The Role of Catechol-O-Methyltransferase in Processing Affective Stimuli



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Thesis submitted for the degree of Master in Science (by Research) in Psychiatry

Trinity Term 2016

ACKNOWLEDGEMENTS

I would first and foremost like to thank my supervisors Elizabeth Tunbridge and Paul Harrison for their tireless help, support and patience throughout the completion of my project. I've learnt so much from having had the opportunity to study with you, and for that I am truly indebted.

I am also extremely grateful to Li Chen and Jess Scaife for their help with technical aspects of the project and to Marieke Martins and Shi Yu Chan for their friendship, and for making my time in the lab so enjoyable. I also wish to thank all those who generously gave their time to participate in the study.

A very special thank you to George, Jamie, Zoe, Thomas and Emma for being the most brilliant, caring and entertaining friends and housemates one could possibly wish for, and to all in the Brasenose College Choir and Oxford University Lightweight Rowing club - you made my time out of the lab so wonderful.

And finally thank you to my family, for always believing in me. Without your love and support none of this would have been possible.

Oxford, 2016

ABSTRACT

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Introduction: Catechol-O-methyltransferase (COMT) is involved in the catabolism of dopamine and is one of the key regulators of dopamine levels in the prefrontal cortex. COMT is known to modulate cognitive functions, such as working memory and executive function; however, its role in other cognitive processes, such as emotional processing, is relatively unexplored. The aim of this thesis is to examine the hypothesis that COMT activity, as influenced by the Val158Met polymorphism or by acute COMT inhibition, influences the processing of affective stimuli.

Methods: I performed a double-blind, randomised, placebo-controlled, experimental medicine study in a sample of healthy males (n=63) looking at the effects of COMT Val158Met genotype and a single, 200mg dose of the COMT inhibitor tolcapone on tests of emotional processing. I used the Oxford P1Vital emotional test battery (ETB) and an affective startle reflex modulation (ASRM) paradigm to detect attentional bias and emotional reactivity in the processing of affective stimuli.

Results: There was no effect of COMT Val158Met or tolcapone on indices of emotional processing assessed using the ETB. Acute administration of tolcapone was associated with a trend for greater startle response towards positive, negative and neutral picture stimuli in the ASRM paradigm.

Conclusion: This study is the first to examine the influence of COMT Val158Met or tolcapone on emotional processing using the ETB and has given insight into possible methodological limitations of using it as a tool to probe the effects of the genetic variant. These results may help further our understanding of COMT's role in underlying normal variation in emotional processing and contributing to penetrance and severity of psychiatric disorders.

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1. INTRODUCTION

1.1 CATECHOL-O-METHYLTRANSFERASE (COMT)

First identified by Axelrod in 1958, catechol-O-methyltransferase (COMT) is an enzyme involved in the degradation of catecholamines such as dopamine, norepinephrine and epinephrine (Axelrod & Tomchick 1958). COMT catalyses the conjugation of a methyl group on S-adenosyl-L-methionine (SAM) to the 3-hydroxy groups of catecholamine and catecholestrogens substrates in the presence of a divalent Mg^{2+} cation (Lotta et al. 1995). The enzyme is distributed extensively around the body, with particularly high activity in the liver and kidneys (Mannisto & Kaakkola, 1999).

