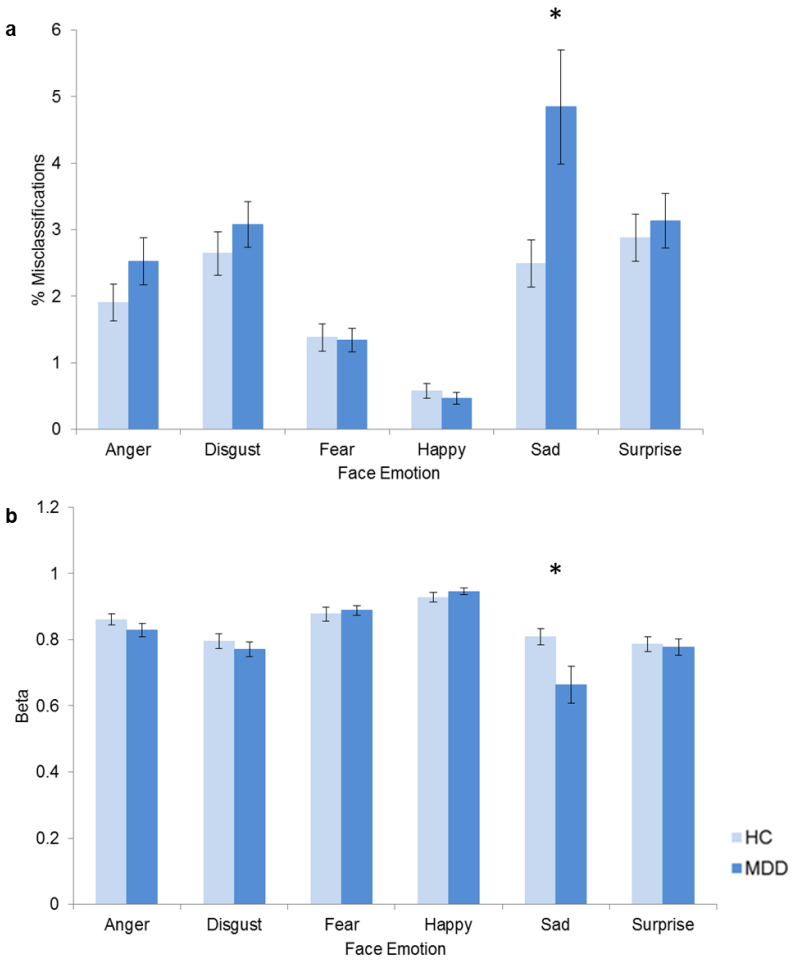


Figure 4.3 – FERT % Misclassifications and Beta: (a) % misclassifications and **(b)** beta for each face emotion for each participant group. Values are reported as means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$).

4.3.1.2 ECAT

The ECAT requires participants to indicate whether they would like or dislike to be described as certain negative and positive self-referent words. A large proportion of individuals had near 100% accuracy, therefore in order to generate more normally distributed data, an arcsine transformation was first applied to the percentage accuracy data prior to analysis. A repeated measures ANOVA with word valence as the within subjects factor and participant group as the between subjects factor was then performed on the

transformed baseline data for each outcome measure of the ECAT. A word valence by group interaction was found for percentage accuracy ($F_{1, 85} = 4.12$, $p < 0.05$). From Figures 4.4a and b, this appeared to be the result of the MDD group having lower percentage accuracy for positive self-referent words compared to the HC group; however, this was not found to be significant in a post hoc independent samples t-test on either the untransformed ($t_{85} = 0.15$, $p = 0.14$) or transformed baseline data ($t_{85} = 1.46$, $p = 0.15$). Although, a paired t-test found that MDD group had significantly lower percentage accuracy for positive compared to negative self-referent words ($t_{44} = 2.18$, $p < 0.05$).

A main effect of group was found for reaction time ($F_{1, 85} = 6.25$, $p < 0.05$), with the MDD group showing significantly slower reaction times than the HC group for both negative and positive self-referent words.

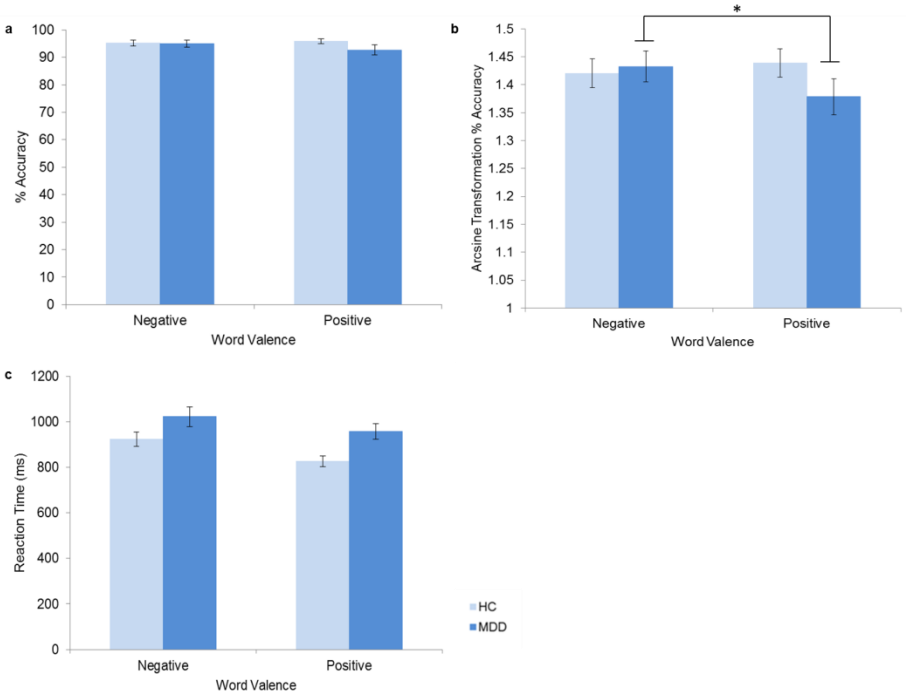


Figure 4.4 – ECAT % Accuracy and Reaction Time: (a) % accuracy, **(b)** arcsine transformation for % accuracy and **(c)** average reaction time for correct responses for each word valence for each participant group. Values are reported as means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$).

4.3.1.3 FDOT

The FDOT requires participants to respond to the alignment of a dot-probe appearing in the place of either a neutral, positive or negative valenced face. Median reaction time to respond to the dot-probe was used to calculate attentional vigilance towards the positively or negatively valenced faces.

For reaction time, a repeated measures ANOVA with three within subjects factors (face emotion, masking and congruency) and participant group as the between subjects factor was performed. There was no main effect of face emotion ($F_{1, 85} = 0.15$, $p = 0.70$), masking ($F_{1, 85} = 3.27$, $p = 0.07$) or congruency ($F_{1, 85} = 1.68$, $p = 0.20$); however, a main effect of group was found ($F_{1, 85} = 8.96$, $p < 0.01$), with the MDD group showing significantly slower reaction times than the HC group across all conditions of the FDOT (Figure 4.5a).

For attentional vigilance, a repeated measures ANOVA with two within subjects factors (face emotion and masking) and participant group as the between subjects factor again found a main effect of group ($F_{1, 85} = 4.43$, $p < 0.05$), with the MDD group displaying overall significantly lower attentional vigilance across all face emotion and masking conditions compared to the HC group (Figure 4.5c and d). On closer inspection of Figures 4.5 c and d, it appeared that the MDD group may have lower vigilance specifically for happy faces and particularly unmasked happy faces. Since no main effect of masking was found ($F_{1, 85} = 0.00$, $p = 0.99$) the data from the masked and unmasked happy face conditions was combined (Figure 4.5b) and significance in a post hoc independent samples t-test was obtained ($t_{85} = 2.06$, $p < 0.05$), suggesting that the MDD group were significantly less vigilant specifically for happy faces compared to the HC group.

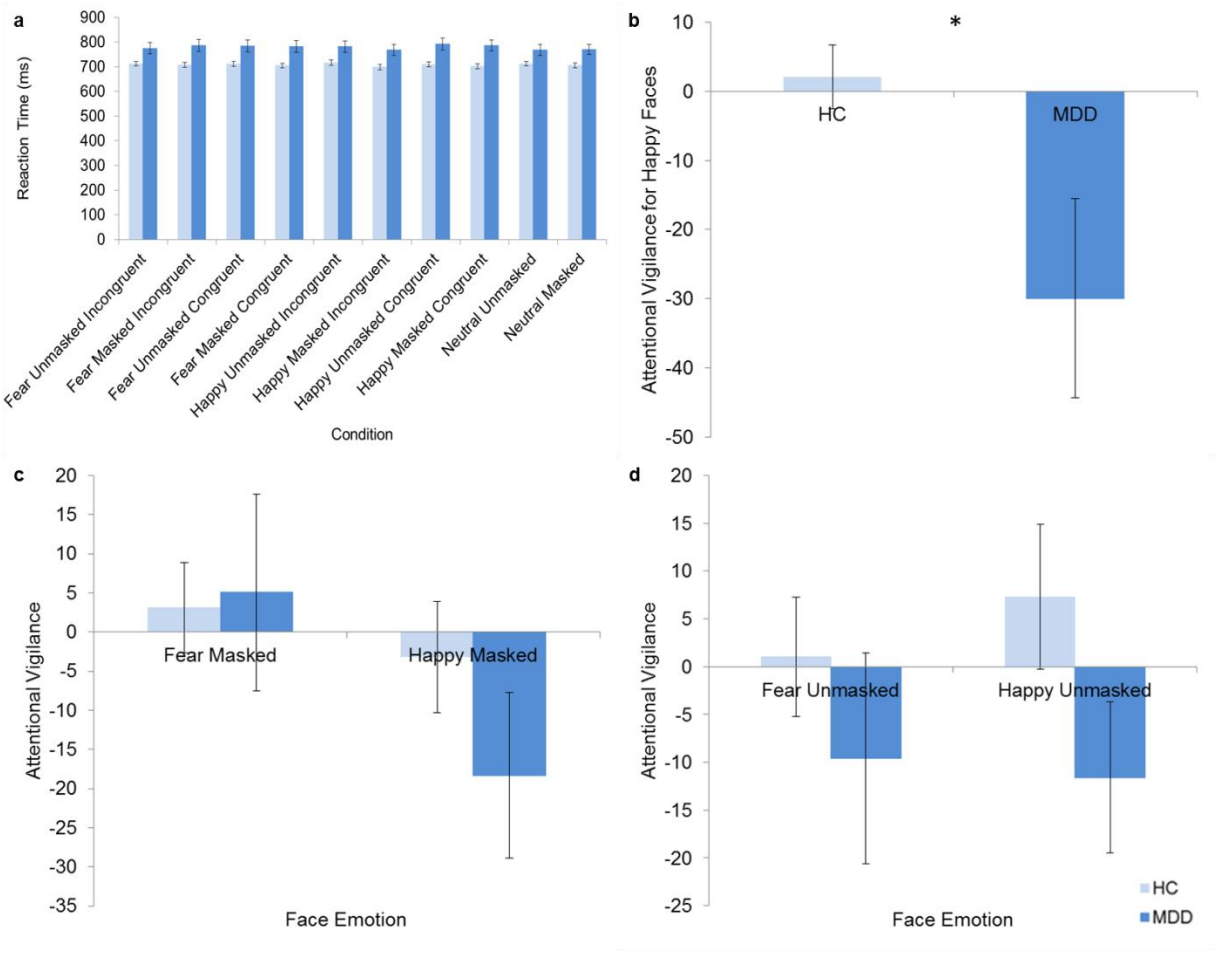


Figure 4.5 – FDOT Reaction Time and Attentional Vigilance: (a) average reaction time for correct responses and attentional vigilance for **(b)** happy faces, **(c)** masked and **(d)** unmasked faces for each condition of the FDOT for each participant group. At the participant level, medians were used then values displayed here are means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*p < 0.05).

4.3.1.4 EREC

The EREC requires participants to write down as many positive and negative self-referent words from the ECAT that they could remember. A repeated measures ANOVA on the baseline data with word valence as the within subjects factor and participant group as the between subjects factor found a significant main effect of word valence for both correctly ($F_{1, 86} = 16.01$, $p < 0.001$) and falsely ($F_{1, 86} = 36.93$, $p < 0.001$) recalled self-referent words, with both groups remembering more positive rather than negative self-referent words (Figure 4.6). Figure 4.6b suggests that the MDD group falsely recalled more negative self-referent words compared to the HC group; however, a significant word valence by group interaction for commission errors was not found ($F_{1, 86} = 1.52$, $p = 0.22$) and a post hoc independent samples t-test did not reach significance ($t_{86} = -1.87$, $p = 0.07$).

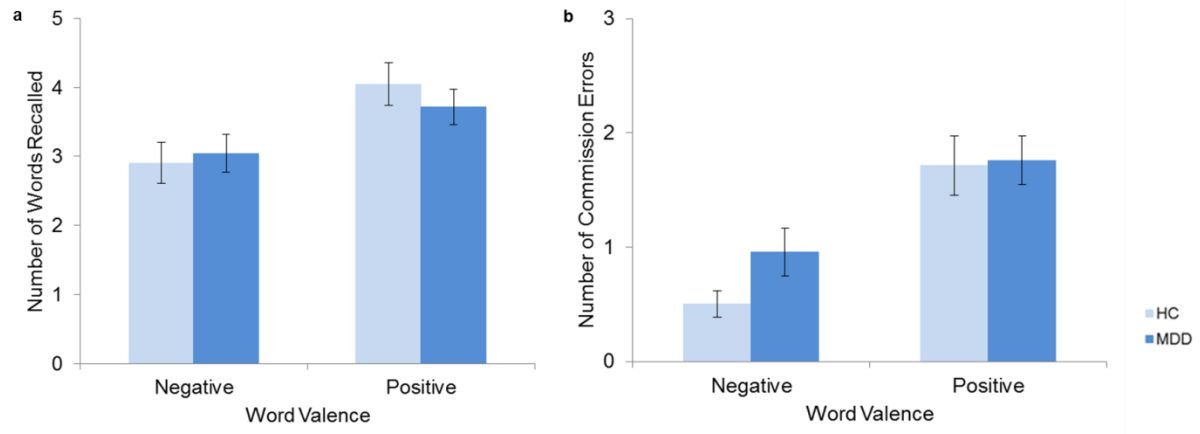


Figure 4.6 – EREC Self-Referent Words Correctly and Falsely Recalled: number of negative and positive **(a)** self-referent words correctly recalled and **(b)** commission errors made for each participant group. Values are reported as means ± standard error of the mean.

4.3.2 Covert Faces Task

4.3.2.1 Behavioural Baseline Differences for the Covert Faces Task

During the covert faces task, participants were required to indicate the gender of a series of emotional facial expressions that were fearful, happy or sad. A large proportion of individuals had near 100% accuracy, therefore in order to generate more normally distributed data, an arcsine transformation was again first applied to the percentage accuracy data prior to analysis. A repeated measures ANOVA with face emotion as the within subjects factor and participant group as the between subjects factor was then performed on the transformed baseline data for each outcome measure of the covert faces task. A main effect of face emotion was found for percentage accuracy ($F_{2, 172} = 3.26$, $p < 0.05$), with participants displaying significantly higher accuracy for sad faces in both the MDD and HC groups (Figure 4.10b). Figure 4.10 suggests that the MDD group was slightly slower and less accurate across all face emotions; however, there was no significant main effect of group for reaction time ($F_{1, 86} = 2.01$, $p = 0.16$) or percentage accuracy ($F_{1, 86} = 1.64$, $p = 0.20$). There was also no face emotion by group interaction for reaction time ($F_{2, 172} = 0.05$, $p = 0.95$) or percentage accuracy ($F_{2, 172} = 0.09$, $p = 0.91$). Overall, all participants showed a high level of accuracy in discriminating the gender of faces, suggesting that all participants were attending to the face stimuli equally well.

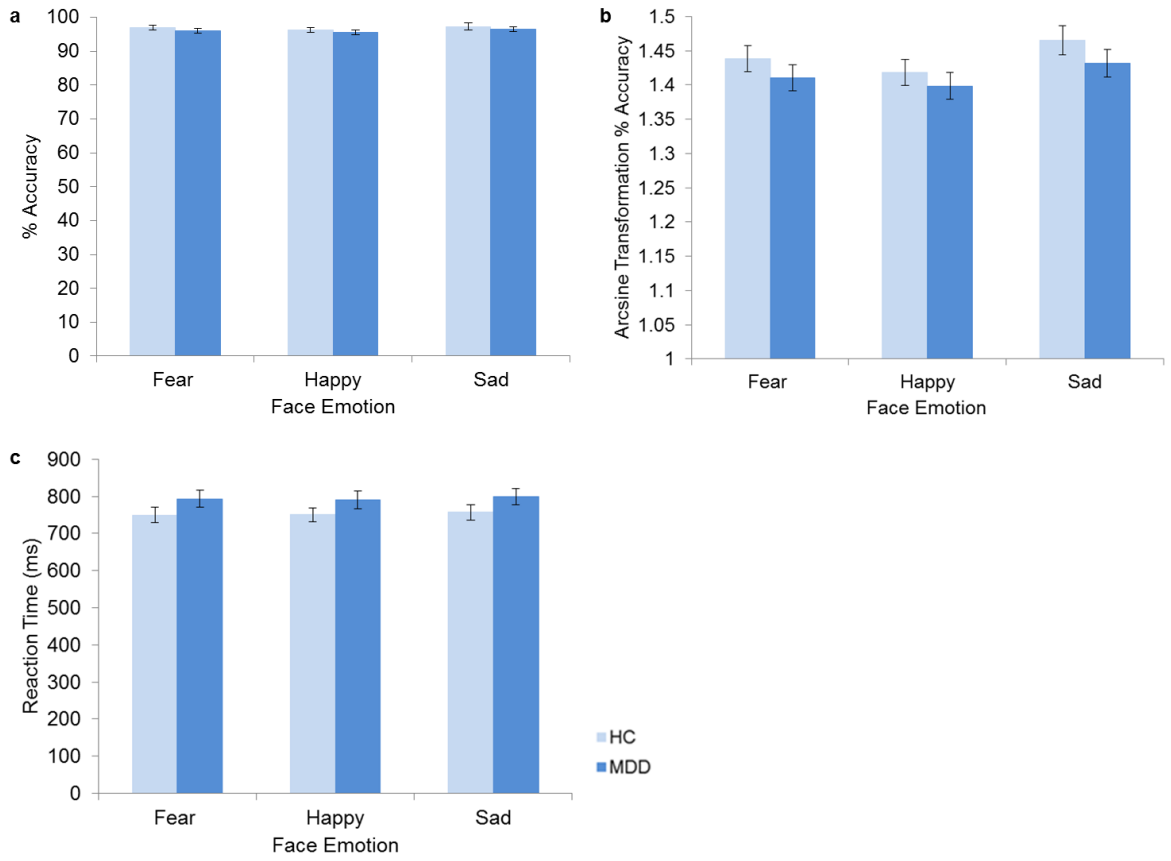


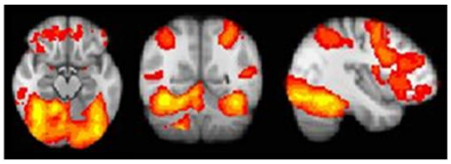
Figure 4.7 – Covert Faces Task % Accuracy and Reaction Time: (a) % accuracy, **(b)** arcsine transformation for % accuracy and **(c)** average reaction time for correct responses for each face emotion for each participant group. Values are reported as means ± standard error of the mean.

4.3.2.2 Neural Baseline Differences for the Covert Faces Task

4.3.2.2.1 Whole Brain Analysis

The whole brain analysis investigating the main effect of the covert faces task across all participants for all faces relative to an implicit baseline showed significant activation of a network of brain regions typically associated with processing of facial expressions, including the occipital fusiform gyrus, insular cortex and amygdala. There were also regions of significant activity for some of the contrasts between the differently valenced facial expressions (Table 4.7).

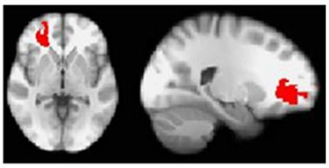
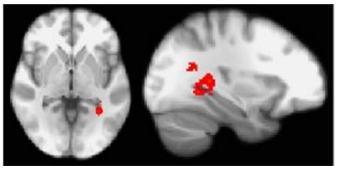
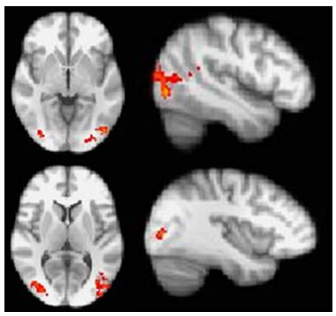
Table 4.7 Main Effects of the Covert Faces Task

Lower-level Contrast	x y z	Z-max	Corrected-*p*	Structure
Happy – Sad	48 -42 40	3.69	< 0.05	Posterior supramarginal gyrus
Happy – Fear				
Sad – Happy	4 58 28	4.42	< 0.05	Frontal pole
Sad – Fear				
Fear – Happy	-6 56 -6	3.41	< 0.001	Frontal pole, frontal medial cortex, paracingulate gyrus
Fear – Sad				
Happy*	-4 8 52 38 -54 -18	8.18 9.46	< 0.001 < 0.001	Juxtapositional lobule cortex, paracingulate gyrus Temporal occipital fusiform cortex
Sad*	40 -56 -18 36 -54 48	9.26 5.77	< 0.001 < 0.001	Temporal occipital fusiform cortex Angular gyrus
Fear*	40 -56 -16 36 -56 46	9.25 5.20	< 0.001 < 0.001	Temporal occipital fusiform cortex Angular gyrus
All Faces*	40 -56 -18	9.58	< 0.001	Temporal occipital fusiform cortex 

* Relative to an implicit baseline

Comparison of the MDD and HC groups at the whole brain level at baseline revealed that the MDD group displayed overall greater activity across all facial expressions in a region of right cerebral white matter including the frontal pole (x y z = 22 38 -2, Z-max = 3.56, *p*-corrected < 0.01) and in response to sad faces in the temporal occipital fusiform cortex (x y z = -32 -50 -2, Z-max = 3.88, *p*-corrected < 0.05). The MDD group also displayed significantly greater activity in the bilateral inferior occipital cortex compared to the HC group in response to sad versus fearful faces (x y z = -46 -76 -4, Z-max = 4.16, *p*-corrected < 0.001; x y z = 40 -78 6, Z-max = 3.43, *p*-corrected < 0.05) (Table 4.8).

Table 4.8 Whole Brain Analysis of Baseline Group Differences for the Covert Faces Task

Lower-level Contrast	Direction of Group Comparison	x y z	Z-max	Corrected-*p*	Image
All Faces *	MDD > HC	22 38 -2	3.56	< 0.01	Right cerebral white matter / frontal pole 
Sad*	MDD > HC	-32 -50 -2	3.88	< 0.05	Left cerebral white matter / temporal occipital fusiform cortex 
Sad – Fear	MDD > HC	-46 -76 -4 40 -78 6	4.16 3.43	< 0.001 < 0.05	Bilateral inferior occipital cortex 

*Relative to an implicit baseline

4.3.2.2.2 SVC / ROI Analysis

In the SVC analysis, the amygdala and OFC showed activation in response to negative facial expressions in the MDD group only; however, no significance was found in the MDD versus HC contrasts. Amygdala activation was of particular interest in this study since previous research has found MDD patients to display hyper- or hypoactive amygdala responses to negative or positive affective stimuli, respectively. Therefore, individual percentage signal change values in response to sad, happy and fearful faces were extracted from the left and right amygdala and compared between MDD and HC groups in an ROI analysis in SPSS. However, this also did not reveal any significant baseline group differences in amygdala activation in response to facial expressions (Figure 4.8).

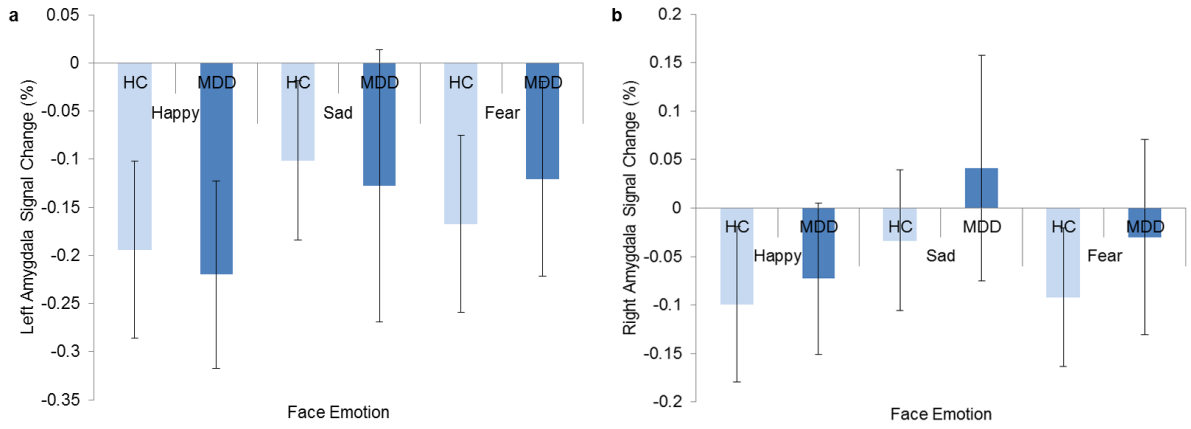


Figure 4.8 – Bilateral Amygdala Activity during the Covert Faces Task: extracted percentage signal change in the **(a)** left and **(b)** right amygdala in response to each face emotion of the covert faces task for each participant group. Values are reported as means ± standard error of the mean.

4.3.3 Reward Task

4.3.3.1 Behavioural Baseline Differences for the Reward Task

During the reward task, participants were presented with two symbols per trial, where each symbol was associated with a certain win, loss or no change outcome probability. Participants were required to choose between the two symbols in order to maximise payoffs. Figure 4.9 shows the average total at the end of the reward task, amount won and amount lost per run as well as averaged across the two runs for each participant group. Analysis of totals was first carried out using repeated measures ANOVAs with run as a within subjects factor; however, no significant main effect of run was found for each outcome condition. Therefore, run was excluded from further analyses and the average across runs was used in an independent samples t-tests for each outcome condition. There was no significant difference between the MDD and HC groups for end total (t_{76} = -0.42, p = 0.67), amount won (t_{76} = 0.69, p = 0.49) or amount lost (t_{76} = -1.26, p = 0.21).

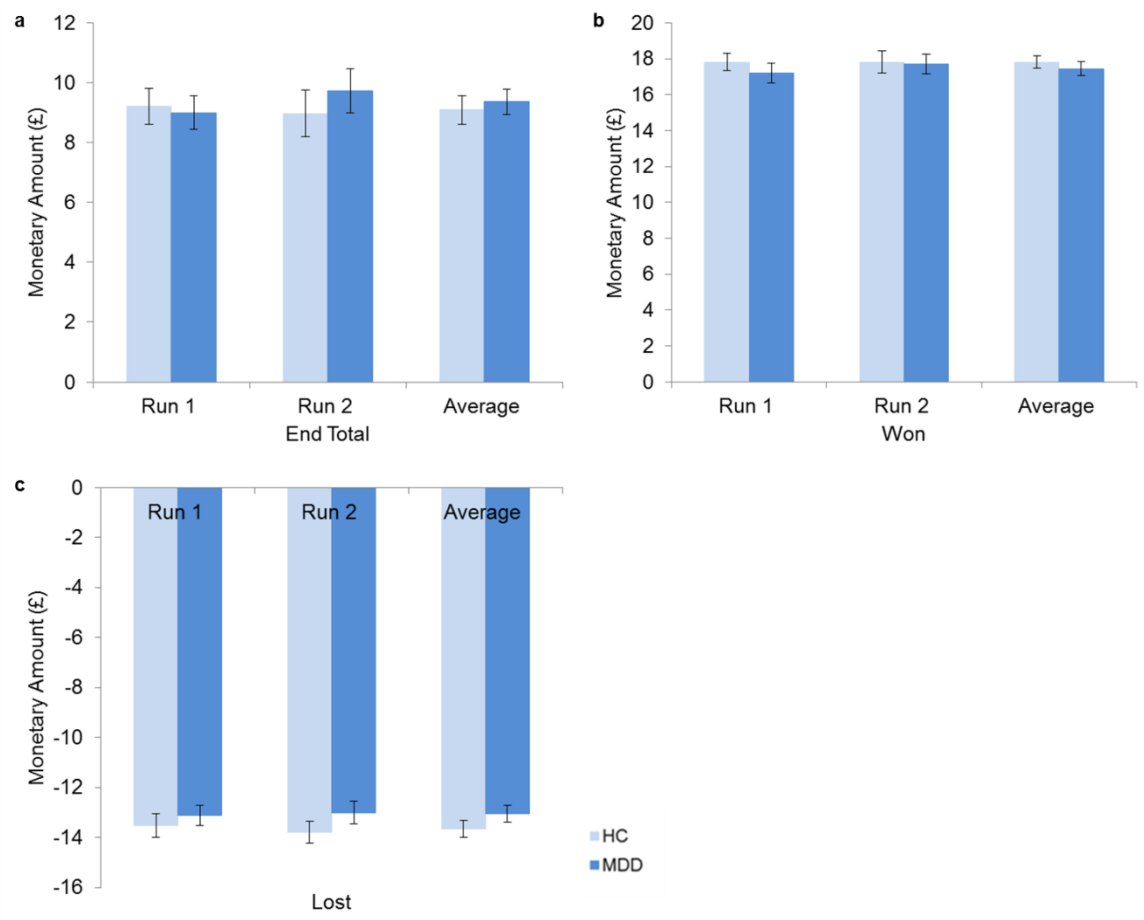


Figure 4.9 – Reward Task Monetary Totals: the monetary amount for run 1, run 2 and averaged across the two runs of the reward task for the **(a)** end total, **(b)** win condition and **(c)** loss condition for each participant group. Values are reported as means ± standard error of the mean.

Figure 4.10a shows the learning curves for each participant group depicting trial-by-trial the proportion of participants that chose the correct symbol in the win condition, associated with high-probably win and the incorrect symbol in the loss condition, associated with high-probability loss. With the exception of those who were excluded (Section 3.4.6.1), participants generally learnt within the 30-trial run for each condition to choose the high-probability win and avoid the high-probability loss. Through visual inspection of Figure 4.10a, there does not appear to be any difference in learning about wins or losses between the MDD and HC groups, consistent with the monetary total results. In the same analysis used

in the acute study (Harrison et al., 2016), the average of the last 50% of the trials (last 15 out of 30) in which participants chose the correct symbol was calculated for both the win and loss conditions for each participant group (Figure 4.10b). Whilst there was no difference between groups in the loss condition, the MDD group appeared to have a lower percentage of correct symbol choice in the win condition compared to the HC group, which was found to be marginally significant ($t_{28} = 2.03$, $p = 0.05$), suggesting possible insensitivity to high-probability win.

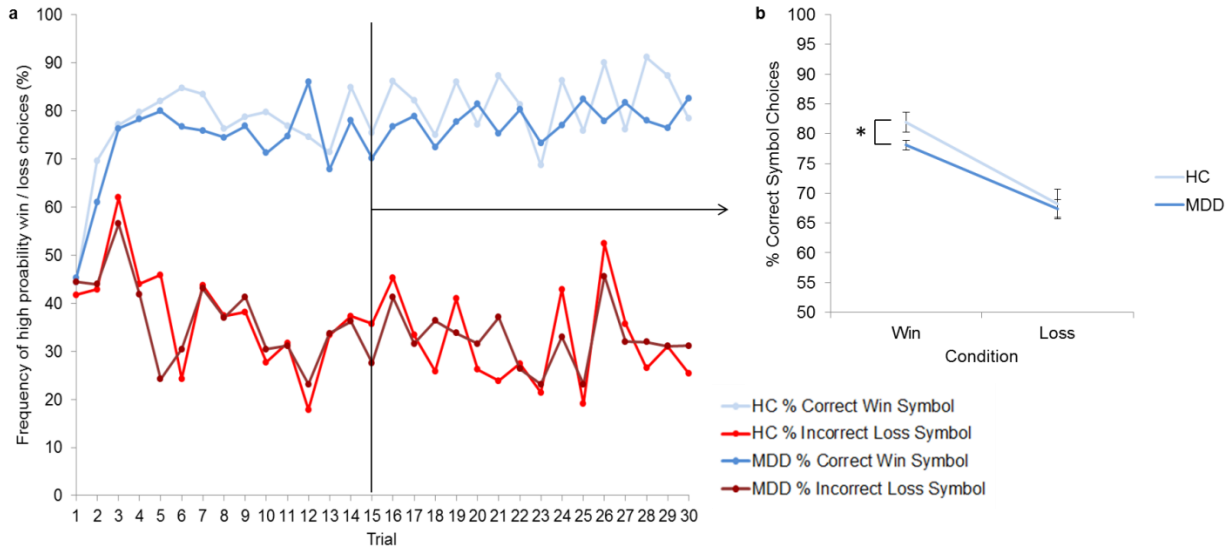


Figure 4.10 – Reward Task Learning: (a) learning curves for each participant group depicting trial-by-trial the proportion of participants that chose the correct symbol in the win condition, associated with high-probability win (top blue lines) and the incorrect symbol in the loss condition, associated with high-probability loss (bottom red lines). Learning curves were very similar for run 1 and run 2 of the reward task; therefore, only the average across the two runs are displayed here. **(b)** Average of the last 50% of trials in which participants chose the correct symbol for both the win and loss conditions for each participant group. Values are reported means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$).

For reaction time, a repeated measures ANOVA with run as a within subjects factor was first performed. A main effect of run was found this time ($F_{1, 86} = 14.52$, $p < 0.001$), with participants responding significantly quicker in the second run in both the MDD and HC groups, likely as a result of practice effects; therefore, run was kept in the analysis. A main effect of condition was also found ($F_{1, 86} = 65.09$, $p < 0.001$), with participants responding significantly quicker in the win condition compared to the loss condition in both the MDD and HC groups but no other significant effects were found (Figure 4.11).

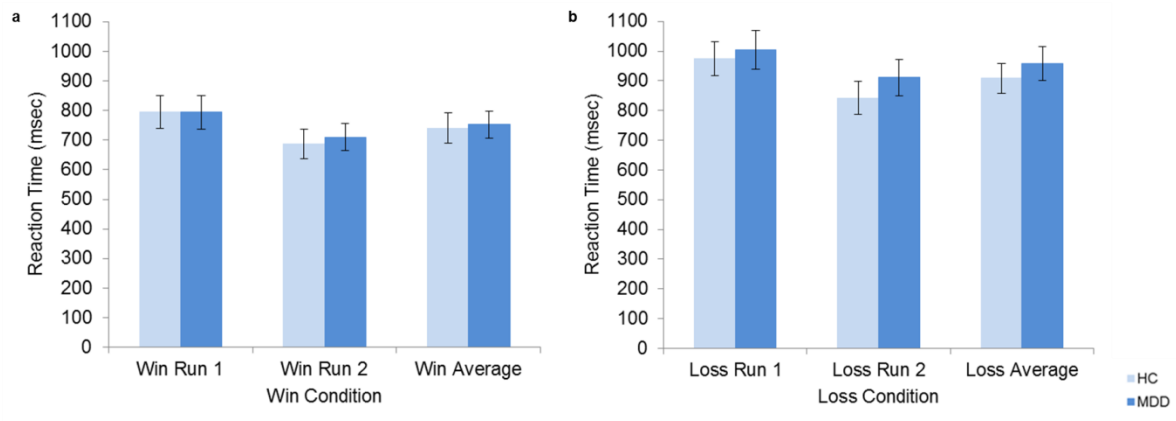


Figure 4.11 – Reward Task Reaction Times: average reaction time for run 1, run 2 and averaged across the two runs for the **(a)** win condition and **(b)** loss condition for each participant group. Values are reported means ± standard error of the mean.

4.3.3.2 Neural Baseline Differences for the Reward Task

The whole brain analysis of baseline neural data across all participants for the reward task showed significant activation of the expected, pre-specified regions of the brain associated with reward processing, including the OFC, VS and ACC. Contrasts between the different task events also revealed brain regions with differing responsiveness to the anticipation or consumption of win vs loss outcomes (Table 4.9).

Table 4.9 Main Effects of the Reward Task

Lower-level Contrast	x y z	Z-max	Corrected-*p*	Structure
Win Anticipation – Loss Anticipation	-8 58 14 54 4 4 -40 6 8 -24 -30 68	4.62 4.71 4.92 4.17	< 0.001 < 0.001 < 0.001 < 0.01	Frontal pole Central opercular cortex Central opercular cortex Postcentral gyrus
Loss Anticipation – Win Anticipation	-30 -62 48 -4 18 48 12 10 8 -12 10 8 46 32 22	5.00 6.37 4.62 4.40 4.02	< 0.001 < 0.001 < 0.01 < 0.05 < 0.05	Superior lateral occipital cortex Paracingulate gyrus Right caudate Left caudate Middle frontal gyrus
Win Anticipation*	-26 -6 54 30 -70 30 -8 -18 6 -4 -22 28 22 10 4	8.81 7.42 6.00 5.32 5.01	< 0.001 < 0.001 < 0.001 < 0.01 < 0.05	Precentral gyrus Superior lateral occipital cortex Left thalamus CC – posterior Right putamen
Loss Anticipation*	-6 14 46 -10 -20 12 -6 -28 28	9.05 5.76 5.32	< 0.001 < 0.001 < 0.05	Paracingulate gyrus Left thalamus CC - posterior
Win – Neutral	12 -90 2 -52 -4 42 48 -2 42	8.90 5.60 4.15	< 0.001 < 0.001 < 0.001	Occipital pole Precentral gyrus Precentral gyrus
Win – Loss	-10 10 -8	8.19	< 0.001	Left NAcc
Loss – Neutral	8 16 66 30 20 -12 -30 18 -12 -14 -92 -4 12 8 10 52 6 46 0 -20 44	7.26 6.98 7.71 7.54 6.00 5.08 4.16	< 0.001 < 0.001 < 0.001 < 0.001 < 0.001 < 0.05 < 0.05	Superior frontal gyrus Frontal orbital cortex Frontal orbital cortex Occipital pole Right caudate Precentral gyrus CC – posterior
Loss – Win	44 28 -4 8 14 66 -28 18 -12 54 -26 -8	4.92 5.89 5.24 4.95	< 0.001 < 0.001 < 0.001 < 0.05	Frontal orbital cortex Superior frontal gyrus Frontal orbital cortex Posterior middle temporal gyrus
Win*	-10 -98 2	10.1	< 0.001	Occipital pole
Neutral*	28 -68 -10 46 32 22 38 20 -4 22 -28 -2	9.58 6.39 6.82 6.39	< 0.001 < 0.001 < 0.001 < 0.05	Occipital fusiform gyrus Middle frontal gyrus Insular cortex Right thalamus
Loss*	28 -70 -10 -28 18 -12 6 38 42	9.33 7.93 6.07	< 0.001 < 0.001 < 0.001	Occipital fusiform gyrus Frontal orbital cortex Superior frontal gyrus
No Win in Win*	14 -98 8 46 34 22 -56 -32 2	9.46 6.03 5.90	< 0.001 < 0.001 < 0.001	Occipital pole Middle frontal gyrus Posterior superior temporal gyrus
No Loss in Loss*	14 -98 8 46 32 20 -54 -40 6 36 20 4	9.30 6.25 5.83 6.37	< 0.001 < 0.001 < 0.001 < 0.001	Occipital pole Middle frontal gyrus Posterior superior temporal gyrus Insular cortex

***Relative to an implicit baseline**

4.3.3.2.1 Anticipation of Win or Loss

4.3.3.2.1.1 Whole Brain Analysis

Comparison of the MDD and HC groups at the whole brain level at baseline did not reveal any group differences in brain activity in response to the anticipation of a win or loss outcome.

4.3.3.2.1.2 SVC Analysis

The SVC analysis did reveal baseline group differences in the anticipation of win versus loss outcomes, with the MDD group displaying significantly less activity in the right lateral OFC (x y z = 30 38 -8, Z-max = 3.24, *p*-corrected < 0.05) during anticipation of win versus loss outcomes. However, extraction of percentage signal change found this to be driven by 3 MDD participants with particularly low right lateral OFC activity (Figure 4.12).

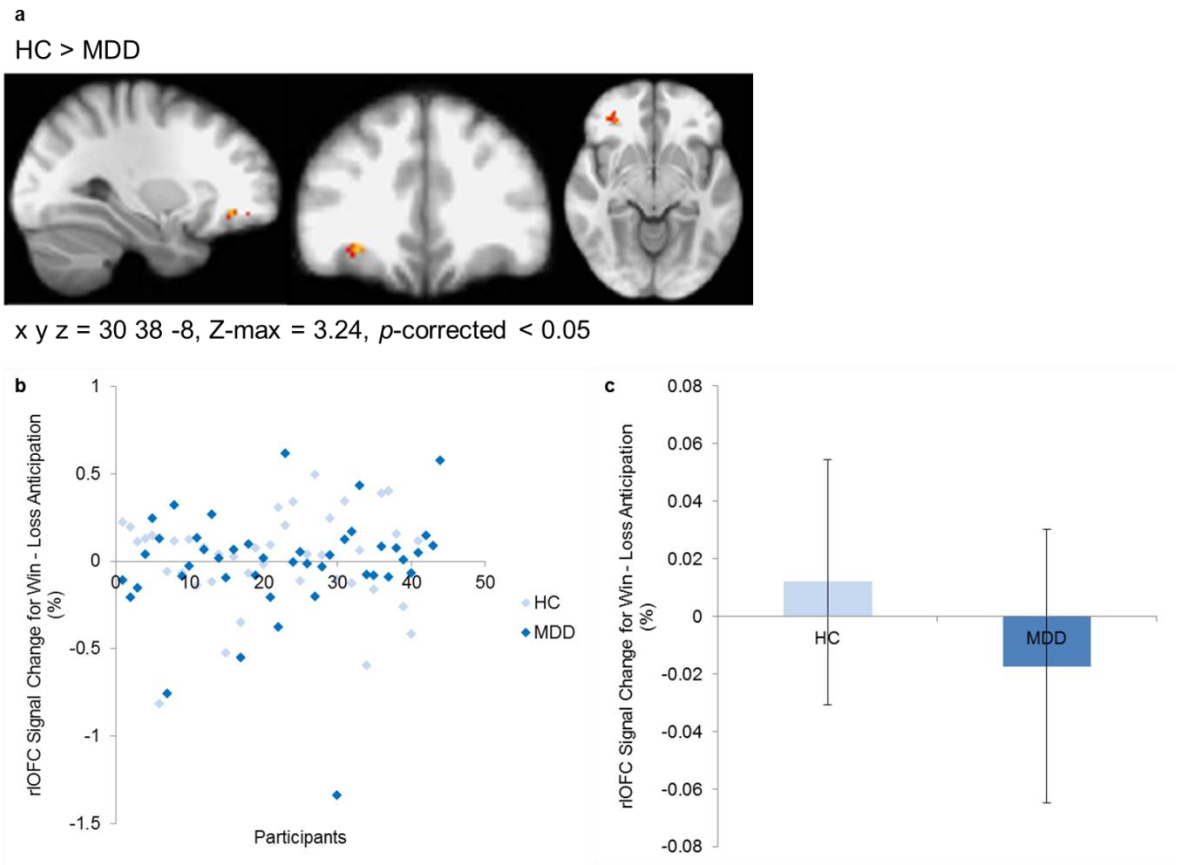


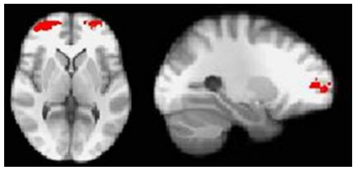
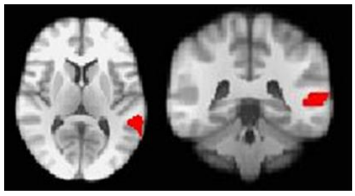
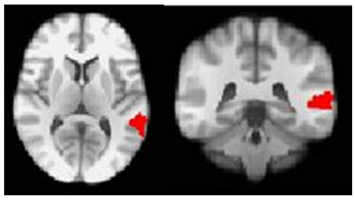
Figure 4.12 – Baseline Right Lateral OFC Activity in Response to Win – Loss Anticipation: (a) SVC analysis demonstrating reduced activity in the right lateral OFC of MDD participants compared to HCs during anticipation of win versus loss outcomes. Extracted percentage signal change in the right lateral OFC in response to anticipation of win versus loss outcomes for each participant group in **(b)** scatter graph form showing individual values for each participant or **(c)** bar chart form reporting means ± standard error of the mean.

4.3.3.2.2 Win or Loss Outcome

4.3.3.2.2.1 Whole Brain Analysis

Comparison of the MDD and HC groups at the whole brain level at baseline revealed that the MDD group displayed an overall reduction in brain activity in response to positive outcomes during reward processing. More specifically, there was an indication that the MDD group displayed reduced activity in the right VS in response to win versus neutral outcomes, which is further explored in the SVC analysis below. The MDD group also displayed significantly less activity in the bilateral frontal pole (x y z = -26 58 2, Z-max = 4.10, *p*-corrected < 0.05; x y z = 42 52 4, Z-max = 3.44, *p*-corrected < 0.05) in response to a win outcome and in the posterior superior temporal gyrus (x y z = -60 -40 8, Z-max = 3.76, *p*-corrected < 0.05) in response to a neutral outcome, derived mainly from a no loss in loss outcome (x y z = -60 -40 8, Z-max = 3.51, *p*-corrected < 0.01). A positive (win and no loss in loss) versus negative (loss and no win in win) outcome contrast revealed significantly less activity in the inferior frontal gyrus (x y z = 48 32 2, Z-max = 3.92, *p*-corrected < 0.05) in the MDD group (Table 4.10).

Table 4.10 Whole Brain Analysis of Baseline Group Differences for the Reward Task

Lower-level Contrast	Direction of Group Comparison	x y z	Z-max	Corrected-*p*	Image
Win *	HC > MDD	-26 58 2 42 52 4	4.10 3.44	< 0.05 < 0.05	Bilateral frontal pole 
Neutral *	HC > MDD	-60 -40 8	3.76	< 0.05	Posterior superior temporal gyrus 
No Loss in Loss *	HC > MDD	-60 -40 8	3.51	< 0.01	Posterior superior temporal gyrus 
Positive (win and no loss in loss) – Negative (loss and no win in win)	HC > MDD	48 32 2	3.92	< 0.05	Inferior frontal gyrus 

*Relative to an implicit baseline

4.3.3.2.2.2 SVC Analysis

The SVC analysis revealed that the MDD group did display significantly reduced activity in the right VS in response to win versus neutral outcomes compared to the HC group (x y z = 28 0 2, Z-max = 3.30, *p*-corrected < 0.05) (Figure 4.13).