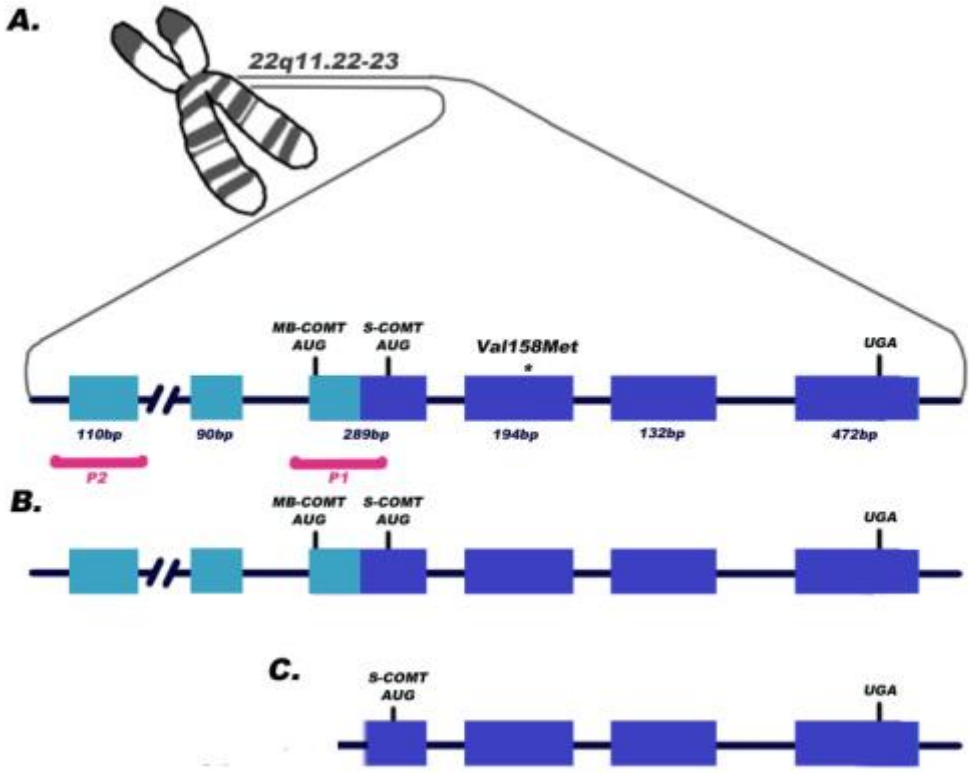


Figure 1.1: *adapted from Lundstrom et al., 1995; Tunbridge et al., 2006* ***A)*** *Schematic of the catechol-O-methyltransferase gene (COMT) on chromosome 22. The gene consists of 6 exons, which are shown as blue boxes connected by lines, which represent introns. The two promoters, P1 and P2 are indicated by pink bars and the position of AUG translation initiation codons and the UGA stop codon is also shown. The Val158Met polymorphism (in exon 4 of the 1.5kb transcript) is also labelled.* ***B)*** *1.5kb COMT mRNA transcript which encodes both MB-COMT and S-COMT. The turquoise boxes represent portions of exon sequence that are unique to the longer transcript.* ***C)*** *The 1.3kb COMT mRNA transcript does not contain the MB-COMT start codon and hence it can only encode S-COMT*

1.1.1 THE COMT GENE

The enzyme is encoded by the COMT gene, found on chromosome 22q11.2 (Lundstrom et al., 1995; Tenhunen et al., 1994). The COMT gene consists of 6 exons, which encode two transcripts of 1.3kb and 1.5kb, from different promoters (Tenhunen & Ulmanen 1993; Tenhunen et al. 1994). Two distinct allozymes arise from the COMT gene depending on the starting point of translation (Tenhunen et al. 1994). A soluble form of the protein, S-COMT, is located in the cytoplasm, whilst insoluble, MB-COMT is associated with membranes. The longer, 1.5kb transcript can encode both MB-COMT and S-COMT, whilst the shorter of the two transcripts only encodes S-COMT (see Fig. 1.1).

S-COMT is made up of 221 amino acids and has a molecular weight of 24.4kDa, whilst MB-COMT, with a weight of 30.0kDa, contains 271 amino acid residues (Mannisto & Kaakkola, 1999). The additional 50 amino acids attached to the N-terminal in MB-COMT provide a hydrophobic, membrane spanning region (Mannisto & Kaakkola 1999; Vidgren et al. 1994).

1.1.2 COMT GENE EXPRESSION

Northern blotting has demonstrated that both short, 1.3kb, and long, 1.5kb, COMT mRNA transcripts are present at varying levels in most tissues (Tenhunen et al. 1994). Both long and short transcripts are particularly abundant in the liver, kidneys and adrenal glands (Tenhunen et al. 1994). Despite expression of both transcripts, in the vast majority of tissues a significant disparity exists in the level of expression of the two allozymes. Generally, the S-COMT allozyme is considerably more highly expressed. The greater abundance of S-COMT protein, combined with its higher catalytic activity, means that the allozyme accounts for ~95% of COMT activity (Grossman et al. 1985; Jeffery & Roth, 1984).

Brain tissue is a notable exception to this. In the brain, MB-COMT is the predominant allozyme and, in comparison to most other tissues, there is a markedly reduced proportion of S-COMT, which only accounts for 30% of cerebral COMT protein (Bonifacio et al. 2007; Karhunen et al. 1994; Tenhunen et al. 1994). This may, in part, result from transcriptional regulation, since the 1.3kb COMT mRNA transcript is undetectable in the CNS (Hong et al., 1998; Tenhunen et al., 1994).

Interestingly, though MB-COMT and S-COMT have broadly the same sequences, their substrate affinities differ quite substantially. In a study of the kinetic properties of the two COMT allozymes, Lotta et al. discovered that membrane bound COMT has significantly higher affinity for dopamine than its soluble

counterpart (Lotta et al. 1995). By calculating K_m values, they estimated that MB-COMT has approximately 10-fold higher affinity for catecholamines than S-COMT. This could suggest why it is more highly expressed in the CNS, where it might be more suited to catabolising breakdown of catecholamine neurotransmitters at the concentrations present there.

A number of transcription factors are thought to help regulate tissue-specific COMT expression. Notably, there is strong evidence to suggest that oestrogen may play a role - it has been demonstrated in cell culture that physiological concentrations of 17-β-estradiol suppress production of 1.3kb COMT mRNA in a dose-dependent manner (Xie et al. 1999). Two oestrogen response elements have been identified in the COMT gene, which may facilitate such regulatory control (Jiang et al. 2003; Xie et al. 1999). Transcriptional regulation by oestrogen is of particular interest as it is believed to contribute to COMT sexual dimorphisms, which itself may be a factor contributing to the sex differences seen in emergence and clinical phenotype of some psychiatric disorders (Harrison & Tunbridge 2008). Other factors such as the extent of DNA methylation may also play a part in regulating COMT expression (Nohesara et al. 2011; Swift-Scanlan et al. 2014).

1.1.3 THE COMT VAL^{158}MET POLYMORPHISM

The COMT gene has a number of single nucleotide polymorphisms (SNPs). Of these, rs4680, involving a G → A substitution in exon 4 (part of the coding sequence of the COMT gene in human populations) is one of the most notable (Lachman et al. 1996; Tunbridge et al. 2006). The polymorphism alters the amino acid codon at position 158 in MB-COMT (corresponding to codon 108 in S-COMT) which, as a result, may encode either Valine (Val) or Methionine (Met). I will hereafter refer to the rs4680 SNP as Val^{158}Met (see Figure 1.1).

Several studies have shown that, by altering the structural integrity of the protein, the Val^{158}Met polymorphism has a significant bearing on the activity of the enzyme (Chen et al. 2004; Lotta et al. 1995). Indeed, Chen et al. found that the COMT activity of Met homozygotes was 38% lower than that of Val homozygotes in post-mortem human prefrontal cortex at physiological temperature (Chen et al. 2004). The difference in enzyme activity results from the fact that Met-MB-COMT has significantly lower thermostability than Val-MB-COMT (Chen et al. 2004; Lotta et al. 1995; Weinshilboum & Dunnette 1981).

The two human COMT alleles are codominant (Floderus and Wetterberg, 1981), resulting in a trimodal distribution of COMT activity within the population (Floderus et al. 1978). Homozygotes (Val/Val and Met/Met) represent individuals with respectively high or low enzyme activity, whilst COMT heterozygotes (Val/Met) have intermediate enzyme activity. The ancestrally younger, low activity Met allele and the high activity Val allele are present in nearly equal frequencies in Caucasian populations (Palmatier et al. 1999). The allelic frequencies do differ depending on which population is being studied. For example, in Asian populations, the Met allele is considerably less prevalent, with a frequency of only 25% (Palmatier et al. 1999).