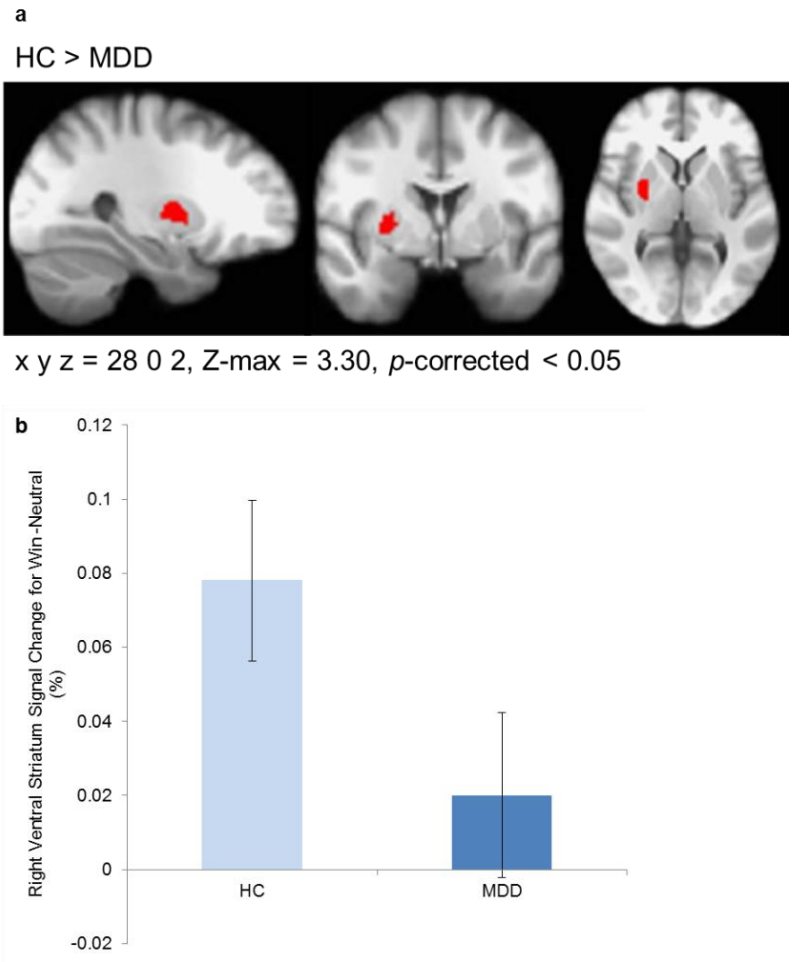


Figure 4.13 - Baseline Right Ventral Striatum Activity in Response to Win – Neutral Outcomes: (a) SVC analysis demonstrating reduced activity in the right VS of MDD participants compared to HCs in response to win versus neutral outcomes. **(b)** Extracted percentage signal change in the right VS in response to win versus neutral outcomes for each participant group. Values are reported as means ± standard error of the mean.

The SVC analysis also found reduced activity in MDD participants in response to a neutral outcome (x y z = 38 46 -18, Z-max = 3.14, *p*-corrected < 0.05), again derived mainly from a no loss in loss outcome (x y z = 38 46 -18, Z-max = 3.18, *p*-corrected < 0.05), similar to the whole brain analysis above, but in the right lateral OFC, which was this time corroborated by the extracted percentage signal change (Figure 4.14).

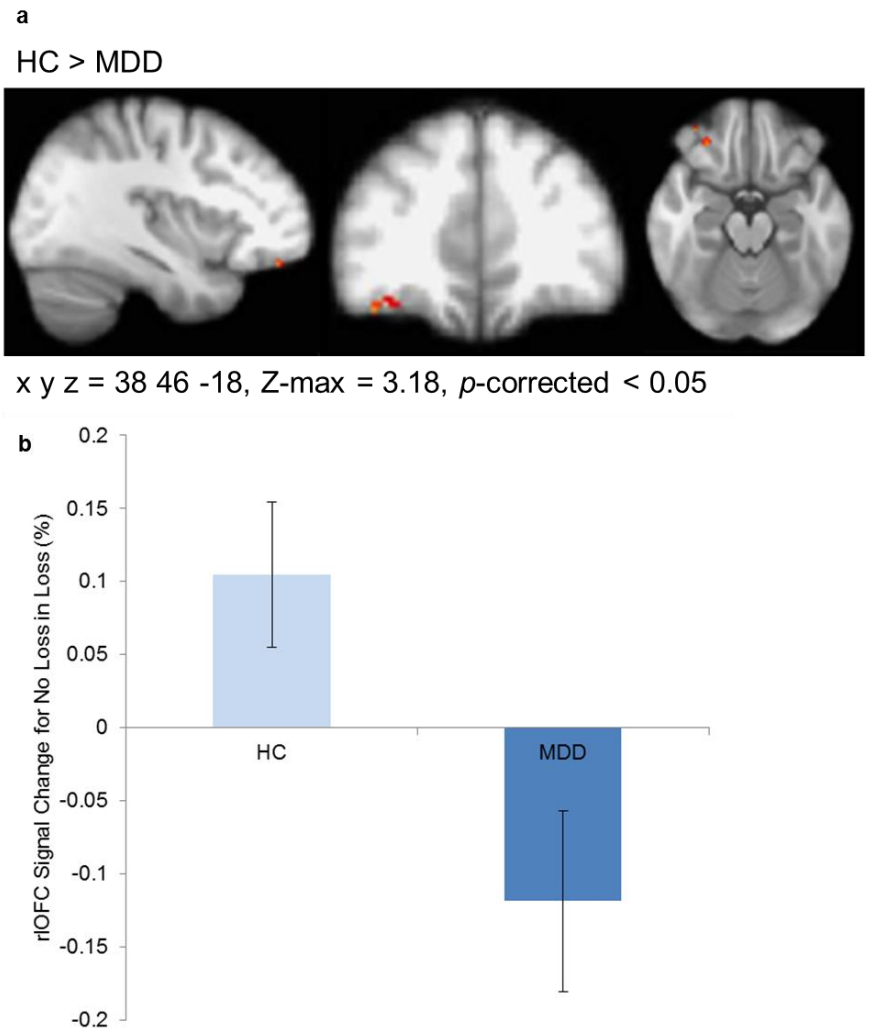


Figure 4.14 - Baseline Right Lateral OFC Activity in Response to No Loss in Loss Outcomes: (a) SVC analysis demonstrating reduced activity in the right lateral OFC of MDD participants compared to HCs in response to no loss in loss outcomes. **(b)** Extracted percentage signal change in the right lateral OFC in response to no loss in loss outcomes for each participant group. Values are reported as means ± standard error of the mean.

Finally, there was also evidence for altered brain activity in response to negative outcomes in the MDD group. Specifically, the MDD group displayed significantly reduced left amygdala activity in response to loss versus neutral outcomes compared to the HC group (x y z = -26 -6 -20, Z-max = 3.11, *p*-corrected < 0.01) (Figure 4.15).

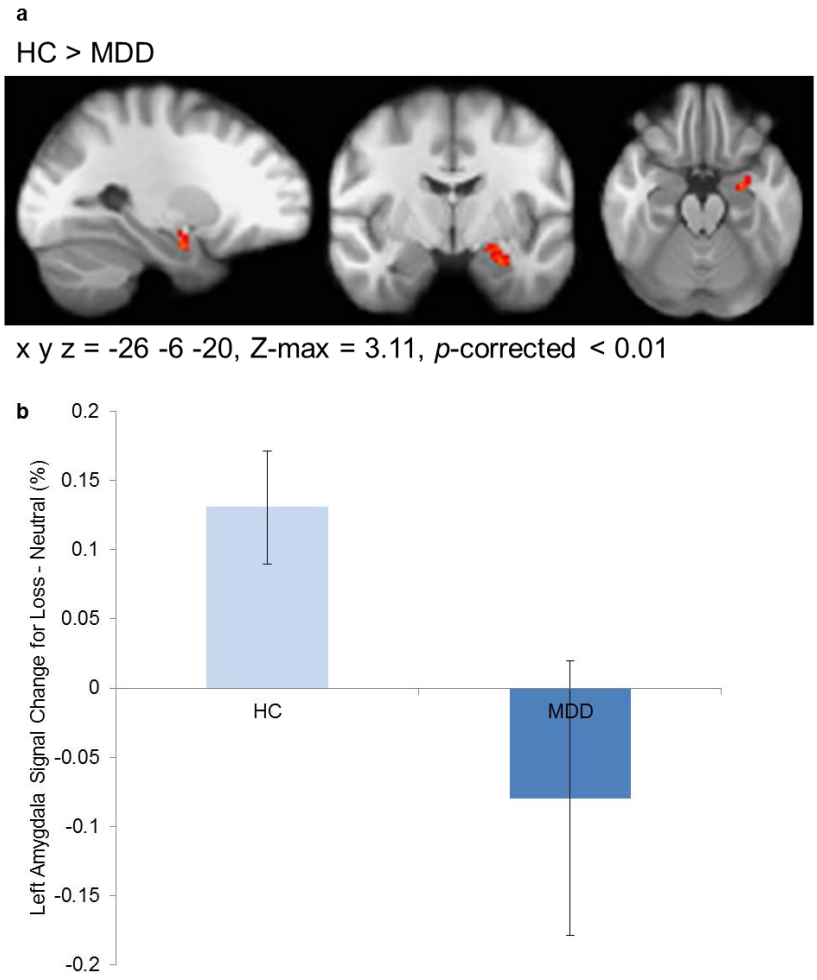


Figure 4.15 – Baseline Left Amygdala Activity in Response to Loss – Neutral Outcomes: (a) SVC analysis demonstrating reduced activity in the left amygdala of MDD participants compared to HCs in response to loss versus neutral outcomes. **(b)** Extracted percentage signal change in the left amygdala in response to loss versus neutral outcomes for each participant group. Values are reported as means ± standard error of the mean.

4.3.4 Summary

This Chapter aimed to investigate whether MDD patients display dissociable abnormalities in behavioural and neural measures of reward and emotional processing at baseline prior to treatment compared to the HC group.

Regarding the behavioural measures, the MDD group displayed a response bias towards sad faces, misclassifying significantly more faces as sad in the FERT, and were

significantly less vigilant of happy faces in the FDOT compared to the HC group. The MDD group also displayed an overall non-valence specific slowing of reaction times across all tasks of the ETB, consistent with psychomotor slowing observed in MDD. Few behavioural group differences were found for the covert faces and reward task, apart from a possible insensitivity to high-probability win found in post hoc tests in the absence of a significant participant group interaction. However, these tasks did reveal overall greater brain activity in response to negative outcomes and reduced brain activity in response to positive outcomes in the MDD group. This included greater activity in the occipital cortex in response to sad faces and reduced activity in the right VS in response to win versus neutral outcomes and in the right lateral OFC in response to no loss in loss outcomes. An exception to this general pattern was reduced activity in the left amygdala in response to loss versus neutral outcomes in the MDD group.

4.3.5 Discussion

Previous research has shown that MDD patients display baseline abnormalities in behavioural and neural measures of emotional and reward processing. Specifically, it has been shown that MDD patients display negative affective biases with amygdala hyperactivity to negative affective stimuli (Fitzgerald et al., 2008) and aberrant processing of reward or other positive stimuli with striatal hypoactivity to reward (Knutson et al., 2008, Pizzagalli et al., 2009, McCabe et al., 2010, McCabe et al., 2012).

4.3.5.1 Baseline Negative Affective Biases

Many studies have shown that MDD patients display negative affective biases across a range of cognitive domains, including perception, attention and memory. MDD patients display an inclination to interpret ambiguous information as negative, preferentially attend to negative affective stimuli and better recall negative information (Mathews and MacLeod, 2005, Leppanen, 2006, Elliott et al., 2011, Roiser et al., 2012). For example, during facial

expression recognition tasks, MDD patients are more sensitive to sad faces, showing increased accuracy at recognising sad faces at lower sad intensities, and have a greater response bias towards sad faces, interpreting ambiguous or neutral facial expressions as sad (Gur et al., 1992, Bouhuys et al., 1999, Gilboa-Schechtman et al., 2002). The same effect was also found in this Chapter with the MDD group displaying a response bias towards sad faces, misclassifying significantly more faces as sad.

Neuroimaging studies have indicated that hyperactivity in various brain regions in response to negative affective stimuli may underlie these negative affective biases. These brain regions include the occipital or extrastriate cortex, involved in the processing of specific features of visual information, the fusiform gyrus, involved in the recognition of visual information and frontal regions, involved in the higher-order control of emotional processing (Lawrence et al., 2004, Surguladze et al., 2005). The MDD group was found to display significantly greater activity in the occipital cortex to sad faces compared to the HC group, suggesting a processing bias for sad visual information. There is evidence to suggest that increased occipital cortex activity in response to negative affective stimuli may involve modulatory influence from the amygdala (Vuilleumier et al., 2004).

Some have also reported hyperactivity in the amygdala itself in response to negative facial expressions in particular (Sheline et al., 2001, Fu et al., 2004, Surguladze et al., 2005, Fu et al., 2008, Victor et al., 2010). No group differences in amygdala activity were found for the covert faces task; however, the MDD group was found to display reduced left amygdala activity in response to loss versus neutral outcomes during the reward task. Rather than a heightened emotional reaction to a loss outcome, this amygdala hypoactivity may be more related to the function of the amygdala in associative learning (Li et al., 2011), with reduced associative learning about losses during the reward task.

4.3.5.2 Baseline Aberrant Processing of Positive Stimuli & Reward

Facial expression recognition tasks have also found MDD patients to display reduced sensitivity for happy facial expressions, requiring higher intensities to accurately recognise

happy faces (Joormann and Gotlib, 2006, Coupland et al., 2004). Whilst this Chapter did not find any group differences in the accuracy for recognising happy faces, the MDD group did display significantly reduced attentional vigilance for happy faces on the FDOT compared to the HC group.

This Chapter also found possible reduced sensitivity to high-probability win in the MDD group, that is, reduced frequency of choosing a stimulus associated with a high probability of winning £1. This is consistent with previous research showing abnormal response selection by MDD patients during probabilistic instrumental learning tasks. In such tasks, healthy controls typically develop a reward response bias, learning to choose the stimulus associated with high-probability win and avoid the stimulus associated with high-probability loss in order to maximise payoffs. It is observed that MDD patients fail to develop a reward response bias resulting in reduced frequency of choosing the high-probability win (Henriques and Davidson, 2000, Pizzagalli et al., 2008, Vrieze et al., 2013). Such reduced sensitivity to reward may be the result of hypoactivity in the VS of MDD patients, which was also reported in this study.

Overall, the results of this Chapter are consistent with well-established previous research, with the MDD group displaying many of the expected baseline abnormalities in behavioural and neural measures of emotional and reward processing compared to the HC group.

CHAPTER 5

LINKING BASELINE REWARD AND EMOTIONAL PROCESSING TO BASELINE SYMPTOMS IN MDD

In section 4.3, the baseline abnormalities in the behavioural and neural measures of reward and emotional processing in the MDD group compared to the HC group were discussed. It was found that at baseline prior to any treatment, the MDD group displayed a response bias towards sad faces, misclassifying significantly more faces as sad in the FERT, and were significantly less vigilant of happy faces in the FDOT. Whilst few baseline behavioural abnormalities were found for the covert faces and reward tasks, underlying brain activity was greater in response to negative outcomes and reduced in response to positive outcomes. For example, the MDD group displayed greater activity in the occipital cortex to sad faces and reduced activity in the right VS in response to win versus neutral outcomes and in the right lateral OFC in response to no loss in loss outcomes.

Previous research has found behavioural and neural abnormalities in MDD to correlate with symptom severity. In particular, behavioural and neural abnormalities in reward processing in MDD have been found to correlate specifically with severity of self-reported anhedonia, independently of overall depression severity (Keedwell et al., 2005, Epstein et al., 2006, Steele et al., 2007, Chase et al., 2010, Vrieze et al., 2013). In addition, the severity of the baseline abnormality has been found to predict treatment outcome (Forbes et al., 2010, Vrieze et al., 2013). Therefore, this Chapter aims to investigate whether baseline subjective experiences of anhedonia and negative affect in MDD relate to dissociable baseline abnormalities in behavioural and neural measures of reward and emotional processing, with a focus on the baseline abnormalities found in the previous Chapter. It is hypothesised that the baseline abnormalities in the behavioural and neural measures of reward and emotional processing in the MDD group correlate with the baseline scores on questionnaires measuring anhedonia and negative affect.

5.1 Do the Baseline Abnormalities in Behavioural Measures of Reward and Emotional Processing Relate to Baseline Symptoms?

The following sections investigate the relationship between baseline symptoms and baseline abnormalities in behavioural measures of reward and emotional processing found in the previous Chapter through the use of correlations with questionnaire scores.

5.1.1 Correlating Behavioural Measures with Questionnaire Scores

The data for the baseline abnormalities in behavioural measures of reward and emotional processing was correlated with the baseline questionnaire scores and the results are discussed in the following sections that consider baseline symptom severity and questionnaires measuring the negative affect and anhedonia symptom clusters. Given the number of correlations performed for the behavioural and questionnaire measures, it is important to highlight the problem of multiple comparisons. A higher significance threshold is general required to compensate for the number of tests being performed; however, since this is a more exploratory analysis, the correlations were not corrected for multiple comparisons.

5.1.1.1 Baseline Symptom Severity

As discussed in Section 4.1.1, the HAM-D is typically used to assess baseline symptom severity. Baseline HAM-D score was not found to correlate with any of the baseline behavioural abnormalities (Figure 5.1). When investigating the remaining behavioural measures not found to differ from HCs at baseline in the previous Chapter, there did seem to be a slight negative correlation between baseline HAM-D score and percentage accuracy for happy faces in the FERT; however, this was not found to be significant ($r_{45} = -0.26$, $p = 0.09$) (Figure 5.1e).

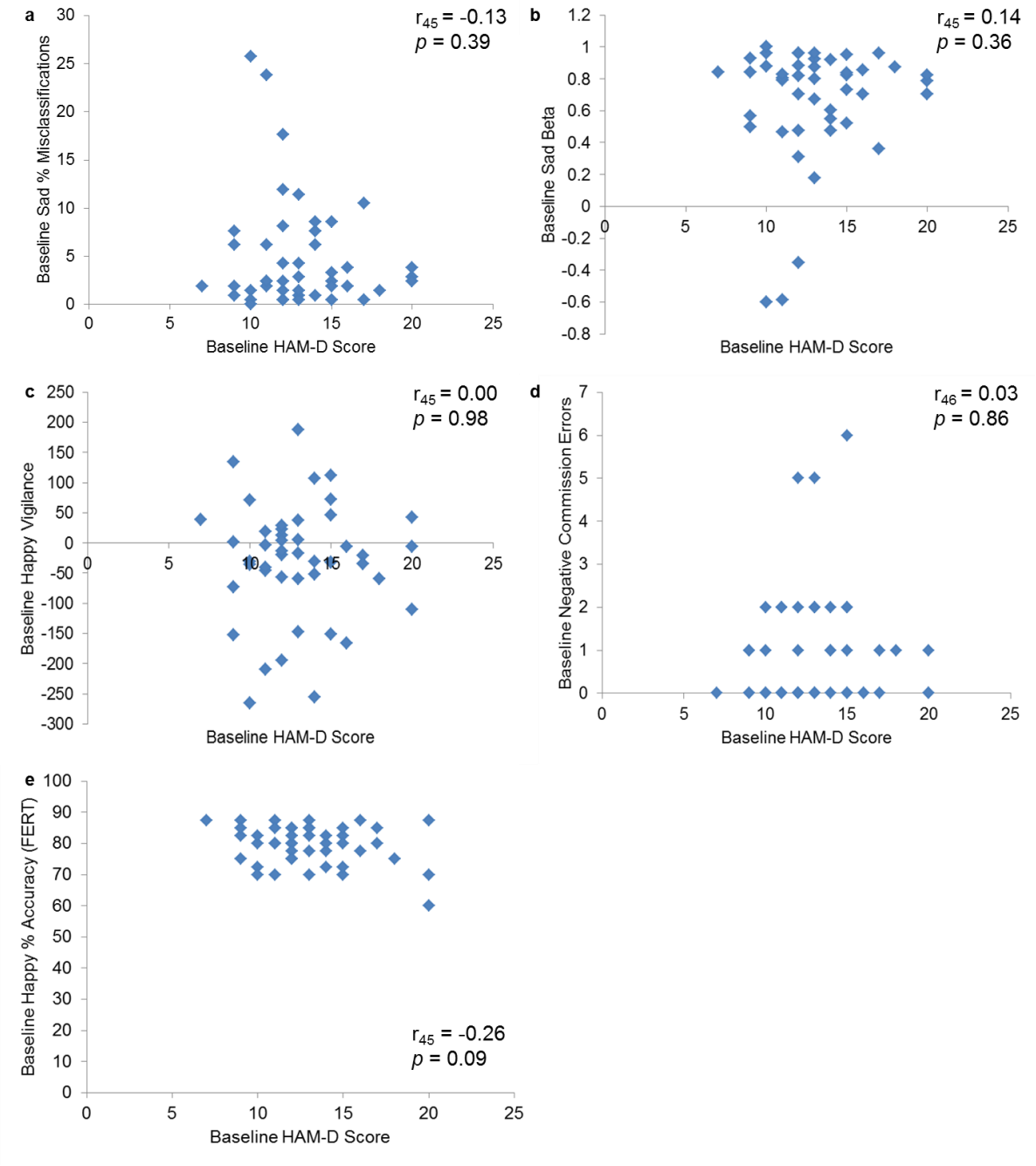


Figure 5.1 – Correlating the Baseline Behavioural Abnormalities with Baseline HAM-D Score: correlations of the baseline HAM-D score with the baseline **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces, **(d)** number of negative commission errors made and **(e)** % accuracy for happy faces in the FERT.

5.1.1.2 Negative Affect

Other questionnaires assessing mood and negative affect included the PANAS and the MASQ general distress: depressive sub-scale. Again, none of the baseline behavioural abnormalities were found to correlate with the baseline score of MASQ general distress: depressive (Figure 5.2), or PANAS negative (Figure 5.3) or positive (Figure 5.4) affect. However, investigation of other behavioural measures did reveal a significant negative correlation between baseline MASQ general distress: depressive score and number of positive self-referent words falsely recalled ($r_{46} = -0.33$, $p < 0.05$) (Figure 5.2e) and baseline PANAS negative affect was found to negatively correlate with both percentage accuracy ($r_{45} = -0.30$, $p < 0.05$) and d' ($r_{45} = -0.34$, $p < 0.05$) for sad faces (Figure 5.3e and f).

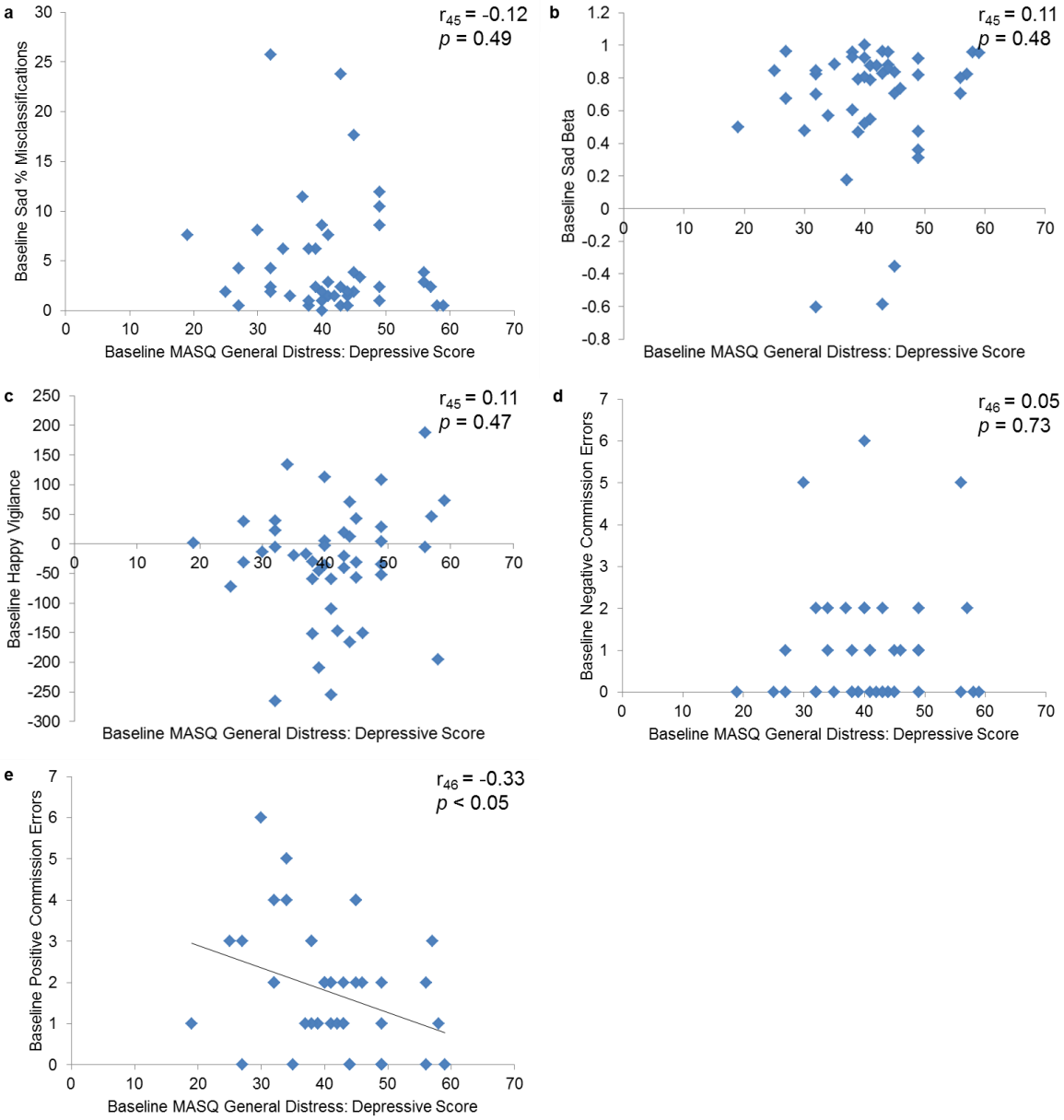


Figure 5.2 – Correlating the Baseline Behavioural Abnormalities with Baseline MASQ General Distress: Depressive Score: correlations of the baseline MASQ general distress: depressive score with the baseline **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces, **(d)** number of negative commission errors made and **(e)** number of positive commission errors made.

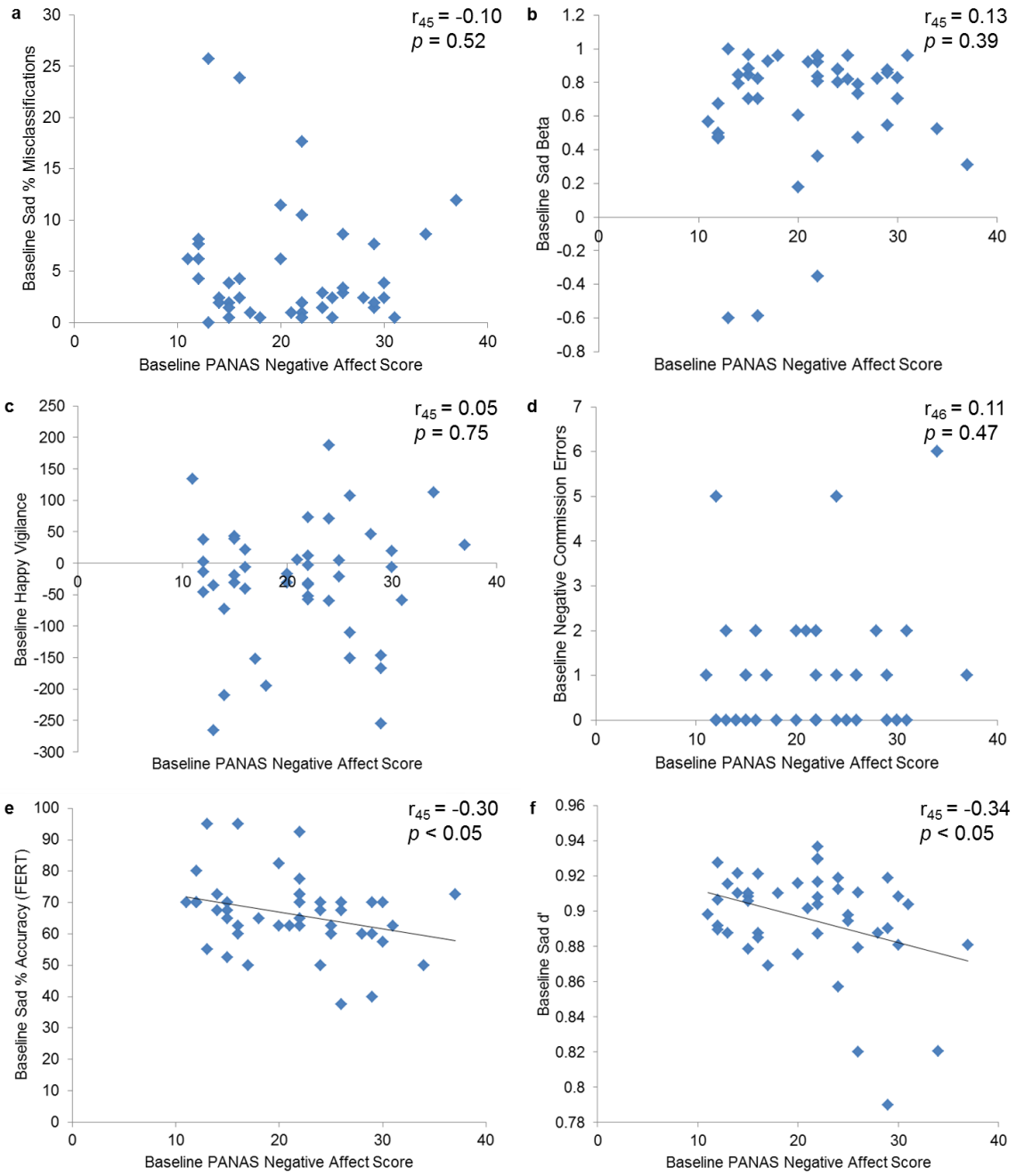


Figure 5.3 – Correlating the Baseline Behavioural Abnormalities with Baseline PANAS Negative Affect Score: correlations of the baseline PANAS negative affect score with the baseline **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces, **(d)** number of negative commission errors made, **(e)** % accuracy for sad faces and **(f)** d' for sad faces.

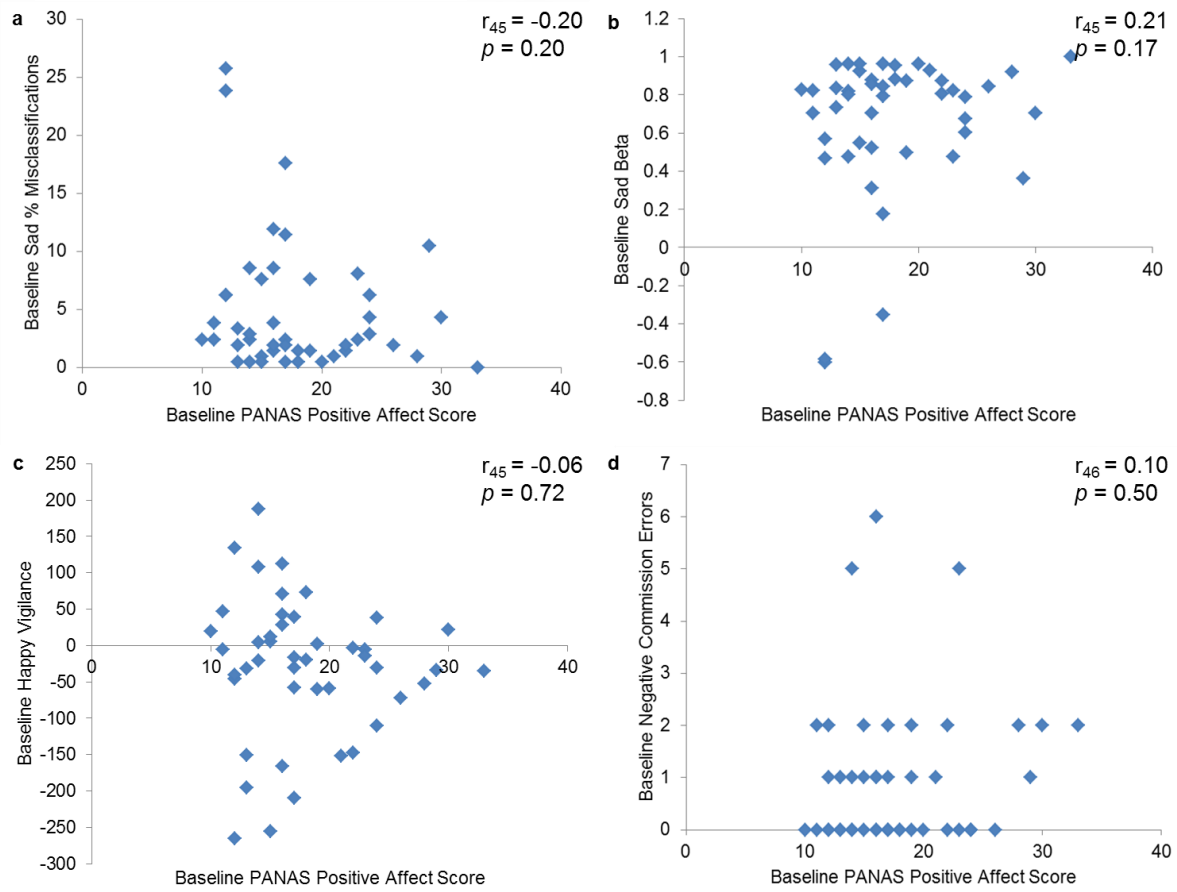


Figure 5.4 – Correlating the Baseline Behavioural Abnormalities with Baseline PANAS Positive Affect Score: correlations of the baseline PANAS positive affect score with the baseline **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces, and **(d)** number of negative commission errors made.

5.1.1.3 Anhedonia

Baseline levels of anhedonia were measured using the SHAPS and the anhedonic depression sub-scale of the MASQ. Again, none of the baseline behavioural abnormalities were found to correlate with the baseline score of the SHAPS (Figure 5.5) or MASQ anhedonic depression (Figure 5.6). However, similarly to the MASQ general distress: depressive score, baseline MASQ anhedonic depression negatively correlated with the number of positive self-referent words falsely recalled (r_{46} = -0.35, $p < 0.05$) (Figure 5.6e).

Given the a priori hypothesis that anhedonia may be related to aberrant reward processing, the relationship between baseline anhedonia and winnings on the reward task was also investigated. Greater end totals did appear to relate to lower baseline MASQ anhedonic depression scores; however, this was not found to be significant (r_{46} = -0.25, p = 0.09) (Figure 5.6f).

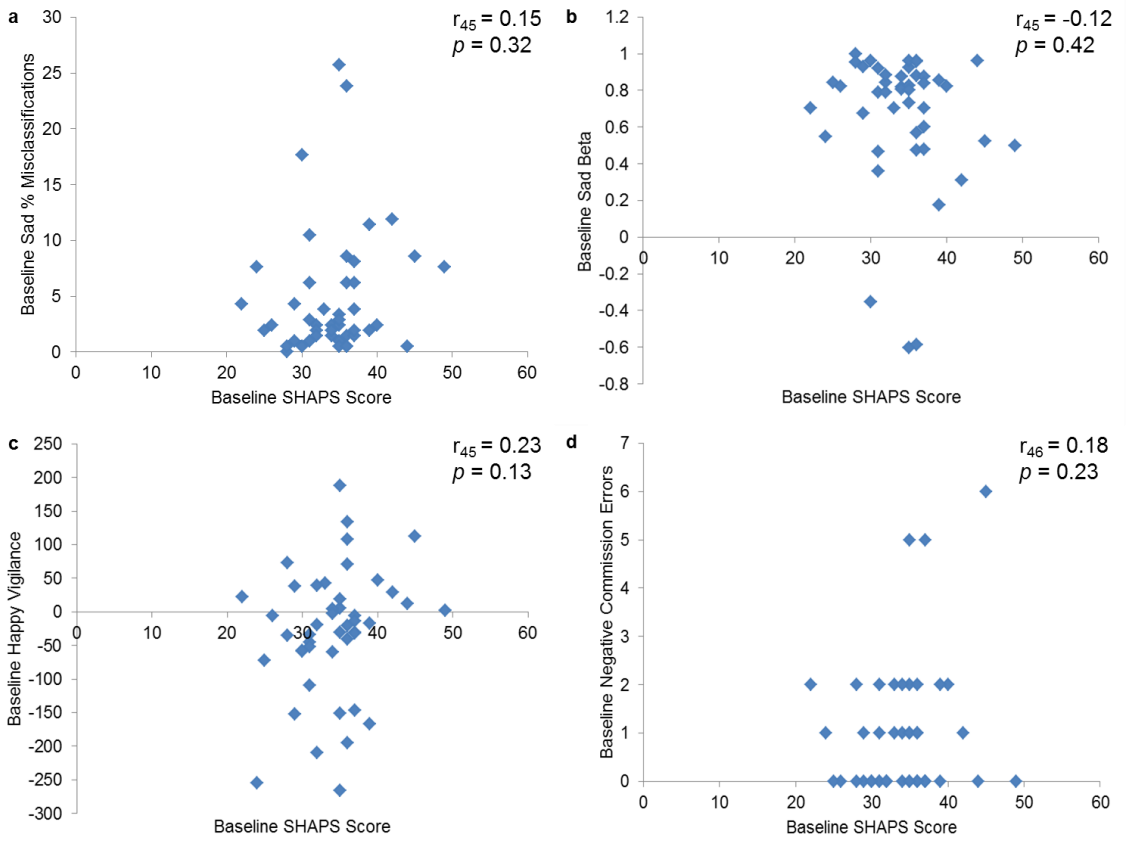


Figure 5.5 – Correlating the Baseline Behavioural Abnormalities with Baseline SHAPS Score: correlations of the baseline SHAPS score with the baseline **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces and **(d)** number of negative commission errors made.

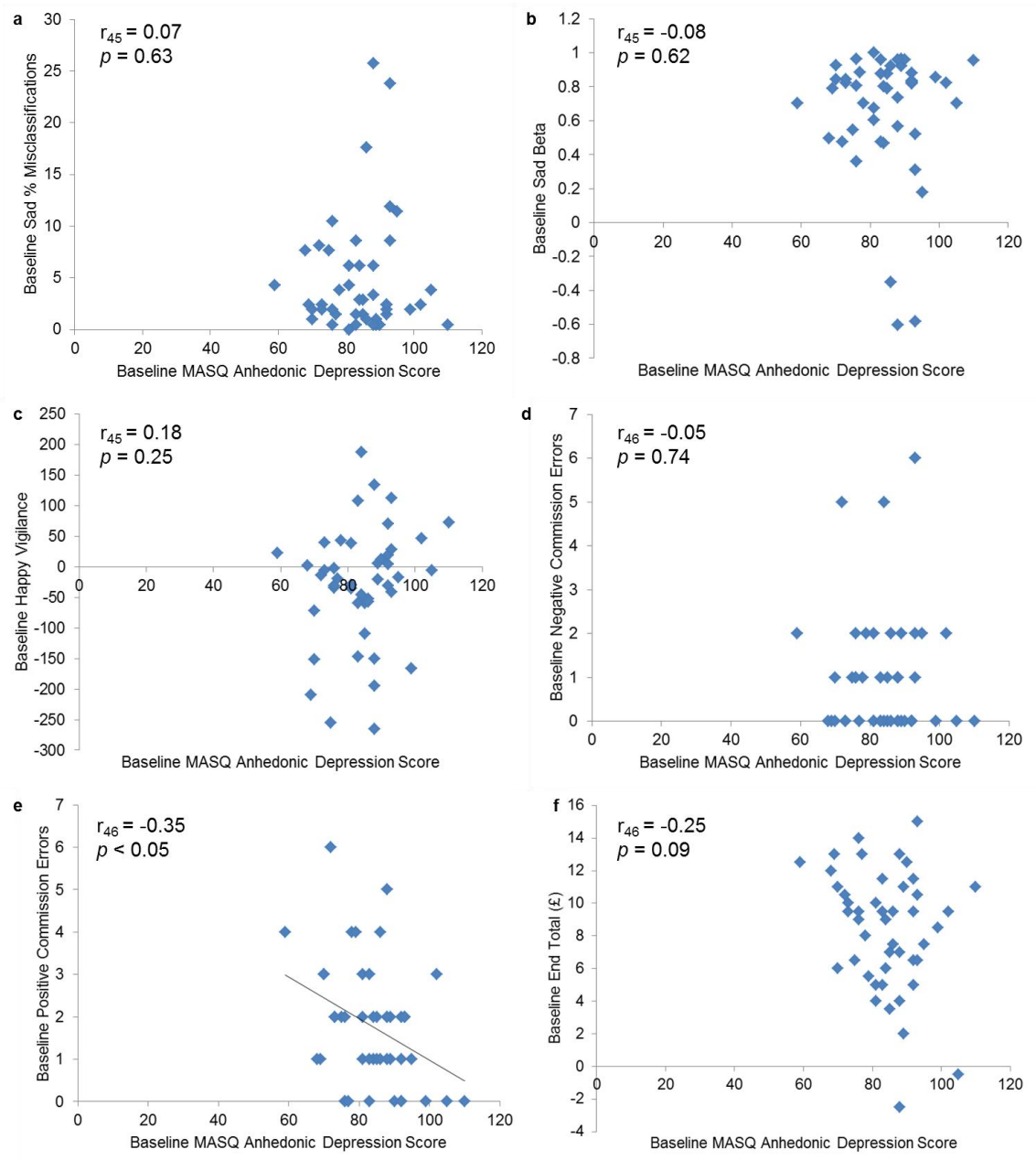


Figure 5.5 – Correlating the Baseline Behavioural Abnormalities with Baseline MASQ Anhedonic Depression Score: correlations of the baseline MASQ anhedonic depression score with the baseline **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces, **(d)** number of negative commission errors made, **(e)** number of positive commission errors made and **(f)** end total.

5.2 Do the Baseline Abnormalities in Neural Measures of Reward and Emotional Processing Relate to Baseline Symptoms?

5.2.1. Correlating Neural Measures with Questionnaire Scores

Contrast maps derived from the first-level analysis of MDD participants were inputted into a second-level analysis along with demeaned baseline questionnaire scores. Significant clusters indicated an association between baseline brain activity and symptoms and are discussed in the following sections that consider baseline symptom severity, negative affect and anhedonia.

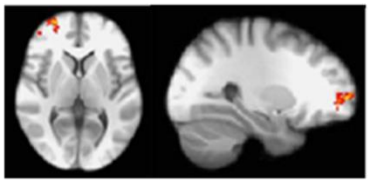
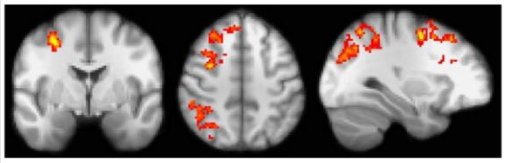
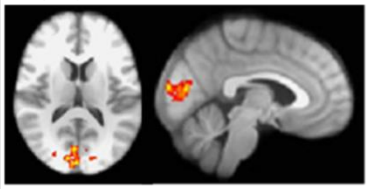
5.2.1.1 Covert Faces Task

5.2.1.1.1 Baseline Symptom Severity

5.2.1.1.1.1 Whole Brain Analysis

The whole brain analysis revealed an association between baseline symptom severity measured with the HAM-D and brain activity in response to happy faces. Baseline HAM-D score was found to negatively correlate with activity in the frontal pole (x y z = 28 62 4, Z-max = 4.00, *p*-corrected < 0.05) in response to happy faces. Baseline HAM-D score was also found to negatively correlate with activity in the middle frontal (x y z = 32 -2 50, Z-max = 4.00, *p*-corrected < 0.001) and angular (x y z = 42 -52 46, Z-max = 3.98, *p*-corrected < 0.001) gyri in response to happy versus sad faces and in the occipital pole (x y z = 4 -92 14, Z-max = 3.36, *p*-corrected < 0.001) in response to happy versus fearful faces. Overall, it appears the greater the baseline HAM-D score, the lower the brain activity to happy faces (Table 5.1).

Table 5.1 Whole Brain Analysis of the Relationship between Baseline Brain Activity for the Covert Faces Task and Baseline HAM-D Score

Lower-level Contrast	Correlation	x y z	Z-max	Corrected-*p*	Image
Happy	Negative	28 62 4	4.00	< 0.05	Frontal pole 
Happy – Sad	Negative	32 -2 50	4.00	< 0.001	Middle frontal gyrus 
		42 -52 46	3.98	< 0.001	Angular gyrus
Happy – Fear	Negative	4 -92 14	3.36	< 0.001	Occipital pole 

5.2.1.1.1.2 SVC Analysis

The SVC analysis confirmed an association between baseline brain activity in response to happy versus fearful faces and baseline HAM-D score. Specifically, baseline HAM-D score was found to negatively correlate with right lateral OFC (x y z = 34 44 -16, Z-max = 3.51, *p*-corrected < 0.05) and left amygdala (x y z = -28 -4 -20, Z-max = 3.19, *p*-corrected < 0.05) activity in response to happy versus fearful faces. The SVC analysis also found baseline HAM-D score to positively correlate with bilateral amygdala (x y z = 28 2 -18, Z-max = 3.19, *p*-corrected < 0.05; x y z = -28 -2 -28, Z-max = 2.94, *p*-corrected < 0.05) activity in response to fearful versus sad faces (Figure 5.6).

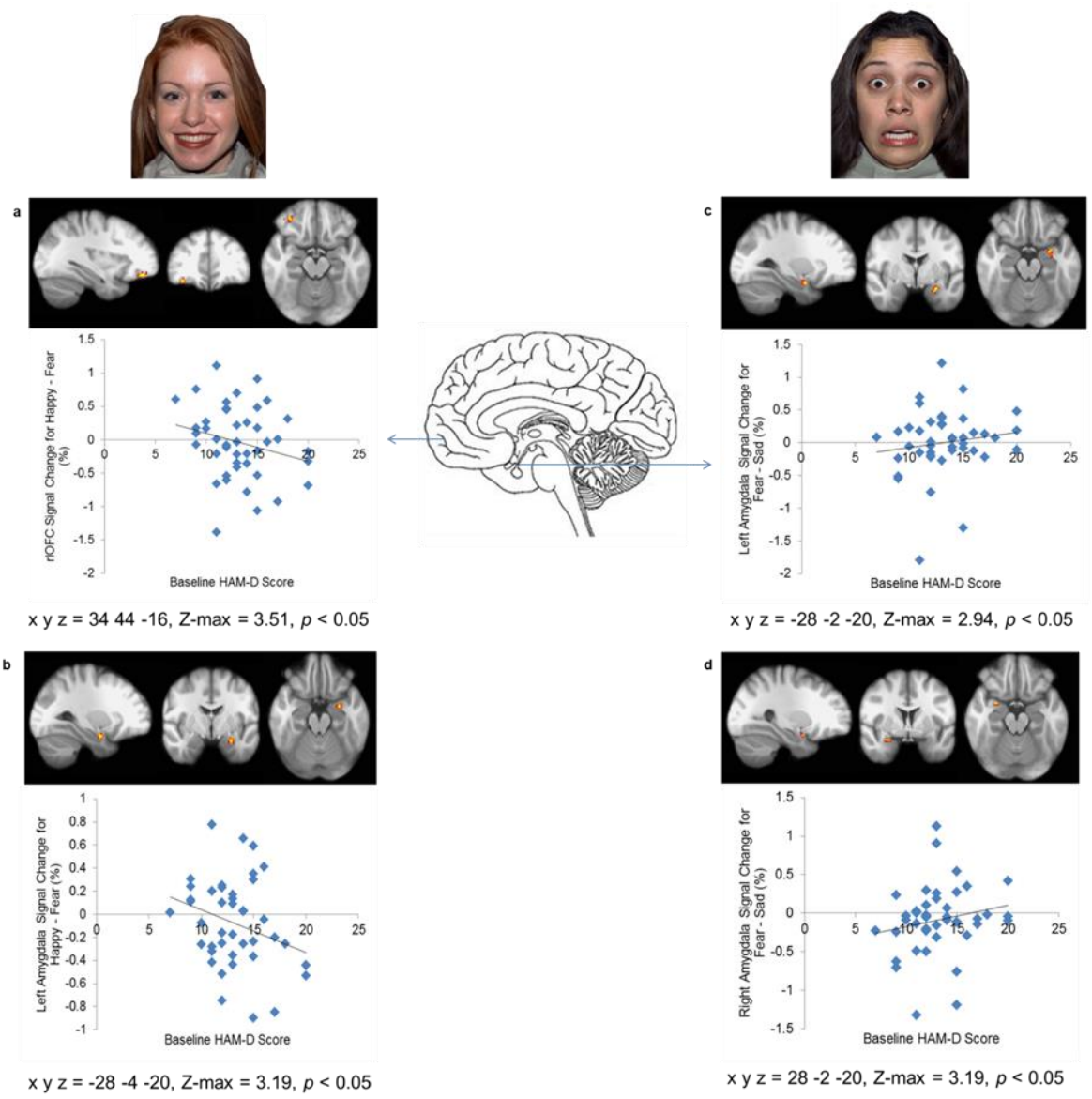


Figure 5.6 – Correlating HAM-D Score with Emotional Neural Activity: with greater baseline symptom severity as measured with the HAM-D, there is lower activity in response to happy versus fearful faces in the **(a)** right lateral OFC and **(b)** left amygdala and higher activity in response to fearful versus sad faces in the **(c-d)** bilateral amygdala.

5.2.1.1.2 Negative Affect

5.2.1.1.2.1 Whole Brain Analysis

Many correlations were found in the whole brain analysis of the relationship between questionnaires measuring negative affect and brain activity during the covert faces task at baseline and are summarised in Table 5.2. The general trend was a negative correlation between baseline negative affect and brain activity in response to all three different emotional facial expressions, that is, the greater the negative affect, the lower the brain activity in response to happy, sad and fearful faces.

Table 5.2 Whole Brain Analysis of the Relationship between Baseline Brain Activity for the Covert Faces Task and Negative Affect

Lower-level Contrast	Correlation	x y z	Z-max	Corrected-*p*	Image
MASQ General Distress: Depressive					
Happy	Negative	2 -46 -22 -2 34 38 -50 -72 8 -32 54 -6 2 -26 32	4.25 4.31 4.20 3.99 3.50	< 0.001 < 0.001 < 0.01 < 0.05 < 0.05	Cerebellum, brainstem Paracingulate gyrus Inferior lateral occipital cortex Frontal pole Posterior cingulate gyrus
Sad	Negative	-56 -24 -12 -52 -74 4 54 -28 12	4.03 4.63 3.88	< 0.001 < 0.001 < 0.001	Posterior middle temporal gyrus Inferior lateral occipital cortex Planum temporale
Fear	Negative	-30 52 -6 -6 32 52 46 26 40 -52 -72 -12	4.53 4.03 3.81 4.80	< 0.001 < 0.01 < 0.05 < 0.05	Frontal pole Superior frontal gyrus Middle frontal gyrus Inferior lateral occipital cortex
PANAS Negative Affect					
Happy	Negative	-48 -72 10	3.49	< 0.01	Inferior lateral occipital cortex
Sad	Negative	16 -84 6 0 -14 48	3.76 3.46	< 0.01 < 0.01	Intracalcarine cortex Juxtapositional lobule cortex
Fear	Negative	-30 -34 50 -58 -22 -12 -60 -60 34 -24 -62 20 -36 22 44	4.38 4.75 4.18 3.60 4.02	< 0.001 < 0.001 < 0.001 < 0.05 < 0.05	Postcentral gyrus Posterior middle temporal gyrus Superior lateral occipital cortex Supracalcarine cortex Middle frontal gyrus
PANAS Positive Affect					
Happy	Positive	-40 -58 -14	3.61	< 0.05	Temporal occipital fusiform cortex
Sad	Positive	-14 -76 -22	3.88	< 0.001	Cerebellum
Fear	Positive	-28 -74 -26 32 16 54 -14 38 58	4.16 4.14 3.52	< 0.001 < 0.001 < 0.05	Cerebellum Middle frontal gyrus Frontal pole

5.2.1.1.2.2 SVC Analysis

The SVC analysis also revealed a general trend for a negative correlation between baseline negative affect and brain activity in response to happy (Figure 5.7), sad (Figure 5.8) and fearful (Figure 5.9) faces, with the exception of the right medial OFC specifically in response to fearful faces (x y z = 24 32 -16, Z-max = 3.15, *p*-corrected < 0.05), which was found to positively correlate with negative affect measured by the MASQ general distress: depressive (Figure 5.9c).

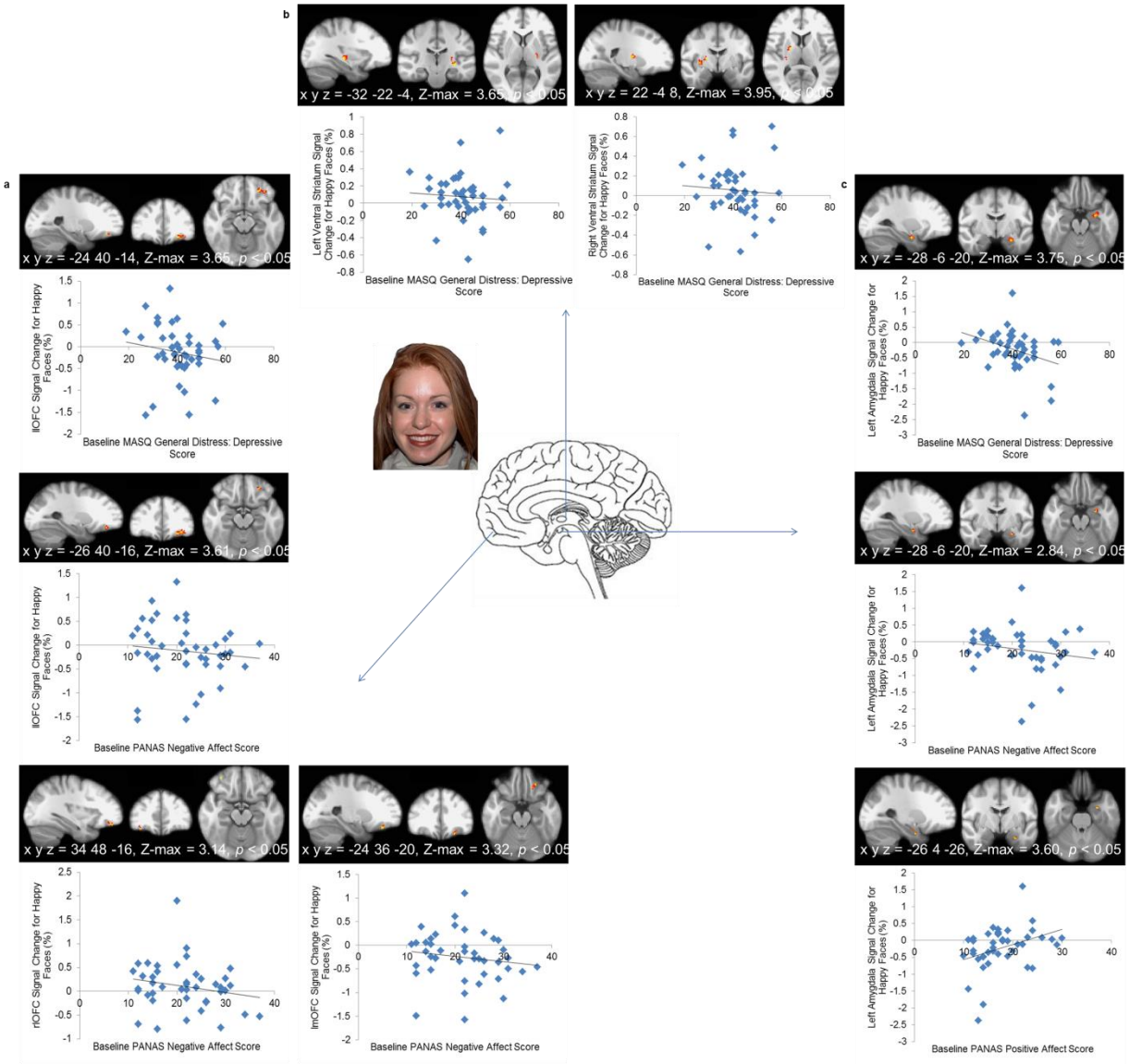


Figure 5.7 - Correlating Negative Affect with Neural Activity in response to Happy Faces: with greater baseline negative affect as measured by the MASQ general distress: depressive sub-scale or the PANAS, there is lower activity in response to happy faces in the **(a)** bilateral OFC, **(b)** bilateral VS and (c) left amygdala.

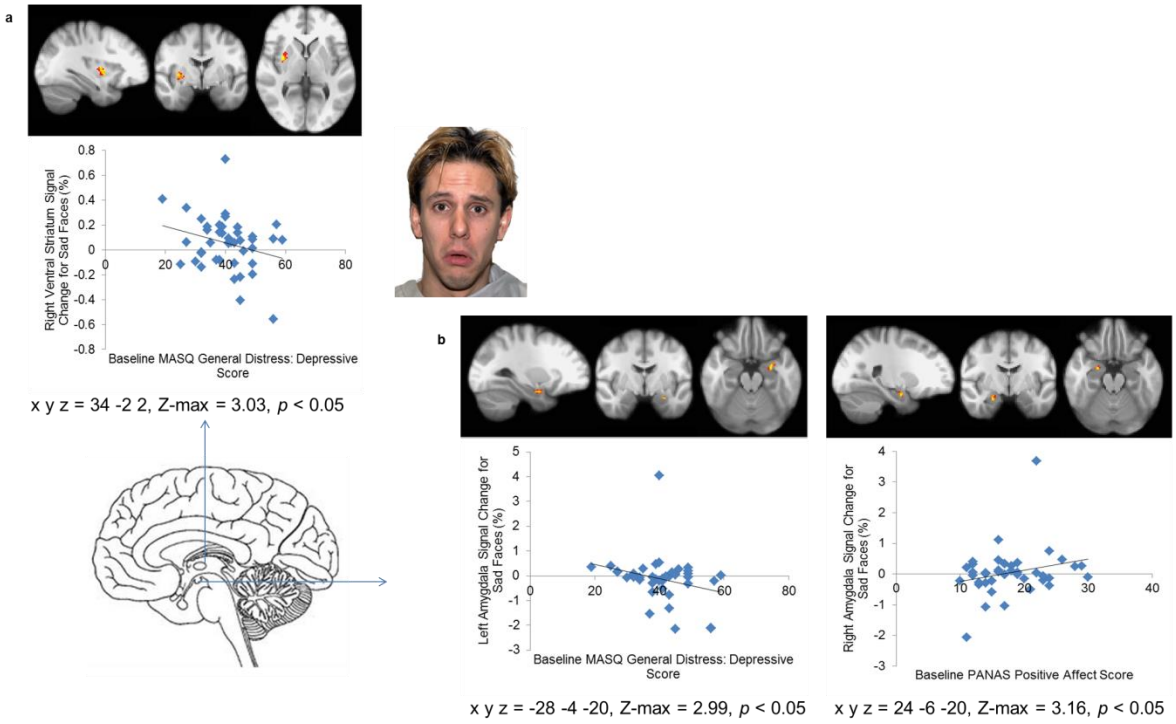


Figure 5.8 - Correlating Negative Affect with Neural Activity in response to Sad Faces: with greater baseline negative affect as measured by the MASQ general distress: depressive sub-scale or the PANAS, there is lower in response activity in response to sad faces in the **(a)** right VS and **(b)** bilateral amygdala.

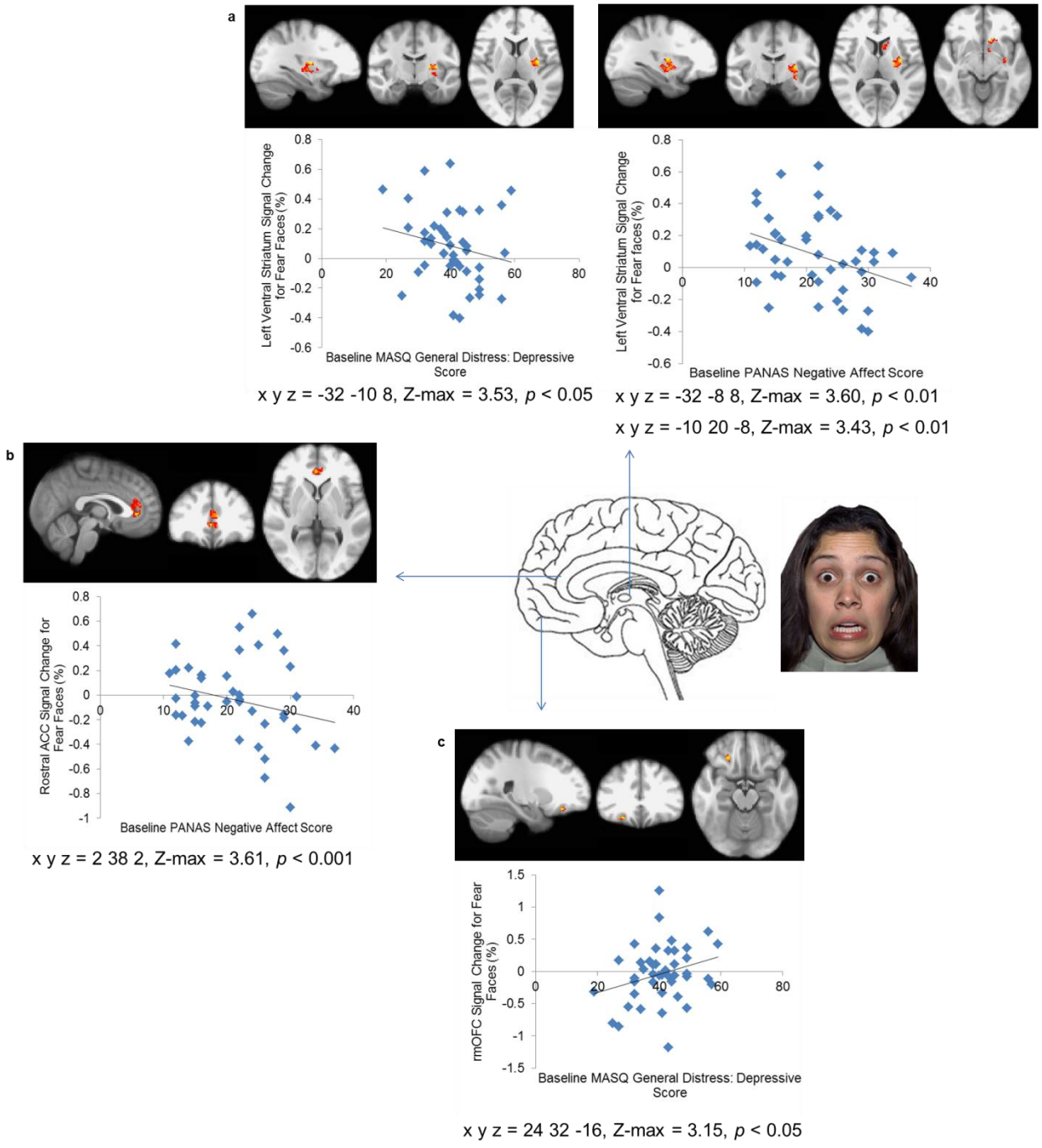


Figure 5.9 - Correlating Negative Affect with Neural Activity in response to Fearful Faces: with greater baseline negative affect as measured by the MASQ general distress: depressive sub-scale or the PANAS, there is lower activity in response to fearful faces in the **(a)** left VS and **(b)** rostral ACC but greater in the **(c)** right medial OFC.

Given the positive correlation between activity in the bilateral amygdala and the right medial OFC in response to fearful faces and depression severity and negative affect, we sought to investigate whether this related more specifically to increased anxiety. An SVC analysis did not find a positive correlation between bilateral amygdala or right medial OFC activity in response to fearful faces and anxiety measured by the MASQ general distress: anxious or anxious arousal sub-scales. However, interestingly, a positive correlation between activity in the bilateral amygdala in response to fearful versus sad faces and neuroticism measured by the EPQ was found (Figure 5.10).

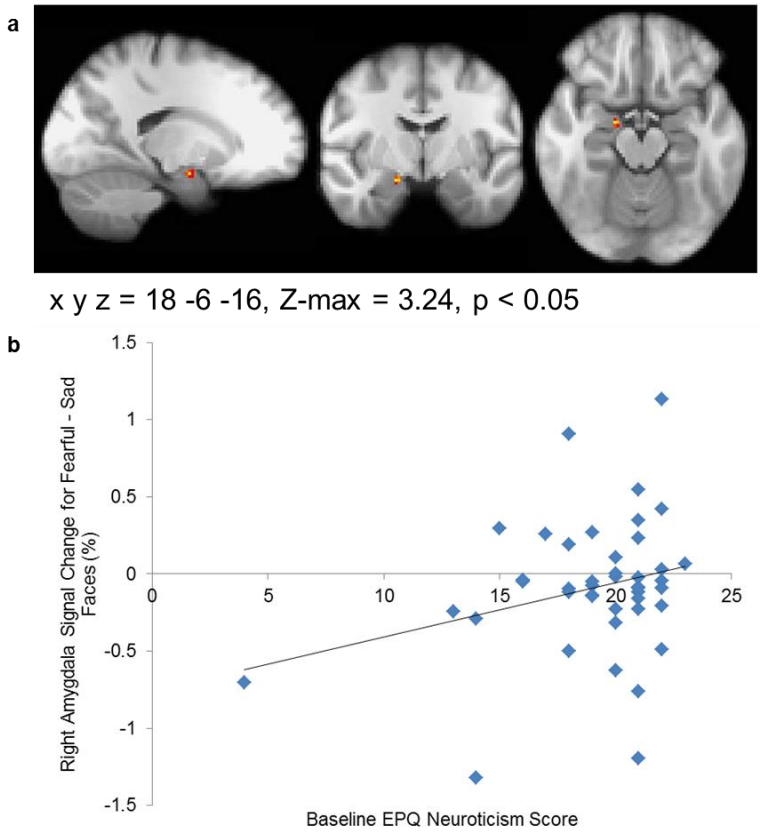


Figure 5.10 – Positive Correlation between Baseline Right Amygdala Activity to Fear and Neuroticism: (a) SVC analysis of right amygdala and **(b)** scatter graph of extracted percentage signal change in the right amygdala demonstrating a positive correlation between baseline activity and baseline neuroticism measured by the EPQ.

5.2.1.1.3 Anhedonia

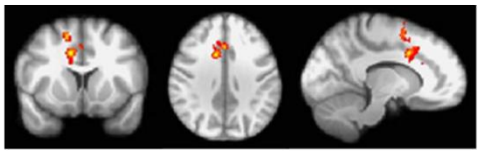
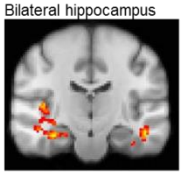
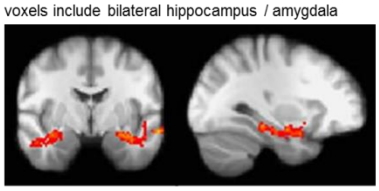
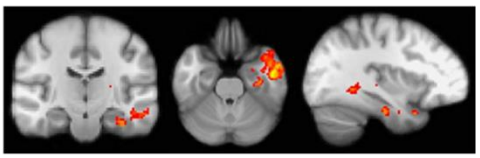
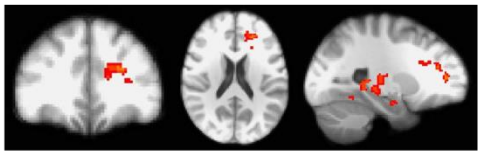
5.2.1.1.3.1 Whole Brain Analysis

The whole brain analysis found a positive correlation between baseline anhedonia measured with the SHAPS and central opercular cortex activity (x y z = 44 10 2, Z-max = 3.91, *p*-corrected < 0.001) in response to sad faces and paracingulate gyrus activity (12 12 36, Z-max = 3.54, *p*-corrected < 0.01) in response to fearful faces. There was also a positive correlation between baseline SHAPS score and brain activity in response to sad versus happy or sad versus fearful faces including activity in the bilateral hippocampus and amygdala, which is considered in more detail in the SVC analysis below. In contrast, there was a negative correlation between baseline anhedonia measured with the SHAPS and middle frontal gyrus (x y z = 40 26 48, Z-max = 4.03, *p*-corrected < 0.05) activity, as well as baseline anhedonia measured with the MASQ anhedonic depression sub-scale and middle temporal gyrus (x y z = -56 -18 -16, Z-max = 3.55, *p*-corrected < 0.01) activity in response to sad faces.

Finally, there was a negative correlation between baseline anhedonia measured with the MASQ anhedonic depression sub-scale and activity in the frontal pole (x y z = -24 38 20, Z-max = 3.78, *p*-corrected < 0.05) and temporal fusiform cortex (x y z = -36 -18 -26, Z-max = 3.78, $p < 0.001$), including activity in the left hippocampus and left amygdala, which is again considered in more detail in the SVC analysis below.

Overall, it seems that the greater the baseline anhedonia, the higher the brain activity in response to negative facial expressions, except the middle frontal and temporal gyri, and the lower the brain activity in response to positive facial expressions (Table 5.3).

Table 5.3 Whole Brain Analysis of the Relationship between Baseline Brain Activity for the Covert Faces Task and Anhedonia

Lower-level Contrast	Correlation	x y z	Z-max	Corrected-*p*	Image
SHAPS					
Sad	Positive	44 10 2	3.91	< 0.001	Central opercular cortex voxels include right hippocampus
	Negative	40 26 48	4.03	< 0.05	Middle frontal gyrus
Fear	Positive	12 12 36	3.54	< 0.01	Paracingulate gyrus, ACC 
Sad – Happy	Positive	32 -20 -20 -48 -18 -18	3.67 3.69	< 0.001 < 0.01	Bilateral hippocampus 
Sad - Fear	Positive	-2 -16 -18 -60 -12 -14 -52 -78 16	3.66 4.01 4.33	< 0.001 < 0.001 < 0.01	Brainstem Posterior middle temporal gyrus Superior lateral occipital cortex voxels include bilateral hippocampus / amygdala 
MASQ Anhedonic Depression					
Happy	Negative	-52 -4 -24 -36 -18 -26	4.82 3.78	< 0.001 < 0.001	Anterior middle temporal gyrus Posterior temporal fusiform cortex, some anterior parahippocampal gyrus, left hippocampus , left amygdala 
		-24 38 20	3.78	0.0451	Frontal pole 
Sad	Negative	-56 -18 -16 -46 -62 -30	3.55 3.91	0.00627 0.0149	Posterior middle temporal gyrus Cerebellum

5.2.1.1.3.2 SVC Analysis

The SVC analysis corroborated with the general trend described and confirmed involvement of the amygdala. Specifically, there was a positive correlation between baseline SHAPS score and right VS (x y z = 22 12 10, Z-max = 3.03, *p*-corrected < 0.05) activity in response to sad faces, dorsal ACC (x y z = 2 24 32, Z-max = 3.47, *p*-corrected < 0.05) activity in response to fearful faces, right medial OFC (x y z = 22 32 -10, Z-max = 3.36, *p* < 0.05) activity in response to sad versus happy faces and right amygdala (x y z = 30 -2 -20, Z-max = 3.06, *p*-corrected < 0.01) activity in response to sad versus fearful faces. There was a negative correlation between the MASQ anhedonic depression sub-scale and left amygdala (x y z = -22 -8 -18, Z-max = 2.72, *p*-corrected < 0.05) activity in response to happy faces (Figure 5.11).

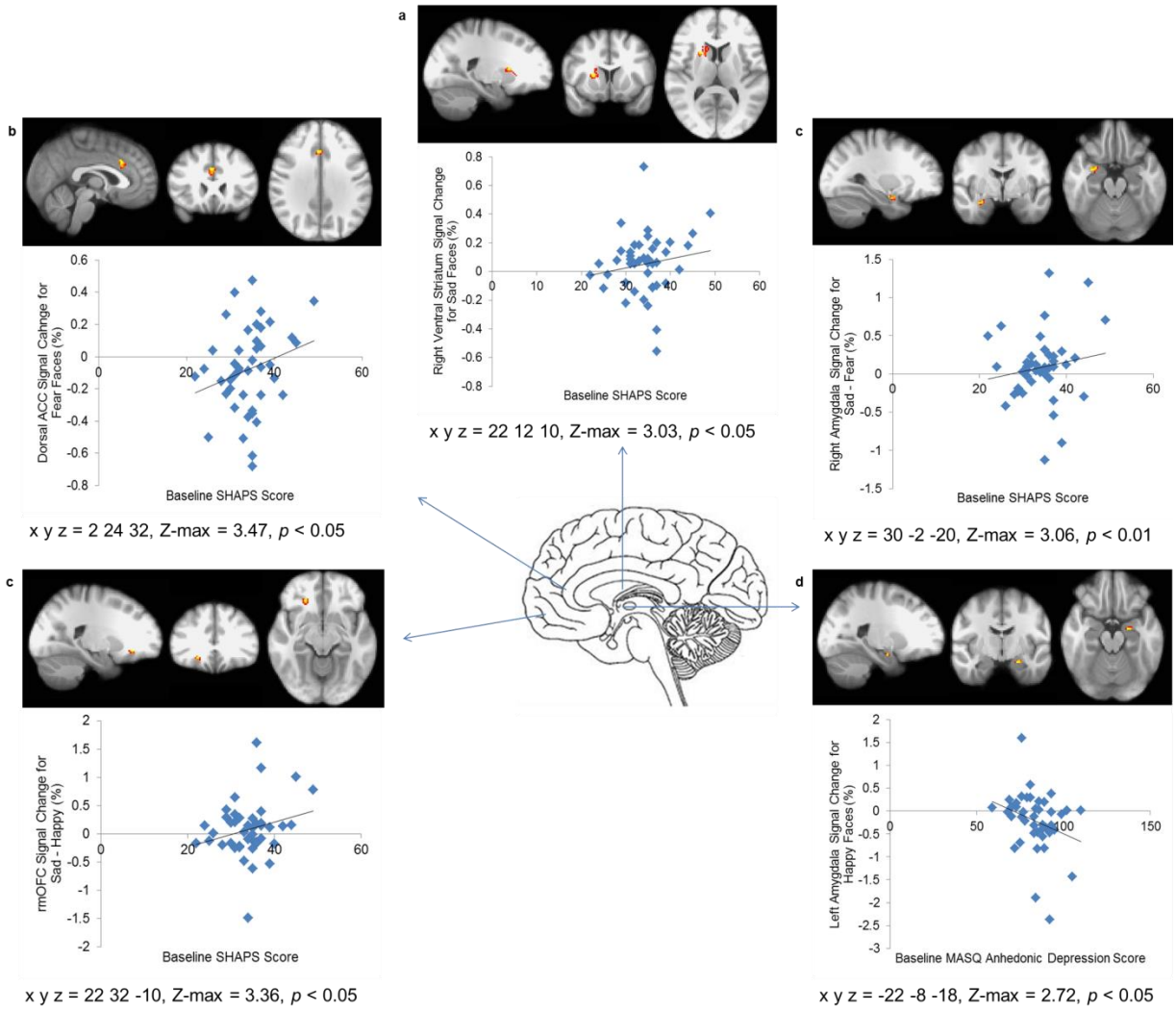


Figure 5.11 – Correlating Anhedonia with Emotional Neural Activity: with greater baseline anhedonia as measured by the SHAPS or the MASQ anhedonic depression sub-scale, there is higher **(a)** right VS activity in response to sad faces, **(b)** dorsal ACC activity in response to fearful faces, **(c)** right medial OFC activity in response to sad versus happy faces, **(d)** right amygdala activity in response to sad versus fearful faces but lower **(e)** left amygdala activity in response to happy faces.

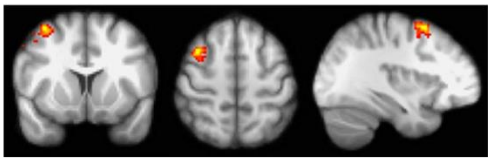
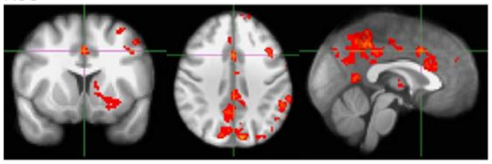
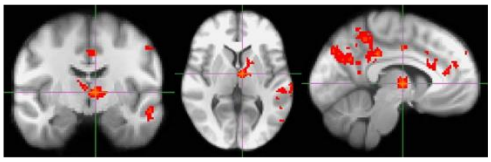
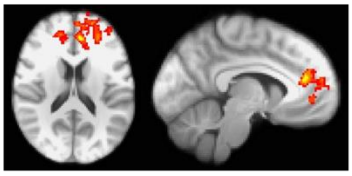
5.2.1.2 Reward Task

5.2.1.2.1 Baseline Symptom Severity

5.2.1.2.1.1 Whole Brain Analysis

The whole brain analysis found a positive correlation between baseline symptom severity measured with the HAM-D and middle frontal gyrus (x y z = 36 12 56, Z-max = 4.10, *p*-corrected < 0.05) activity in response to the anticipation of a win outcome. Otherwise, there was a general trend for a negative correlation between baseline HAM-D score and brain activity in response to both positive and negative outcomes. Specifically, there was a negative correlation between baseline HAM-D score and ACC (x y z = 0 12 38, Z-max = 3.49, *p*-corrected < 0.001) and left thalamus (x y z = -6 -8 4, Z-max = 3.81, *p*-corrected < 0.01) activity in response to win outcomes, ACC (x y z = -6 40 18, Z-max = 4.41, *p*-corrected < 0.001) activity in response to loss outcomes and frontal pole activity in response to loss versus neutral (x y z = 30 54 -6, Z-max = 4.03, *p*-corrected < 0.001) or loss versus win (x y z = 26 58 8, Z-max = 3.76, *p*-corrected <0.01) contrasts (Table 5.4).

Table 5.4 Whole Brain Analysis of the Relationship between Baseline Brain Activity for the Reward Task and Baseline HAM-D Score

Lower-level Contrast	Correlation	x y z	Z-max	Corrected-*p*	Image
Win Anticipation	Positive	36 12 56	4.10	< 0.05	Middle frontal gyrus 
Win Anticipation – Loss Anticipation	Positive	18 -70 36	3.87	< 0.001	Precuneous cortex
Win	Negative	14 -80 42 -52 -36 48 0 12 38 -6 -8 4 -50 -4 46	4.94 5.25 3.49 3.81 4.02	< 0.001 < 0.001 < 0.001 < 0.01 < 0.05	Superior lateral occipital cortex Anterior supramarginal gyrus ACC  Left thalamus  Precentral gyrus
Neutral (No Loss in Loss and No Win in Win)	Negative	-58 -34 50 -34 -88 26 8 -34 42	4.25 4.46 3.66	< 0.001 < 0.05 < 0.05	Anterior supramarginal gyrus Superior lateral occipital cortex Posterior cingulate gyrus
Loss	Negative	-6 40 18 -58 -36 52	4.41 4.23	< 0.001 < 0.05	ACC  Anterior supramarginal gyrus
Loss – Win	Negative	26 58 8	3.76	< 0.01	Frontal pole
Loss – Neutral	Negative	30 54 -6	4.03	< 0.001	Frontal pole

5.2.1.2.1.2 SVC Analysis

The SVC analysis also found a general negative correlation between HAM-D score and reward-related brain activity at baseline. Specifically, there was a negative correlation between baseline HAM-D score and activity in the dorsal (x y z = 0 12 38, Z-max = 3.49, *p*-corrected < 0.01) and rostral (x y z = -6 38 16, Z-max = 3.58, *p*-corrected < 0.05) ACC, bilateral VS (x y z = -26 6 14, Z-max = 3.21, *p*-corrected < 0.01; x y z = 14 8 6, Z-max = 3.07, *p*-corrected < 0.01) and left lateral OFC (x y z = -36 42 -8, Z-max = 2.88, *p*-corrected < 0.05) in response to positive outcomes (Figure 5.12) and activity in the rostral ACC (x y z = 6

42 6, Z-max = 3.21, *p*-corrected < 0.05; x y z = -6 38 16, Z-max = 4.29, *p*-corrected < 0.01) and left lateral OFC (x y z = -36 42 -8, Z-max = 2.76, *p*-corrected < 0.05) only in response to negative outcomes (Figure 5.13). An exception was a positive correlation between baseline HAM-D score and left amygdala (x y z = -26 -8 -14, Z-max = 2.92, *p*-corrected < 0.05) activity in response to a no win in win outcome (Figure 5.12c).

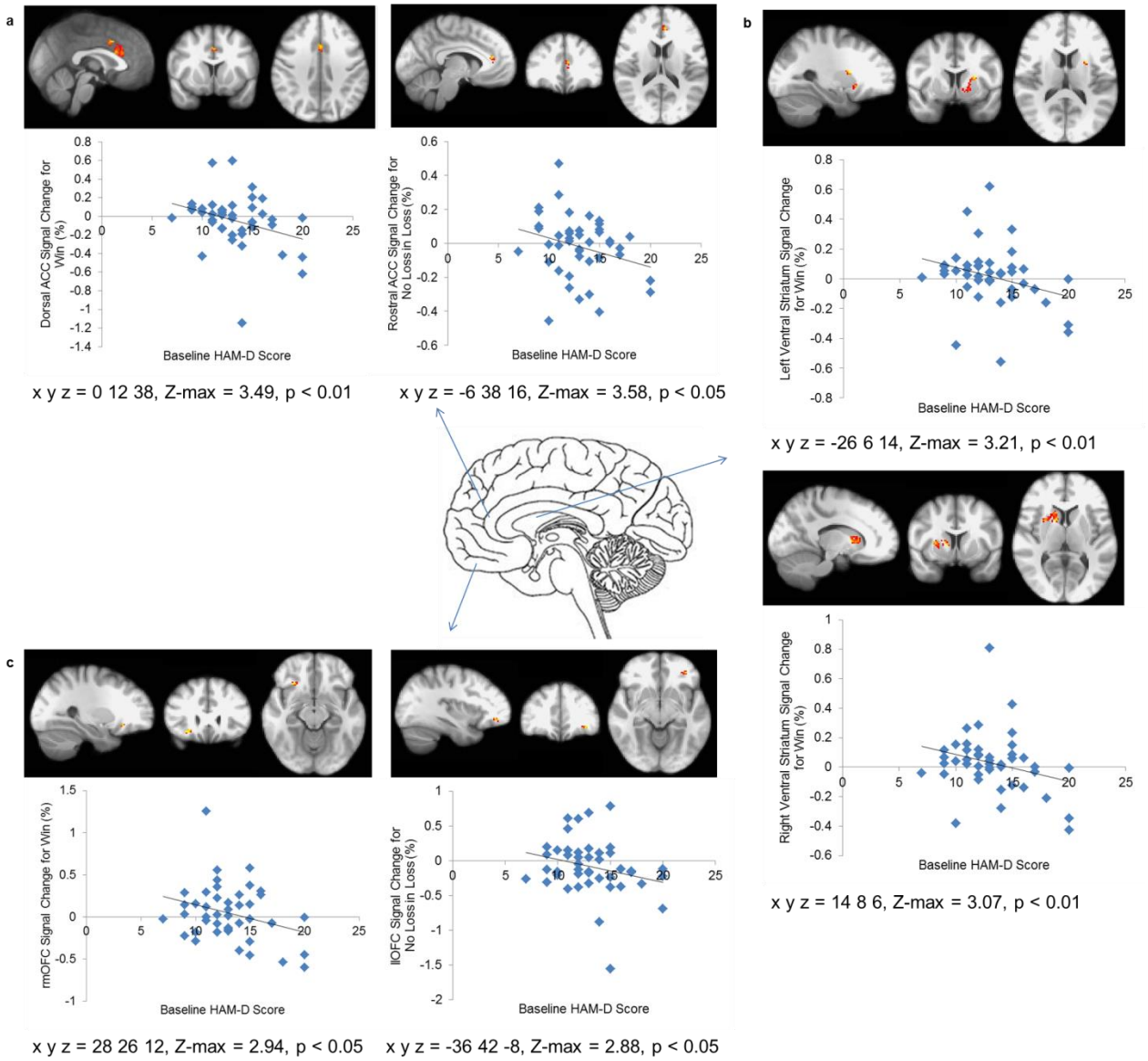


Figure 5.12 – Correlating HAM-D Score with Neural Activity in response to Positive Outcomes: with greater baseline symptom severity as measured with the HAM-D, there is lower activity in response to positive win or no loss in loss outcomes in the **(a)** dorsal and rostral ACC, **(b)** bilateral VS and **(c)** OFC.

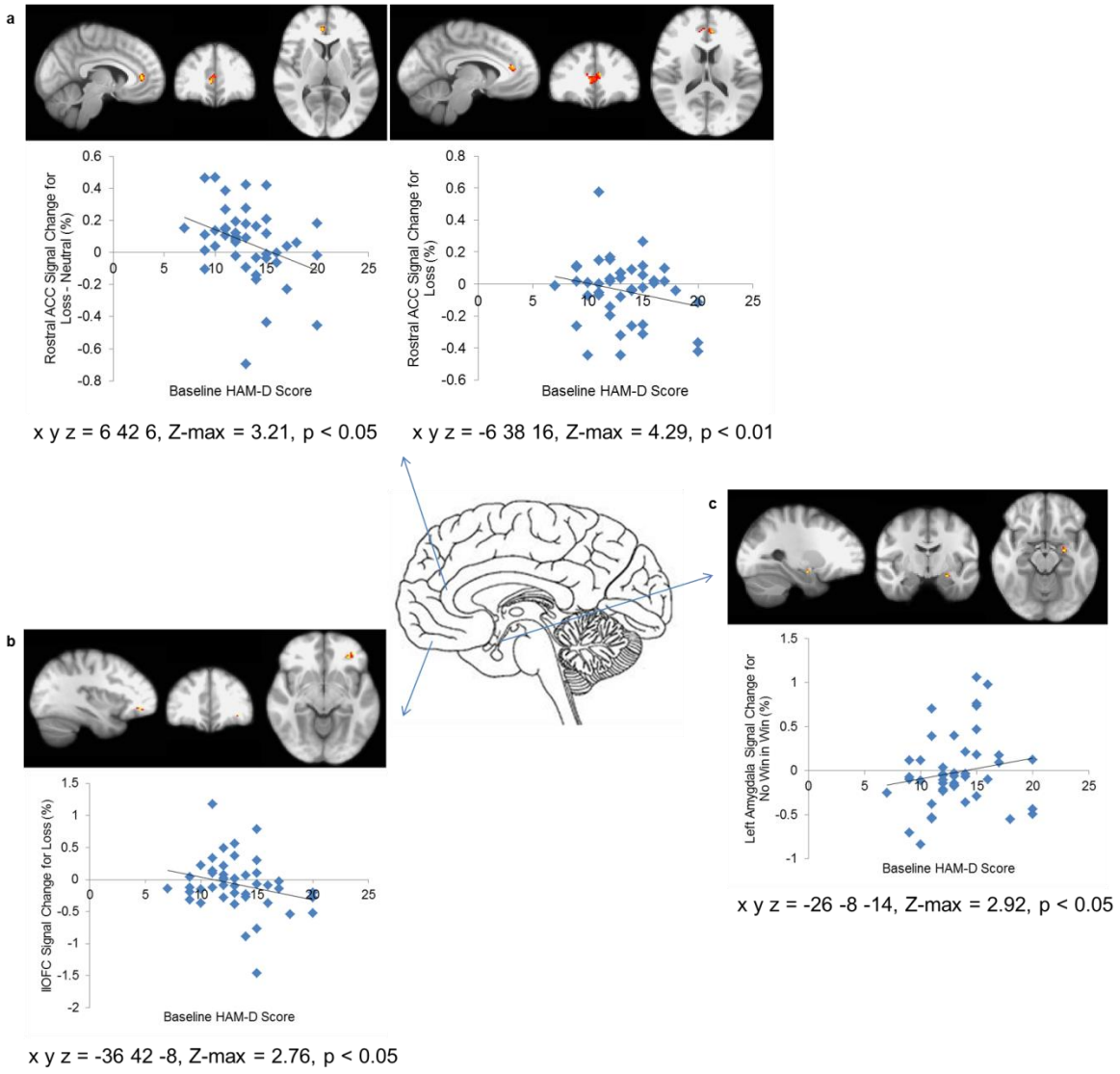


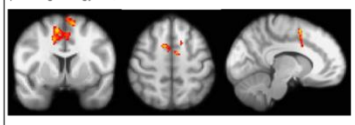
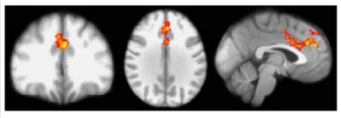
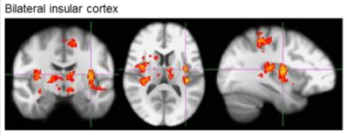
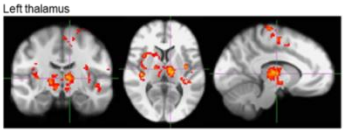
Figure 5.13 – Correlating HAM-D Score with Neural Activity in response to Negative Outcomes: with greater baseline symptom severity as measured with the HAM-D, there is lower activity in response to loss outcomes in the **(a)** rostral ACC and **(b)** OFC but higher activity in response to no win in win outcomes in the **(c)** left amygdala.

5.2.1.2.2 Negative Affect

5.2.1.2.2.1 Whole Brain Analysis

Many correlations were found in the whole brain analysis of the relationship between questionnaires measuring negative affect and brain activity during the reward task at baseline and are summarised in Table 5.5. There was a general trend for negative affect to positively correlate with brain activity in response to negative outcomes and negatively correlate with brain activity in response to positive outcomes, that is, the greater the negative affect, the higher the brain activity in response to negative outcomes and the lower the brain activity in response to loss outcomes.

Table 5.5 Whole Brain Analysis of the Relationship between Baseline Brain Activity for the Reward Task and Negative Affect

Lower-level Contrast	Correlation	x y z	Z-max	Corrected-*p*	Image
MASQ General Distress: Depressive					
Loss - Neutral	Positive	8 -22 40	3.55	< 0.001	Posterior cingulate gyrus
Loss	Positive	-14 -24 46 -26 -80 -14	3.97 3.79	< 0.01 < 0.05	Precentral gyrus Occipital fusiform gyrus
PANAS Negative Affect					
Win Anticipation	Positive	10 6 54	3.81	< 0.05	Juxtapositional lobule cortex stretching inferiorly to include paracingulate gyrus , ACC 
Neutral (No Win in Win and No Loss in Loss)	Positive	-10 -88 36 36 16 28 -60 -52 -12 -24 -24 66 6 -10 42 -38 -66 30	4.60 3.68 3.87 3.80 3.60 3.43	< 0.001 < 0.001 < 0.001 < 0.01 < 0.01 < 0.05	Occipital pole Middle frontal gyrus Middle temporal gyrus Precentral gyrus Anterior to posterior cingulate gyrus Superior lateral occipital cortex
Loss	Positive	20 -82 -16 -20 2 34 4 48 44 40 4 26 48 30 16	4.82 3.39 4.57 3.72 4.33	< 0.001 < 0.01 < 0.05 < 0.05 < 0.05	Occipital fusiform gyrus Left cerebral white matter Superior frontal gyrus Precentral gyrus Inferior frontal gyrus
Loss – Win	Positive	-26 -76 40 0 -48 50	3.63 3.57	< 0.001 < 0.01	Superior lateral occipital cortex Precuneous cortex
Positive – Negative	Negative	-2 34 30	3.53	< 0.001	Paracingulate gyrus, ACC 
PANAS Positive Affect					
Win	Negative	-16 -24 76 -36 -8 12 38 -2 12 -10 -12 6	3.85 4.01 3.63 3.69	< 0.001 < 0.001 < 0.01 < 0.001	Precentral gyrus Bilateral insular cortex  Left thalamus 
No Win in Win	Negative	48 -52 22	4.19	< 0.001	Angular gyrus

5.2.1.2.2.2 SVC Analysis

The SVC analysis corroborated with the general trend described above. For example, there was a positive correlation between baseline negative affect and activity in the dorsal ACC (x y z = -4 -12 40, Z-max = 3.32, *p*-corrected < 0.05) and right medial OFC (x y z = 20 34 -18, Z-max = 2.97, *p*-corrected < 0.05) in response to a loss outcome (Figure 5.14). In contrast, during the anticipation of a win outcome, left medial OFC activity was found to negatively correlate with negative affect measured with either the MASQ general distress: depressive sub-scale (x y z = -22 28 -12, Z-max = 3.01, *p*-corrected < 0.05) or the PANAS (x y z = -22 26 -14, Z-max = 2.97, *p*-corrected < 0.05); however, left VS (x y z = -20 10 8, Z-max = 3.41, *p*-corrected < 0.05) activity positively correlated with negative affect (Figure 5.15). A further incongruence was that the direction of these correlations for positive or negative outcomes was not reversed when considering the positive affect sub-scale of the PANAS.

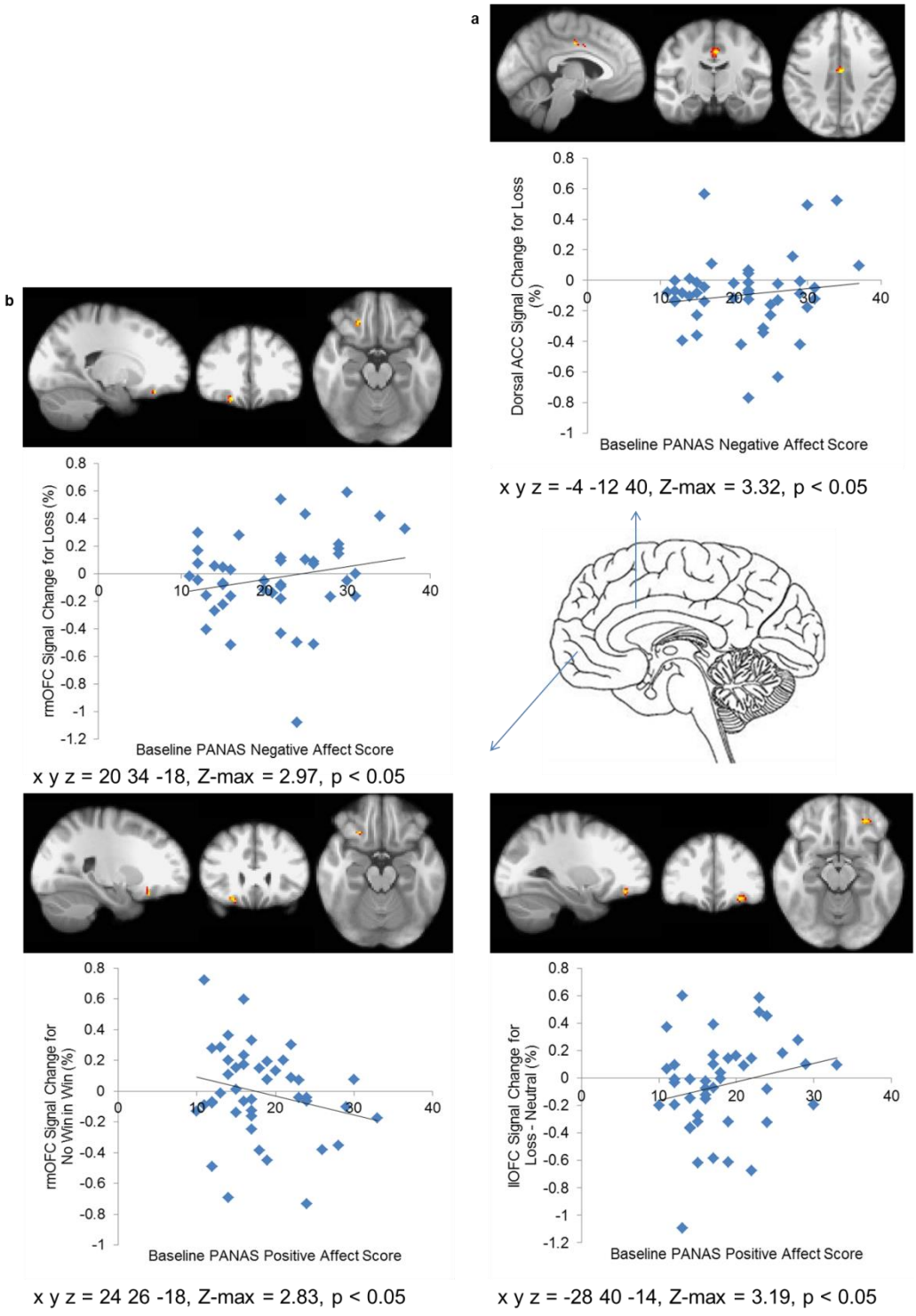


Figure 5.14 – Correlating Negative Affect with Neural Activity in response to Negative Outcomes: with greater baseline negative affect as measured with the MASQ general distress: depressive sub-scale or the PANAS, there is higher activity in response to loss or no win in win negative outcomes in the **(a)** dorsal ACC and **(b)** OFC.