So, what effects, if any, does the genotypic difference in COMT activity arising from the Val^{158}Met polymorphism have? I will now discuss the literature on putative manifestations of COMT Val^{158}Met.

1.2 COMT AND COGNITION

1.2.1 COMT: A MODULATOR OF PREFRONTAL FUNCTION?

In most regions of the brain, dopamine transporters (DAT) are the predominant means of dopamine clearance from the synapse (Kaenmaki et al. 2010). However, due to the relative sparsity of DAT in the prefrontal cortex (Lewis et al. 2001; Sesack et al. 1998), COMT represents the principal mechanism of dopamine removal from the synapse in this region of the brain (Kaenmaki et al. 2010; Tunbridge et al. 2004; Yavich et al., 2007). As a result, the level of COMT activity is believed to be a key determinant of dopamine flux and, as such, genotypic differences in activity are thought to have a considerable impact on function of the prefrontal cortex (Tunbridge et al. 2006).

There is accumulating evidence in support of this hypothesis. Notably, in 2001, Egan and colleagues performed a study to determine the effect of COMT genotype on PFC-dependent neuropsychological functioning in a cohort of individuals with schizophrenia, the patients' siblings and healthy controls (Egan et al. 2001). Using the Wisconsin Card Sorting Test (WCST), a paradigm to test executive functioning that activates the dorsolateral prefrontal cortex (Hashimoto et al. 2007; Sumitani et al. 2006), the study demonstrated a significant influence of COMT genotype on PFC function. Specifically, it found that COMT genotype accounted for ≈4% variance (P= 0.001) in the frequency of perseverative errors in the task, with an association between the high activity Val COMT allele and poorer performance. Furthermore, the study used functional magnetic resonance imaging (fMRI) to assess PFC activation during the performance of a simple working memory task. This identified a relationship between COMT genotype

and PFC activity. It was found that a greater degree of Val allele loading was associated with higher PFC activity, for the same level of performance. As greater activity is required to produce the same level of attainment in Val allele carriers, we can deduce that the Val allele may contribute to less efficient prefrontal functioning than the Met allele.

Although there is some controversy (Dickerson et al., 2007; Ho et al. 2005; Szoke et al. 2006), several studies have gone on to confirm the findings of Egan et al. (Goldberg et. al. 2003; Joober 1992; Malhotra et al. 2002) and further examine the relationship between COMT genotype and prefrontal functioning. For example, Goldberg et al. reported that COMT genotype was linked to performance in the N-back task of working memory. Val/Val individuals had poorest performance in the task whilst Met/Met individuals performed best, findings that were consistent in both the 1-back and 2-back condition (Goldberg et al. 2003). COMT genotype has also been studied in relation to brain response to amphetamine, an inhibitor of monoamine reuptake in the synapse (Mattay et al. 2003). Using an fMRI assay during the n-back task, it was found that the administration of 0.25mg/kg amphetamine improved prefrontal efficiency and task performance in high activity Val homozygotes. In contrast, the prefrontal efficiency and performance of low activity Met homozygotes remained the same, or even deteriorated in the high working memory load (2-back) condition. A follow up study using a larger sample size failed to replicate this interaction (Wardle et al. 2013); however, it is possible that subtle differences in the experimental set-up, such as the difficulty of the task, could have caused the discrepancy in results, especially if the true effect size is smaller than that suggested by the Mattay study. Further investigation, ideally using more sensitive techniques, would be useful to clarify whether or not COMT genotype does have a role in determining brain response to Amphetamine.

More consistent evidence of a relationship between COMT genotype and PFC function comes from studies using COMT inhibitors such as Tolcapone. At this stage I will only mention a couple of particularly relevant findings, but I will go on to discuss Tolcapone and various studies in which it has been used in more detail later (see section 1.3). One key study is that of Apud et al. who found a significant drug x genotype interaction for tasks involving intradimensional set shifting ($F = 4.9$, $df = 2$. $p < 0.05$) and a trend effect on verbal episodic memory ($F = 2.34$, $df = 2$. $p = 0.1$) (Apud et al. 2007). This interaction was such that the performance of Val/Val individuals receiving Tolcapone markedly improved, whilst performance of Met/Met individuals receiving the drug worsened.