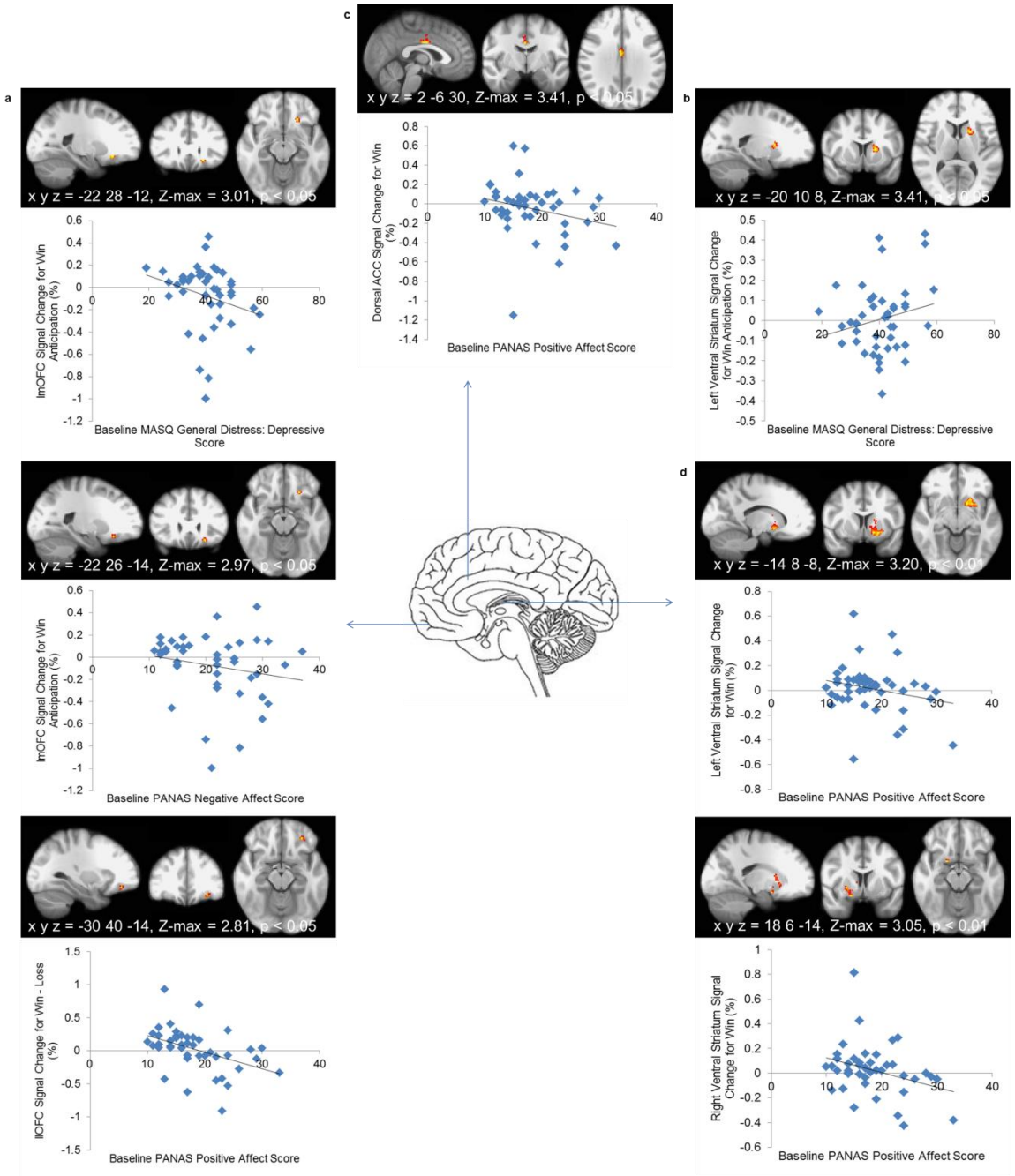


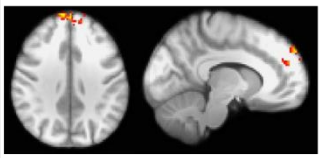
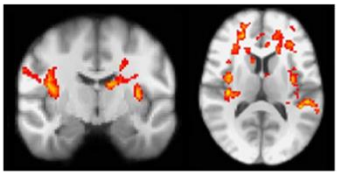
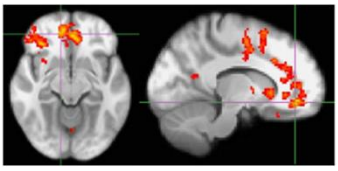
Figure 5.15 – Correlating Negative Affect with Neural Activity in response to Positive Outcomes: with greater baseline negative affect as measured with the MASQ general distress: depressive sub-scale or the PANAS, there is lower **(a)** left medial OFC activity but higher **(b)** left VS activity during anticipation of a win outcome. Correlations with the positive affect sub-scale of the PANAS are shown in **(c-d)**.

5.2.1.2.3 Anhedonia

5.2.1.2.3.1 Whole Brain Analysis

The whole brain analysis revealed an association between anhedonia measured with the SHAPS, but not the MASQ anhedonic depression sub-scale, and several regions of reward-related brain activity at baseline summarised in Table 5.6. Frontal activity (x y z = 10 60 36, Z-max = 3.41, *p*-corrected < 0.01) during the anticipation of a win outcome was found to negatively correlate with SHAPS score, indicating reduced anticipatory activity with greater anhedonia at baseline. Conversely, brain activity in response to receipt of either a positive or negative outcome, including frontal (x y z = 12 44 -10, Z-max = 4.09, *p*-corrected < 0.001) and bilateral insular (x y z = -34 -16 18, Z-max = 3.86, *p*-corrected < 0.001; x y z = 38 -6 16, Z-max = 3.76, *p*-corrected < 0.001) activity, was found to positively correlate with anhedonia at baseline.

Table 5.6 Whole Brain Analysis of the Relationship between Baseline Brain Activity for the Reward Task and Anhedonia

Lower-level Contrast	Correlation	x y z	Z-max	Corrected-*p*	Image
SHAPS					
Win Anticipation	Negative	10 60 36	3.41	< 0.01	Frontal pole 
Win Anticipation – Loss Anticipation	Negative	64 -16 0	3.85	< 0.05	Posterior superior temporal gyrus
Win	Positive	-34 -16 18 38 -6 16	3.86 3.76	< 0.001 < 0.001	Bilateral insular cortex 
Neutral	Positive	12 44 -10 -10 0 36 -38 -18 26 -8 -24 74 -18 32 54	3.89 3.50 3.82 3.67 3.70	0.000287 0.000541 0.00678 0.03 0.0418	Frontal medial cortex ACC (quite posterior) Central opercular cortex Precentral gyrus Superior frontal gyrus
No Win in Win	Positive	12 44 -10 62 14 14 -14 -60 30 48 -58 28	4.09 4.08 3.82 3.55	< 0.001 < 0.001 0.00066 0.0427	Frontal medial cortex  Inferior frontal gyrus Precuneous cortex Angular gyrus
Loss - Win	Positive	-44 -64 46	3.96	< 0.01	Superior lateral occipital cortex
Loss - Neutral	Positive	4 -82 2	3.72	< 0.01	Intracalcarine cortex
MASQ Anhedonic Depression					

5.2.1.2.3.2 SVC Analysis

The SVC analysis corroborated with the whole brain analysis whereby OFC activity during the anticipation of win or loss outcomes was found to negatively correlate with baseline anhedonia but brain activity in response to the receipt of either a positive or negative outcome was found to positively correlate with baseline anhedonia (Figures 5.16 and 5.17).

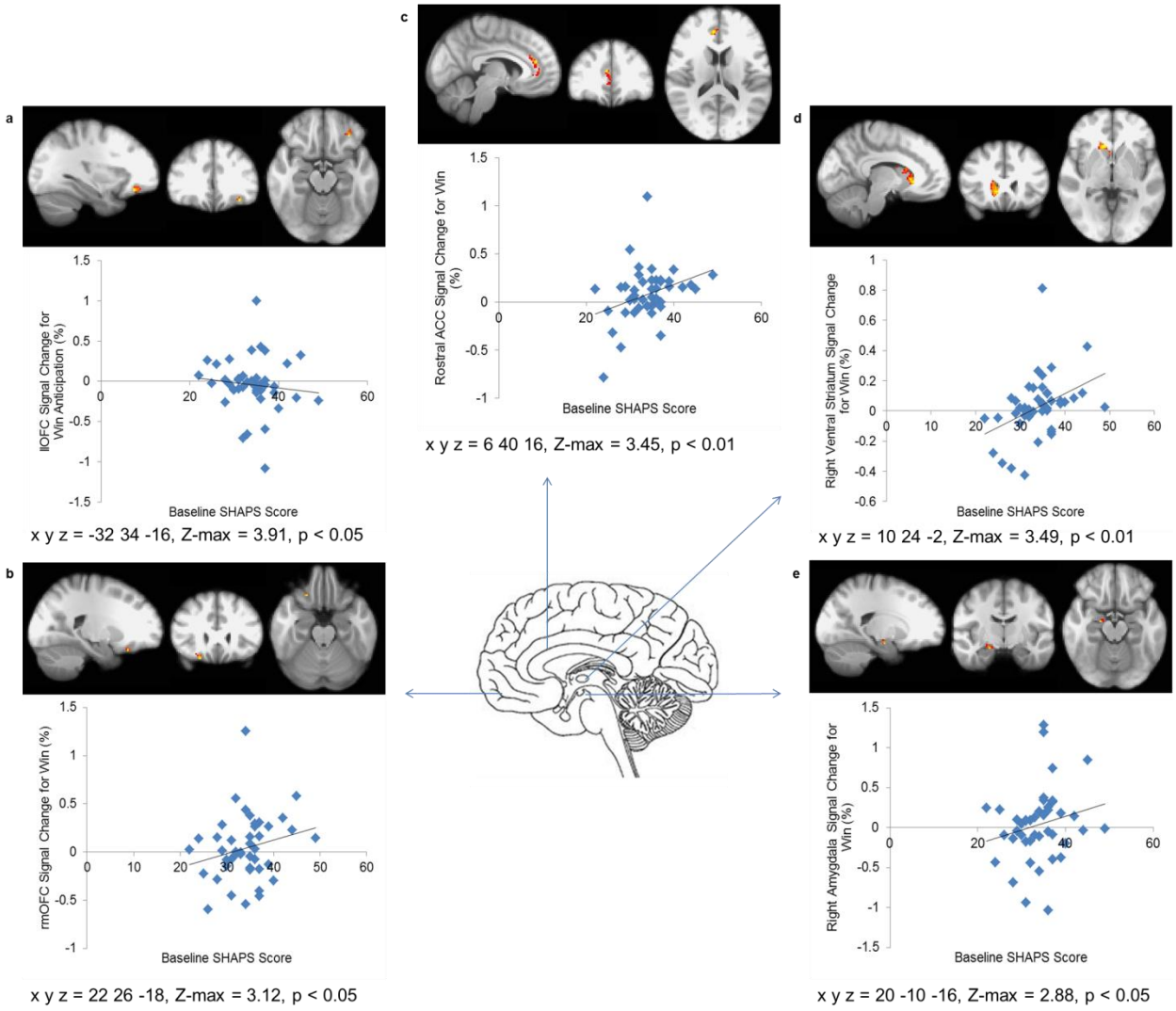


Figure 5.16 – Correlating Anhedonia with Neural Activity in response to Positive Outcomes: with greater baseline anhedonia as measured with the SHAPS or the MASQ anhedonic depression sub-scale, there is lower activity during the anticipation of a win outcome in the **(a)** left lateral OFC but higher activity in response to receipt of a win outcome in the **(b)** right medial OFC, **(c)** rostral ACC, **(d)** right VS and **(e)** right amygdala.

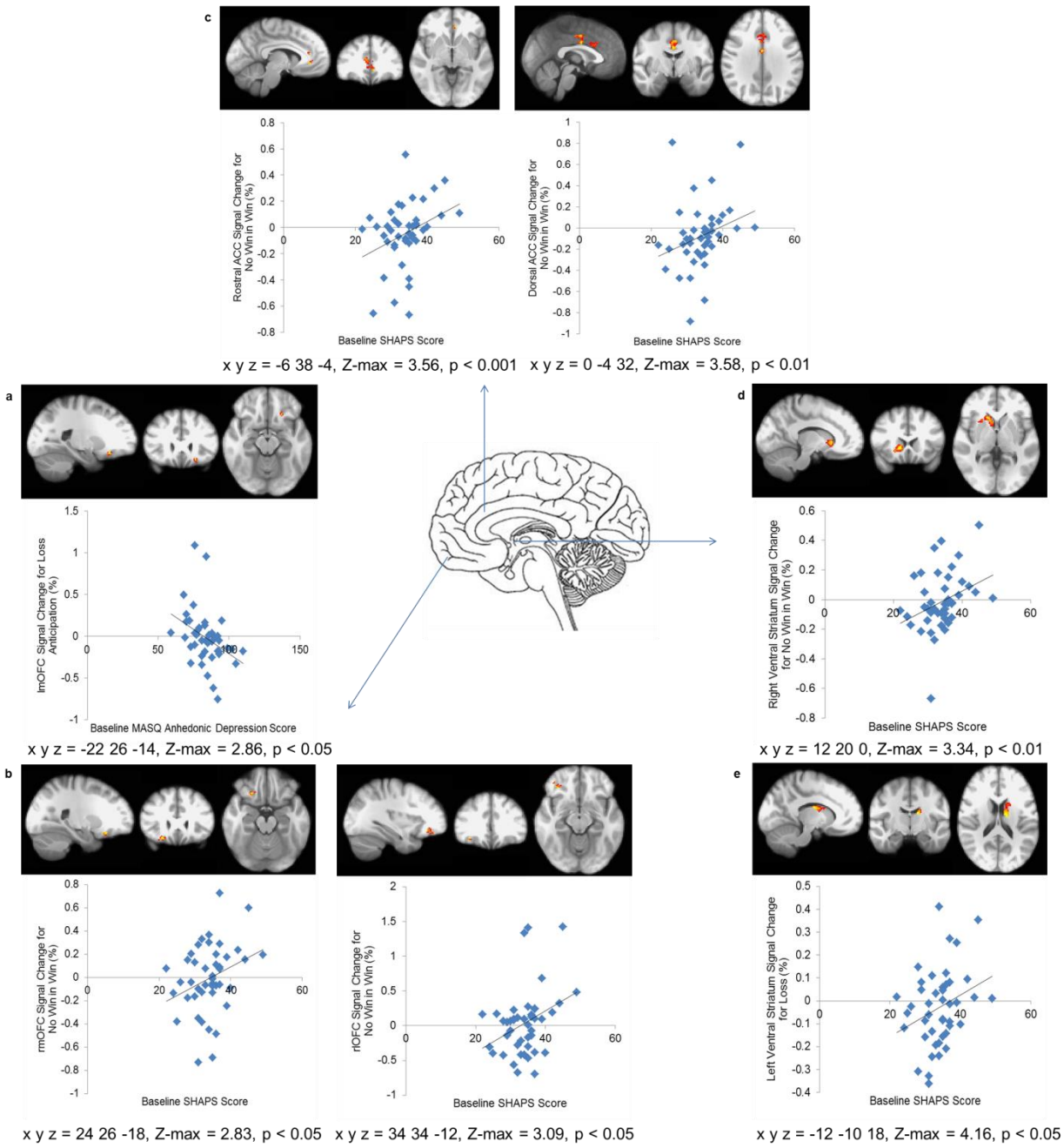


Figure 5.17 – Correlating Anhedonia with Neural Activity in response to Negative Outcomes: with greater baseline anhedonia as measured with the SHAPS or the MASQ anhedonic depression sub-scale, there is lower activity during the anticipation of a loss outcome in the **(a)** left medial OFC but higher activity in response to a loss or no win in win outcome in the **(b)** right OFC, **(c)** rostral and dorsal ACC and **(d-e)** bilateral VS.

5.3 Are there any Distinct Baseline Abnormalities Related to Negative Affect versus Anhedonia?

From the results above, it does not appear that negative affect and anhedonia can be differentiated from each other with no clear pattern of distinct baseline abnormalities related to negative affect versus anhedonia.

Regarding baseline abnormalities in behavioural measures of emotional and reward processing, there were few significant correlations with baseline symptoms and those that were significant were related to both negative affect and anhedonia. For example, the number of positive commission errors made in the EREC was found to negatively correlate with both negative affect measured by the MASQ general distress: depressive sub-scale and anhedonia measured by the MASQ anhedonic depression sub-scale. If the number of positive commission errors is correlated with either MASQ sub-scale whilst controlling for the other in a partial correlation, the significance is lost (MASQ general distress: depressive controlling for MASQ anhedonic depression: $t_{43} = -0.18$, $p = 0.23$; MASQ anhedonic depression controlling for MASQ general distress: depressive: $t_{43} = -0.14$, $p = 0.36$).

Regarding baseline abnormalities in neural measures of emotional and reward processing, there were many correlations between brain activity in response to sad faces and SHAPS or the MASQ anhedonic depression sub-scale suggesting that brain activity in response to sad faces may be particularly related to anhedonia rather than negative affect; however further research is required.

One reason for the lack of differentiation between negative affect and anhedonia may be the presence of significant correlations between negative affect and anhedonia themselves on the scales used to measure them in this study (Figure 5.18).

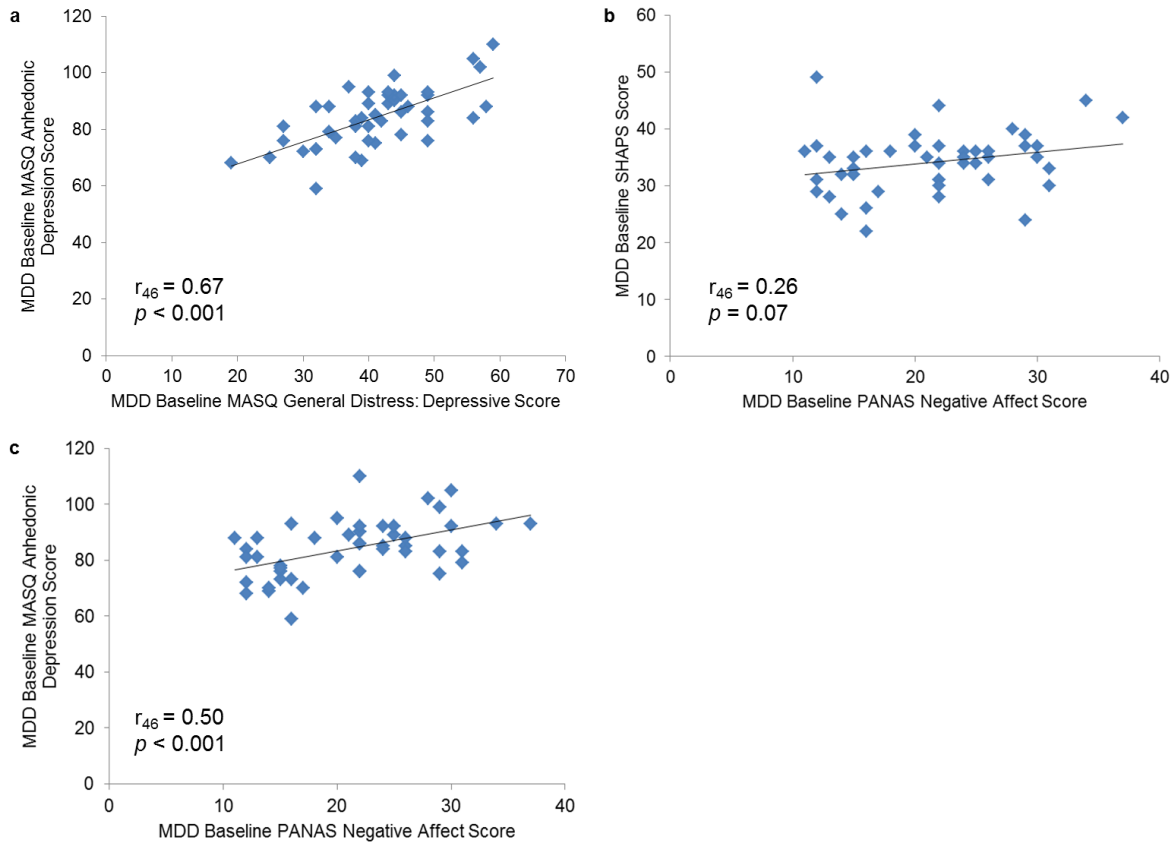


Figure 5.18 – Correlations between Negative Affect and Anhedonia: a significant correlation was present between scores on the **(a)** MASQ general distress: depression sub-scale and MASQ anhedonic depression sub-scale, **(b)** PANAS negative affect and SHAPS and **(c)** PANAS negative affect and MASQ anhedonic depression sub-scale. Scores are taken from the MDD group at baseline.

5.4 Summary

This Chapter aimed to investigate whether baseline subjective experiences of anhedonia and negative affect in MDD relate to dissociable baseline abnormalities in behavioural and neural measures of reward and emotional processing. It was hypothesised that the baseline abnormalities in the behavioural and neural measures of reward and emotional processing in the MDD group would correlate with the baseline scores on questionnaires measuring anhedonia and negative affect.

None of the baseline behavioural abnormalities were found to correlate with any of the baseline scores on questionnaires measuring anhedonia and negative affect. Other behavioural measures of reward and emotional processing that were not previously found to be different from the HC group at baseline were also investigated. The number of positive commission errors made in the EREC was found to negatively correlate with both negative affect measured by the MASQ general distress: depressive sub-scale and anhedonia measured by the MASQ anhedonic depression sub-scale, indicating reduced positive intrusions with greater negative affect and anhedonia.

Many correlations were found between baseline neural measures of reward and emotional processing and baseline scores on questionnaires measuring anhedonia and negative affect. Regarding emotional processing during the covert faces task, there was a general trend for lower brain activity in response to happy, sad and fearful faces with greater symptom severity and negative affect scores, with the exception of higher bilateral amygdala activity in response to fearful versus sad faces with greater symptom severity. Regarding reward processing during the reward task, there was a general trend for lower ACC, VS and OFC activity in response to both positive and negative outcomes with greater symptom severity. When considering just negative affect, there was a distinction between brain activity in response to positive and negative outcomes whereby greater negative affect was associated with lower brain activity to positive outcomes but higher brain activity to negative outcomes. Finally, higher levels of anhedonia were associated with greater brain activity during both the covert faces task and the reward task, with the exception of left amygdala activity in response to happy faces and OFC activity during anticipation of win or loss outcomes, which was lower with greater anhedonia.

5.5 Discussion

As previously discussed, MDD patients display abnormalities in behavioural and neural measures of emotional and reward processing, including biases towards negative information and away from positive information. These information processing biases are

also exhibited by individuals who have never been depressed but are at high risk of the disorder by virtue of high neuroticism (Chan et al., 2007, Haas et al., 2007, Robinson et al., 2010) and family history (Joormann et al., 2007). They have also been found to correlate with later depression severity (Hale, 1998, Vrieze et al., 2013), predict later relapse (Bouhuys et al., 1999) and persist following remission (Joormann and Gotlib, 2007, Bhagwagar and Cowen, 2008, Kerestes et al., 2012). Whilst information processing biases have been reliably demonstrated and clearly play a role in the onset of MDD, their exact relationship to depressive symptomatology remains unclear. Therefore it was interesting to investigate whether the information processing biases found at baseline were associated with overall symptom severity measured by HAM-D as well as the two symptom clusters of negative affect and anhedonia in this larger than average study.

5.5.1 Symptom Severity

Unfortunately, none of the abnormalities in the behavioural measures of emotional or reward processing, such as increased misclassification of other faces as sad, reduced attentional vigilance for happy faces or insensitivity to high-probability gain were found to correlate with baseline HAM-D score. Regarding the neural measures of emotional processing, a greater baseline HAM-D score was found to be associated with lower right lateral OFC activity in response to happy faces, lower left amygdala activity in response to happy versus fearful faces and higher bilateral amygdala activity in response to fearful versus sad faces. This is consistent with reports of lower brain activity in response to positive affective stimuli and higher brain activity in response to negative affective stimuli, including threat stimuli, in MDD, observed particularly in frontal regions and the amygdala. Conversely, during reward processing, a greater baseline HAM-D score was found to be associated with lower reward-related brain activity in the OFC, VS and ACC in response to both positive and negative outcomes, suggesting an overall decrease in responsiveness in MDD. However, this may be the result of blunted responses specifically to reinforcement, whether it is positive or negative feedback (Eshel and Roiser, 2010).

5.5.2 Negative Affect

It has previously been demonstrated that the increased misclassification of other faces as sad and reduced sensitivity for happy faces correlates with severity of negative affect measured by the PANAS (Gur et al., 1992). However, again, unfortunately none of the abnormalities in the behavioural measures of emotional or reward processing found at baseline, including the increased misclassification of other faces as sad or reduced attentional vigilance for happy faces, were found to correlate with baseline negative affect. Investigation of other behavioural measures did find a negative correlation between number of positive self-referent words falsely recalled and baseline negative affect, indicating reduced positive intrusions with greater negative affect. Indeed, the strongest evidence for information processing biases in MDD comes from memory studies, in which MDD patients consistently display an absence of the memory advantage for positive words (Matt et al., 1992).

With regards to the neural measures of emotional processing, there was a general trend for lower brain activity in response to happy, sad and fearful faces with greater baseline negative affect, with the exception of greater right medial OFC in response to fearful faces. This may relate to the higher bilateral amygdala activity observed in response to fearful versus sad faces. Since the increased brain activity appeared specific to fearful rather than sad faces, associations with the more anxious aspects of negative affect were investigated, since anxiety is often co-morbid with MDD. The higher right medial OFC or bilateral amygdala activity was not found to be associated with higher anxiety, but was found to be associated with higher neuroticism, a possible risk factor for MDD (Chan et al., 2007)

With regards to the neural measures of reward processing, when considering just negative affect, this time there was a distinction between brain activity in response to positive and negative outcomes, with the typical association of lower brain activity in response to positive outcomes and higher brain activity in response to negative outcomes with greater baseline negative affect, particularly in the left and right medial OFC.

5.5.3 Anhedonia

There is evidence that abnormalities in behavioural and neural measures of reward processing correlate specifically with anhedonia, independently of overall depression severity. In particular, reduced choice frequency of high-probability gain (Steele et al., 2007, Chase et al., 2010, Vrieze et al., 2013, Steele et al., 2004) and reduced VS activity and increased vmPFC activity in response to reward (Keedwell et al., 2005, Epstein et al., 2006) have been found to correlate with anhedonia severity. However, in this study, the insensitivity to high-probability gain was not found to correlate with baseline anhedonia and brain activity including the VS, ACC, amygdala and insula, in response to receipt of either a win or loss outcome was actually found to positively correlate with baseline anhedonia. One exception was an association between lower OFC activity during the anticipation of win or loss outcomes and greater baseline anhedonia, suggesting that the anhedonia observed, at least in this group of MDD patients, may be more related to aberrant processing during the anticipatory rather than consummatory aspects of reward.

CHAPTER 6

THE EFFECT OF BUPROPION

Chapter 2 investigated the acute effects of bupropion on behavioural measures of reward and emotional processing in healthy volunteers. It was found that bupropion significantly reduced the misclassification of other faces as sad and increased the recognition of happy faces and familiarity of positive words in healthy volunteers. Others have also investigated the effects of bupropion on neural measures of reward processing in healthy volunteers. For example, in a recently published study (Dean et al., 2016), healthy volunteers completed an fMRI reward task following 7 day treatment with bupropion or placebo. The task covered all aspects of reward processing with a cue for the anticipation of reward, effort exerted to receive the reward and actual receipt of the reward. Whilst no behavioural effects were observed, bupropion was found to increase activity during anticipation of the reward in the vmPFC and caudate, exertion of effort for the reward in the vmPFC, VS and ACC and finally receipt of the reward in the mOFC compared to placebo. Bupropion was also found to increase activity to an aversive stimulus in the vmPFC, mOFC, VS and amygdala compared to placebo.

Therefore, there is some preliminary evidence that bupropion does have effects on behavioural and neural measures of reward and emotional processing at least in healthy volunteers, but it remains to be seen whether bupropion can remediate aberrant reward processing and negative affective biases observed in MDD patients in line with the cognitive neuropsychological theory of antidepressant action. Therefore, this Chapter aims to investigate whether bupropion affects behavioural and neural measures of reward and emotional processing in MDD patients. It was hypothesised that bupropion would act to remediate the baseline abnormalities observed in the previous two Chapters. Given the results of the acute study, it was also more specifically hypothesised that both an increase in positive emotional processing and a decrease in negative emotional processing would be observed.

6.1 The Effect of Bupropion on Behavioural Measures of Reward and Emotional Processing

Given that the behavioural measures of reward and emotional processing were tested three times throughout the course of the study, both the early effects of bupropion at 2 weeks between baseline and test visit 2 and the longer-term effects of bupropion at 6 weeks between baseline and test visit 3 were considered for each task. To assess the early effects of bupropion, the repeated measures ANOVA included visit as a within subjects factor with 2 levels (baseline and test visit 2). To assess the longer-term effects of bupropion, the repeated measures ANOVA included visit as a within subjects factor with 3 levels (baseline, test visit 2 and test visit 3).

6.1.1 ETB

6.1.1.1 FERT

The FERT requires participants to indicate whether an emotional facial expression is one of anger, disgust, fear, happy, sad, surprise or neutral (neutral was again excluded from the following statistical analyses). Outcome measures were percentage accuracy, d', percentage misclassification, response bias and average reaction time for correct responses. Separate repeated measures ANOVAs with face emotion and visit as the within subjects factors and participant group as the between subjects factor were performed for each outcome measure of the FERT.

6.1.1.1.1 Early Effects of Bupropion at Week 2

For percentage accuracy, a significant main effect of face emotion was found ($F_{5, 80} = 90.97$, $p < 0.001$), along with a face emotion by visit interaction ($F_{5, 400} = 9.27$, $p < 0.001$); however no face emotion by visit by group interaction was found ($F_{5, 400} = 0.88$, $p = 0.50$). This indicated that percentage accuracy did change between baseline and test visit 2, differentially for each face emotion, likely as a result of different stimuli used for each test visit or practice effects, but with no difference between the two participant groups. Indeed, it can be seen from Figure 6.1a that the MDD and HC groups displayed the same change in

percentage accuracy between baseline and test visit 2 for each face emotion, perhaps with the exception of fearful faces, for which percentage accuracy appeared to increase in just the MDD group. Therefore, a post hoc analysis considering just fearful faces was performed. A repeated measures ANOVA did not find a visit by group interaction for percentage accuracy for fearful faces ($F_{1, 80} = 0.48$, $p = 0.49$); however, paired t-tests found only the bupropion-treated MDD group to show a significant increase in percentage accuracy for fearful faces between baseline and test visit 2 ($t_{41} = -2.14$, $p < 0.05$) (Figure 6.1b). A similar pattern of results was observed for d' (Figure 6.1 c and d). In addition, Figure 6.1c indicated that whilst d' for angry faces remained similar between baseline and test visit 2 in the MDD group, it increased in the HC group, suggesting that the MDD group had reduced sensitivity to angry faces compared to the HC group at 2 weeks. A post hoc repeated measures ANOVA considering just angry faces found the effect of group to trend towards significance ($F_{1, 80} = 3.52$, $p = 0.06$) but no visit by group interaction ($F_{1, 80} = 1.13$, $p = 0.29$). Given the lack of a visit by group interaction and only marginal significance obtained in these post hoc analyses for percentage accuracy and d', these results should be treated with caution.

For percentage misclassifications, the repeated measures ANOVA found a visit by group interaction ($F_{1, 80} = 4.09$, $p < 0.05$). Inspection of Figure 6.1e suggested this was derived from a large group difference in the change in percentage misclassification of other faces as sad between baseline and test visit 2. Therefore, a post hoc analysis considering just sad faces was performed. A repeated measures ANOVA found a trend towards significance for a visit by group interaction for percentage misclassification of other faces as sad ($F_{1, 80} = 3.62$, $p = 0.06$). Paired t-tests found only the bupropion-treated MDD group to show a significant decrease in the percentage misclassification of other faces as sad between baseline and test visit 2 ($t_{41} = 2.72$, $p < 0.05$), such that the group difference in the percentage misclassification of other faces as sad at baseline ($t_{85} = -2.47$, $p < 0.05$) was no longer significant at test visit 2 ($t_{81} = -1.54$, $p = 0.13$) (Figure 6.1f). A similar pattern of results was observed for beta for sad faces with a trend towards significance for a visit by group interaction ($F_{1, 80} = 3.35$, $p = 0.07$) and paired t-tests finding only the bupropion-treated MDD

group to show a significant increase in the beta for sad faces between baseline and test visit 2 (t_{41} = -2.85, p < 0.01), such that the group difference in beta for sad faces at baseline (t_{85} = 2.32, p < 0.05) was no longer significant at test visit 2 (t_{81} = 1.58, p = 0.12) (Figure 6.1 g and h). These results appear to be similar to the acute effect of bupropion on the percentage misclassification of other faces as sad or beta for sad faces observed in the healthy volunteer study (Chapter 2). Finally, a repeated measures ANOVA found a main effect of visit on reaction times ($F_{1, 78}$ = 101.03, p < 0.001) with a significant reduction in reaction times across all participants and face emotions between baseline and test visit 2, likely as a result of practice effects (Figure 6.1i).

6.1.1.1.2 Longer-Term Effects of Bupropion at Week 6

Figure 6.1 suggested the effects displayed by the bupropion-treated MDD group at week 2 were heightened at week 6. However, for percentage accuracy, repeated measures ANOVAs with 3 levels of visit considering all face emotions ($F_{10, 780}$ = 0.76, p = 0.67) or just fearful faces ($F_{2, 156}$ = 1.08, p = 0.34) still did not find any visit by group interactions. While paired t-tests found only the bupropion-treated MDD group to show a significant increase in the percentage accuracy between baseline and test visit 3 (t_{39} = -2.69, p < 0.05), as this effect was not pre-specified, these results should again be treated with caution. For percentage misclassification of other faces as sad, the repeated measures ANOVA with 3 levels of visit still found a trend towards significance for a visit by group interaction ($F_{2, 156}$ = 2.87, p = 0.06). Paired t-tests found only the bupropion-treated MDD group to show a significant decrease in the percentage misclassification of other faces as sad between baseline and test visit 3 (t_{39} = 2.21, p < 0.05), such that the group difference in the percentage misclassification of other faces as sad at baseline (t_{85} = -2.47, p < 0.05) was no longer significant at test visit 3 (t_{79} = -0.77, p = 0.45). Finally, with 3 levels of visit, a significant group by visit interaction was found for reaction times ($F_{2, 152}$ = 5.07, p < 0.01), suggesting that across all face emotions, the MDD group displayed a smaller reduction in reaction times over 6 weeks than the HC group.

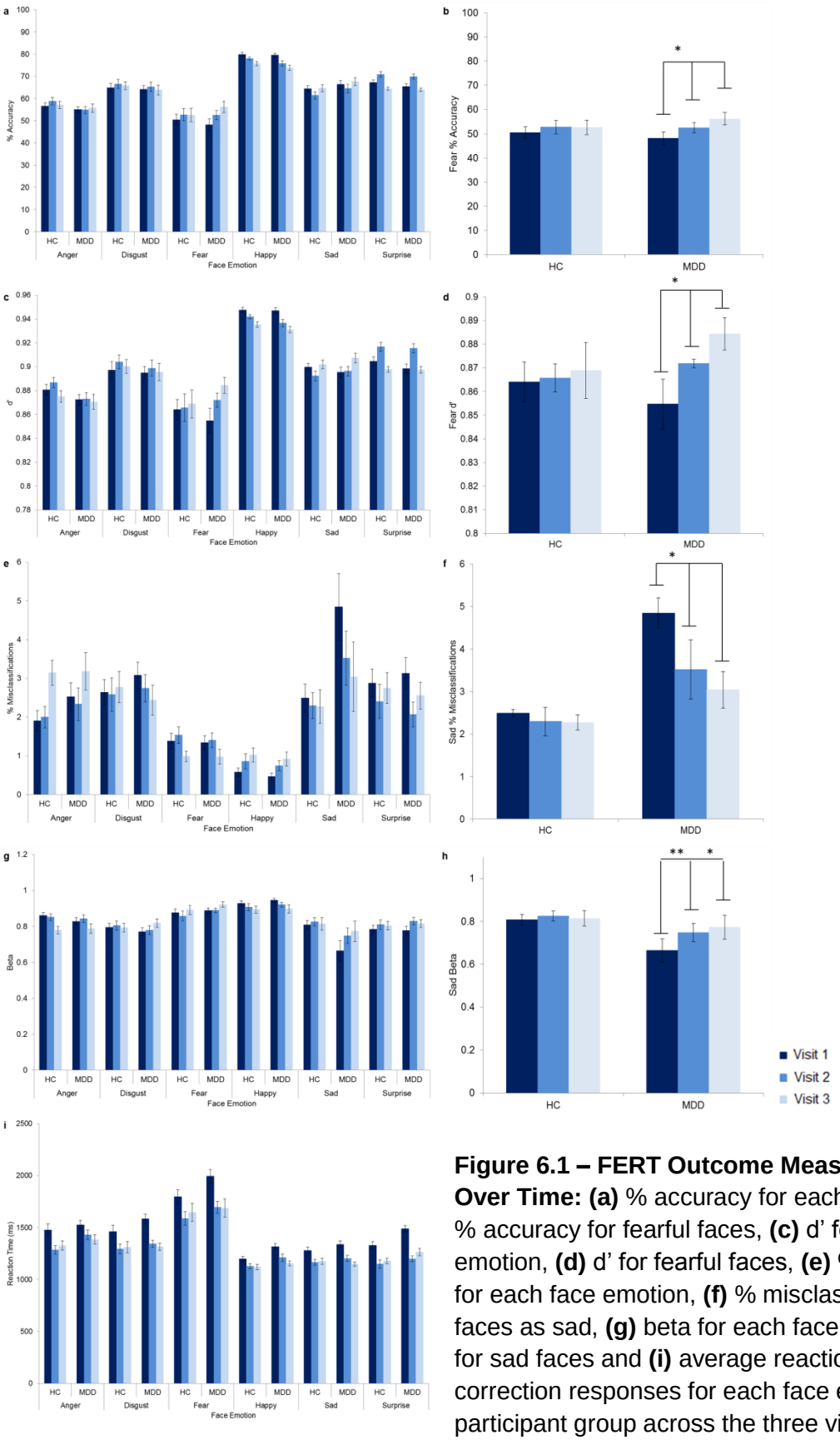


Figure 6.1 – FERT Outcome Measures Change Over Time: (a) % accuracy for each face emotion, **(b)** % accuracy for fearful faces, **(c)** d' for each face emotion, **(d)** d' for fearful faces, **(e)** % misclassification for each face emotion, **(f)** % misclassification of other faces as sad, **(g)** beta for each face emotion, **(h)** beta for sad faces and **(i)** average reaction time for correction responses for each face emotion for each participant group across the three visits. Values are reported as means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$, **$p < 0.01$).

6.1.1.2 ECAT

The ECAT requires participants to indicate whether they would like or dislike to be described as certain negative and positive self-referent words. Outcome measures were percentage accuracy and average reaction time for correct responses. A large proportion of individuals had near 100% accuracy, therefore in order to generate more normally distributed data, an arcsine transformation was again first applied to the percentage accuracy data prior to analysis. Separate repeated measures ANOVAs with word valence and visit as the within subjects factors and participant group as the between subjects factor were then performed for each outcome measure of the ECAT.

6.1.1.2.1 Early Effects of Bupropion at Week 2

A repeated measures ANOVA with 2 levels of visit did not find a main effect of word valence ($F_{1, 80} = 0.18$, $p = 0.67$), visit ($F_{1, 80} = 0.13$, $p = 0.72$) or group ($F_{1, 80} = 0.50$, $p = 0.48$) or word valence by visit by group interaction ($F_{1, 80} = 0.39$, $p = 0.53$) for arcsine transformed percentage accuracy (Figure 6.2a). A visit by group interaction was found for reaction time ($F_{1, 80} = 6.55$, $p < 0.05$), with an increase in reaction time for the HC group and decrease in reaction time for the MDD group across both word valences between baseline and test visit 2 (Figure 6.2b).

6.1.1.2.2 Longer-term Effects of Bupropion at Week 6

A repeated measures ANOVA with 3 levels of visit found a trend towards significance for a word valence by visit by group interaction ($F_{2, 154} = 2.46$, $p = 0.09$) for arcsine transformed percentage accuracy. Inspection of Figure 6.2a suggests this is derived from a group difference in the change in percentage accuracy for positive self-referent words across the three visits. Whilst the MDD group displays an increase in the accuracy for positive self-referent words, the HC group displays a decrease in the accuracy for positive self-referent words across the three visits. Therefore, a post hoc analysis considering just positive self-referent words was performed. A repeated measures ANOVA found a significant visit by group interaction ($F_{2, 156} = 3.94$, $p < 0.05$); however, post hoc paired t-tests only found the

reduction in percentage accuracy for positive self-referent words in the HC group between baseline and test visit 3 to be significant ($t_{39} = 2.64$, $p < 0.05$).

A visit by group interaction was again found for reaction time ($F_{2, 156} = 5.24$, $p < 0.01$), with the HC group displaying an increase in reaction time across the three visits for both positive ($t_{39} = -4.73$, $p < 0.001$) and negative ($t_{39} = -3.53$, $p < 0.01$) self-referent words (Figure 6.2b). Such an increase in reaction time with multiple testing is most likely the result of the HC group actively trying to remember the words for the EREC, which is less of a surprise with each visit.

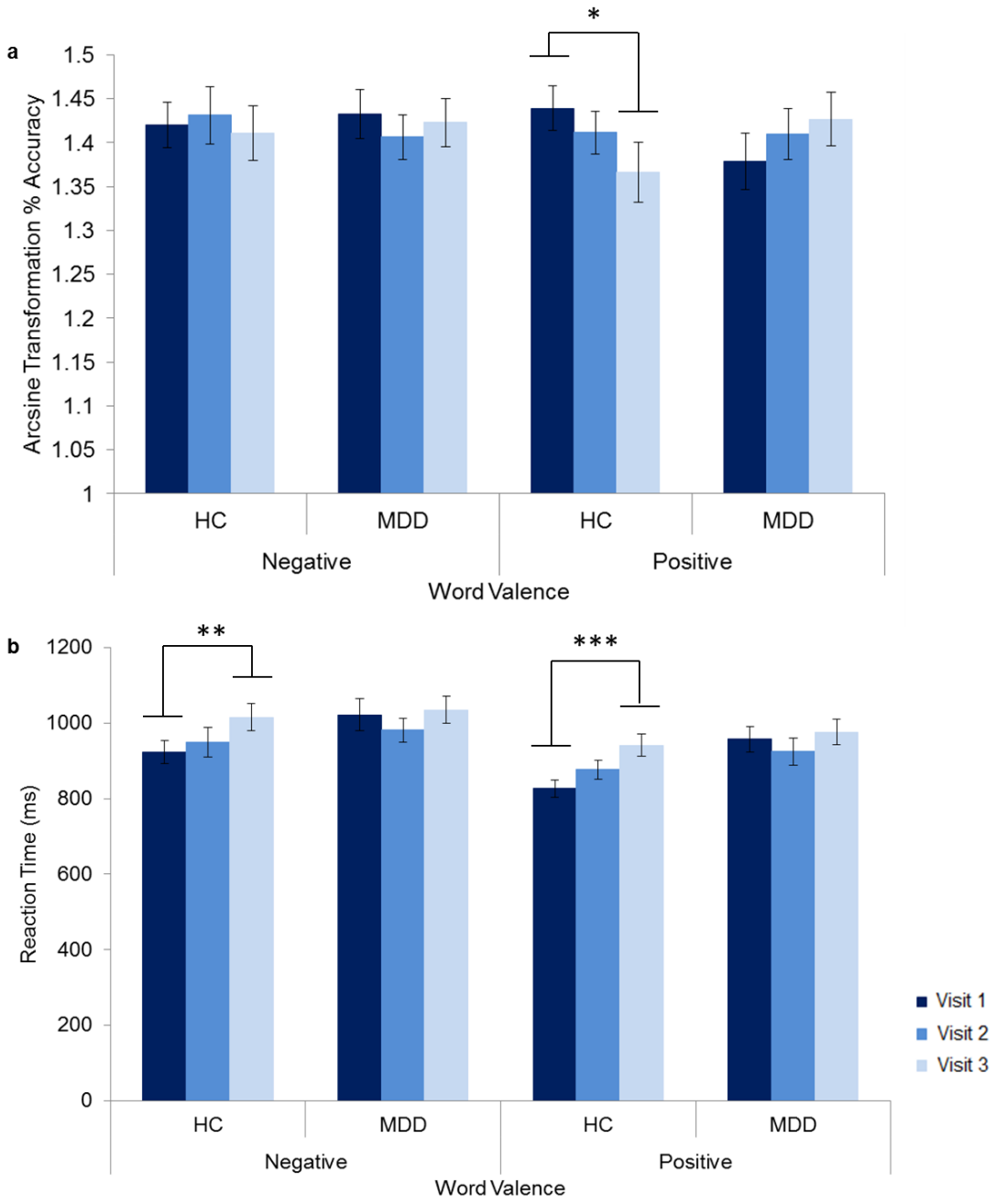


Figure 6.2 – ECAT Outcome Measures Change Over Time: (a) arcsine transformed % accuracy and **(b)** average reaction time for correct responses for each word valence for each participant group across the three visits. Values are reported as means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*p < 0.05, **p < 0.01, ***p < 0.001).

6.1.1.3 FDOT

The FDOT requires participants to respond to the alignment of a dot-probe appearing in the place of either a neutral, positively or negative valenced face. Median reaction time to respond to the dot-probe was used to calculate attentional vigilance towards the positively or negatively valenced faces. Separate repeated measures ANOVAs were performed for each outcome measure. The between subjects factor was participant group and within subjects factors were face emotion, masking and visit, with an additional within subjects factor of congruency for reaction time.

6.1.1.3.1 Early Effects of Bupropion at Week 2

For attentional vigilance, only a main effect of face emotion was found ($F_{1, 80} = 4.48$, $p < 0.05$) with a significantly greater vigilance for fearful faces across all participants and the two test visits. In a post hoc analysis considering the two face emotions separately, the repeated measures ANOVA for attentional vigilance for happy faces revealed a main effect of group ($F_{1, 80} = 6.02$, $p < 0.05$); however, this was most likely the result of the significantly lower attentional vigilance across masked and unmasked happy faces in the MDD group compared to the HC group found at baseline (Section 4.3.1.3) (Figure 6.3a – c).

A main effect of group was also found for reaction time ($F_{1, 80} = 5.03$, $p < 0.05$), again as a result of the significantly higher reaction times in the MDD group compared to the HC group at baseline. As a result of the baseline difference, the MDD group displayed a larger reduction in reaction times between baseline and test visit 2 compared to the HC group, manifesting as a visit by group interaction ($F_{1, 80} = 16.53$, $p < 0.001$) (Figure 6.3d).

6.1.1.3.2 Longer-term Effects of Bupropion at Week 6

A repeated measures ANOVA with 3 levels of visit for attentional vigilance again only revealed a main effect of face emotion ($F_{1, 78} = 4.94$, $p < 0.05$) for the greater vigilance for fearful faces across all participants and visits. However, inspection of Figure 6.3a suggests that attentional vigilance for both unmasked and masked happy faces increased between baseline and test visit 3 in the MDD group only. Since there was no significant main effect of

masking, data from the masked and unmasked happy face conditions was combined (Figure 6.3c). A repeated measures ANOVA considering just attentional vigilance for happy faces with only visit and not masking as the within subjects factor did find a visit by group interaction ($F_{2, 156} = 4.29$, $p < 0.05$), with only the bupropion-treated MDD group showing a significant increase in attentional vigilance for happy faces from baseline to test visit 3 ($t_{39} = -2.27$, $p < 0.05$).

For reaction time, again a visit by group interaction was found due to the greater reduction in reaction time from baseline to subsequent visits in the MDD group ($F_{2, 156} = 13.18$, $p < 0.001$).

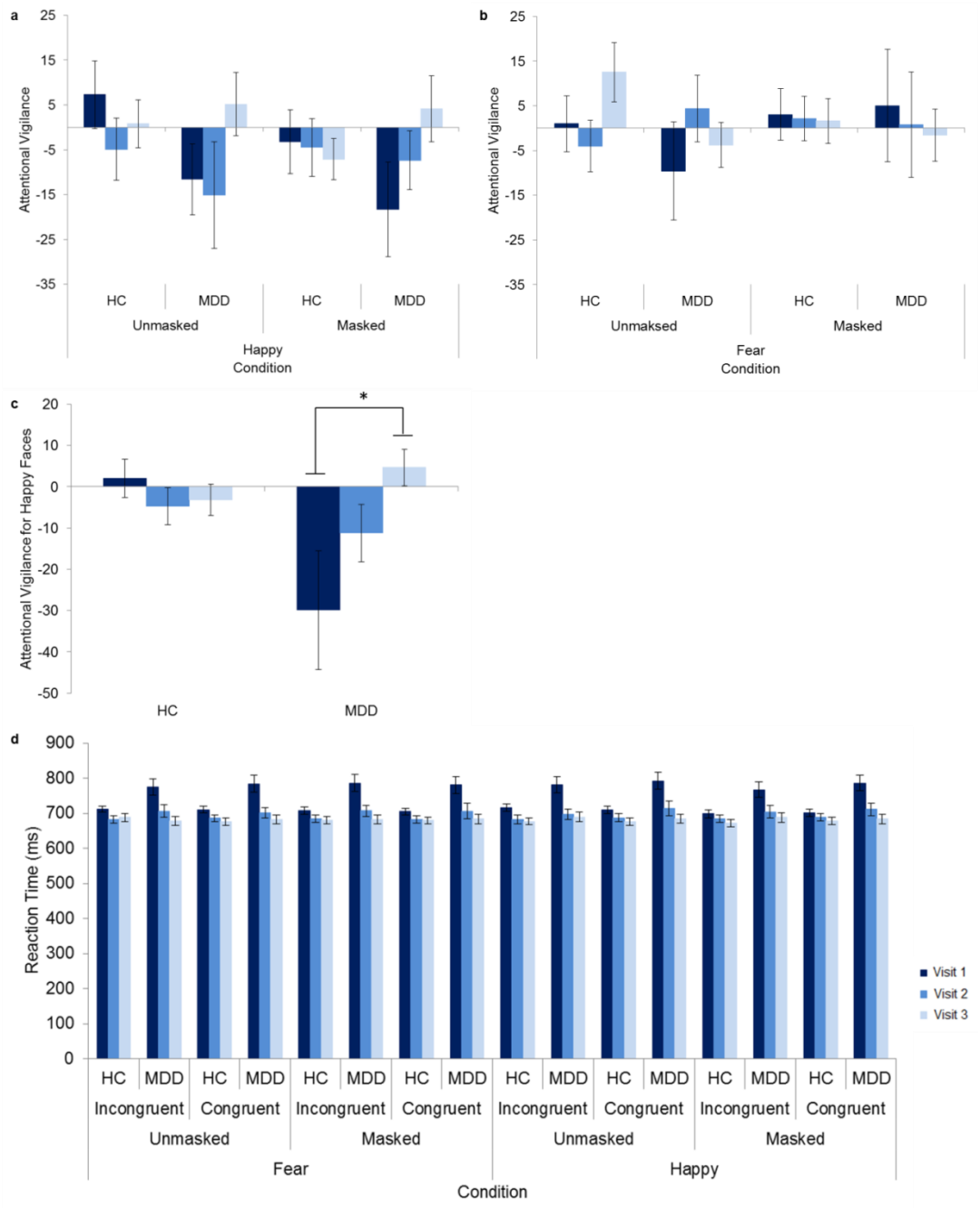


Figure 6.3 – FDOT Outcome Measures Change over Time: attentional vigilance for **(a)** unmasked and masked happy faces, **(b)** unmasked and masked fearful faces and **(c)** all happy faces for each participant group across the three visits. **(d)** Average reaction time for correct responses for each condition of the FDOT for each participant group across the three visits. At the participant level, medians were used then values displayed here are means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$).

6.1.1.4 EREC

The EREC requires participants to write down as many positive and negative self-referent words from the ECAT that they could remember. Outcome measures were number of self-referent words correctly and falsely recalled. Separate repeated measures ANOVAs with word valence and visit as the within subjects factors and participant group as the between subjects factor were performed for each outcome measure of the EREC.

6.1.1.4.1 Early Effects of Bupropion at Week 2

There was a main effect of word valence for the number of self-referent words both correctly ($F_{1, 81} = 84.21$, $p < 0.001$) and falsely ($F_{1, 81} = 62.87$, $p < 0.001$) recalled, with all participants remembering significantly more positive self-referent words across the two test visits.

For number of words correctly recalled, there was also a word valence by visit interaction ($F_{1, 81} = 20.31$, $p < 0.001$), with a significant increase in the number of positive self-referent words correctly recalled between baseline and test visit 2 in both the MDD ($t_{42} = -3.44$, $p < 0.01$) and HC ($t_{39} = -3.03$, $p < 0.01$) groups (Figure 6.4a). This was most likely due to the EREC being less of a surprise at test visit 2 so participants consciously tried to remember the words during the ECAT. No significant main effect of group ($F_{1, 81} = 0.20$, $p = 0.65$) or word valence by visit by group interaction was found ($F_{1, 81} = 1.00$, $p = 0.32$).

For number of words falsely recalled, there was a word valence by visit by group interaction ($F_{1, 81} = 5.73$, $p < 0.05$). This interaction remained in a repeated measures ANOVA considering just negative self-referent words ($F_{1, 81} = 4.60$, $p < 0.05$) and paired t-tests found this to be the result of a significant decrease in the number of negative self-referent words falsely recalled between baseline and test visit 2 in the bupropion-treated MDD group only ($t_{42} = 2.12$, $p < 0.05$) (Figure 6.4b).

6.1.1.4.2 Longer-term Effects of Bupropion at Week 6

A repeated measures ANOVA with 3 levels of visit again found a main effect of word valence for the number of self-referent words both correctly ($F_{1, 79} = 85.06$, $p < 0.001$) and

falsely ($F_{1, 79}$ = 69.64, p < 0.001) recalled driven by a greater memory for positive words across all participants and visits. However, between tests visits 2 and 3 there was a significant decrease in the number of positive self-referent words correctly recalled in both the MDD (t_{40} = 4.31, p < 0.001) and HC (t_{39} = 4.32, p < 0.001) groups. It could be argued that this was due to the longer break of 4 weeks rather than 2 weeks between these visits such that the EREC was more of a surprise again; however, if this was true there would be a general decrease in recall of both positive and negative self-referent words. No significant main effect of group or word valence by visit by group interaction was found for either number of words correctly ($F_{1, 79}$ = 0.01, p = 0.91; $F_{2, 158}$ = 0.50, p = 0.61) or falsely recalled ($F_{1, 79}$ = 0.43, p = 0.51; $F_{2, 158}$ = 0.06, p = 0.95).

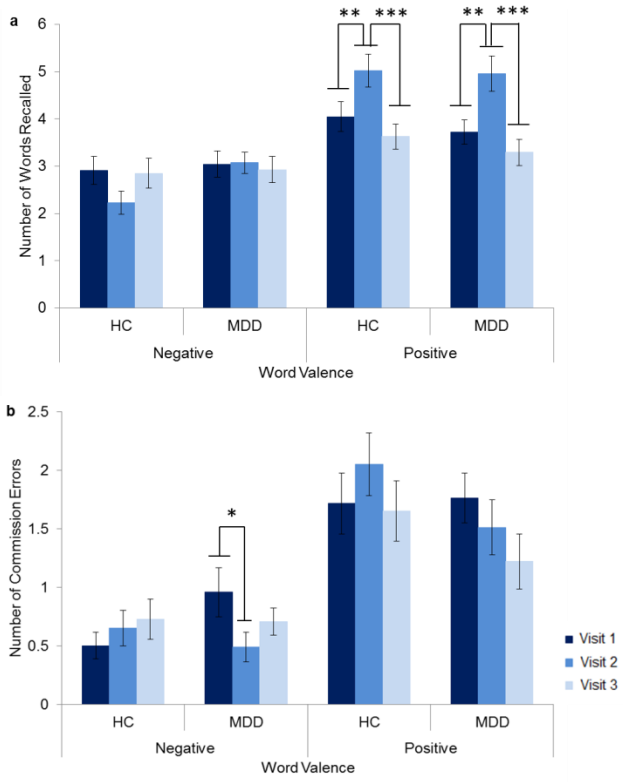


Figure 6.6 – EREC Outcome Measures Changes over Time: number of negative and positive **(a)** self-referent words correctly recalled and **(b)** commission errors made for each participant group across the three visits. Values are reported as means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*p < 0.05, **p < 0.01, ***p < 0.001).

6.1.2 Covert Faces Task

During the covert faces task, participants are required to indicate the gender of a series of emotional facial expressions that are fearful, happy or sad. Outcome measures were percentage accuracy and average reaction time for correct responses. A large proportion of individuals had near 100% accuracy, therefore in order to generate more normally distributed data, an arcsine transformation was again first applied to the percentage accuracy data prior to analysis. Separate repeated measures ANOVAs with face emotion and visit as the within subjects factors and participant group as the between subjects factor were then performed for each outcome measure of the covert faces task.

6.1.2.1 Early Effects of Bupropion at Week 2

No significant effects were found for arcsine transformed percentage accuracy, with percentage accuracy similar for each face emotion and between baseline and test visit 2 for both participant groups (Figure 6.5a).

A visit by group interaction was found for reaction time ($F_{1, 80} = 5.86$, $p < 0.05$). It can be seen from Figure 6.5b that only the bupropion-treated MDD group displayed a reduction in reaction time between baseline and test visit 2 across all face emotions, possibly as a result of higher reaction times at baseline compared to the HC group.

6.1.2.2 Longer-term Effects of Bupropion at Week 6

Arcsine transformed percentage accuracy was also similar between baseline and test visit 3, again with no significant effects in either participant group (Figure 6.5a).

A main effect of visit was found for reaction time ($F_{2, 156} = 149.14$, $p < 0.001$), with both groups displaying a significant decrease in reaction times between test visits 2 and 3, most likely as a result of performing the covert faces task outside of the scanner at week 6 (Figure 6.5b).

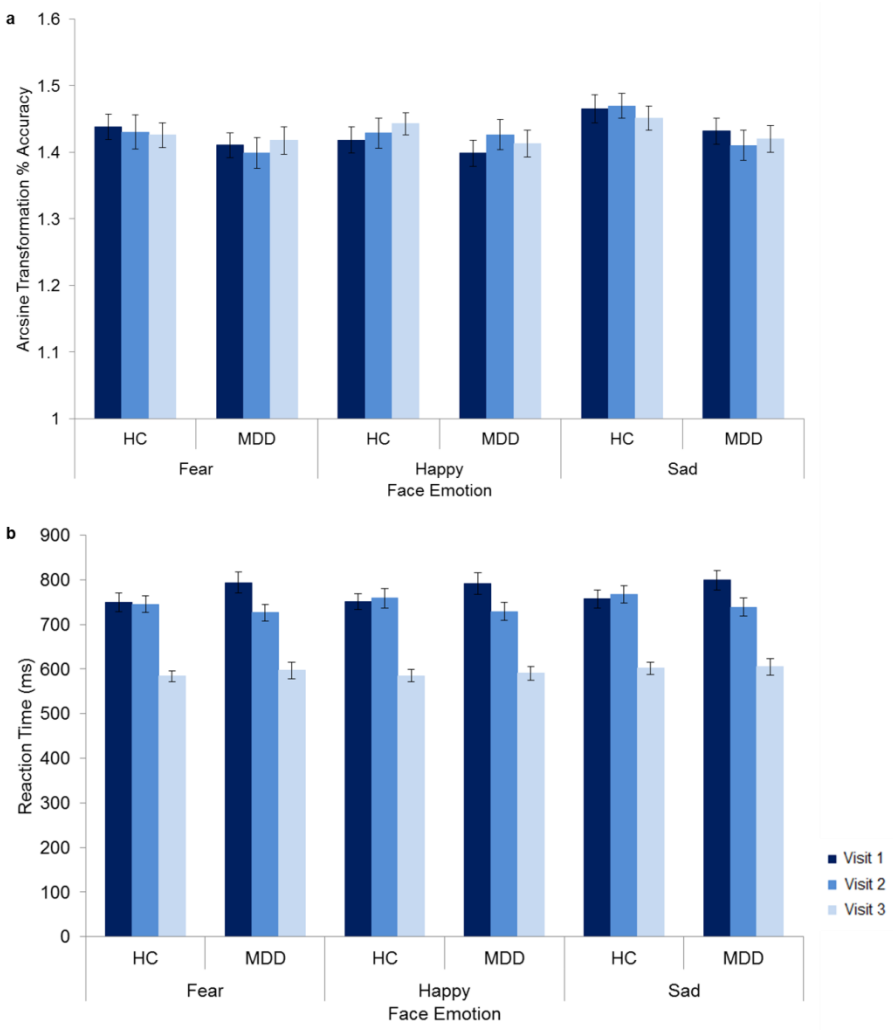


Figure 6.5 – Covert Faces Task Outcome Measures Change over Time: (a) arcsine transformed % accuracy and **(b)** average reaction time for correct responses for each face emotion for each participant group across the three visits. Values are reported as means ± standard error of the mean.

6.1.3 Reward Task

During the reward task, participants were presented with two symbols per trial, where each symbol was associated with a certain win, loss or no change outcome probability. Outcome measures were monetary totals (end total, amount won and amount lost) and reaction time. Separate repeated measures ANOVAs were performed for each outcome measure of the reward task. The between subjects factor was participant group. Run was

first included as an additional within subjects factor along with visit; however, no significant main effect of run was found at any point; therefore, run was no longer included and the average across runs was used in all subsequent analyses.

6.1.3.1 Early Effects of Bupropion at Week 2

A main effect of visit was found for the end total ($F_{1, 58} = 5.92$, $p < 0.05$), with a significant increase in the end total between baseline and test visit 2 across both participant groups (Figure 6.6a). An increase in the end total could be the result of an increase in the amount won, decrease in the amount lost or both. There was no main effect of visit for the amount won (Figure 6.6b) but there was a trend towards significance for visit for the amount lost ($F_{1, 58} = 3.47$, $p = 0.07$), with a slight decrease in the amount lost between baseline and test visit 2 in both participant groups (Figure 6.6c), which is most likely to be the contributor to the increase in end total. There was also a trend towards significance for group for the amount won ($F_{1, 58} = 3.59$, $p = 0.06$), with the MDD group appearing to win slightly less than the HC group at both baseline and test visit 2 (Figure 6.6b).

The totals do not provide much temporal information about responses made during the course of the reward task therefore learning curves were also produced depicting trial-by-trial the proportion of participants that chose the correct symbol in the win condition, associated with high-probably win and the incorrect symbol in the loss condition, associated with high-probability loss for each visit. It appeared that the bupropion-treated MDD group displayed a reduction in the likelihood of choosing the correct symbol in the win condition between baseline and test visit 2 (Figure 6.7a and b). To allow for statistical analysis, the average of the last 50% of the trials (last 15 out of 30) in which participants chose the correct symbol was calculated for both the win and loss conditions for each participant group (Harrison et al., 2016). A repeated measures ANOVA did find a visit by group interaction for the win condition ($F_{1, 28} = 10.11$, $p < 0.01$). Post hoc paired t-tests confirmed a significant reduction in the likelihood of choosing the correct symbol in the win condition between visits 1 and 2 for the bupropion-treated MDD group ($t_{14} = 4.17$, $p < 0.01$) but not the HC group (t_{14}

= -0.91, $p = 0.38$), such that the MDD group was significantly less likely than the HC group to choose the correct symbol in the win condition at test visit 2 ($t_{28} = 8.11$, $p < 0.001$), even more so than at baseline ($t_{28} = 2.03$, $p = 0.05$) (Figure 6.7d). These results add to the study investigating the acute effects of bupropion in healthy volunteers (Chapter 2), which, whilst there was no group interaction, also suggested that bupropion reduces the likelihood of choosing the correct symbol in the win condition.

6.1.3.2 Longer-term Effects of Bupropion at Week 6

A main effect of visit was again found for the end total ($F_{2, 108} = 7.77$, $p < 0.01$) and the amount lost ($F_{2, 108} = 4.32$, $p < 0.05$) , with a significant increase in the end total as a result of a significant decrease in the amount lost across all three visits (Figure 6.6a and c).

Regarding symbol choice during the course of the reward task, a repeated measures ANOVA found a visit by group interaction for the win condition ($F_{2, 56} = 17.59$, $p < 0.001$). Post hoc paired t-tests indicated a significant increase in the likelihood of choosing the correct symbol in the win condition between test visits 2 and 3 for both the bupropion-treated MDD group ($t_{14} = -10.5$, $p < 0.001$) and the HC group ($t_{14} = -3.35$, $p < 0.01$) but to a much greater extent for the bupropion-treated MDD group, such that the group difference observed at baseline ($t_{28} = 2.03$, $p = 0.05$) and test visit 2 ($t_{28} = 8.11$, $p < 0.001$) was no longer significant at test visit 3 ($t_{28} = -0.25$, $p = 0.80$) (Figure 6.7c). Finally, a main effect of visit was found for the loss condition ($F_{2, 56} = 40.83$, $p < 0.001$), with both participant groups displaying an increase in the likelihood of choosing the correct symbol in the loss condition over the three visits, consistent with the decrease in the overall amount lost over the three visits and likely the result of practice effects (Figure 6.7d).

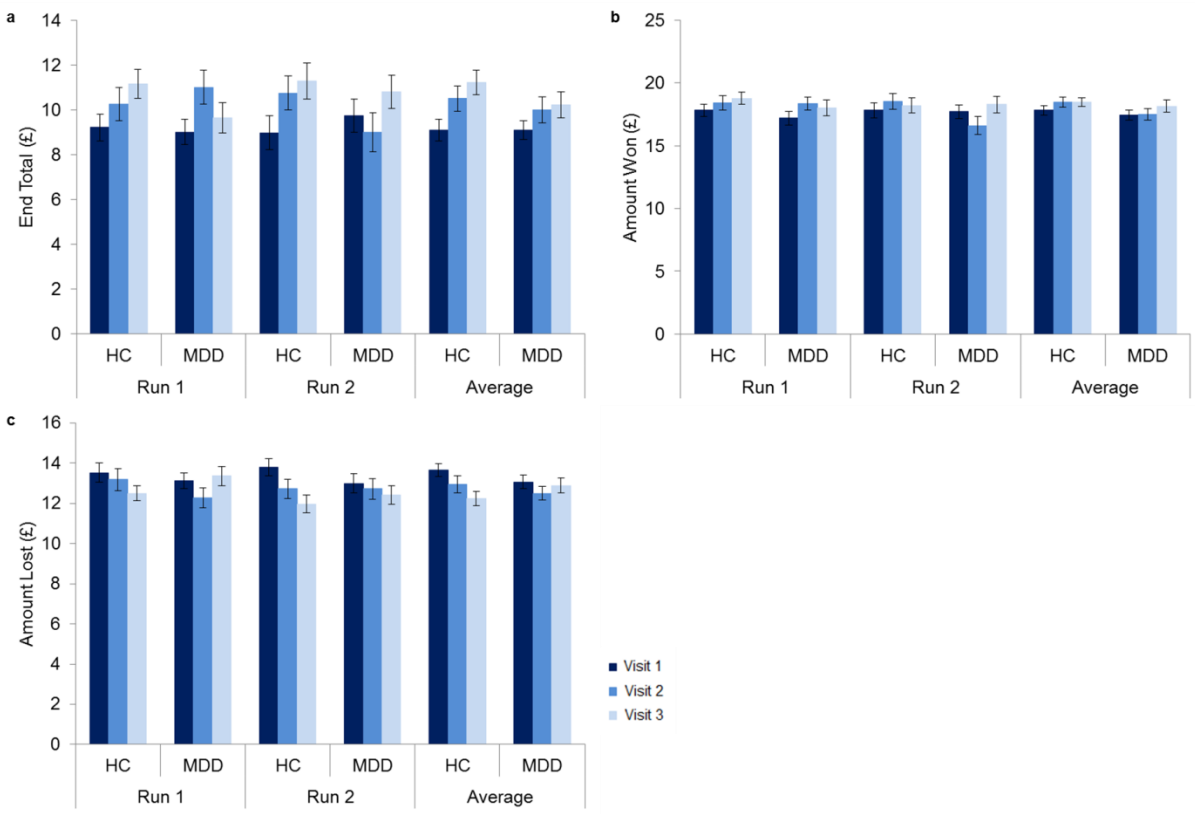


Figure 6.6 – Reward Task Monetary Totals Change Over Time: the monetary amount for run 1, run 2 and averaged across the two runs of the reward task for the **(a)** end total, **(b)** win condition and **(c)** loss condition for each participant group across the three visits. Values are reported as means ± standard error of the mean.

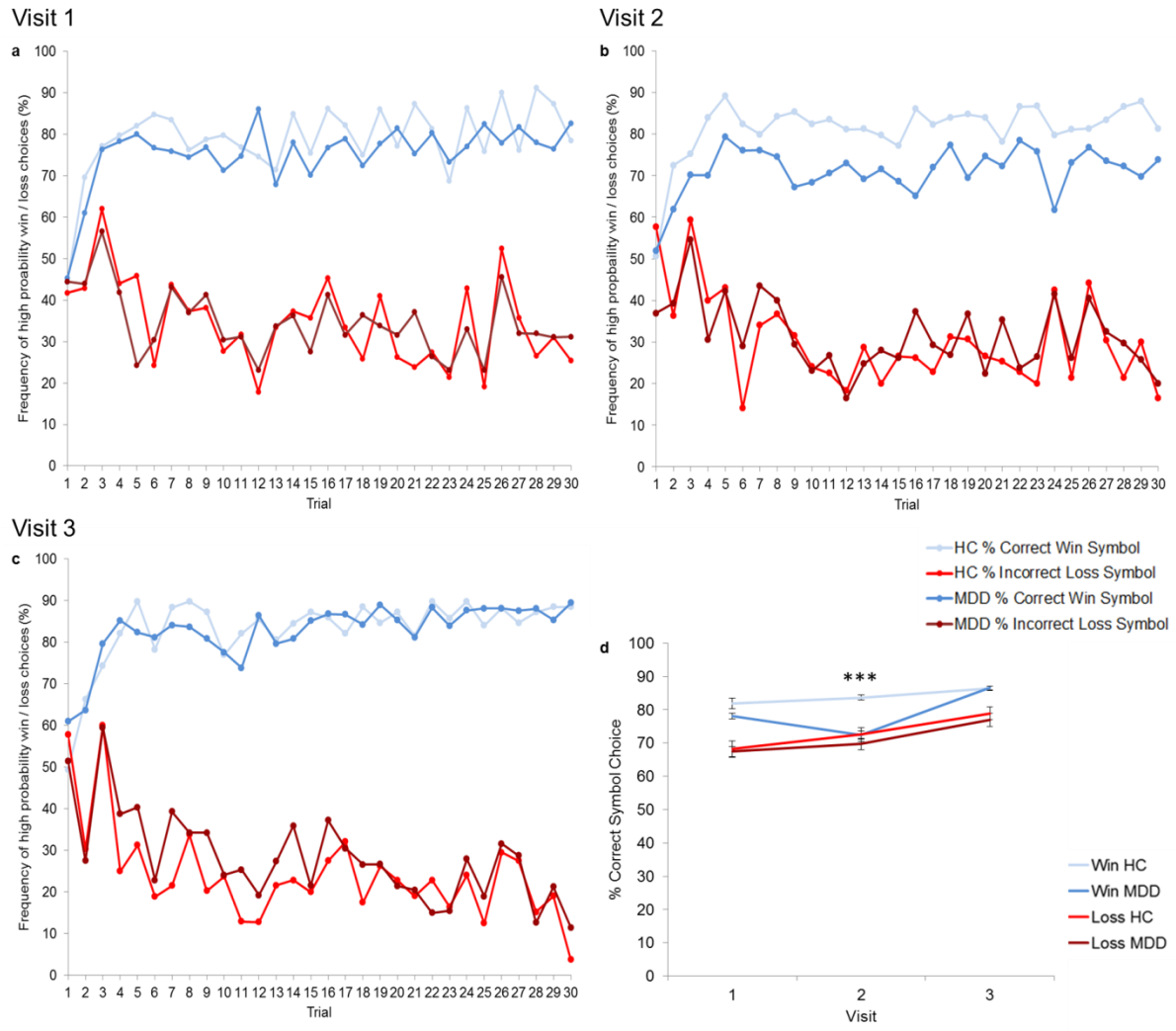


Figure 6.7 – Reward Task Learning Change over Time: learning curves for each participant group depicting trial-by-trial the proportion of participants that chose the correct symbol in the win condition, associated with high-probability win (top blue lines) and the incorrect symbol in the loss condition, associated with high-probability loss (bottom red lines) for test visits **(a)** 1, **(b)** 2 and **(c)** 3. **(d)** Average of the last 50% of trials in which participants chose the correct symbol for both the win and loss conditions for each participant group for each visit. Values are reported means ± standard error of the mean. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

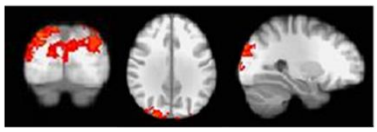
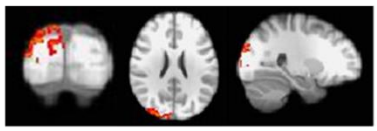
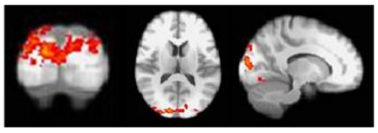
6.2 The Effect of Bupropion on Neural Measures of Reward and Emotional Processing

6.2.1 Covert Faces Task

6.2.1.1 Whole Brain Analysis

The whole brain analysis of changes in brain activity between baseline and test visit 2 for the covert faces task found that compared to the HC group, the bupropion-treated MDD group displayed a significantly greater decrease in occipital pole activity in response to faces (x y z = 28 -90 32, Z-max = 3.76, *p*-corrected < 0.001), in particular happy (x y z = 26 -92 24, Z-max = 3.57, *p*-corrected < 0.01) and sad faces (x y z = 14 -92 14, Z-max = 3.83, *p*-corrected < 0.001) (Table 6.1).

Table 6.1 Whole Brain Analysis of the Effect of Bupropion for the Covert Faces Task

Lower-level Contrast	Direction of Brain Activity	Direction of Group Comparison	x y z	Z-max	Corrected-*p*	Image
All Faces	Decrease	MDD > HC	28 -90 32	3.76	< 0.001	Occipital pole / superior lateral occipital cortex 
Happy	Decrease	MDD > HC	26 -92 24	3.57	< 0.01	Occipital pole 
Sad	Decrease	MDD > HC	14 -92 14	3.83	< 0.001	Occipital pole 

6.2.1.2 SVC / ROI Analysis

The SVC analysis of changes in brain activity between baseline and test visit 2 found that compared to the HC group, the bupropion-treated MDD group displayed a significantly greater increase in left medial OFC activity (x y z = -22 34 -16, Z-max = 3.12, *p*-corrected < 0.05) in response to sad versus happy faces, likely driven by an increase in left medial OFC

activity (x y z = -22 34 -16, Z-max = 3.41, *p*-corrected < 0.05) in response to sad faces (Figure 6.8).

The SVC analysis did not find any group differences in the change in amygdala activity. Change in amygdala activity was of particular interest in this study since previous research has found antidepressants to normalise hyper- or hypoactive responses to negative or positive affective stimuli, respectively. Therefore, individual percentage signal change values in response to sad, happy and fearful faces were extracted from the left and right amygdala at baseline and test visit 2 and the change in activity compared between MDD and HC groups in an ROI analysis in SPSS. However, this also did not reveal any significant group differences in the change in amygdala activation in response to emotional facial expressions.

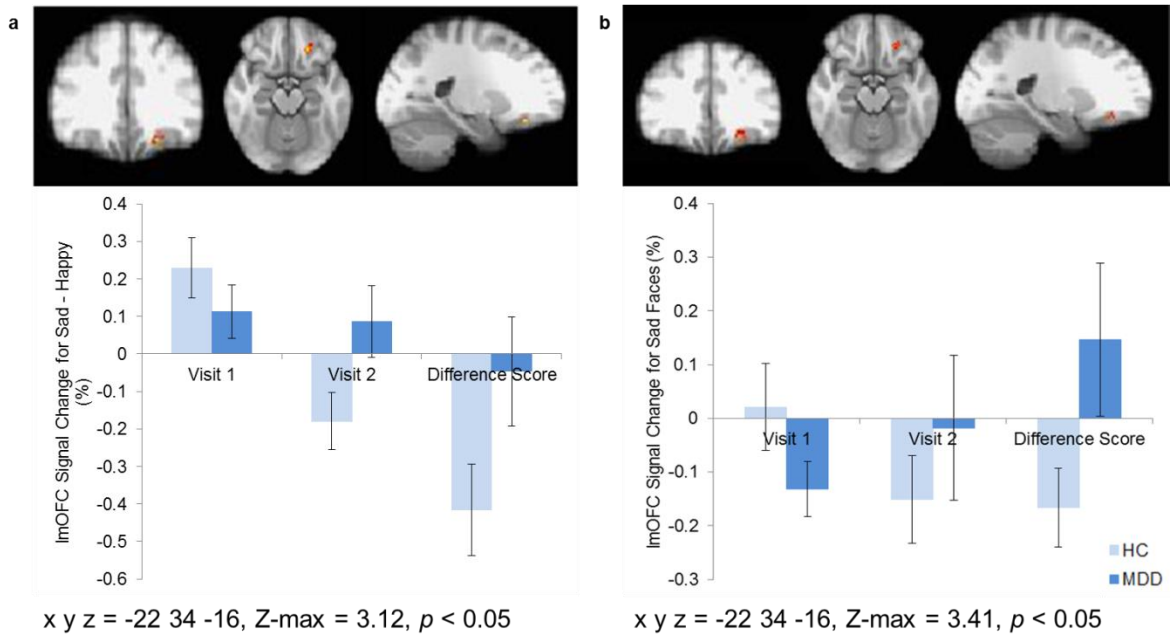


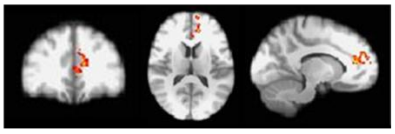
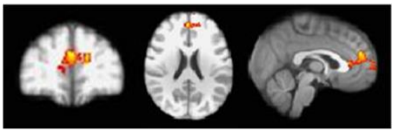
Figure 6.8 – Effect of Bupropion on Neural Activity during the Covert Faces Task: compared to the HC group, the bupropion-treated MDD group displayed a significantly greater increase in left medial activity in response to **(a)** sad versus happy faces and **(b)** sad faces. Values are reported as means ± standard error of the mean.

6.2.2 Reward Task

6.2.2.1 Whole Brain Analysis

The whole brain analysis of changes in brain activity between baseline and test visit 2 for the reward task found that compared to the HC group, the bupropion-treated MDD group displayed a significantly greater increase in paracingulate gyrus activity (x y z = -12 40 14, Z-max = 3.21, *p*-corrected < 0.05) in response to win verses neutral outcomes, likely driven by an increase in paracingulate gyrus activity (x y z = 2 50 22, Z-max = 3.40, *p*-corrected < 0.001) in response to win outcomes (Table 6.2).

Table 6.2 Whole Brain Analysis of the Effect of Bupropion for the Reward Task

Lower-level Contrast	Direction of Brain Activity	Direction of Group Comparison	x y z	Z-max	Corrected-*p*	Image
Win - Neutral	Increase	MDD > HC	-12 40 14	3.21	< 0.05	Paracingulate gyrus 
Win	Increase	MDD > HC	2 50 22	3.40	< 0.001	Paracingulate gyrus 

6.2.2.2 SVC Analysis

The SVC analysis found a group difference in the change in rostral ACC activity in response to win versus neutral outcomes (x y z = -12 40 14, Z-max = 3.21, *p*-corrected < 0.05) and win outcomes (x y z = 2 50 22, Z-max = 3.40, *p*-corrected < 0.001). Extraction of individual percentage signal change values from the rostral ACC clusters found this to be driven by an absence of a decrease in rostral ACC activity in response to win outcomes between baseline and test visit 2 in the bupropion-treated MDD group (Figure 6.9a). The SVC analysis also found the bupropion-treated MDD group to display a significantly greater increase in right medial OFC (x y z = 26 26 -12, Z-max = 2.65, *p*-corrected < 0.05) activity in response to no win in win outcomes (Figure 6.9b).

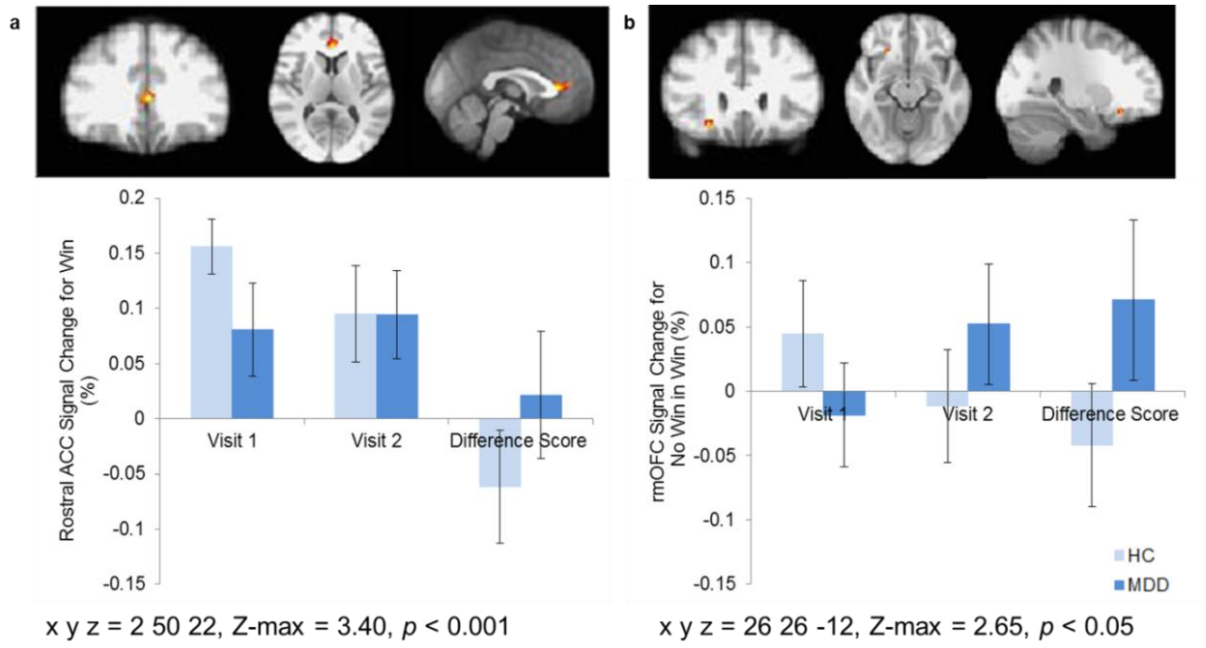


Figure 6.9 – Effect of Bupropion on Neural Activity during the Reward Task: compared to the HC group, the bupropion-treated MDD group **(a)** did not display a decrease in rostral ACC activity in response to win outcomes and **(b)** displayed a significantly greater increase in right medial OFC activity in response to no win in win outcomes. Values are reported as means ± standard error of the mean.

6.3 Summary

This Chapter aimed to investigate whether the DA antidepressant bupropion would affect behavioural and neural measures of reward and emotional processing in MDD patients. It was hypothesised that bupropion would act to remediate the negative affective biases and aberrant reward processing observed in the previous Chapters in line with the cognitive neuropsychological theory of antidepressant action.

Compared to the HC group, between baseline and test visit 2, the bupropion-treated MDD group was found to display significantly reduced response bias for or misclassification of other faces as sad, reduced number of negative self-referent words falsely recalled, reduced likelihood of choosing the correct symbol in the win condition associated with high-probability win, a greater increase in left medial OFC activity in response to sad faces and

greater rostral ACC activity in response to win outcomes. The effects on response bias or misclassification of other faces as sad were heightened between baseline and test visit 3 and between test visits 2 and 3, the bupropion-treated MDD group displayed restoration of choice frequency for high-probability win back to similar levels observed in the HC group. There was also some evidence for increased percentage accuracy for recognising fearful faces in the bupropion-treated MDD group over the 3 visits; however, significance was only found in post hoc t-tests without a prior significant interaction so these results should be treated with caution.

6.4 Discussion

The cognitive neuropsychological theory of antidepressant action suggests that antidepressants do not act as direct mood enhancers but instead act to alter the balance between positive and negative emotional processing prior to mood improvement. As previously discussed this theory of antidepressant action is supported by extensive evidence from studies investigating the effect of TRP depletion and repeated, subchronic (7 day) or acute antidepressant treatment on emotional processing in HCs and MDD patients. However, it remains unclear how the initial antidepressant effects on biases in emotional processing relate to effects on aberrant reward processing in MDD patients and whether they are also targeted by antidepressants with DA activity, such as bupropion.

6.4.1 Effect of Bupropion on Emotional Processing

Many studies have previously found acute and subchronic administration of both SSRIs and SNRIs to alter the balance between positive and negative emotional processing, including reducing the interpretation of ambiguous or neutral faces as sad, reducing the memory for negative self-referent words and increasing sensitivity to happy faces (Harmer et al., 2004, Harmer et al., 2009b). This Chapter also found the bupropion-treated MDD group to display a significant reduction in the response bias for sad faces, misclassifying

significantly fewer faces as sad and a significant reduction in the number of negative self-referent words falsely recalled by 2 weeks, as well as an increase in the attentional vigilance for happy faces by 6 weeks. These results appear to be similar to the acute effect of bupropion compared to placebo observed in the healthy volunteer study (Chapter 2), as well as the effects of other antidepressants. As discussed in Chapter 2, the profile of effects of bupropion on emotional processing is very similar to that of reboxetine.

An acute dose of an antidepressant, particularly an SSRI, has also been shown to actually increase fear processing, which is then reduced following repeated administration, consistent with the clinical observation of an initial increase in anxiety prior to the production of long-term anxiolytic effects. For example, a single 20 mg dose of the SSRI citalopram has been found to enhance the startle response (Grillon et al., 2007) and increase the percentage accuracy for recognising fearful faces (Browning et al., 2007). After 7 days citalopram treatment, the percentage accuracy for recognising fearful faces is decreased, along with activity in the amygdala, hippocampal and medial PFC in response to fearful stimuli (Harmer et al., 2006). Therefore, there is previous evidence to support the suggestion that bupropion may also increase the percentage accuracy for the recognition of fearful faces; however, there are a few inconsistencies that need to be considered. Firstly, the initial increase in fear processing is an effect often observed with SSRIs but not SNRIs. Therefore, it is surprising to observe an increase in fear processing with an antidepressant that acts to increase NA and DA without any effect on 5-HT activity. Secondly, whilst the increase in fear processing is typically only observed with acute administration, in this Chapter it was observed at 2 weeks and heightened at 6 weeks. However, the side-effects of bupropion do include agitation and anxiety that some participants did report to worsen throughout the course of the study. Further work is needed to disentangle these effects and consider the relationship between changes in anxiety and arousal versus changes in negative affect and anhedonia in relation to these cognitive measures.

Regarding neural measures of emotional processing, most studies report that antidepressant treatment decreases hyperactivity in various brain regions in response to

negative affective stimuli. These brain regions include the amygdala, involved in emotional reactivity and memory, the occipital or extrastriate cortex, involved in the processing of specific features of visual information, the fusiform gyrus, involved in the recognition of visual information and frontal regions, involved in the higher-order control of emotional processing (Sheline et al., 2001, Fu et al., 2004, Lawrence et al., 2004, Surguladze et al., 2005, Fu et al., 2008, Victor et al., 2010). However, this Chapter found the bupropion-treated MDD group to display a greater increase in left medial OFC activity in response to sad faces compared to the HC group. This may relate to the role of frontal regions in the top-down control of emotional processing and inverse coupling with limbic regions, including the amygdala. In the healthy brain, downregulation of negative affect involves increased medial frontal activation with decreased amygdala activation (Urry et al., 2006). This inverse coupling may be altered in MDD, which may be the cause of the inability to downregulate negative affect and the amygdala hyperactivity (Johnstone et al., 2007, Veer et al., 2010). In this way, the increase in left medial OFC activity in response to sad faces in the bupropion-treated MDD group may allow reduced amygdala activity in response to sad faces and subsequent reduced negative affect.

6.4.2 Effect of Bupropion on Reward Processing

Administration of drugs enhancing DA activity has been found to alter behavioural and neural measures of reward processing, at least in healthy volunteers. For example, during a probabilistic instrumental learning task, the metabolic precursor of DA, L-DOPA was found to significantly increase the likelihood of choosing the stimulus associated with high-probability win, the amount of money won and VS activity in response to reward compared to the DA receptor antagonist haloperidol. Neither L-DOPA nor haloperidol were found to alter the amount of money lost or brain activity in response to losses (Pessiglione et al., 2006). Administration of L-DOPA has also been found to restore blunted reward-related brain activity in old age (Chowdhury et al., 2013).

A recently published study has also investigated the specific effects of bupropion on neural measures of reward processing in healthy volunteers. Healthy volunteers completed an fMRI reward task following 7 day treatment with bupropion or placebo. The task covered all aspects of reward processing with a cue for the anticipation of reward, effort exerted to receive the reward and actual receipt of the reward. Whilst no behavioural effects were observed, bupropion was found to increase activity during anticipation of the reward in the vmPFC and caudate, exertion of effort for the reward in the vmPFC, VS and ACC and finally receipt of the reward in the mOFC compared to placebo. Bupropion was also found to increase activity to an aversive stimulus in the vmPFC, mOFC, VS and amygdala compared to placebo (Dean et al., 2016). This suggests that bupropion can increase reward-related neural activity and therefore be potentially beneficial for MDD patients with aberrant reward processing.

From these studies investigating the effects of DA enhancing drugs, it was hypothesised that bupropion would act to restore the aberrant reward processing observed in MDD patients. Specifically, increase the likelihood of choosing the stimulus associated with high-probability win and increase reward-related brain activity, particularly VS activity. The bupropion-treated MDD group were found to have greater activity in the paracingulate gyrus and rostral ACC in response to win outcomes and right medial OFC activity in response to no win in win outcomes compared to the HC group at 2 weeks. However, bupropion actually further decreased the likelihood of choosing the correct symbol in the win condition associated with high-probability win at 2 weeks compared to the HC group. One possible explanation for this could be an autoreceptor response to the enhanced DA activity. The enhanced DA activity could activate presynaptic autoreceptors, such as DA D2-autoreceptors, which would in turn reduce the release of DA by the presynaptic neuron, thereby inducing a negative feedback loop (Ford, 2014). A further possible explanation could be the phasic versus tonic firing nature of DA neurons. Phasic firing refers to a transient burst of firing following presynaptic input in response to a stimulus and as previously discussed, plays a crucial role in associative reward learning. Tonic firing refers to sustained

firing at a constant frequency regulated by frontal activity in order to set the background level of DA and subsequently the responsivity of the DA system (Grace, 1991). Administration of bupropion may act to increase tonic firing of DA neurons and the general background levels of DA but as a result decrease the responsivity of the DA system such that phasic firing is actually reduced. In this way, associative reward learning is disrupted and bupropion-treated MDD patients fail to or are slower to learn the association of a particular stimulus with high-probability win. By week 6, homeostatic mechanisms may result in increased responsivity of the DA system allowing restoration of performance on the reward task back to HC levels. Finally, especially given that the effects of bupropion on emotional processing were similar to SSRIs / SNRIs, bupropion could also affect reward processing in a similar way to SSRIs, acting to initially exacerbate the loss of response bias towards reward (McCabe et al., 2010).

Overall, bupropion was found to have effects on behavioural and neural measures of emotional and reward processing. However, it is important to highlight a few limitations in this Chapter. Firstly, there is the problem of multiple comparisons, whereby the more statistical tests that are performed, the greater the likelihood that some *p* values less than 0.05 have occurred purely by chance creating a false positive result. In the current Chapter, statistical analyses were not corrected for multiple comparisons and post hoc tests without prior justification from a significant interaction were performed. This was due to the exploratory nature of the study. Whilst there is increased likelihood for false positive results, there is some evidence that the reported results are true. For example, the effects of bupropion appear to be similar to those reported in the study investigating the acute effects of bupropion in healthy controls as well as the effects of other antidepressants. However, replication of these effects of bupropion in an independent study is certainly still required.