A similar drug x genotype interaction was also observed when Tolcapone was administered to healthy male Val^{158}Met homozygotes in a study by Farrell et al. (Farrell et al. 2012). In this randomised, placebo-controlled, double-blind, between-subjects study, participants were given either 200mg of tolcapone or placebo. They were then asked to complete both an N-back task of working memory (1,2 and 3-back conditions) and a monetary decision making task (Gehring & Willoughby 2002), which had been used previously in a number of studies of risky decision making (Marco-Pallares et al. 2009). It was observed that in the placebo group, Met-COMT homozygotes outperformed Val-COMT homozygotes on the N-back task (particularly in the 2-back condition ($p = .006$)) and were more risk averse in the gambling task. However, in subjects given a dose of tolcapone, the genotypic differences observed in subjects given placebo were reversed. Of those given tolcapone, Val homozygotes outperformed Met homozygotes in the N-back task, and were found to be more risk averse.

These results were interpreted by the authors as being consistent with the proposed inverted-U model of cortical dopamine function (Cools & D'Esposito 2011; Williams & Goldman-Rakic 1995). This model suggests that dopamine influences the function of the prefrontal cortex in agreement with an inverted U-shaped dose-response curve. Hence, there exists an optimum level of dopamine for PFC function, above or below which PFC function is adversely affected.

In the Farrell study it was suggested that Met-COMT individuals, with lower COMT activity and correspondingly higher baseline dopamine levels, sit further towards the peak of the inverted U-shaped curve than Val-COMT individuals. Val-COMT individuals have lower baseline dopaminergic tone and so they sit further to the left of the curve, with dopamine levels that may be considered 'suboptimal' for PFC functioning in the context of the study's tasks. When tolcapone is administered dopamine catabolism is reduced, meaning that both Val-COMT and Met-COMT individuals are shifted rightward on the curve. For Val-COMT individuals this moves them further towards the peak of the curve, meaning that their performance on tasks requiring prefrontal function is improved. Met-individuals on the other hand, are shifted beyond the optimum level of DA required for PFC functioning and as a result, their performance in PFC-dependent tasks is adversely affected (see Fig. 1.2).

The effects of COMT inhibition have also been examined in the context of animal studies; the activity of rodent COMT is comparable to, or possibly greater than activity of the human Val isoform (Chen et al. 2004; Risbrough et al. 2014) and studies have shown that COMT inhibition enhances cognitive function in wild-type rodents (Barkus et al. 2016; Lapish et al. 2009; Tunbridge et al, 2004). Furthermore, promising studies using a novel transgenic mouse model, engineered to express human-COMT on a COMT

null background, indicate that the Met-COMT transgenic mice do not show the same improvements in cognitive function following tolcapone administration as their wild-type littermates (Barkus et al. 2016), in line with human studies (Farrell et al. 2012).

Considering the apparent detriment to prefrontal cognition arising from the Val form of the enzyme, it seems somewhat surprising that the allele is so prevalent. As mentioned previously, in Caucasian populations both alleles are present at roughly equal frequencies and in Asian populations the Val allele is considerably more prevalent (Palmatier et al. 1999). This prevalence would be more consistent if the Val allele were able to compensate for its detriment to PFC function by conferring some other advantage to its carriers. Certainly, COMT plays a role in metabolising catecholestrogens, and there is some evidence, albeit inconsistent, that the low activity Met allele may reduce an individual's ability to detox these compounds, which might predispose Met carriers to estrogen-induced cancers (Lavigne et al. 1997; Mitrunen et al 2001; Yim et al, 2001). However, another suggestion is that the Val allele may offer advantages in terms of emotional processing, making its carriers more emotionally resilient. I will now consider the evidence that COMT genotype has an influence on emotional processing.