Secondly, the study design meant that all participants who received bupropion were depressed, whereas all participants who received no drug treatment were not depressed, introducing a confound into the study. This makes it challenging to determine whether group by visit interactions result from drug effects or from differences between the depressed and healthy groups. For example, some of the effects of bupropion were found on measures that

had large group differences at baseline and could simply be the result of regression to the mean; although other effects of bupropion were found on measures without any obvious group differences at baseline, such as the reduction in the number of negative self-referent words falsely recalled. An investigation of the association between the potential effects of bupropion and clinical outcome with bupropion would not be influenced by this group difference confound with drug effect as it involves looking at associations within the same group of participants and is therefore discussed in the following Chapter.

CHAPTER 7

PREDICTING CLINICAL OUTCOME

The previous Chapter showed that bupropion has early effects on behavioural and neural measures of reward and emotional processing, within 2 weeks of treatment. The question remains as to whether it is possible to predict how effective a particular antidepressant will be for a particular individual in the long-term from the magnitude of such early effects of an antidepressant on behavioural and neural measures of reward and emotional processing. Initial results are promising, for example, the increase in the accuracy of recognising happy facial expressions produced by 2 weeks treatment with citalopram or reboxetine was found to be associated with the clinical improvement seen after 6 weeks of continued treatment (Tranter et al., 2009). Increased accuracy of happy facial expression recognition following 7 days citalopram treatment has also been found to be a marginally significant predictor of antidepressant response after 8 weeks in later-life MDD (Shiroma et al., 2014). At the neural level, changes in left amygdala activity have been found to differentiate between responders and non-responders, with responders showing reduced activity and non-responders showing increased activity in response to fearful compared to happy facial expressions after 1 week of treatment with an SSRI (Godlewska et al., 2013).

This Chapter aims to investigate whether changes in the behavioural and neural measures of reward and emotional processing in the bupropion-treated MDD group at week 2 are associated with clinical outcome at week 6, with a focus on the changes found in the previous Chapter. This will be useful for the interpretation of the results from the previous Chapter without influence of the group difference confound with drug effect as well as having important clinical implications. It was expected that the early changes in behavioural and/or neural measures at 2 weeks would correlate with the change in scores on questionnaires measuring clinical outcome, anhedonia and negative affect at 6 weeks.

7.1 Are the Changes in Behavioural Measures of Reward and Emotional Processing Associated with Clinical Outcome in MDD?

In section 6.1, the changes in the behavioural measures of reward and emotional processing in the bupropion-treated MDD were discussed and it was found that the bupropion-treated MDD group displayed significantly reduced misclassification of other faces as sad or increased beta for sad faces, increased attentional vigilance for happy faces, reduced false recall of negatively valenced self-referent words and reduced likelihood of choosing the correct symbol in the win condition of the reward task. There was also a suggestion of increased accuracy in the recognition of fearful faces. The following sections investigate whether these behavioural effects as well as changes in any of the other behavioural measures of reward and emotional processing not found to differ between the HC and bupropion-treated MDD groups are associated with clinical outcome and change in subjective experiences of anhedonia and negative affect, firstly through the use of correlations with questionnaire scores then by dividing the MDD group into responders versus non-responders based on the HAM-D (as discussed in Section 4.2.1).

7.1.1 Correlating Behavioural Measures with Questionnaire Scores

The relationship between each change in behavioural measure at 2 weeks and the change in questionnaire scores at 6 weeks was assessed by calculating difference scores and inputting these into partial correlations with the baseline scores of the questionnaire of interest included as a covariate. The results for questionnaires measuring clinical outcome, negative affect and anhedonia are discussed in the following sections. Given the number of correlations performed for the behavioural and questionnaire measures, it is important to highlight the problem of multiple comparisons. A higher significance threshold is general required to compensate for the number of tests being performed; however, since this is a more exploratory analysis, the correlations were not corrected for multiple comparisons and results should be interpreted with caution.

7.1.1.1 Clinical Outcome based on the HAM-D

As discussed in Section 4.2, the HAM-D is typically used to assess clinical outcome. None of the changes in behavioural measures either at 2 weeks or 6 weeks were found to correlate with the change in HAM-D score (Figure 7.1) or with the percentage improvement in HAM-D (Figure 7.2) at 6 weeks.

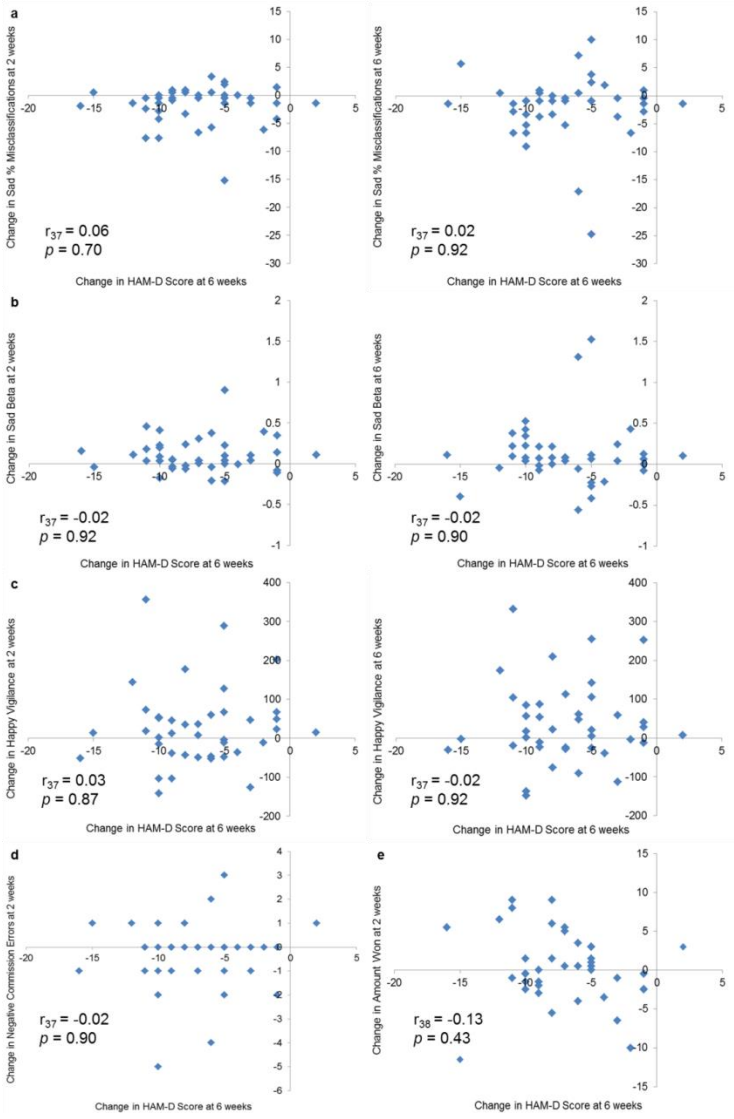


Figure 7.1 - Correlating the Changes in Behavioural Measures with the Change in HAM-D Score: correlations of the change in HAM-D score at 6 weeks with the change in the **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces at 2 and 6 weeks, **(d)** negative commission errors and **(e)** amount won at 2 weeks only.

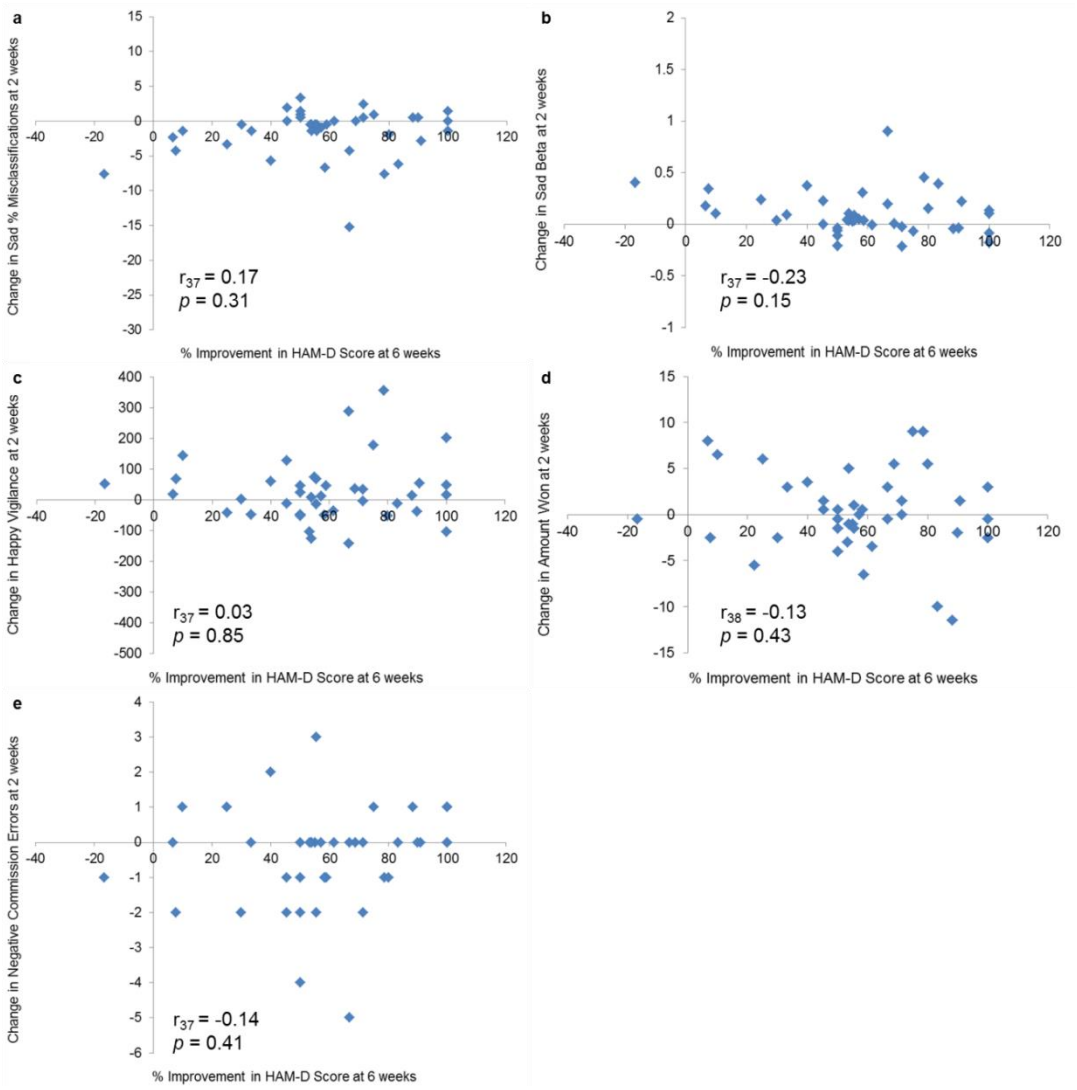


Figure 7.2 – Correlating the Changes in Behavioural Measures with the % Improvement in HAM-D score: correlations of the % improvement in HAM-D score at 6 weeks with the change in the **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces, **(d)** amount won and **(e)** negative commission errors at 2 weeks.

7.1.1.2 Negative Affect

Other questionnaires assessing mood and negative affect were included in this study and therefore also considered. Again, none of the changes in behavioural measures either at 2 weeks or 6 weeks were found to correlate with the change in score of the MASQ general distress: depressive sub-scale (Figure 7.3), PANAS negative affect (Figure 7.4) or PANAS positive affect (Figure 7.5). When investigating other behavioural measures, a negative correlation was found between the change in the number of positive self-referent words correctly recalled at 2 weeks and the change in score of the MASQ general distress: depressive sub-scale at 6 weeks ($r_{37} = -0.39$, $p < 0.05$) (Figure 7.3f).

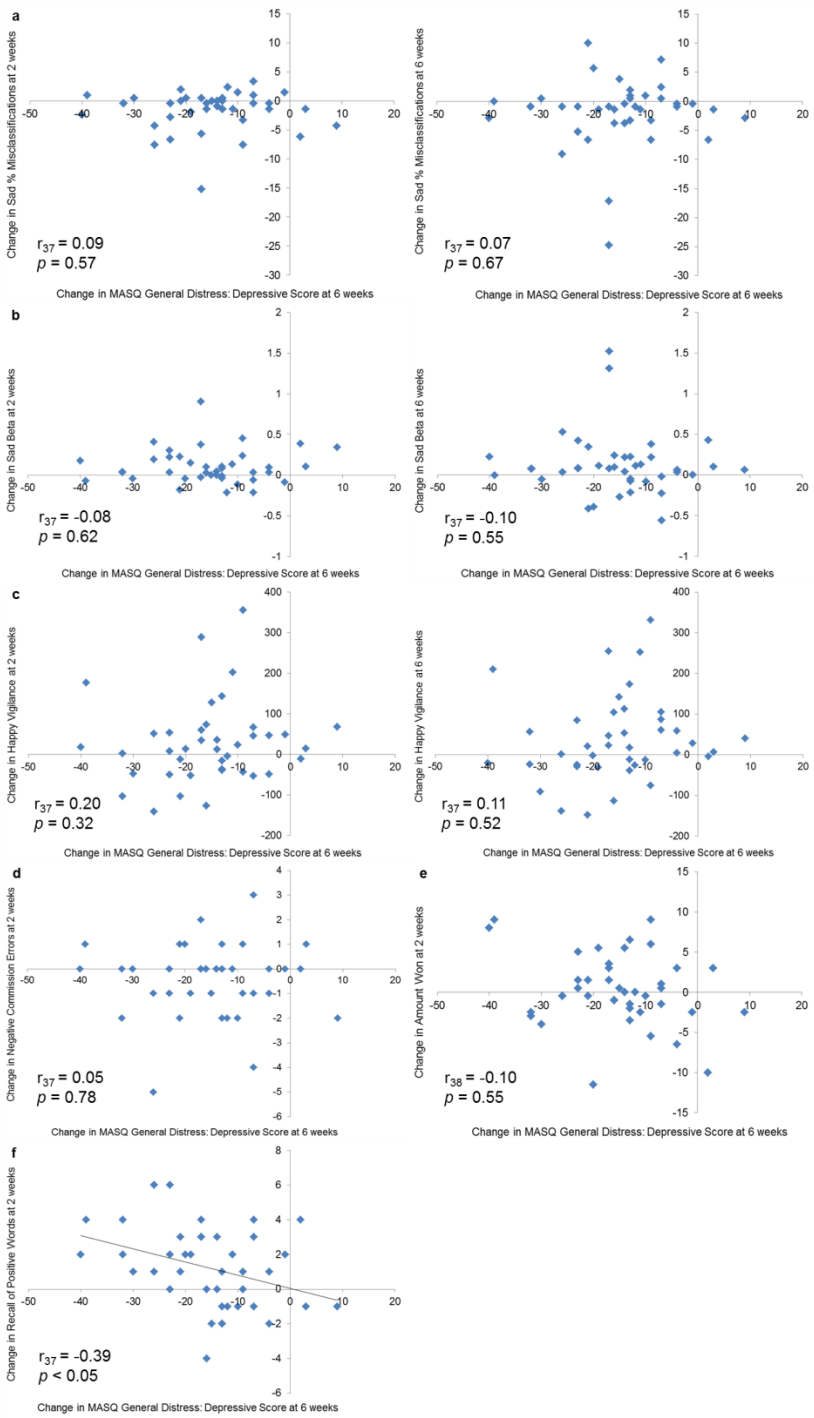


Figure 7.3 - Correlating the Changes in Behavioural Measures with the Change in MASQ General Distress: Depressive Score: correlations of the change in MASQ general distress: depressive score at 6 weeks with the change in the **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces at 2 and 6 weeks, **(d)** negative commission errors, **(e)** amount won and **(f)** number of positive self-referent words correctly recalled at 2 weeks only.

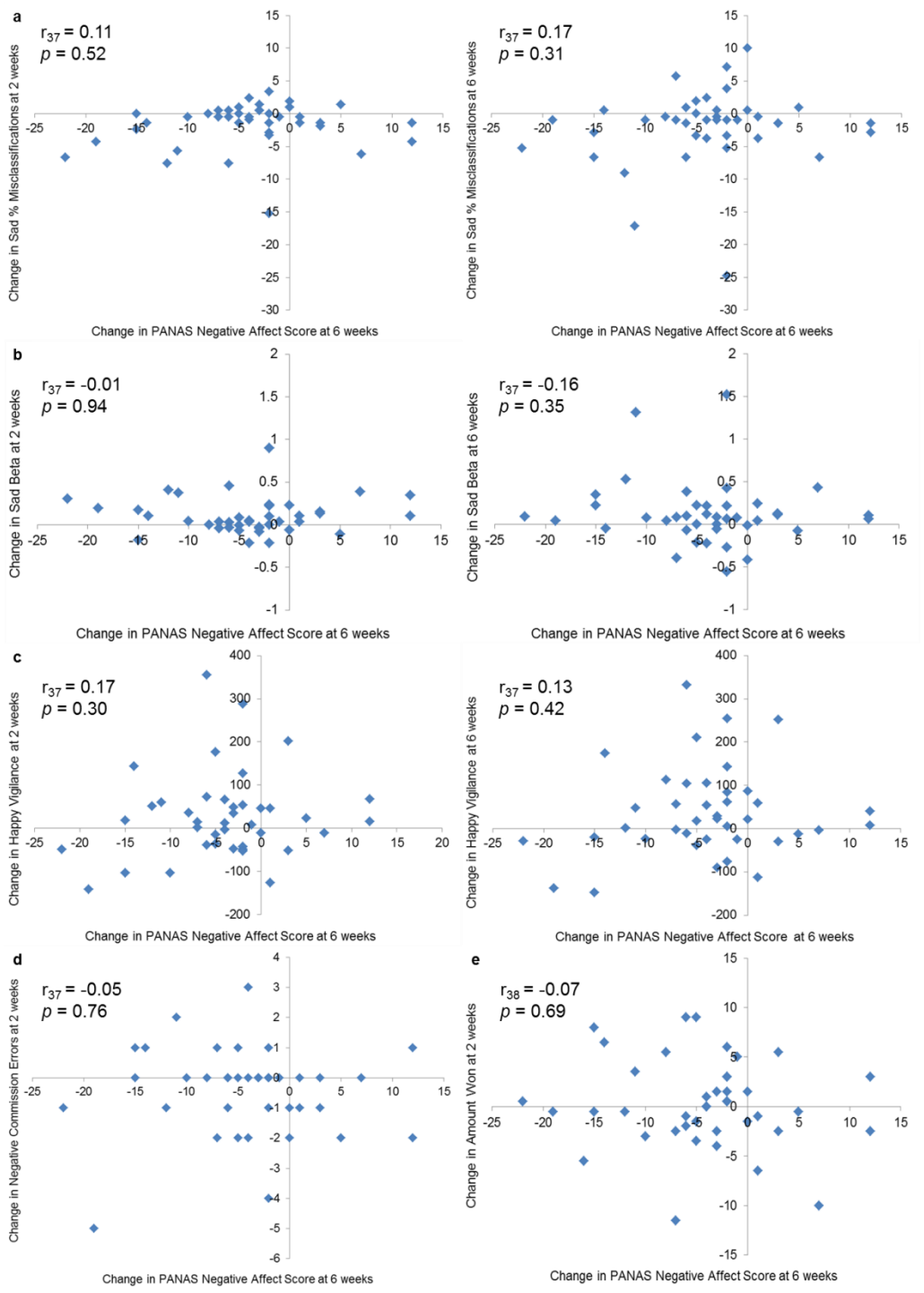


Figure 7.4 - Correlating the Changes in Behavioural Measures with the Change in PANAS Negative Affect Score: correlations of the change in PANAS negative affect score at 6 weeks with the change in the **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces at 2 and 6 weeks, **(d)** negative commission errors and **(e)** amount won at 2 weeks only.

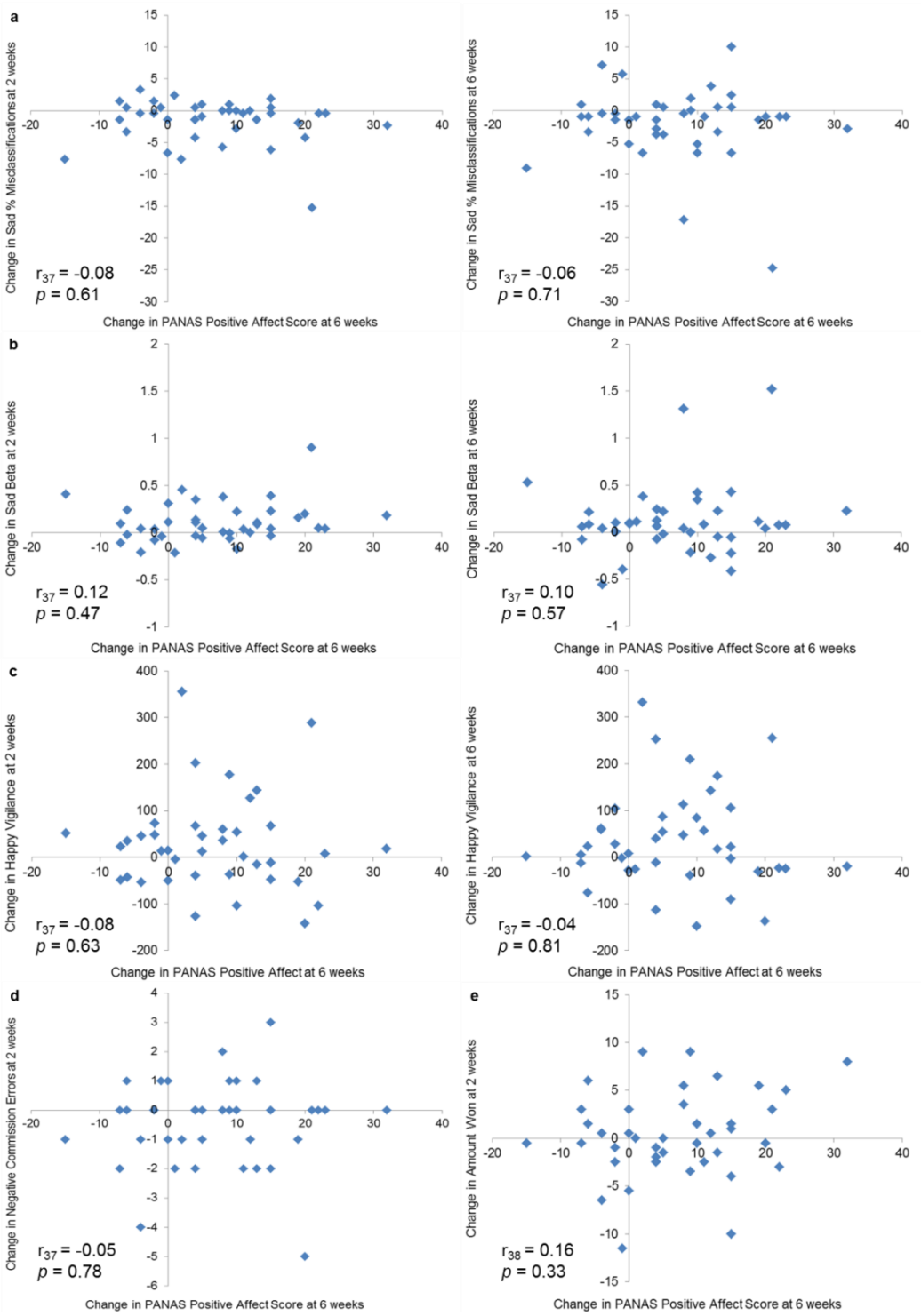


Figure 7.5 - Correlating the Changes in Behavioural Measures with the Change in PANAS Positive Affect Score: correlations of the change in PANAS positive affect score at 6 weeks with the change in the **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces at 2 and 6 weeks, **(d)** negative commission errors and **(e)** amount won at 2 weeks only.

7.1.1.3 Anhedonia

The SHAPS was the main questionnaire used to measure anhedonia; however, the MASQ includes a sub-scale that also measures anhedonia. Again, none of the changes in behavioural measures either at 2 weeks or 6 weeks were found to correlate with the change in score of the SHAPS (Figure 7.6) or MASQ anhedonic depression (Figure 7.7). When investigating other behavioural measures, a negative correlation was found between the change in the number of positive self-referent words correctly recalled at 2 weeks and the change in score of the MASQ anhedonic depression sub-scale at 6 weeks ($r_{37} = -0.32$, $p < 0.05$) (Figure 7.7f).

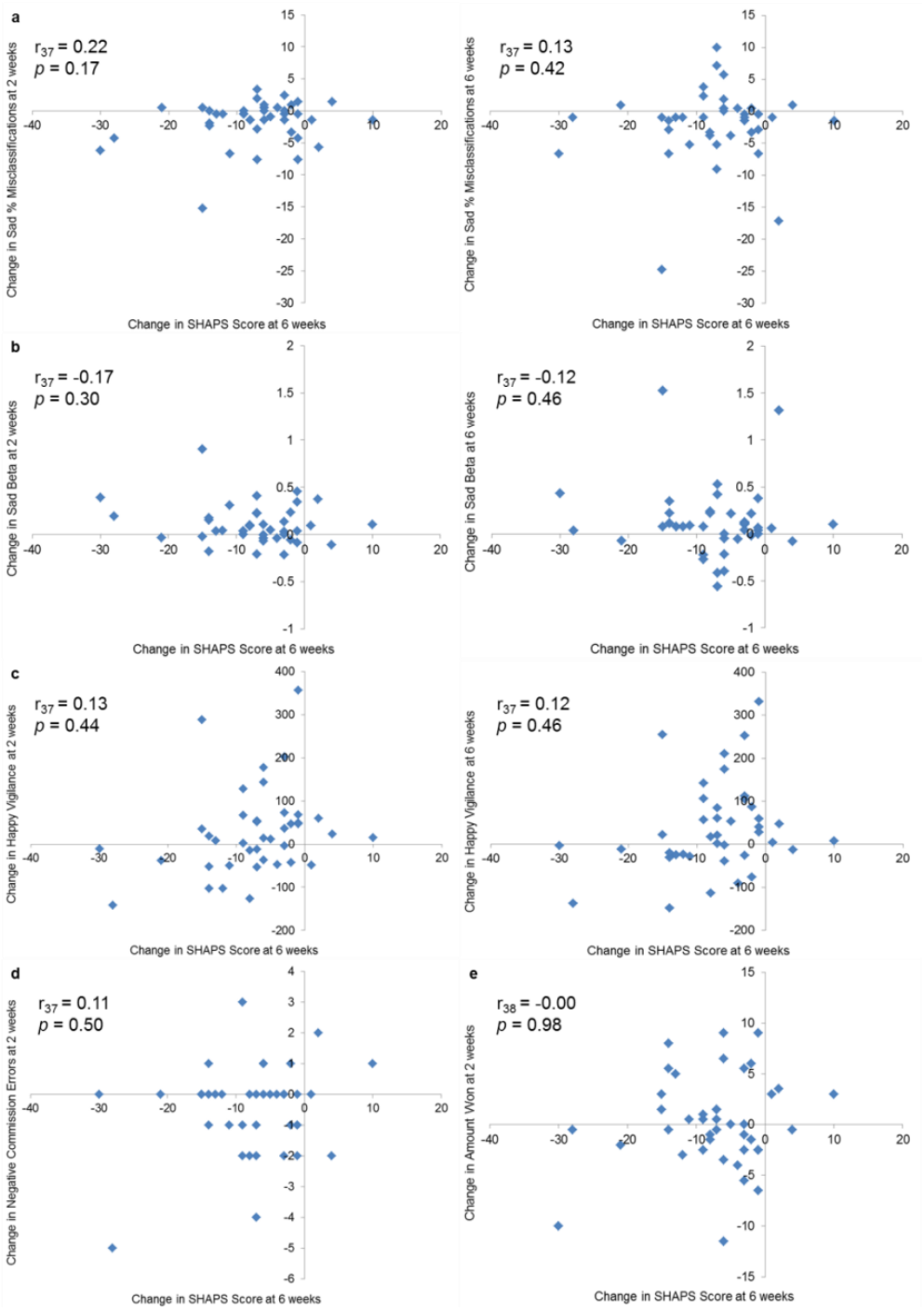


Figure 7.6 - Correlating the Changes in Behavioural Measures with the Change in SHAPS Score: correlations of the change in SHAPS score at 6 weeks with the change in the **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces at 2 and 6 weeks, **(d)** negative commission errors and **(e)** amount won at 2 weeks only.

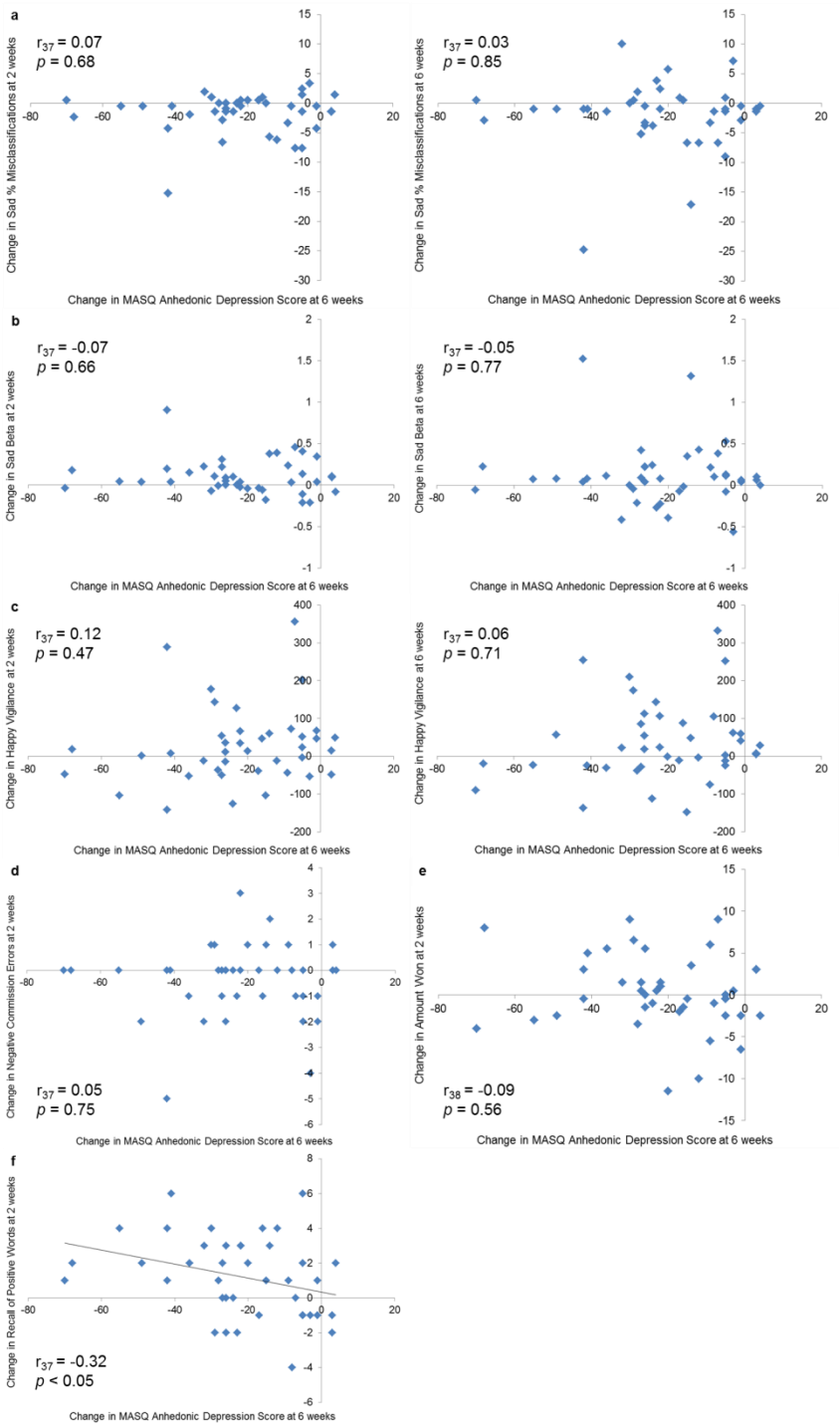


Figure 7.7 - Correlating the Changes in Behavioural Measures with the Change in MASQ Anhedonic Depression Score: correlations of the change in MASQ anhedonic depression score at 6 weeks with the change in the **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces at 2 and 6 weeks, **(d)** negative commission errors, **(e)** amount won and **(f)** number of positive self-referent words correctly recalled at 2 weeks only.

7.1.1.4 Anxiety

As discussed at the end of Chapter 6, previous research has shown that acute administration of some antidepressants, particularly SSRIs, can enhance the startle response (Grillon et al., 2007) and recognition of fearful faces (Browning et al., 2007) that is reduced following repeated treatments, consistent with the clinical observation of an initial increase in anxiety prior to the production of longer-term anxiolytic effects. Given that the bupropion-treated MDD group was shown to display a possible increase in the accuracy for recognising fearful faces, the change in this measure was correlated with the change in measures of anxiety (MASQ anxious arousal and MASQ general distress: anxious sub-scales). The change in the percentage accuracy for fearful faces at 2 weeks was not found to correlate with the change in MASQ Anxious Arousal or MASQ General Distress: Anxious scores at either 2 weeks (r_{39} = 0.08, p = 0.63; r_{39} = 0.10, p = 0.52) or 6 weeks (r_{37} = -0.03, p = 0.87; r_{37} = 0.08, p = 0.62) (Figure 7.8).

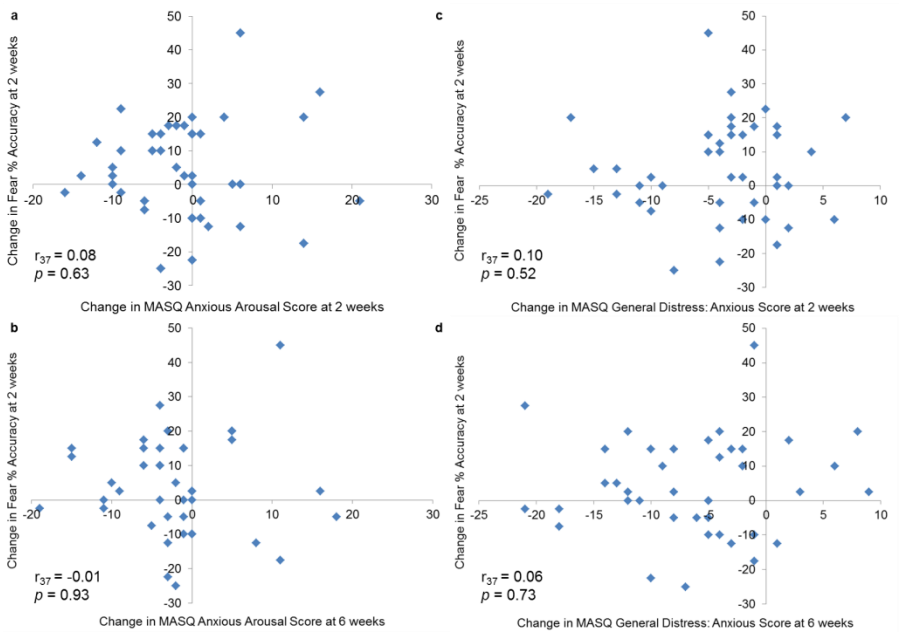


Figure 7.8 – Correlating the Change in % Accuracy for Fearful Faces with the Change in MASQ Anxiety Scores: correlations of the change in % accuracy for fearful faces at 2 weeks with the change in MASQ anxious arousal score at **(a)** 2 weeks and **(b)** 6 weeks and the change in MASQ general distress: anxious at **(c)** 2 weeks and **(d)** 6 weeks.

7.1.2 Responders versus Non-Responders

Another way to investigate whether the changes in behavioural measures were associated with clinical outcome was to divide the MDD group into responders and non-responders (based on the criteria discussed in Section 4.2) and compare how the behavioural measure changed between the two groups. In order to do this, a repeated measures ANOVA with visit as a within subjects factor (2 levels: baseline and test visit 2) and clinical response group as the between subjects factor was performed for each change in behavioural measure.

A significant main effect of visit, but no visit by clinical response group interaction was found for the percentage misclassification of other faces as sad, ($F_{1, 39} = 6.07$, $p < 0.05$; $F_{1, 39} = 1.70$, $p = 0.20$), beta for sad faces ($F_{1, 39} = 7.43$, $p < 0.01$; $F_{1, 39} = 0.48$, $p = 0.49$) and the number of negative commission errors made ($F_{1, 40} = 5.03$, $p < 0.05$; $F_{1, 40} = 0.56$, $p = 0.46$), with both the responders and non-responders showing the increase in beta for sad faces, the decrease in percentage misclassification of other faces as sad and the decrease in the number of negative commission errors made between baseline and test visit 2. Although, Figure 7.9a does suggest that the responders showed a greater reduction than the non-responders in the percentage misclassification of other faces as sad between baseline and test visit 2; however, an independent samples t-test performed on the difference score was not found to be significant ($t_{39} = -1.30$, $p = 0.20$). Figure 7.9c also suggests that the increase in attentional vigilance for happy faces between baseline and test visit 2 was derived mainly from the responders, whilst the non-responders actually showed a slight decrease; however a visit by clinical response group interaction was not found ($F_{1, 39} = 1.31$, $p = 0.26$). A visit by clinical response group interaction was found for the amount won ($F_{1, 40} = 4.47$, $p < 0.05$), with the responders displaying an increase and the non-responders displaying a decrease in the amount won between baseline and test visit 2, resulting in significantly different difference scores ($t_{40} = 2.11$, $p < 0.05$). Such improved performance on a reward task with bupropion treatment is consistent with the expected effect of bupropion

on reward processing, in contrast with results found in previous Chapters indicating that bupropion worsens aberrant reward processing.

Finally, when investigating other behavioural measures, again for the number of positive self-referent words recalled, a visit by clinical group interaction was found ($F_{1, 39}$ = 4.20, $p < 0.05$), with the responders displaying an increase in the number of positive self-referent words between baseline and test visit 2 that was not observed for the non-responders, resulting in significantly different difference scores (t_{39} = 2.05, $p < 0.05$).

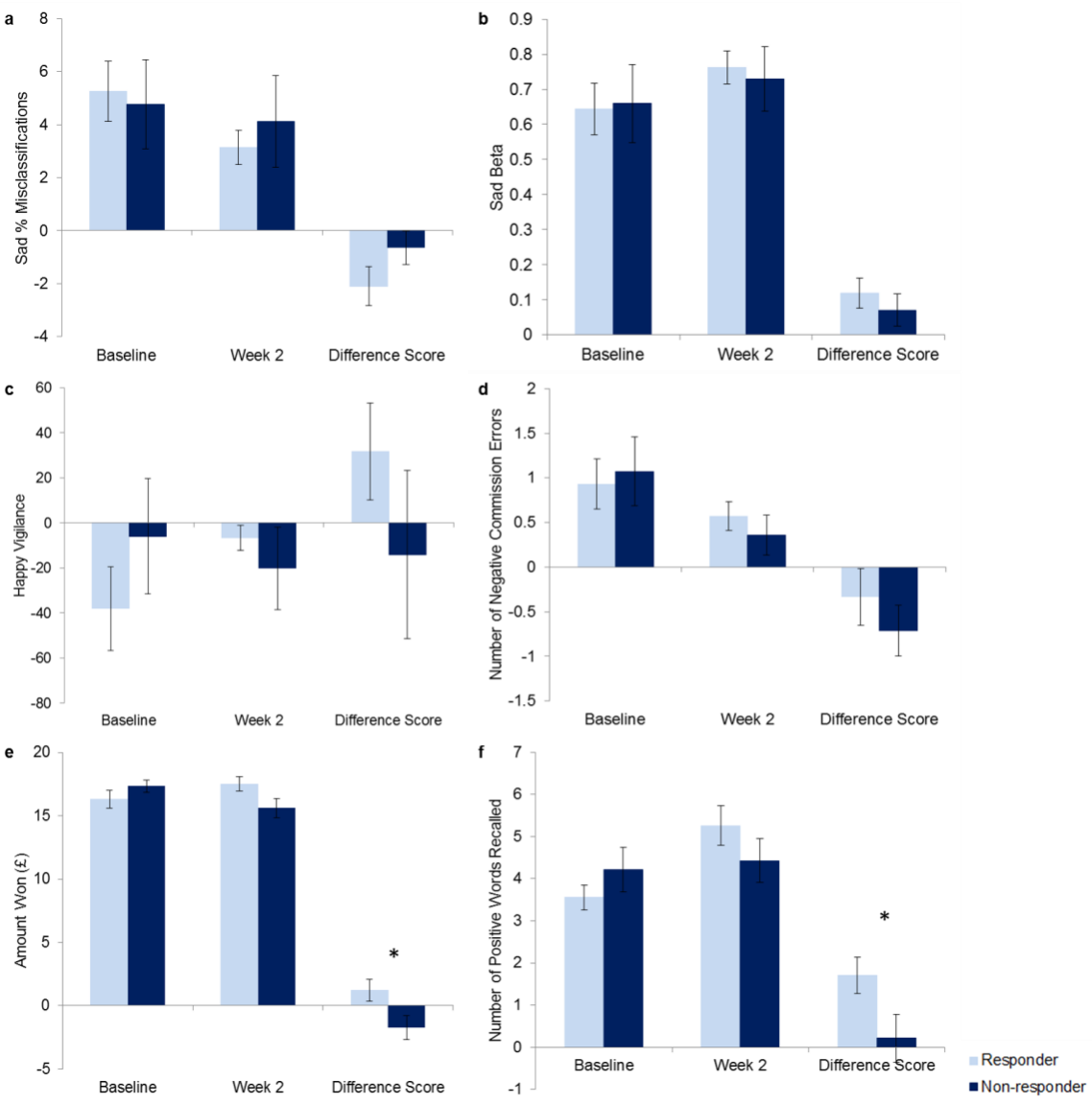


Figure 7.9 – The Difference between Responders and Non-responders for the Changes in Behavioural Measures: the difference between responders and non-responders at baseline, week 2 and week 2 – baseline difference score for **(a)** % misclassification of other faces as sad, **(b)** beta for sad faces, **(c)** attentional vigilance for happy faces, **(d)** number of negative commission errors, **(e)** amount won and **(f)** number of positive self-referent words correctly recalled. Asterisks denote the degree of significance obtained for planned comparisons (*$p < 0.05$).

7.2 Are the Changes in Neural Measures of Reward and Emotional Processing Associated with Clinical Outcome in MDD?

7.2.1 Correlating Neural Measures with Questionnaire Scores

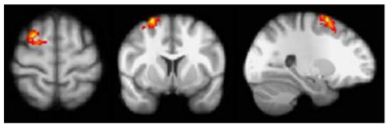
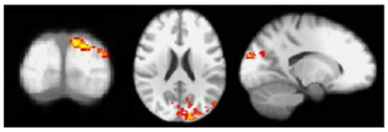
7.2.1.1 Covert Faces Task

7.2.1.1.1 Clinical Outcome based on the HAM-D

7.2.1.1.1.1 Whole Brain Analysis

The whole brain analysis of the relationship between the change in brain activity for the covert faces task between baseline and test visit 2 and the change in HAM-D score at 6 weeks in the MDD group was performed. Activity in response to happy faces in brain regions involved in general attention, including the right superior frontal gyrus (x y z = 26 8 64, Z-max = 3.88, *p*-corrected < 0.05) and right occipital pole (x y z = -16 -94 22, Z-max = 3.58, *p*-corrected < 0.001) at 2 weeks was found to negatively correlate with change in HAM-D score at 6 weeks (Table 7.1); that is, the greater the decrease in HAM-D score or symptom severity, the greater the increase in attention-related brain activity in response to happy faces.

Table 7.1 Whole Brain Analysis of the Relationship between the Change in Brain Activity for the Covert Faces Task and the Change in HAM-D Score

Lower-level Contrast	Direction of Questionnaire Score	Direction of Brain Activity	Correlation	x y z	Z-max	Corrected-*p*	Image
Happy	Decrease	Increase	Negative	26 8 64	3.88	< 0.05	Superior frontal gyrus 
Happy – Fear	Decrease	Increase	Negative	-16 -94 22	3.58	< 0.001	Occipital pole 

7.2.1.1.1.2 SVC Analysis

The SVC analysis found the negative correlation between the change in frontal activity to happy faces and change in HAM-D score considered above to include the right lateral OFC (x y z = 28 40 -14, Z-max = 3.16, *p*-corrected < 0.01). The SVC analysis also found right amygdala activity (x y z = 22 -2 -20, Z-max = 2.53, *p*-corrected < 0.05) in response to sad faces compared to fearful faces at 2 weeks to positively correlate with change in HAM-D score at 6 weeks (Figure 7.10); that is the greater the decrease in HAM-D score or symptom severity, the greater the decrease in right amygdala activity in response to sad versus fearful faces.

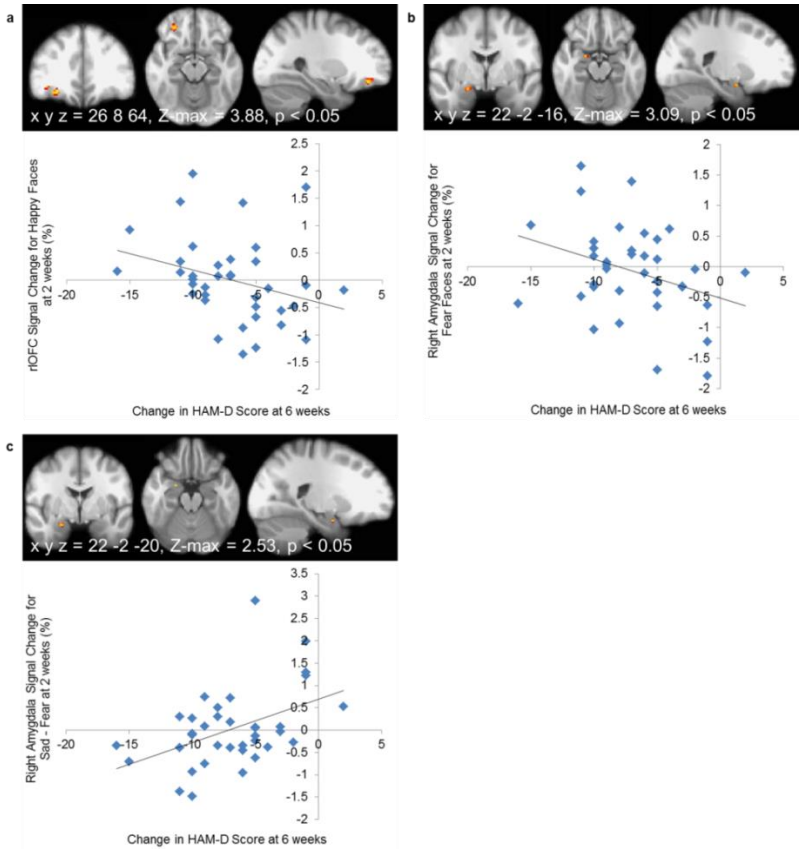


Figure 7.10 - Correlating the Changes in Neural Measures at 2 Weeks with the Change in HAM-D Score at 6 weeks: change in activity in the **(a)** right lateral OFC in response to happy faces, **(b)** right amygdala in response to fearful faces and **(c)** right amygdala in response to sad versus fearful faces at 2 weeks correlated with change in HAM-D score at 6 weeks.

7.2.1.1.2 Negative Affect

7.2.1.1.2.1 Whole Brain Analysis

Similarly to above, the whole brain analysis of the relationship between the changes in brain activity for the covert faces task and the change in negative affect found a correlation between change in brain activity in response to happy faces at 2 weeks and change in negative affect at 6 weeks. However, this time a positive correlation was found, that is, the decrease in negative affect at 6 weeks was associated with a decrease in lingual gyrus activity (x y z = 16 -60 -14, Z-max = 4.13, *p*-corrected < 0.01) in response to happy faces at 2 weeks. The same pattern was observed between postcentral gyrus activity in response to happy versus fearful faces and negative affect, when measured with either the MASQ general distress: depressive sub-scale (x y z = 64 -12 36, Z-max = 4.25, *p*-corrected < 0.01) or PANAS (x y z = 64 -12 36, Z-max = 3.89, *p*-corrected < 0.01). Conversely, the increase in positive affect at 6 weeks was associated with an increase in precentral gyrus activity (x y z = -20 -24 70, Z-max = 3.93, *p*-corrected < 0.01) in response to happy faces and frontal pole (x y z = 22 40 26, Z-max = 4.28, *p*-corrected < 0.001) and left putamen (x y z = -28 -4 16, Z-max = 3.84, *p*-corrected < 0.001) activity in response to happy versus fearful faces at 2 weeks (Table 7.2).

Table 7.2 Whole Brain Analysis of the Relationship between the Change in Neural Activity for the Covert Faces Task and the Change in Negative Affect

Lower-level Contrast	Direction of Questionnaire Score	Direction of Brain Activity	Correlation	x y z	Z-max	Corrected-*p*	Image
MASQ General Distress: Depressive							
Happy – Fear	Decrease	Decrease	Positive	64 -12 36	4.25	< 0.01	Postcentral gyrus
PANAS Negative Affect							
Happy	Decrease	Decrease	Positive	16 -60 -14	4.13	< 0.01	Lingual gyrus, cerebellum
Happy – Fear	Decrease	Decrease	Positive	64 -12 36	3.89	< 0.01	Postcentral gyrus
PANAS Positive Affect							
Happy	Increase	Increase	Positive	-20 -24 70	3.93	< 0.01	Precentral gyrus
Fear	Increase	Increase	Positive	-64 -40 -14	4.50	< 0.01	Posterior middle temporal gyrus
Happy – Fear	Increase	Increase	Positive	22 40 26 -28 -4 16	4.28 3.84	< 0.001 < 0.001	Frontal pole Left putamen

7.2.1.1.2.2 SVC Analysis

In contrast to the whole brain analysis, the SVC analysis found an association between change in brain activity in response to sad faces, rather than happy faces, at 2 weeks and change in negative affect at 6 weeks. More specifically, decreased right amygdala activity in response to sad versus fearful faces at 2 weeks was associated with decreased negative affect at 6 weeks, when measured with either the MASQ general distress: depressive sub- scale (x y z = 32 2 -20, Z-max = 3.91, *p*-corrected < 0.05) or the PANAS (x y z = 22 -4 -20, Z-max = 2.62, *p*-corrected < 0.05). Decreased right medial OFC activity in response to sad faces or sad versus happy faces at 2 weeks was also associated with decreased negative affect at 6 weeks, when measured with either the MASQ general distress: depressive sub-scale or the PANAS. Conversely, increased right medial OFC activity (x y z = 2 36 -18, Z-max = 3.32, *p*-corrected < 0.05) in response to fearful faces at 2 weeks was associated with decreased negative affect at 6 weeks (Figure 7.11).

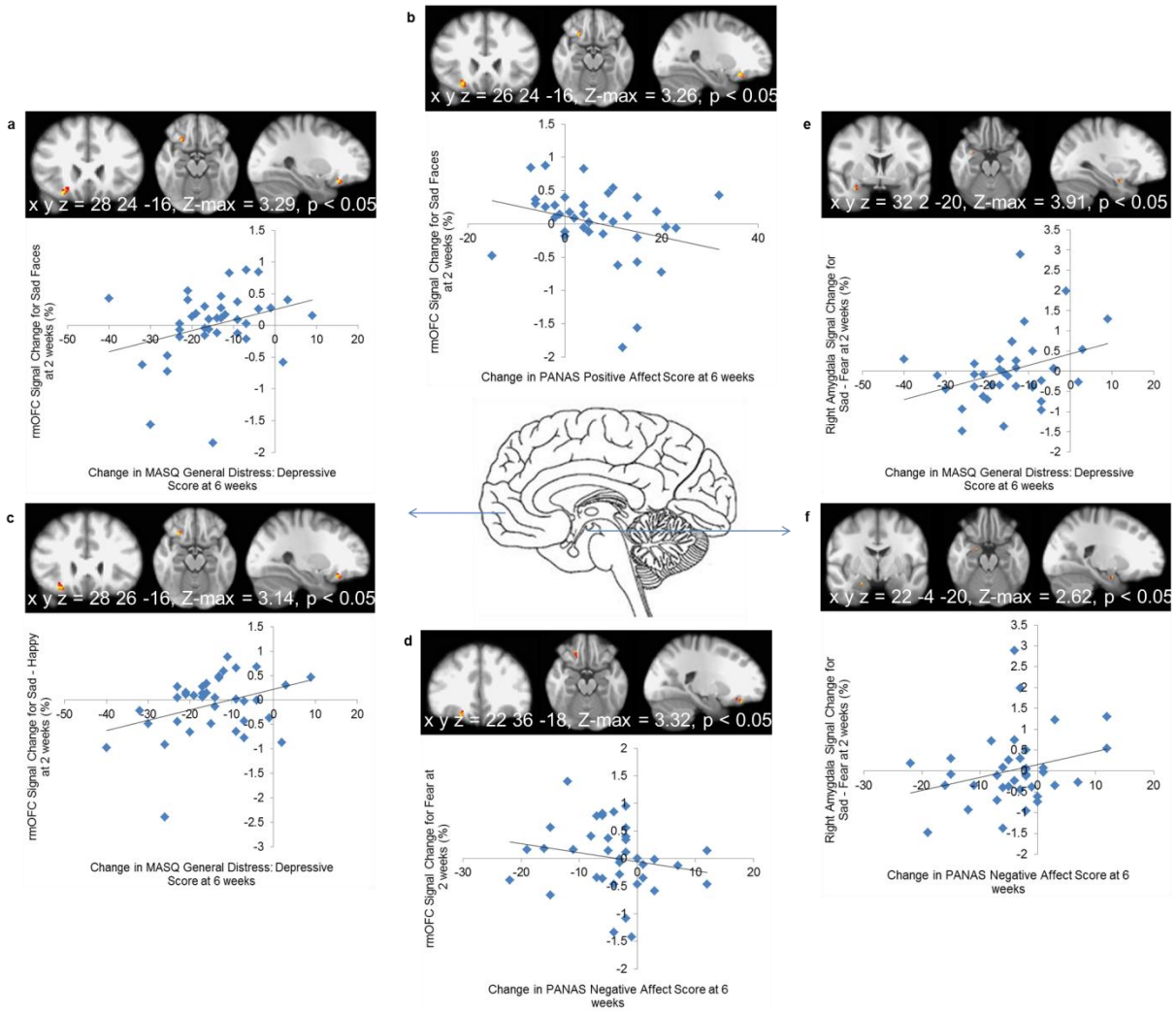


Figure 7.11 - Correlating the Changes in Neural Measures at 2 Weeks with the Change in Negative Affect at 6 weeks: change in activity in the right medial OFC in response to **(a-b)** sad faces, **(c)** sad versus happy faces and **(d)** fearful faces and change in activity in the **(e-f)** right amygdala in response to sad versus fearful faces at 2 weeks correlated with change in negative affect at 6 weeks, measured by the MASQ general distress: depressive sub-scale or the PANAS.

7.2.1.1.3 Anhedonia

7.2.1.1.3.1 Whole Brain Analysis

The whole brain analysis of the relationship between the change in brain activity for the covert faces task between baseline and test visit 2 and the change in anhedonia at 6 weeks only found an association between decreased temporal activity in response to faces, in particular fearful faces, and decreased anhedonia measured by the SHAPS (Table 7.3).

Table 7.3 Whole Brain Analysis of the Relationship between the Change in Neural Activity for the Covert Faces Task and the Change in Anhedonia

Lower-level Contrast	Direction of Questionnaire Score	Direction of Brain Activity	Correlation	x y z	Z-max	Corrected-*p*	Image
SHAPS							
Fear	Decrease	Decrease	Positive	-64 -32 4	3.54	< 0.01	Posterior superior temporal gyrus
All Faces	Decrease	Decrease	Positive	-36 -44 -20 -50 -52 2	4.26 4.02	< 0.01 < 0.05	Posterior temporal fusiform cortex Middle temporal gyrus
MASQ Anhedonic Depression							

7.2.1.1.3.2 SVC Analysis

The SVC analysis further revealed a general decrease in activity in the right medial OFC, right VS and right amygdala in response to sad faces at 2 weeks was associated with decreased anhedonia at 6 weeks (Figure 7.12). This was surprising since reduced frontal and striatal activity is generally associated with greater anhedonia.

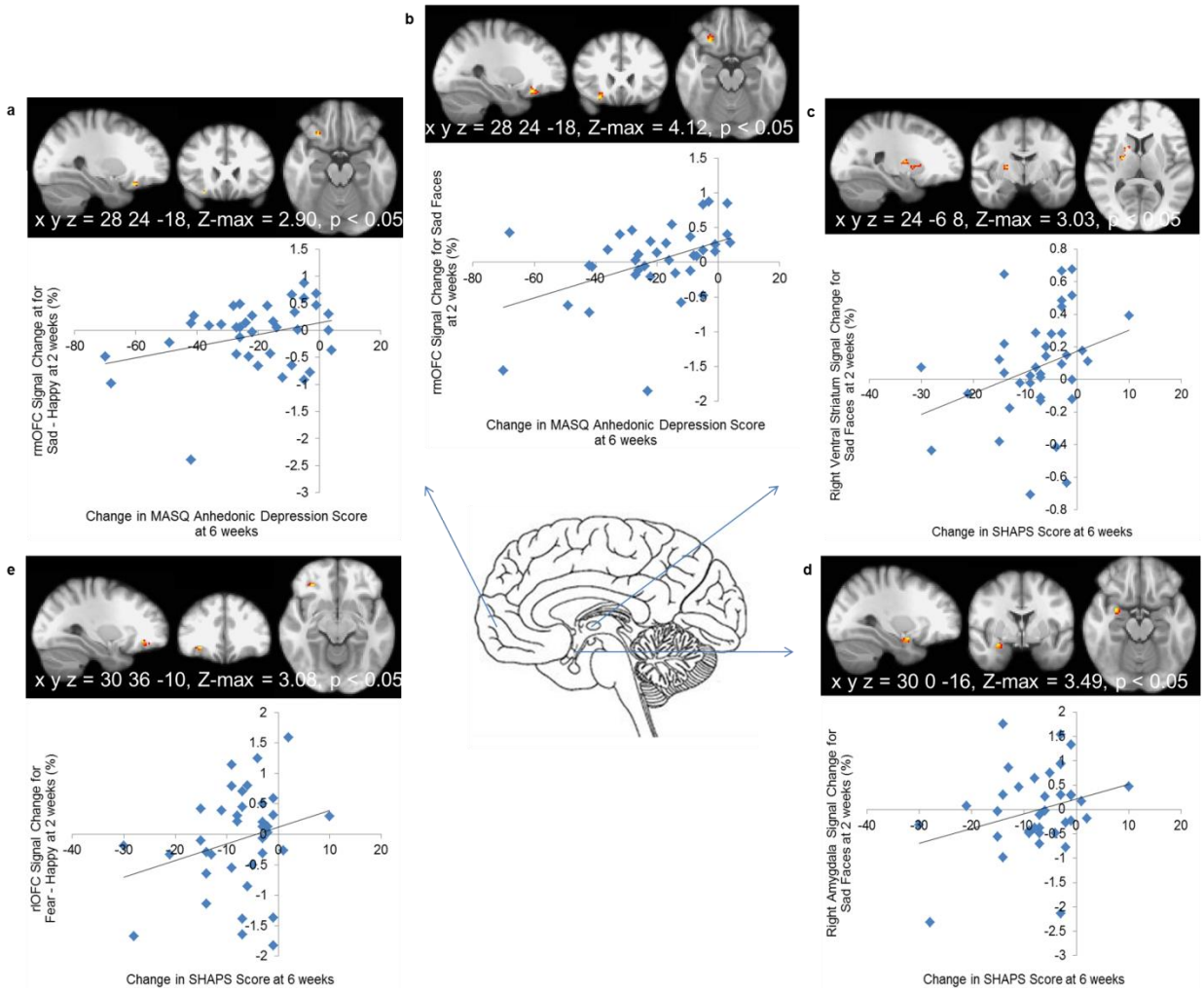


Figure 7.12 - Correlating the Changes in Neural Measures at 2 Weeks with the Change in Anhedonia at 6 weeks: change in activity in the **(a)** right medial OFC in response to sad versus happy faces, the **(b)** right medial OFC, **(c)** right VS and **(d)** right amygdala in response to sad faces and the **(e)** right lateral OFC in response to fear versus happy faces at 2 weeks positively correlated with change in anhedonia at 6 weeks, measured by the SHAPS or MASQ anhedonic depression sub-scale.

7.2.1.1.4 Anxiety

7.2.1.1.4.1 Whole Brain Analysis

Whilst the accuracy of determining the gender of fearful faces in the covert faces task was unchanged over time, it was still interesting to investigate whether any changes in brain activity in response to fearful faces at 2 weeks were associated with change in anxiety at 6 weeks. The whole brain analysis found an increase in activity in the brainstem (x y z = -8 -18 -24, Z-max = 3.72, *p*-corrected < 0.05) in response to fear versus sad faces at 2 weeks to be associated with reduced anxiety at 6 weeks but other associations involved brain activity in response to happy faces (Table 7.4).

Table 7.4 Whole Brain Analysis of the Relationship between the Change in Neural Activity for the Covert Faces Task and the Change in Anxiety

Lower-level Contrast	Direction of Questionnaire Score	Direction of Brain Activity	Correlation	x y z	Z-max	Corrected-*p*	Image
MASQ Anxious Arousal							
Fear – Sad	Decrease	Increase	Negative	-8 -18 -24	3.72	< 0.05	Brainstem
MASQ General Distress: Anxious							
Happy – Sad	Decrease	Decrease	Positive	2 52 42	3.56	< 0.01	Superior frontal gyrus
Happy	Decrease	Decrease	Positive	42 -54 -26	4.14	< 0.05	Temporal occipital fusiform cortex / cerebellum

7.2.1.1.4.2 SVC Analysis

The SVC analysis revealed a broader pattern with an increase in activity in the right medial OFC and bilateral amygdala specifically in response to fearful faces at 2 weeks to be associated with decreased anxiety at 6 weeks (Figure 7.13).

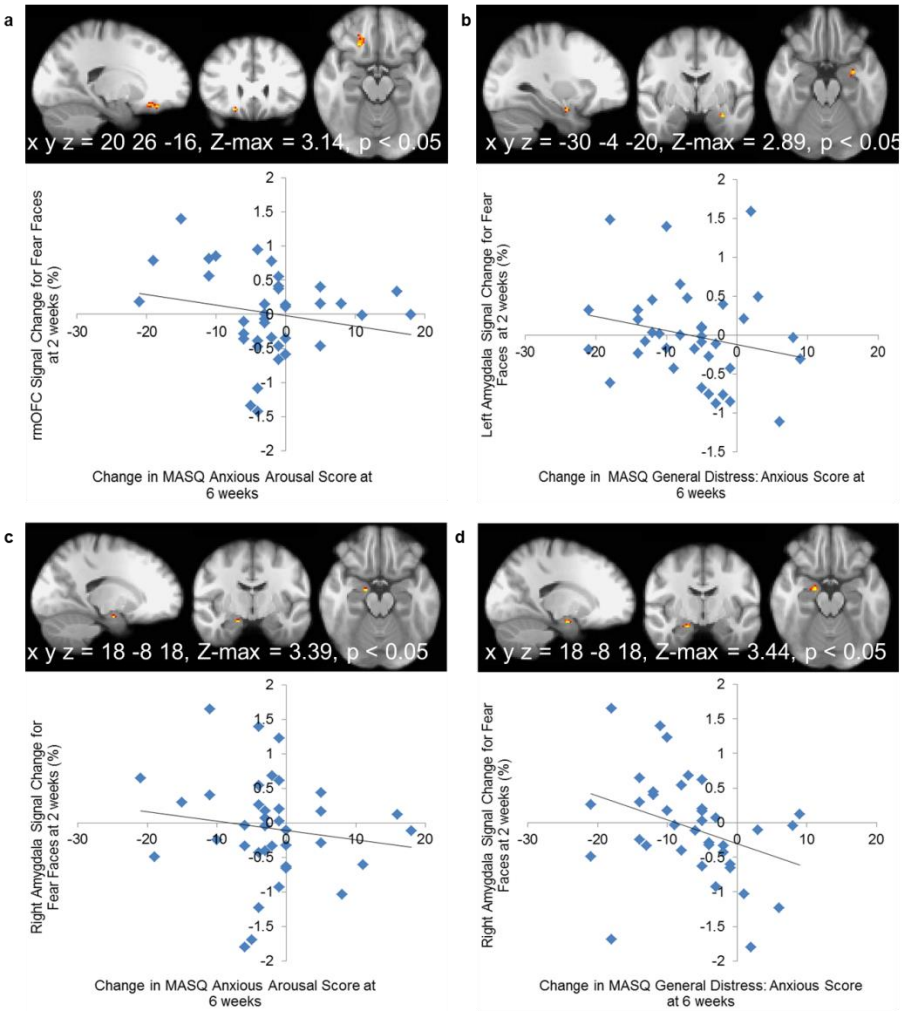


Figure 7.13 - Correlating the Changes in Neural Measures at 2 Weeks with the Change in Anxiety at 6 weeks: change in activity in the **(a)** right medial OFC and **(b-d)** bilateral amygdala in response to sad faces at 2 weeks negatively correlated with change in anxiety at 6 weeks, measured by the MASQ anxious arousal or general distress: anxious sub-scales.

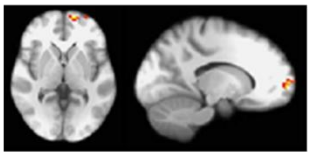
7.2.1.2 Reward Task

7.2.1.2.1 Clinical Outcome based on the HAM-D

7.2.1.2.1.1 Whole Brain Analysis

The whole brain analysis of the relationship between the change in brain activity for the reward task between baseline and test visit 2 and the change in HAM-D score over 6 weeks found a general decrease in brain activity with the decrease in HAM-D score across all aspects of the task including anticipation and win and loss outcomes (Table 7.5). Of particular interest was a positive correlation between frontal pole activity and HAM-D score in response to a loss outcome (x y z = -14 64 0, Z-max = 3.73, *p*-corrected < 0.05), since this could involve the OFC, included in the pre-specified regions of interest.

Table 7.5 Whole Brain Analysis of the Relationship between the Change in Brain Activity for the Reward Task and the Change in HAM-D Score

Lower-level Contrast	Direction of Questionnaire Score	Direction of Brain Activity	Correlation	x y z	Z-max	Corrected-*p*	Image
Win Anticipation – Loss Anticipation	Decrease	Decrease	Positive	-10 -58 20	3.38	< 0.05	Precuneous cortex, posterior cingulate gyrus
Win	Decrease	Decrease	Positive	24 -42 -28	3.51	< 0.05	Cerebellum
Neutral	Decrease	Decrease	Positive	20 -82 -8 -18 68 14	3.88 3.33	< 0.001 < 0.05	Occipital fusiform gyrus (including lingual gyrus below) Frontal pole
No loss in loss	Decrease	Decrease	Positive	8 -88 -4 30 -76 28	3.97 3.43	< 0.001 < 0.05	Lingual gyrus
Loss	Decrease	Decrease	Positive	-14 64 0	3.73	< 0.05	Frontal pole 

7.2.1.2.1.2 SVC Analysis

The SVC analysis found the positive correlation between the change in frontal activity to loss outcomes and change in HAM-D score considered above to include the right medial OFC (x y z = 28 28 -20, Z-max = 3.05, *p*-corrected < 0.05). The SVC analysis also consistently found a positive correlation between change in brain activity in response to a no loss in loss outcome at 2 weeks and change in HAM-D score over 6 weeks, in regions including the subgenual ACC (x y z = 2 22 -10, Z-max = 3.40, *p*-corrected < 0.01) and left amygdala (x y z = -20 -6 -12, Z-max = 3.24, *p*-corrected < 0.05) (Figure 7.14).

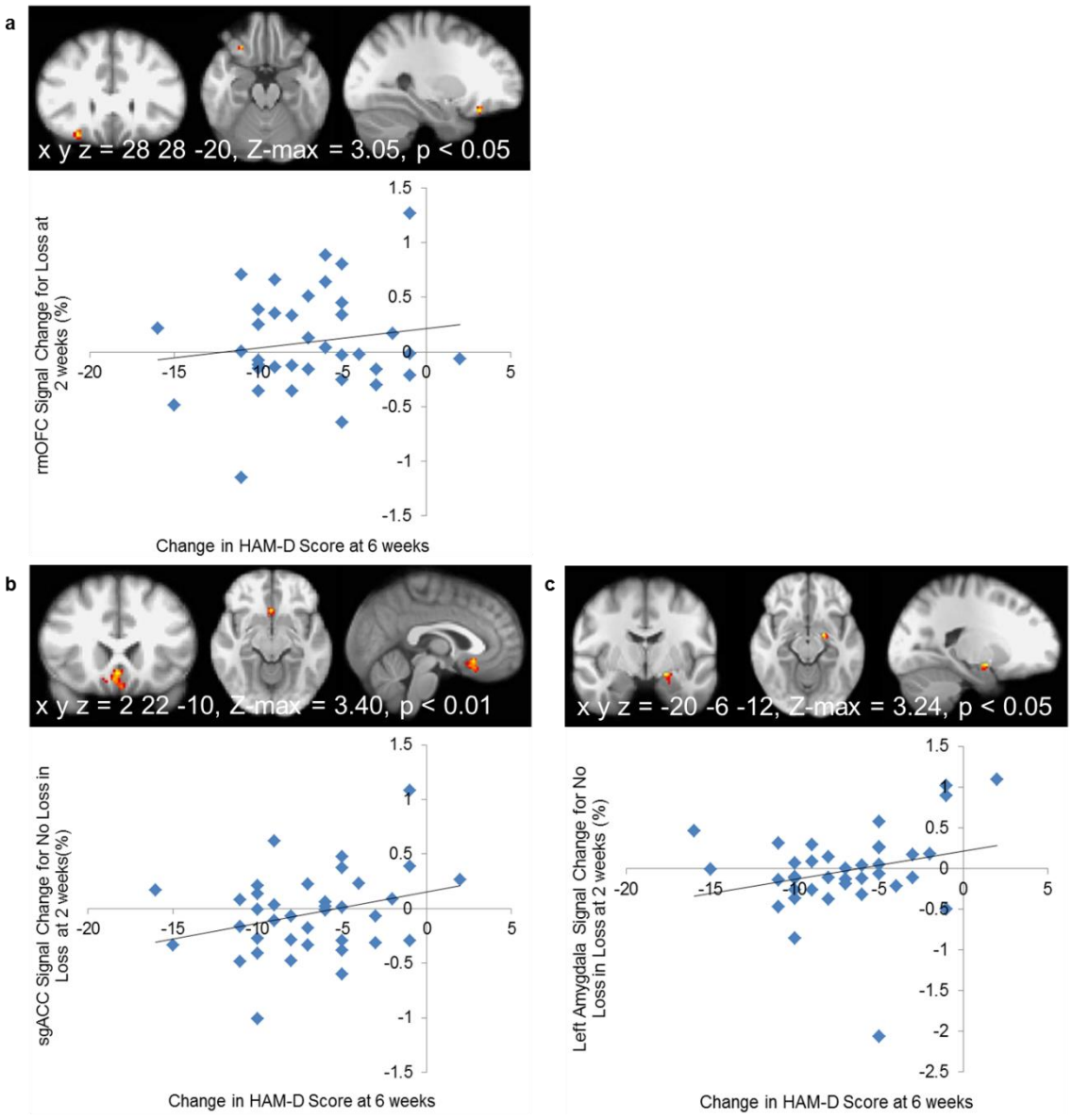


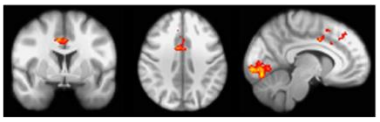
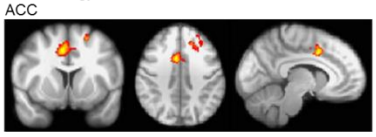
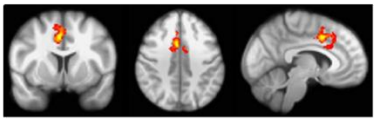
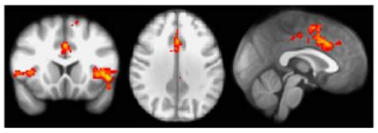
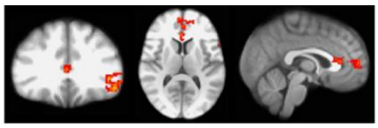
Figure 7.14 - Correlating the Changes in Neural Measures at 2 Weeks with the Change in HAM-D Score at 6 weeks: change in activity in the **(a)** right medial OFC in response to loss outcomes and **(b)** subgenual ACC and **(c)** left amygdala in response to no loss in loss outcomes at 2 weeks positively correlated with change in HAM-D score at 6 weeks.

7.2.1.2.2 Negative Affect

7.2.1.2.2.1 Whole Brain Analysis

Many correlations were found in the whole brain analysis of the relationship between the change in brain activity for the reward task between baseline and test visit 2 and the change in negative affect over 6 weeks. These are summarised in Table 7.6. Generally, decreased brain activity in various regions at 2 weeks was associated with decreased negative affect or increased positive affect at 6 weeks, apart from ACC activity specifically in response to win versus neutral or win versus loss outcomes. Instead, increased ACC activity in response to win verses neutral (x y z = 8 10 40, Z-max = 3.91, *p*-corrected < 0.05) or win versus loss (x y z = 6 8 42, Z-max = 3.73, *p*-corrected < 0.05) outcomes at 2 weeks was associated with decreased negative affect at 6 weeks.

Table 7.6 Whole Brain Analysis of the Relationship between the Change in Neural Activity for the Reward Task and the Change in Negative Affect

Lower-level Contrast	Direction of Questionnaire Score	Direction of Brain Activity	Correlation	x y z	Z-max	Corrected-*p*	Image
MASQ General Distress: Depressive							
Win	Decrease	Decrease	Positive	24 -42 -28 10 -84 46	3.96 3.38	< 0.001 < 0.05	Cerebellum Superior lateral occipital cortex
Neutral (No win in win and no loss in loss)	Decrease	Decrease	Positive	20 -82 -8 30 -92 12	4.69 3.67	< 0.001 < 0.01	Occipital fusiform gyrus Occipital pole
Win – Neutral	Decrease	Decrease	Positive	-26 -34 -18	4.24	< 0.001	Parahippocampal gyrus
Loss – Neutral	Decrease	Increase	Negative	48 -32 -8	3.48	< 0.01	Middle temporal gyrus
PANAS Negative Affect							
Win	Decrease	Decrease	Positive	-30 -74 -14 20 -70 -14	3.89 4.38	< 0.001 < 0.001	Bilateral occipital fusiform gyrus
Neutral	Decrease	Decrease	Positive	22 -80 -6 -18 72 14	4.48 3.47	< 0.001 < 0.05	Occipital fusiform gyrus Occipital pole
No Loss in Loss	Decrease	Decrease	Positive	8 -78 -12 8 2 38	4.12 3.43	< 0.001 < 0.05	Lingual gyrus ACC 
Win – Neutral	Decrease	Increase	Negative	-26 -6 66 8 10 40	3.74 3.91	< 0.01 < 0.05	Precentral gyrus ACC 
Win – Loss	Decrease	Increase	Negative	6 8 42	3.73	< 0.05	ACC 
Loss	Decrease	Decrease	Positive	-18 -82 -18 -24 -60 50 24 -70 -18 2 -70 48 -50 10 38	4.40 4.09 3.56 3.64 4.16	< 0.001 < 0.001 < 0.01 < 0.05 < 0.05	Bilateral occipital fusiform cortex Superior lateral occipital fusiform cortex Precuneous cortex Middle frontal gyrus
PANAS Positive Affect							
Win	Increase	Decrease	Negative	-48 18 -2 0 18 34 52 16 4	4.11 3.66 3.57	< 0.001 < 0.001 < 0.05	Frontal operculum cortex ACC  Inferior frontal gyrus
Win – Neutral	Increase	Decrease	Negative	-50 20 -4 2 32 10	4.01 3.19	< 0.001 < 0.01	Inferior frontal gyrus ACC 
Win – Loss	Increase	Decrease	Negative	-6 12 68	3.39	< 0.05	Superior frontal gyrus
Loss – Neutral	Increase	Decrease	Negative	38 -52 -26 -32 -60 38 -34 -52 -34	3.47 3.39 3.53	< 0.05 < 0.05 < 0.05	Bilateral cerebellum Superior lateral occipital cortex

7.2.1.2.2.2 SVC Analysis

The SVC analysis corroborated the results of the whole brain analysis above, with reduced left lateral OFC, rostral and dorsal ACC and left VS activity at 2 weeks associated with reduced negative affect (Figure 7.15) or increased positive affect (Figure 7.16) at 6 weeks, apart from dorsal ACC activity specifically in response to win versus neutral outcomes. Instead, again, increased ACC activity (x y z = 6 10 40, Z-max = 3.86, *p*-corrected < 0.05) in response to win versus neutral outcomes as well as increased left VS activity (x y z = -12 18 0, Z-max = 3.09, *p*-corrected < 0.05) in response to win versus loss outcomes at 2 weeks was associated with decreased negative affect at 6 weeks (Figure 7.15 d and e).

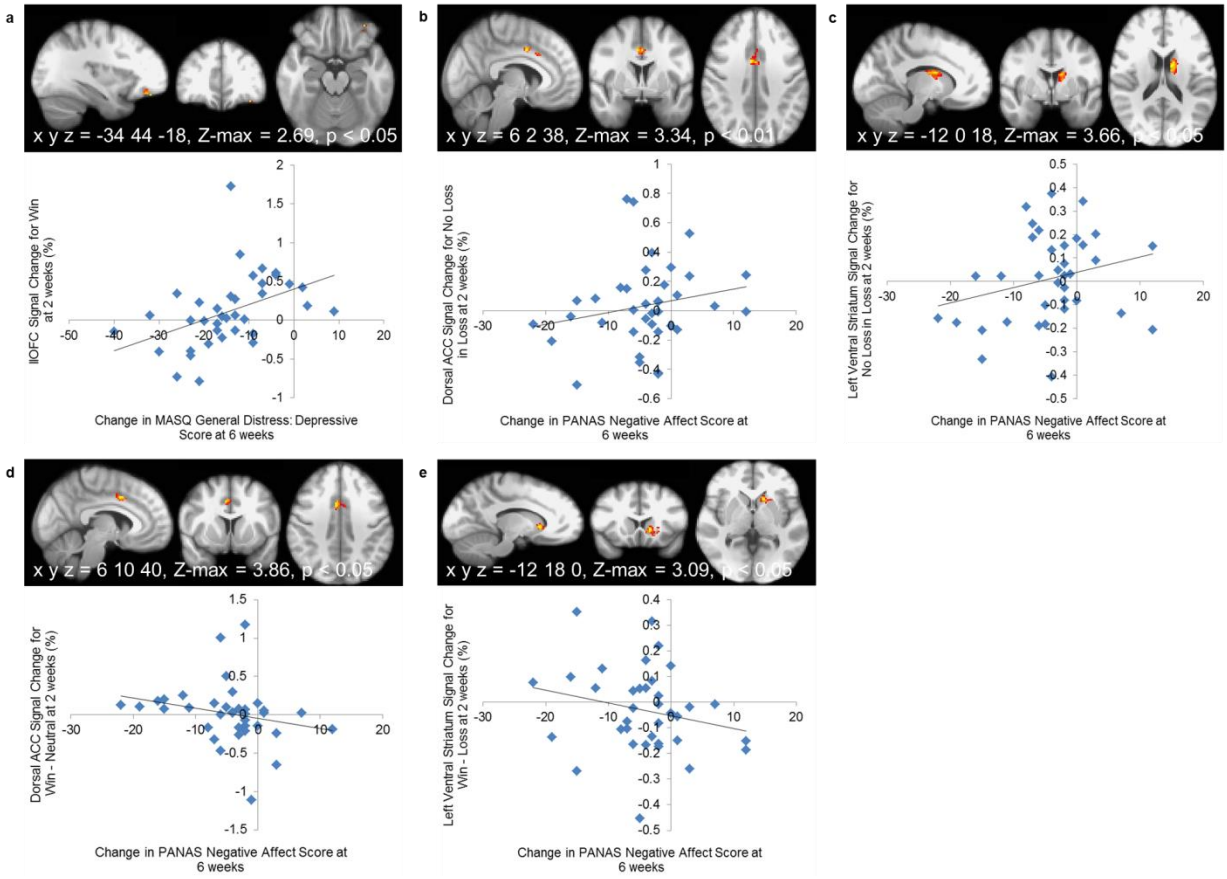


Figure 7.15 - Correlating the Changes in Neural Measures at 2 Weeks with the Change in Negative Affect at 6 weeks: change in activity in the **(a)** left lateral OFC in response to win outcomes and **(b)** dorsal ACC and **(c)** left VS in response to no loss in loss outcomes at 2 weeks positively correlated with change in negative affect at 6 weeks, measured by the MASQ general distress: depressive and PANAS negative affect sub-scales. Change in activity in the **(d)** dorsal ACC in response to win versus neutral outcomes and **(e)** left VS in response to win versus loss outcomes at 2 weeks negatively correlated with change in PANAS negative affect at 6 weeks.

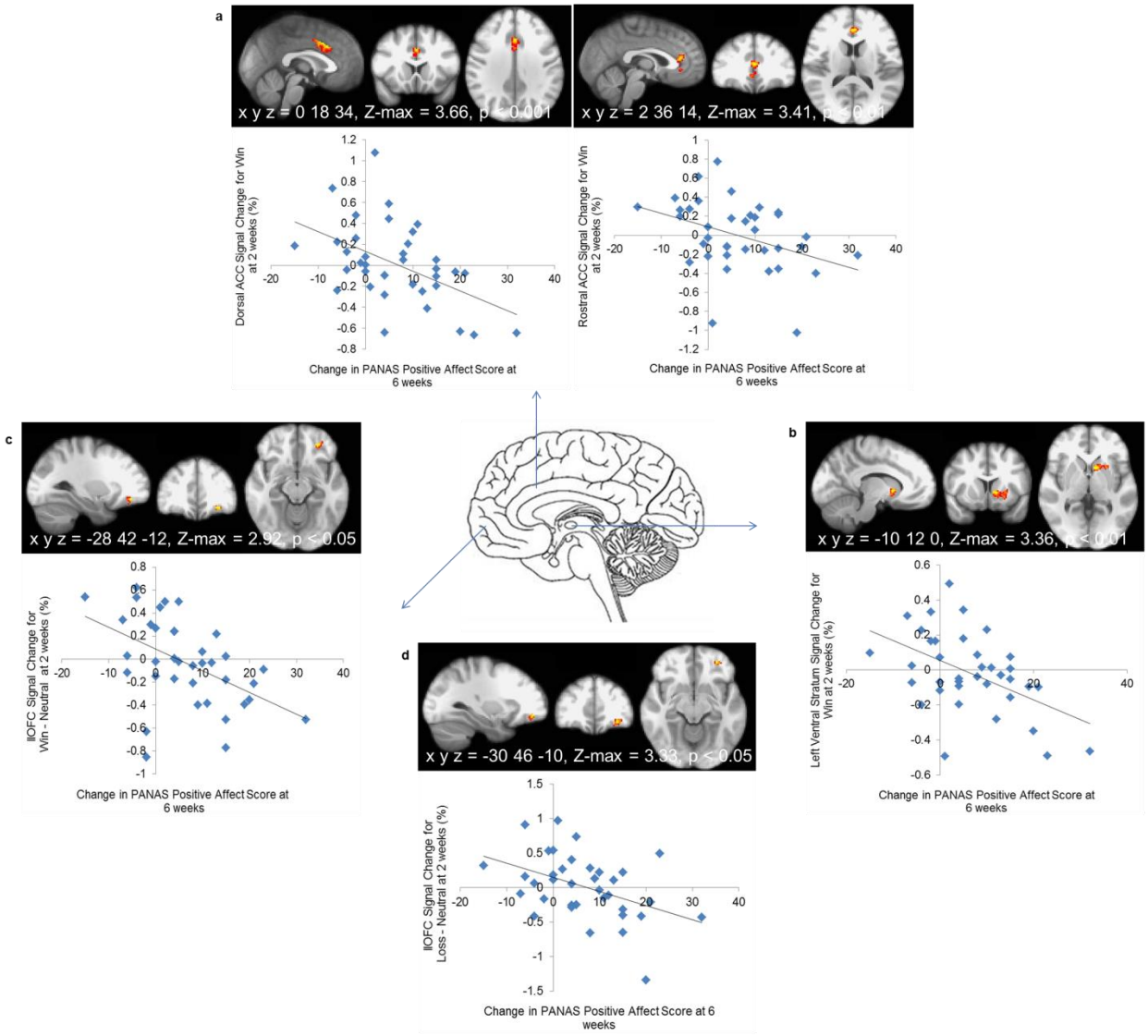


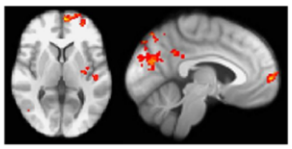
Figure 7.16 - Correlating the Changes in Neural Measures at 2 Weeks with the Change in PANAS Positive Affect at 6 weeks: change in activity in the **(a)** dorsal and rostral ACC and **(b)** left VS in response to win outcomes, **(c)** left lateral OFC in response to win versus neutral outcomes and **(d)** left lateral OFC in response to loss versus neutral outcomes at 2 weeks negatively correlated with change in PANAS positive affect score at 6 weeks.

7.2.1.2.3 Anhedonia

7.2.1.2.3.1 Whole Brain Analysis

Many correlations were also found in the whole brain analysis of the relationship between the change in brain activity for the reward task between baseline and test visit 2 and the change anhedonia over 6 weeks. These are summarised in Table 7.7. Generally, again, decreased brain activity in various regions during anticipation and response to win and loss outcomes at 2 weeks was associated with decreased anhedonia at 6 weeks. Of particular interest was the association between reduced frontal (x y z = -4 62 4, Z-max = 3.63, *p*-corrected <0.01), insular (x y z = -38 -20 14, Z-max = 3.52, *p*-corrected < 0.05) and right hippocampal (x y z = 36 -16 -18, Z-max = 3.34, *p*-corrected < 0.05) activity in response to loss outcomes at 2 weeks and reduced anhedonia at 6 weeks, since these regions have previously been reported to be affected by antidepressant treatment.

Table 7.7 Whole Brain Analysis of the Relationship between the Change in Neural Activity for the Reward Task and the Change in Anhedonia

Lower-level Contrast	Direction of Questionnaire Score	Direction of Brain Activity	Correlation	x y z	Z-max	Corrected-*p*	Image
SHAPS							
Neutral	Decrease	Decrease	Positive	6 -90 -2	3.95	< 0.001	Occipital pole
No Loss in Loss	Decrease	Decrease	Positive	6 -90 -2	4.11	< 0.001	Occipital pole
Loss	Decrease	Decrease	Positive	-20 -86 38 -4 62 4 -38 -20 14 36 -16 -18	3.86 3.63 3.52 3.34	< 0.001 < 0.01 < 0.05 < 0.05	Lateral occipital cortex Frontal pole  Insular cortex Right hippocampus
Loss – Neutral	Decrease	Decrease	Positive	8 -74 26	3.50	< 0.01	Cuneal cortex
MASQ Anhedonic Depression							
Win Anticipation	Decrease	Decrease	Positive	0 -16 58 -20 -76 8	3.86 3.47	< 0.05 < 0.05	Precentral gyrus Intracalcarine cortex
Win	Decrease	Decrease	Positive	-4 -16 46 -6 -56 -16 -2 -84 16 -60 6 6 -56 -48 16	3.97 3.43 3.83 3.45 3.33	< 0.001 < 0.001 < 0.001 < 0.001 < 0.05	Posterior cingulate cortex Cerebellum Supracalcarine cortex Precentral gyrus Posterior supramarginal gyrus
Win – Neutral	Decrease	Decrease	Positive	-58 12 4 54 20 -2 -26 -34 -18 36 -58 -26 -58 -22 30	4.09 3.63 4.10 3.72 3.50	< 0.001 < 0.001 < 0.001 < 0.05 < 0.05	Bilateral inferior frontal gyrus Parahippocampal gyrus Cerebellum Postcentral gyrus

7.2.1.2.3.2 SVC Analysis

The SVC analysis also found a general trend for decreased brain activity during the reward task at 2 weeks to be associated with decreased anhedonia at 6 weeks; however, associations were mainly for brain activity in response to just win outcomes. For example, decreased medial and lateral OFC activity in response to win versus neutral or win outcomes at 2 weeks was associated with decreased anhedonia at 6 weeks. Decreased dorsal ACC (x y z = 0 8 36, Z-max = 3.53, p-corrected < 0.01) and left amygdala (x y z = -26 -8 -16, Z-max = 2.86, *p*-corrected < 0.05) activity in response to win outcomes at 2 weeks was also associated with decreased anhedonia at 6 weeks. Finally, the only association with decreased anhedonia involving brain activity in response to loss outcomes was decreased left amygdala activity (x y z = -26 -8 -14, Z-max = 2.90, *p*-corrected < 0.05) at 2 weeks (Figure 7.17).

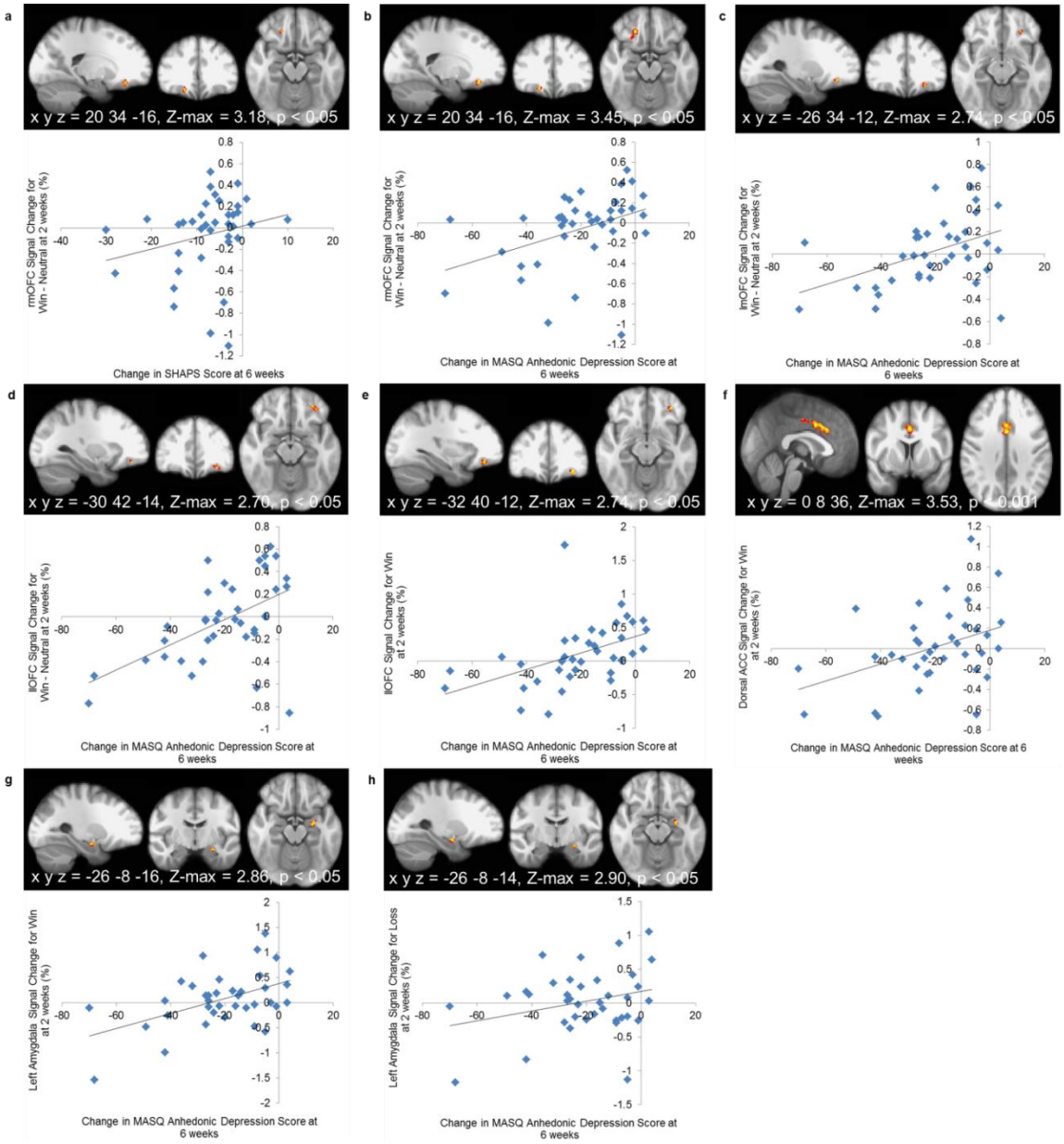


Figure 7.17 - Correlating the Changes in Neural Measures at 2 Weeks with the Change in Anhedonia at 6 weeks: change in activity in the **(a-c)** right and left medial OFC and **(d)** left lateral OFC in response to win versus neutral outcomes, **(e)** left lateral OFC, **(f)** dorsal ACC and **(g)** left amygdala in response to win outcomes and **(h)** left amygdala in response to loss outcomes at 2 weeks positively correlated with change in anhedonia at 6 weeks, measured with the SHAPS or MASQ anhedonic depression sub-scale.

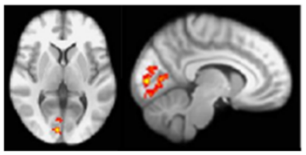
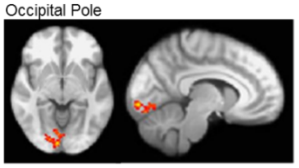
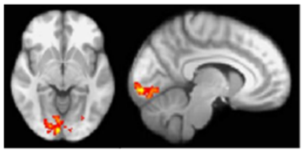
7.2.2 Responders versus Non-Responders

Another way to investigate whether the changes in neural measures were associated with clinical outcome was to divide the MDD group into responders and non-responders (based on the criteria discussed in Section 4.2) and compare how the neural measure changed between the two groups. In order to do this, contrast images of the difference in brain activity between test visits 1 and 2 obtained from the fixed effects analysis of MDD participants were inputted into a second-level analysis and a contrast of responders versus non-responders was performed. Significant clusters indicated a difference in the change in brain activity between responders and non-responders.

7.2.2.1 Whole Brain Analysis

Regarding the covert faces task, the whole brain analysis did not find any difference in the change in brain activity between responders and non-responders. However, for the reward task, the whole brain analysis did find responders to show a greater decrease in brain activity in the intracalcarine cortex (x y z = 8 -86 4, Z-max = 3.62, *p*-corrected < 0.001) during the anticipation of win outcomes and the occipital pole (x y z = 8 -90 -6, Z-max = 3.67, *p*-corrected <0.001) in response to neutral outcomes, particularly no loss in loss outcomes, than non-responders. This is interesting given that decreased occipital cortex activity in response to neutral outcomes, particularly no loss in loss outcomes, was found to be associated with decreased symptom severity, negative affect and anhedonia in the above sections.

Table 7.8 Whole Brain Analysis of Responders versus Non-responders for the Changes in Neural Activity

Lower-level Contrast	Direction of Brain Activity	Direction of Group Comparison	x y z	Z-max	Corrected-*p*	Image
Covert Faces Task						
Reward Task						
Win Anticipation	Decrease	Responders > Non-responders	8 -86 4	3.62	< 0.001	Intracalcarine cortex 
Neutral	Decrease	Responders > Non-responders	8 -90 -6	3.46	< 0.01	Occipital Pole 
No Loss in Loss	Decrease	Responders > Non-responders	8 -90 -6	3.67	< 0.001	Occipital Pole 

7.2.2.2 SVC Analysis

Conversely, the SVC analysis did not find any difference in the change in brain activity between responders and non-responders for the reward task but did for the covert faces task. Whilst responders displayed relatively stable right lateral OFC activity in response to faces over time, non-responders displayed a greater reduction in right lateral OFC activity in response to faces, particularly happy faces. This corroborates the negative correlation between change in right lateral OFC activity (x y z = 38 40 -8, Z-max = 2.84, *p*-corrected < 0.05) in response to happy faces and change in HAM-D score but may be associated with the large baseline difference in right lateral OFC (x y z = 32 38 -14, Z-max = 2.84, *p*-corrected < 0.05) in response to faces between responders and non-responders (Figure 7.18a). When considering baseline differences, non-responders also displayed significantly reduced right VS activity (x y z = 22 4 0, Z-max = 3.12, *p*-corrected < 0.05) during the anticipation of win versus loss outcomes; however, this did not manifest as a significant difference in the change in VS activity between responders and non-responders (Figure 7.18b).

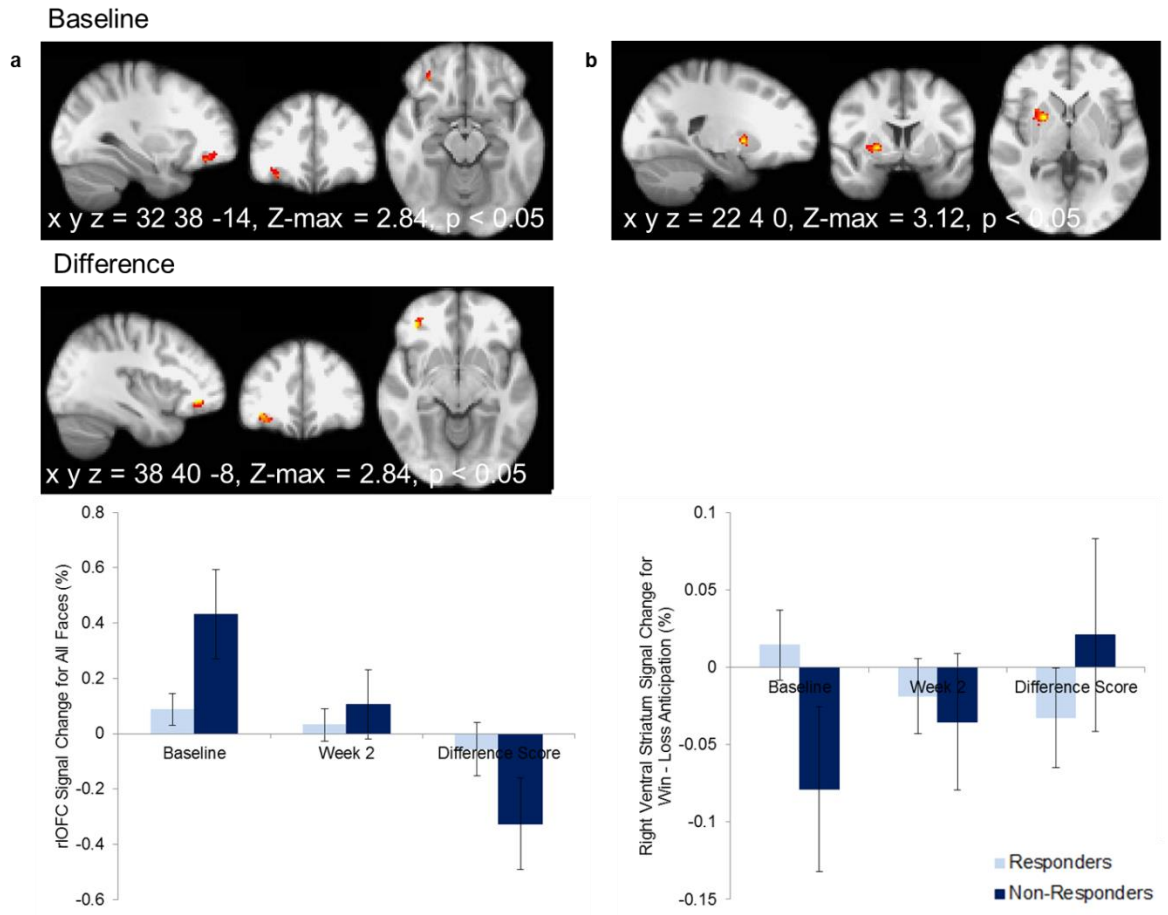


Figure 7.18 – The Difference between Responders and Non-responders for the Changes in Neural Measures: non-responders displayed **(a)** greater right lateral OFC activity in response to faces at baseline, greater reduction in right lateral OFC activity in response to faces between baseline and test visit 2 and **(b)** lower right VS activity during anticipation of win versus loss outcomes at baseline compared to responders.

7.3 Are there any Distinct Predictors Related to Negative Affect versus Anhedonia?

From the results above, again, it does not appear that negative affect and anhedonia can be differentiated from each other with no clear pattern of distinct changes in behavioural or neural measures of emotional and reward processing related to change in negative affect versus anhedonia.

Regarding changes in behavioural measures of emotional and reward processing, there were few significant correlations with changes in negative affect or anhedonia and those that were significant were related to both negative affect and anhedonia. For example, the change in the number of positive self-referent words correctly recalled in the EREC was found to negatively correlate with change in both negative affect measured by the MASQ general distress: depressive sub-scale and anhedonia measured by the MASQ anhedonic depression sub-scale.

Regarding changes in neural measures of emotional and reward processing, again, there were many correlations involving brain activity in response to sad faces; however, this time, decreased right amygdala and OFC activity was associated with both decreased negative affect and anhedonia at 6 weeks.

7.4 Summary

This Chapter aimed to investigate whether the early changes in behavioural and neural measures of reward and emotional processing in the bupropion-treated MDD group found at test visit 2 were associated with clinical outcome. It was expected that the early changes in behavioural and/or neural measures at 2 weeks would correlate with the change in scores on questionnaires measuring clinical outcome, anhedonia and negative affect over 6 weeks.

None of the early changes in behavioural measures of reward and emotional processing at 2 weeks were found to correlate with the change in scores on questionnaires measuring clinical outcome, anhedonia and negative affect at 6 weeks. When investigating other behavioural measures, the number of positive self-referent words correctly recalled was found to negatively correlate with both negative affect measured by the MASQ general distress: depression sub-scale and anhedonia measured by the MASQ anhedonic depression subscale as well as differentiate between responders and non-responders.

Changes in neural measures of reward and emotional processing at 2 weeks were much more related to clinical outcome at 6 weeks. With regards to neural measures of emotional processing during the covert faces task, higher right lateral OFC activity in response to happy faces at 2 weeks was associated with decreased HAM-D score at 6 weeks and was also found to differentiate between responders and non-responders. In addition, decreased right amygdala activity in response to sad versus fearful faces at 2 weeks was associated with decreased HAM-D score and negative affect at 6 weeks. Decreased activity in the right amygdala, right medial OFC and right VS in response to sad faces was also associated with decreased anhedonia at 6 weeks.

With regards to neural measures of reward processing during the reward task, a general decrease in brain activity in regions including the OFC, ACC, VS and amygdala across all aspects of the reward task including anticipation and receipt of win and loss outcomes was associated with decreased HAM-D score and negative affect or increased positive affect at 6 weeks. Exceptions were increased dorsal ACC activity specifically in response to win versus neutral outcomes and increased left VS activity specifically in response to win versus loss outcomes. There was also a general trend for decreased brain activity, particularly decreased medial and lateral OFC and left amygdala activity, during the reward task to be associated with decreased anhedonia at 6 weeks.

7.5 Discussion

Research on predicting clinical outcome from the magnitude of initial antidepressant effects is in its relatively early stages, particularly with regards to DA antidepressants. From research currently available there appears to be a general trend for clinical outcome to be particularly associated with an initial increase in the processing of happy faces. For example, the increase in the accuracy of recognising happy faces produced by 2 weeks treatment with citalopram or reboxetine was found to be associated with the clinical improvement seen after 6 weeks of continued treatment (Tranter et al., 2009). Increased accuracy of recognising happy faces following 7 days citalopram treatment has also been found to be a marginally

significant predictor of antidepressant response after 8 weeks in later-life MDD (Shiroma et al., 2014). Whilst the bupropion treated-MDD group did not exhibit increased accuracy for recognising happy faces, they did have increased attentional vigilance for happy faces with responders appearing to show a greater increase in attentional vigilance for happy faces than non-responders at 2 weeks but this was not found to be significant. Responders also appeared to show a greater reduction in the misclassification of other faces as sad compared to non-responders at 2 weeks but again this was not found to be significant. Given the trends in the right direction, there is some support that these effects of bupropion are not simply the result of regression to the mean and are related to clinical outcome, with a greater increase in positive or decrease in negative emotional processing associated with better clinical outcome. However, further research aimed at replicating the effects on the specific measures above is required, perhaps with a larger sample size, particularly for the non-responder group.

All the other early changes in behavioural measures of reward and emotional processing at 2 weeks that differed between the HC and bupropion-treated MDD groups were not found to correlate with clinical outcome, negative effect or anhedonia at 6 weeks or differentiate between responders and non-responders. Whilst change in correct recall of positive self-referent words was not found to differ between the HC and bupropion-treated MDD groups in the previous Chapter, it was found to negatively correlate with both negative affect and anhedonia and differentiate between responders and non-responders suggesting a possible candidate behavioural marker of clinical outcome with bupropion and should also be further investigated in an independent study.

At the neural level, higher right lateral OFC activity in response to happy faces at 2 weeks was associated with clinical outcome at 6 weeks and was also found to differentiate between responders and non-responders. The OFC has roles in the higher-order control of emotional processing, including attention towards and representing the value of affective stimuli thereby controlling the influence of emotion in associative learning and decision making (Bechara et al., 2000). Such higher right lateral OFC activity in response to happy

faces may be related to increased attention towards and higher affective value placed on happy faces. In addition, considering this result in the context of connectivity with other brain regions, interestingly others have found altered OFC connectivity to differentiate responders from non-responders (Lisiecka et al., 2011).

There has also been particular interest in whether remediation of amygdala hyperactivity in response to negative affective stimuli is associated with clinical outcome. Changes in left amygdala activity have been found to differentiate between responders and non-responders, with responders showing reduced activity and non-responders showing increased activity in response to fearful compared to happy facial expressions after 1 week of treatment with the SSRI escitalopram (Godlewska et al., 2013). This Chapter found that the greater the decrease in right amygdala activity in response to sad versus fearful faces at 2 weeks, the better the clinical outcome at 6 weeks. This does add further evidence for remediation of amygdala hyperactivity in response to negative affective stimuli to be associated with clinical outcome; however, there is an inconsistency with the previous research indicating reduced amygdala activity in response to a negative (fearful) versus a positive (happy) facial expression and the current research indicating reduced activity for the difference between two negative (sad – fearful) facial expressions. Amygdala activity in response to sad faces is more relevant to MDD; however, this argument still would not explain why reduced amygdala activity specifically to sad versus fearful faces rather than sad versus happy faces was associated with clinical outcome. The inconsistency is most likely the result of the different antidepressants used, with 1 week escitalopram acting to decrease fear processing but some evidence from this study that even longer-term bupropion administration may act to increase fear processing.

Given that reduced reward processing has previously been associated with resistance to treatment (Vrieze et al., 2013), it was rather surprising to find that a general decrease in brain activity in regions including the OFC, ACC, VS and amygdala across all aspects of the reward task including anticipation and receipt of win and loss outcomes at 2 weeks was associated with clinical outcome and decreased anhedonia at 6 weeks.

However, this is consistent with the behavioural findings of worsened performance on the reward task at week 2 but improved performance similar to the HC group by week 6 of bupropion treatment. There were two exceptions with an increase in reward-related brain activity in the dorsal ACC specifically in response to win versus neutral outcomes and left VS specifically in response to win versus loss outcomes associated with clinical response.

Overall, very few of the early changes in behavioural measures of reward and emotional processing at 2 weeks were associated with clinical outcome at 6weeks, except perhaps change in the number of positive self-referent words recalled; however, this behavioural measure was not found to differ significantly between the HC and bupropion-treated MDD groups at week 2. Early changes in neural measures of reward and emotional processing at 2 weeks were much more related to clinical outcome at 6 weeks, with a greater increase in attention-related brain activity in response to happy faces, a greater decrease in right amygdala activity in response to sad versus fearful faces and a greater decrease in reward-related brain activity associated with greater clinical response. The majority of these results were in the expected direction with a greater increase in positive or decrease in negative processing associated with greater clinical response, with the exception of reward-related brain activity; however, this was found to be consistent with behavioural findings. Therefore, this Chapter has demonstrated a few possible candidate markers of clinical outcome with bupropion but further research is required, such as a placebo-controlled investigation into the predictive performance of the specific behavioural measures or pre-specified brain regions highlighted here in a new group of MDD patients treated with bupropion.

CHAPTER 8

GENERAL DISCUSSION

This thesis aimed to delineate the role of the DA reward system in the cognitive neurobiological processes underlying the two symptom clusters of low mood and anhedonia in MDD. In order to do this, two experimental studies manipulating DA activity with the antidepressant bupropion were completed and the effects on reward and emotional processing biases implicated in MDD were assessed. The two experimental studies were: a double-blind randomised trial in which non-clinical participants received acute bupropion treatment, placebo or no treatment prior to behavioural assessment of reward and emotional processing (Chapter 2) and a large, repeated measures, open-label, experimental medicine study in which a cohort of MDD patients received bupropion and their reward and emotional processing was assessed at baseline, 2 weeks and 6 weeks via behavioural and fMRI methods (Chapter 3 – 7).

8.1 Summary of Main Findings

8.1.1 Chapter 2: Acute Effects of Bupropion in Healthy Volunteers

In Chapter 2, the acute effects of bupropion on behavioural measures of emotional and reward processing were assessed in a non-clinical population. This study found that, similar to other antidepressants, the antidepressant bupropion acts to alter the balance between negative and positive emotional processing with just a single dose, without any change in mood. Specifically, compared to placebo, an acute dose of bupropion increases the recognition of happy faces at lower happy intensities, increases the familiarity of positive compared to negative self-referent words and decreases the response bias or misclassification of other faces as sad (Figure 8.1). This is a similar profile of effects on emotional processing to the SNRI reboxetine, suggesting the effects may be mediated primarily by the additional NA enhancing activity of bupropion. The study also detected a

very subtle decrease in the likelihood of choosing the correct symbol in the win condition of the reward task in the bupropion group. Such a subtle effect on reward processing by a DA antidepressant may have been the result of sample size, greater NA versus DA activity, insensitivity of the task to detect changes in reward processing, a roof level of reward processing in healthy controls or the general complexity of the relationship between DA manipulations and reward processing that need to be further unravelled. The question remained as to whether bupropion would remediate negative affective biases and aberrant reward processing in a clinical population and whether this was related to clinical outcome, which was covered in the remaining Chapters.

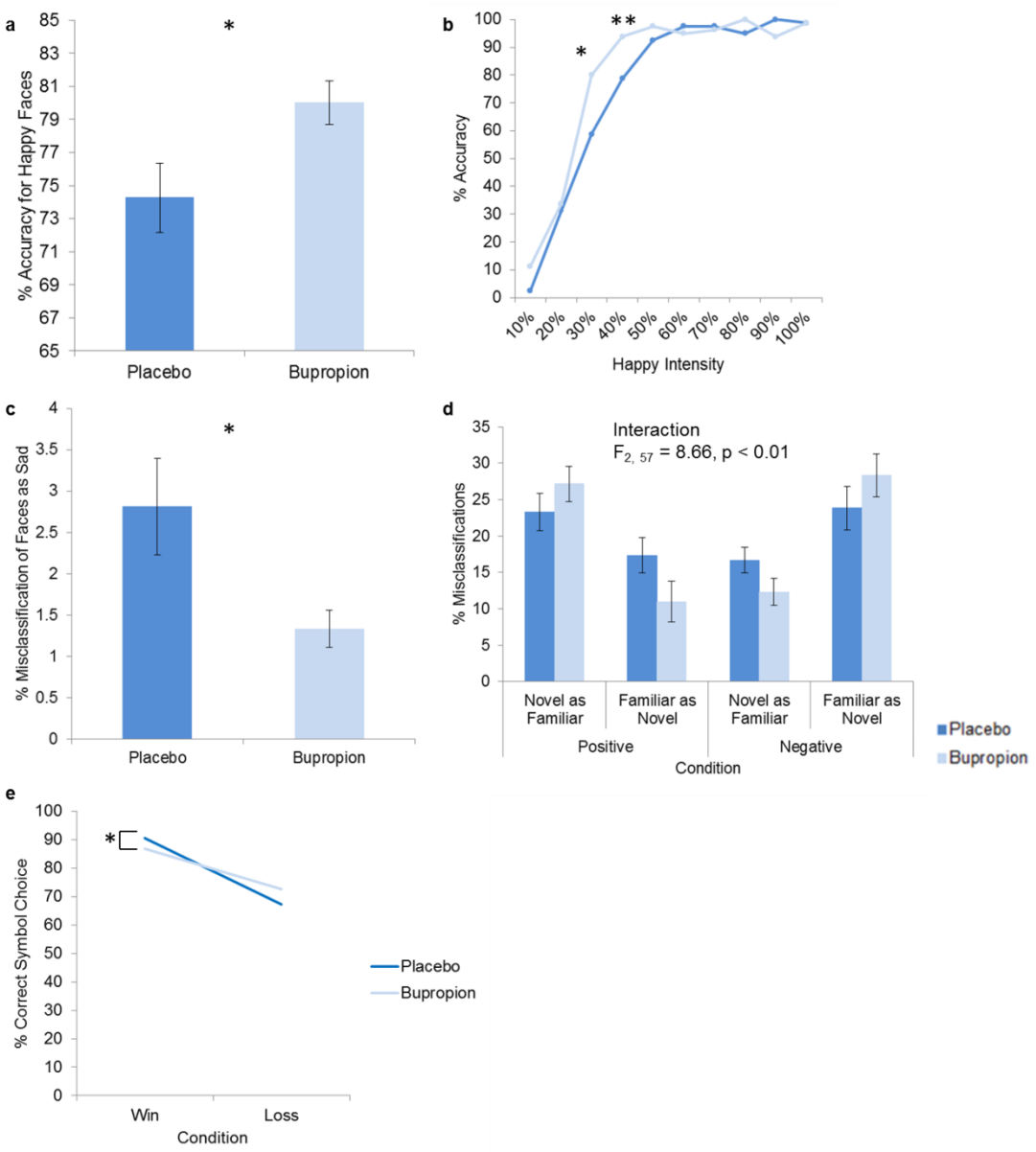


Figure 8.1 – Summary of Main Findings for the Acute Effects of Bupropion in Healthy Volunteers: compared to placebo, bupropion **(a)** increases the % accuracy for the recognition of happy faces at **(b)** lower happy intensities, **(c)** decreases the misclassification of other faces as sad, **(d)** increases the familiarity of positive versus negative self-referent words and **(e)** decreases the likelihood of choosing the correct symbol in the win condition.

8.1.2 Chapters 3 – 7: Effects of Bupropion in MDD

In Chapters 3 – 7, the early and more long-term effects of bupropion on behavioural and neural measures of emotional and reward processing were assessed in MDD patients. There were three main aims: to confirm that the MDD patients displayed negative affective biases and aberrant reward processing (Chapter 4) and that these were associated with the two symptom clusters of negative affect and anhedonia at baseline (Chapter 5), to investigate whether bupropion would remediate the negative affective biases and aberrant reward processing at 2 weeks (Chapter 6) and whether this was associated with clinical outcome at 6 weeks (Chapter 7).

8.1.2.1 Baseline Group Differences and Link to Baseline Symptoms

It was confirmed that the MDD group displayed negative affective biases and aberrant reward processing compared to the HC group, consistent with well-established previous research. Specifically, the MDD group displayed a response bias towards sad faces, misclassifying more faces as sad, decreased attentional vigilance towards happy faces and a possible insensitivity to high-probability wins. At the neural level, the MDD group displayed greater occipital cortex activity in response to sad faces and lower VS activity in response to win versus neutral outcomes (Figure 8.2).

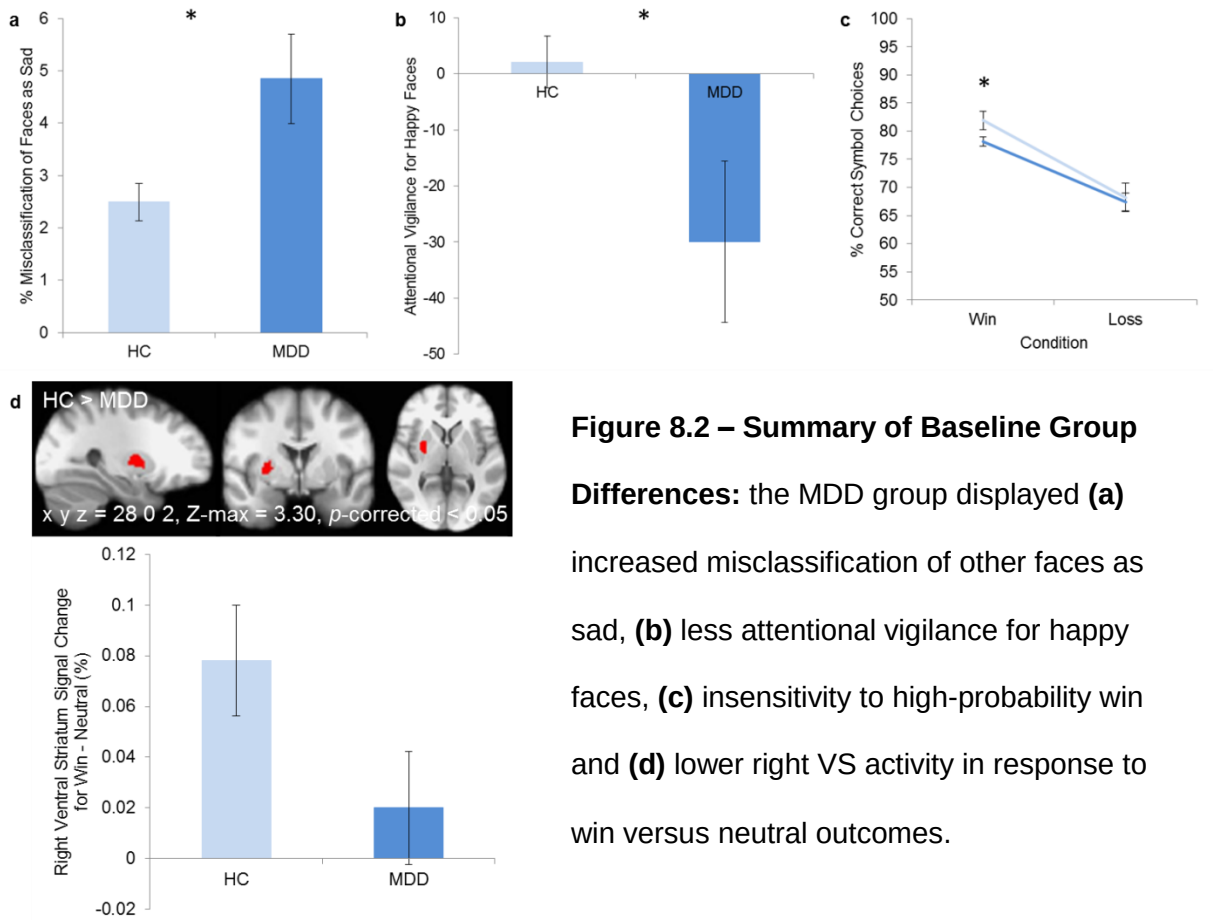


Figure 8.2 – Summary of Baseline Group Differences: the MDD group displayed **(a)** increased misclassification of other faces as sad, **(b)** less attentional vigilance for happy faces, **(c)** insensitivity to high-probability win and **(d)** lower right VS activity in response to win versus neutral outcomes.

None of these baseline abnormalities in reward and emotional processing were found to be associated with overall depression severity, negative affect or anhedonia. This was particularly surprising for the insensitivity to high-probability wins (Keedwell et al., 2005, Epstein et al., 2006) and lower VS activity (Keedwell et al., 2005, Epstein et al., 2006), which have been shown to be associated with severity of self-reported anhedonia. Instead, an association between lower OFC activity during the anticipation of win or loss outcomes and greater anhedonia was found at baseline, suggesting that the anhedonia observed, at least in this group of MDD patients, may be more related to aberrant processing during the anticipatory rather than consummatory aspects of reward. An association between lower right lateral OFC activity in response to happy faces and greater overall depression severity was also found, the importance of which in the differentiation of responders and non-responders came to light in the final Chapter.

8.1.2.2 Effects of Bupropion

It appeared that bupropion did act to alter the balance between negative and positive emotional processing in MDD patients, which may be instrumental in its therapeutic effects. Specifically, compared to the untreated HC group, the bupropion-treated MDD group displayed reduced response bias for sad faces, misclassifying significantly fewer faces as sad, reduced memory for negative self-referent words and increased attentional vigilance for happy faces, consistent with both previous research of other antidepressants and the acute effects of bupropion observed in the healthy volunteer study. The bupropion-treated MDD group also displayed a possible increase in the percentage accuracy for recognising fearful faces, perhaps consistent with the side-effects of bupropion that include agitation and anxiety; however, significance was only found in post hoc t-tests without a prior significant interaction so these results should be treated with caution.

At the neural level, compared to the untreated HC group, the bupropion-treated MDD group displayed a greater increase in left medial OFC activity in response to sad faces, which was suggested to allow greater higher-order control over negative affect.

With regards to reward processing, surprisingly, the bupropion-treated MDD group displayed a further reduction in choice frequency for high-probability to win at 2 weeks, which was then restored back to similar levels observed in the HC group at 6 weeks, possibly as a result of homeostatic mechanisms in an autoreceptor response or the tonic versus phasic firing of DA neurons or bupropion acting in a similar way to SSRIs. At the neural level, the bupropion-treated MDD group displayed greater rostral ACC activity in response to win outcomes compared to the HC group at 2 weeks (Figure 8.3). It has been proposed that the ACC is involved in error detection allowing for performance evaluation and optimisation of future response selection. Increased rostral ACC activity is typically associated with the occurrence of an error (Holroyd and Coles, 2002, Polli et al., 2005); therefore, greater rostral ACC activity in response to a correct, win outcome may result in a

failure of a feedback process and provide the neural substrate for the reduced choice frequency for high-probability win at 2 weeks.

It is important to note that for ethical reasons, a separate MDD group receiving placebo was not included in this study. A lack of comparison with a placebo-treated MDD group and large group differences at baseline means that these effects cannot be definitively attributed to true effects of bupropion. They could conceivably be caused by regression to the mean or by differential learning of the tasks between the two groups of participants. This uncertainty in part prompted the final data Chapter, which investigated the relationship between these effects and clinical outcome. Such a relationship would provide evidence against the alternative interpretations of the effects of bupropion.

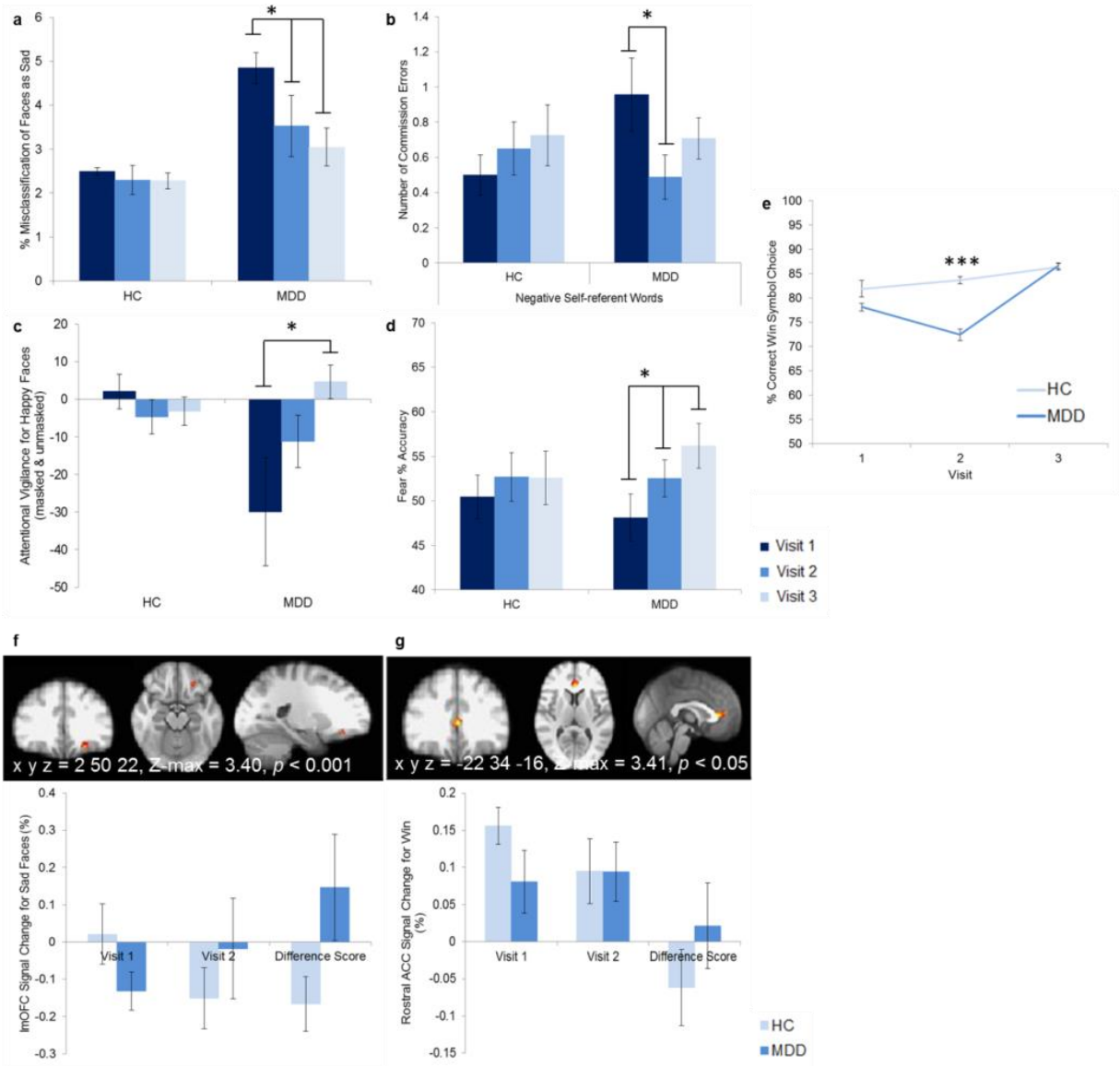


Figure 8.3 – Summary of the Effects of Bupropion: compared to the HC group, the bupropion-treated MDD group displayed **(a)** decreased misclassification of other faces as sad, **(b)** fewer commission errors for negative self-referent words at week 2, **(c)** increased attentional vigilance for happy faces, **(d)** increased % accuracy for fearful faces, **(e)** decreased choice frequency for high-probability win at week 2, increasing to HC levels at week 6, **(f)** a greater increase in left medial OFC activity in response to sad faces and **(g)** greater rostral ACC in response to win outcomes.

8.1.2.3 Predicting Clinical Outcome

None of the early changes in behavioural measures of reward and emotional processing found to differ between the HC and bupropion-treated MDD groups at 2 weeks were found to significantly correlate with the change in scores on questionnaires measuring clinical outcome, anhedonia and negative affect over 6 weeks. Whilst change in correct recall of positive self-referent words was not found to differ between the HC and bupropion-treated MDD groups at 2 weeks, it was found to negatively correlate with both negative affect and anhedonia and differentiate between responders and non-responders.

Changes in neural measures of reward and emotional processing at 2 weeks were related to clinical outcome at 6 weeks. Increased right lateral OFC activity in response to happy faces was associated with reduced HAM-D score and differentiated between responders and non-responders. Decreased right amygdala activity in response to sad versus fearful faces was associated with reduced HAM-D score and negative affect. Decreased OFC activity in response to sad faces was associated with reduced negative affect and anhedonia. Finally, increased dorsal ACC activity for win versus neutral outcomes was associated with reduced negative affect.

8.2 Outstanding Questions

8.2.1 Are Reward and Emotional Processing Abnormalities Distinct in MDD?

In the introduction it was suggested that reduced positive affect or aberrant reward processing in anhedonia and the increased negative effect in low mood are distinct dimensions independent of each other and associated with disturbances in different neurotransmitter systems; the former the result of under-functioning in NA or the DA reward system and the latter the result of under-functioning in the 5-HT emotion system (Argyropoulos and Nutt, 2013, Pringle et al., 2013). However, in the current thesis, it was difficult to differentiate between anhedonia and negative affect, with no clear pattern of distinct reward or emotional processing abnormalities related to anhedonia versus negative

affect. In addition, the DA / NA antidepressant bupropion with no 5-HT activity was found to affect behavioural and neural measures of both reward and emotional processing in MDD. The dual action of bupropion on DA and NA also complicated matters. Since SNRIs have also been found to affect emotional processing in MDD and bupropion has similar effects to such SNRIs, bupropion may mediate its effects on emotional processing via enhancement of NA activity. Previous research has suggested that the roles of NA and 5-HT can be dissociated with NA acting more specifically to increase positive biases in emotional processing (Pringle et al., 2013); however, this would not explain how bupropion also reduced negative biases in emotional processing. This thesis therefore suggests that reward and emotional processing abnormalities are not distinct in MDD. It could be that aberrant reward processing is in fact a general bias away from positive information during emotional processing or if reward and emotional processing are two distinct systems, MDD affects both at the same time through a common mechanism. There is clearly an intricate interplay between various neurotransmitter systems in the modulation of processing biases implicated in MDD that needs to be further unravelled possibly in a study directly comparing the effects of different classes of antidepressants on reward and emotional processing

8.2.2 Does Bupropion specifically affect Reward Processing and Anhedonia?

This was one of the first studies to look at the effect of a DA / NA antidepressant on reward and emotional processing in MDD but given the effects of other DA / NA enhancing drugs in previous research, there was some level of expectation that bupropion would specifically affect reward processing and anhedonia or if bupropion was to also affect emotional processing, it would specifically increase positive emotional processing. However, as mentioned above, bupropion was found to affect behavioural and neural measures of both reward and emotional processing, with both an increase in positive emotional processing and a decrease in negative emotional processing. Furthermore, surprisingly, similarly to SSRIs (Kumar et al., 2008, McCabe et al., 2010), bupropion may actually also

exacerbate aberrant reward processing prior to normalisation with more long-term treatment possibly as a result of its reuptake inhibition actions on tonic versus phasic DA firing. This evidenced the complexity within the DA system itself and of the relationship between DA manipulations and reward processing that also needs to be further unravelled.

Therefore, it does not appear that bupropion specifically affects aberrant reward processing and anhedonia and has a similar profile of effects to other antidepressants. This suggests that either antidepressants which target one neurotransmitter relative to another don't have any cognitive specificity or that the actions of bupropion are too general and that a further study comparing the effects of drugs enhancing tonic and phasic DA release is required.

8.2.3 Is There any Evidence for a Specific Profile of Effects for Bupropion?

As mentioned above, it appears that bupropion has a similar profile of effects to other antidepressants. Regarding the effects of bupropion on behavioural measures of emotional processing, bupropion displayed a similar profile of perceptual and memory effects to the SNRI reboxetine, with both acting to increase sensitivity to happy faces and altering the balance for memory of self-referent words, causing an increase in recall of positive self-referent words or decrease in the recall of negative self-referent words. Bupropion treatment also decreased the misclassification of other faces as sad in both studies conducted in this thesis, an effect often observed with various antidepressants on facial expression recognition tasks.

The time course of the effects of bupropion on reward processing, with an initial decrease in sensitivity for high-probability wins at 2 weeks that is then normalised at 6 weeks may be specific to bupropion; however, the effects of other antidepressants on this particular reward task have not been assessed yet and since there is evidence that SSRIs initially exacerbate aberrant reward processing (Kumar et al., 2008, McCabe et al., 2010), it is conceivable that they may also have this time course of effects on sensitivity for high-probability gains.

Finally, whilst the initial increase in fear processing observed with an acute dose of other antidepressants (Browning et al., 2007, Grillon et al., 2007) is reduced with longer-term treatment (Harmer et al., 2004), there was a suggestion that increased fear processing continues even with longer-term treatment with bupropion. This included potentially increased accuracy for fearful face recognition at 6 weeks and reduced amygdala activity in response to sad versus fearful faces at 2 weeks rather than sad versus happy or fearful versus happy faces as observed with escitalopram.

Therefore, bupropion may have some specific effects on fear processing, which has implications for the provision of the drug to those with comorbid anxiety disorders; however, a study directly comparing bupropion and escitalopram on more specific measures of fear processing is required.

8.3 Clinical Implications

Overall, the results of this thesis showed that the DA / NA antidepressant bupropion does have early effects on behavioural and neural measures of reward and emotional processing biases in both healthy volunteers and MDD patients. This provides further evidence to support the cognitive neuropsychological theory of antidepressant action and crucially, evidence that it applies to antidepressants across a range of neurotransmitter systems, including 5-HT, NA and DA. Since emotional and reward processing is altered by a wide range of antidepressants in healthy volunteers, individuals at risk from MDD and MDD patients, it seems that behavioural and neural measures of emotional and reward processing could be a useful predictor of the therapeutic potential of novel, candidate antidepressants. In this way, if the accuracy and reliability of using these measures in efficacy testing is high enough, this could potentially reduce the time and costs associated with failed clinical trials.

In addition, there was also some evidence that early changes in at least neural measures of emotional and reward processing were associated with clinical outcome. If this is the case, it may be possible to predict how effective a particular antidepressant will be for a particular individual in the longer-term from the magnitude of the initial effects of the

antidepressant on behavioural or neural measures of emotional and reward processing. Such a stratified approach to treatment could significantly increase response and remission rates. In order to test this, an investigation into the predictive performance of the specific behavioural measures or pre-specified brain regions highlighted here in a new group of MDD patients treated with bupropion is required.

8.4 Study Limitations

8.4.1 Study Design

Ideally, the clinical study would have employed a double-blind design involving a separate control group of MDD patients receiving placebo for 6 weeks; however, ethical concerns over the length of time patients would be treated with a placebo instead of a true medication prevented this. Instead, an open-label, repeated measures design was used and a separate group of HCs were recruited who did not receive bupropion, placebo or any other intervention. Comparison with the untreated HC group allowed investigation of baseline difference between MDD and HC groups and controlled for practice effects. Effects of bupropion were inferred from changes over time that occurred in the bupropion-treated MDD group but not in the HC group. However, without comparison with a placebo-treated MDD group it was still challenging to determine whether group by visit interactions were true effects of bupropion or simply an effect of time or regression to the mean. To counteract this, associations between changes in behavioural and neural measures of reward and emotional processing and changes in symptoms were investigated. Given that some studies have treated MDD patients with placebo for 2 weeks, a separate control group of MDD patients receiving placebo for just the first 2 weeks of the study could have been employed here; however, this would have made the study more complicated. A future study could just investigate the effects of bupropion versus placebo over 2 weeks in MDD.

8.4.2 Baseline HAM-D

Inclusion into the study was based solely on the psychiatric assessment and no baseline criteria for the HAM-D score were stated in this study. Therefore, a single MDD patient with a baseline HAM-D score within the normal range was still included and the average baseline HAM-D score of the MDD group was 13.09, which is relatively lower than that reported in some other clinical studies of MDD patients. This low average baseline HAM-D score is, in part, a reflection of the population recruited to the study, which were community based individuals with symptoms of depression who were not receiving treatment. It is also likely to reflect the approach taken in the study itself -- as a central objective of the study was to relate changes in symptoms across time with neural and behavioural measures, particular attention was made to reduce baseline inflation of HAM-D scores during the screening visit. The rationale for this approach was to ensure, as far as possible, that the measured reduction in symptoms across the study reflected real change in the underlying experience of the patients as opposed to expectation effects at enrolment which are known to complicate treatment studies in depression. A future study could also include a clinical self-report questionnaire of symptom severity, such as the self-report version of the (Quick) Inventory of Depressive Symptomatology ((Q)IDS) (Rush et al., 1986) rather than just a researcher administered questionnaire.

8.4.3 Division of the MDD Group

One way to investigate whether the changes in behavioural and neural measures of reward and emotional processing were associated with clinical outcome was to divide the MDD group into responders and non-responders and compare how the measure changed between the two groups. Responders were classed as those MDD participants displaying a greater than 50% decrease between their baseline and final HAM-D scores and a final HAM-D score of less than 7 indicating remission. Non-responders were classed as those MDD participants displaying a less than 50% decrease between their baseline and final HAM-D

scores. However, a large proportion of the MDD group was found to respond to bupropion leaving just 14 individuals in the non-responder group. A sample size of 14 was sufficient to perform statistical analysis; however, ideally, a larger sample size would be preferable. Indeed, whilst responder and non-responders did appear to show differences in behavioural measures in the expected directions, they were not found to be significant due to the small non-responder sample size and subsequent low power. A double-blind randomised placebo controlled study would likely have produced fewer responders and a larger sample of non-responders; however, such a study design was not possible for this particular study for the reasons considered above.

The MDD group could also have been divided based on severity of self-reported anhedonia to investigate whether low versus high anhedonic symptoms had any differential effects on reward processing. For example, a previous study has showed that MDD patients with high anhedonic symptoms displayed reduced reward processing compared with MDD patients with low anhedonic symptoms (Vrieze et al., 2013). The division of the MDD group was based on average baseline SHAPS scores with a SHAPS score greater than 7 indicating high anhedonic symptoms and a SHAPS score less than 7 indicating low anhedonic symptoms; however, in this study, there was a limited range of SHAPS scores with all MDD patients scoring significantly higher than 7.

8.4.4 The Multiple Comparisons Problem and Post hoc Analyses

All of the statistical analyses on the various perceptual, attentional and memory outcomes measured were not corrected for multiple comparisons and some of the post hoc tests were performed without prior justification from a significant interaction. This introduces the problem of multiple comparisons into the study, whereby the more statistical tests that are performed, the greater the likelihood that some *p* values less than 0.05 have occurred purely by chance creating a false positive result. As a result of the exploratory nature of the thesis, a relatively lenient approach was adopted but whilst there is increased likelihood for false positive results in this study, there is some evidence that the reported results are true.

For example, the effects of bupropion were similar in both the healthy volunteer and clinical studies as well as to the effects of other antidepressants. However, replication of the positive results in an independent study is certainly required.

8.5 Future Directions

The exploratory nature of the study also meant a lot of data was obtained but analyses were restricted to those determined a prior in a statistical analysis plan. Therefore extension of the work described in this thesis would firstly involve further, more in-depth analysis or alternative analysis approaches for the data already obtained. For example, further information could certainly be extracted from the data obtained from the reward task. Firstly, the participants who totalled an average across the two runs of the reward task below the starting £5 or had chosen the incorrect symbol all the way through the reward task were assumed to have misunderstood the task and were excluded from the analysis. However, this could actually have been a result of individual differences in associative learning during the reward task and would be interesting to further explore particularly in relation to MDD. The associative learning design of the reward task could have also allowed computational modelling of symbol choice (Pessiglione et al., 2006); however, given the almost identical response of the groups to this task a computational approach is unlikely to further our understanding of the results. Regarding the analysis of neural data, given potential coupling between the amygdala and occipital cortex or frontal regions, a connectivity analysis could be performed to investigate the interplay between these brain regions during bupropion treatment. With the high number of associations found between neural measures of reward and emotional processing and symptom clusters and the inherent overlap in constructs targeted by different measures, it could also be interesting to perform a factor analysis to investigate which neural responses and underlying circuits are most important for different symptom clusters; however, this is also unlikely to further our understanding of the results as most state a sample size of at least 300 is required for a worthwhile factor analysis (Gie Yong and Pearce, (2013)).

There is also clearly a lot more to understand about the effects of the DA antidepressant bupropion on reward processing in both healthy volunteers and MDD patients. When simply assessing the effects of bupropion on neural activity during anticipation, receipt and effort to receive a chocolate reward, bupropion appears to increase reward-related brain activity. However, when assessing the effects of bupropion on behavioural measures of associative reward learning, bupropion appears to initially worsen performance before restoring it back to HC levels. The reward task used in the present thesis was also relatively simple, only allowing investigation of the acquisition of stimulus-outcome associations. More dynamic aspects of reward learning that require flexibility could be investigated using a reversal reward learning task. Overall, it would be interesting to investigate the effect of bupropion at a range of different time points on a range of different reward tasks in order to more definitively describe its effects on reward processing in MDD. There are also some remaining questions over the contribution of DA or NA to the effects of bupropion on reward and emotional processing. A future study including parallel groups of MDD patients treated with either placebo, bupropion, a SNRI or a SSRI would allow direct comparison of the effects of the different classes of antidepressants.

Finally, with some evidence that early changes in at least neural measures of emotional and reward processing are associated with clinical outcome with bupropion, an investigation into the predictive performance of the specific behavioural measures or pre-specified brain regions highlighted here in a new group of MDD patients treated with bupropion is required.

8.6 Conclusion

The current thesis aimed to delineate the role of the DA reward system in the cognitive neurobiological processes underlying the two symptom clusters of low mood and anhedonia in MDD. In order to do this, two experimental studies manipulating DA activity with the antidepressant bupropion were completed and the effects on reward and emotional processing biases implicated in MDD were assessed.

The first study investigated the acute effects of bupropion on behavioural measures of reward and emotional processing in a non-clinical population. Compared to placebo, an acute dose of bupropion was found to reduce negative and increase positive emotional processing with little effect on reward processing.

The second study investigated the early and longer-term effects of bupropion on behavioural and neural measures of reward and emotional processing in individuals suffering with MDD. Compared to untreated healthy controls, bupropion was again found to reduce negative and increase positive emotional processing but was found to worsen aberrant reward processing at 2 weeks prior to normalisation after the full 6 week treatment. Higher right lateral orbitofrontal cortex activity in response to happy faces and decreased right amygdala activity in response to sad faces at 2 weeks was associated with decreased HAM-D score at 6 weeks. This suggests a causal role for the early neural effects of bupropion in later change in subjective symptoms and has clinical implications providing further evidence for the potential to predict clinical outcome from the early cognitive effects of antidepressants.

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