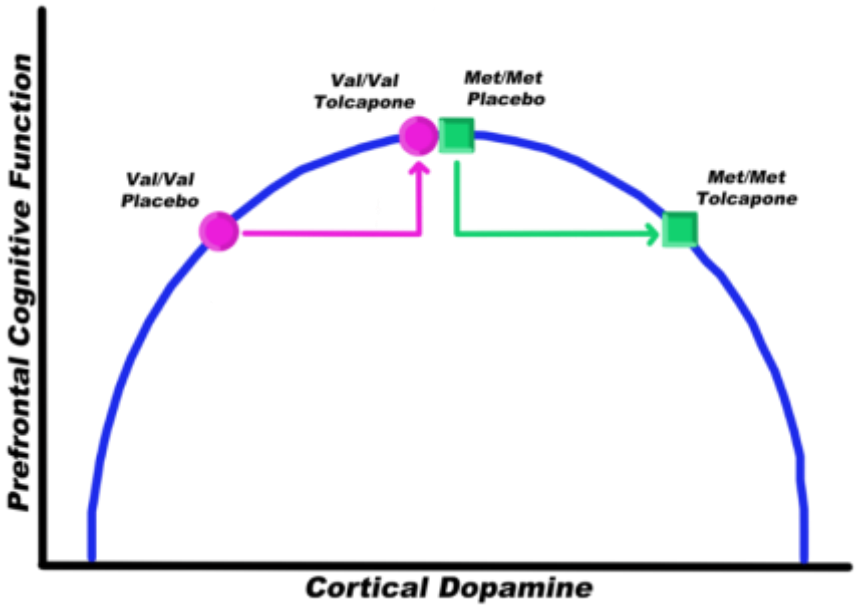


Figure 1.2: *adapted from Farrell et al, 2012 and Williams & Goldman-Rakic 1995. Schematic showing the hypothesised inverted-U shaped relationship between cortical DA levels and prefrontal cognitive functioning. Val-COMT subjects with higher COMT activity sit further to the left of the curve at baseline because they have lower dopamine levels than Met-COMT subjects, who sit further towards the peak of the curve. The Farrell et al study found that the effects of Tolcapone administration on N-back performance were genotype dependent and they interpreted this as being in line with the inverted-U hypothesis. Performance of Val-COMT subjects improved, as it is thought that they were moved closer towards the optimum level of dopamine, whereas performance by Met-COMT homozygotes worsened, it is thought this could because Tolcapone administration shifted them beyond the optimum.*

1.2.2 COMT AND EMOTIONAL PROCESSING

Historically, a great deal of research has focused on the role of dopamine in emotional processing and the neurotransmitter is considered to play a critical role in both cognitive and affective behavioural processes (Bishop et al. 2006; Egan et al. 2001; Goldberg et al. 2003; Heinz & Smolka 2006; Smolka et al. 2005) . DA is known to modulate a number of neural regions consistently implicated in the processing of emotional sensory information including the PFC, Basolateral Amygdala (BLA) and Nucleus Accumbens (NAcc) (Grace & Rosenkranz 2002; Karreman & Moghaddam 1996). Furthermore, aberrant DA signalling is thought to contribute to the emergence and maintenance of affective dysregulation in a range of psychiatric disorders. Considering this, it seems plausible that the COMT Val^{158}Met polymorphism, which

influences dopamine neurotransmission, could underlie some of the interindividual variation seen in the processing of emotional stimuli and risk for affective disorders.

Though there is mounting (albeit controversial) evidence to suggest that the Met allele confers an advantage in terms of prefrontal cognitive function (Egan et al. 2001; Farrell et al. 2012), recently it has emerged that this advantage may be offset by detriments in emotional processing and a slight increase in tendency towards development of a range of psychiatric disorders. To date, studies have linked the low-activity Met allele with panic disorder (Olsson et al. 2005; Woo et al. 2002), heightened anxiety in women (Enoch et al. 2003), obsessive compulsive disorder in males (Pooley et al. 2007; Katerberg et al. 2010), bipolar affective disorder (Mynett-Johnson et al. 1998), augmented pain sensitivity (Zubieta et al. 2003) and major depressive disorder (Ohara, Nagai, Suzuki & Ochiai, 1998) among others. Many of these associations remain somewhat tenuous and, in some cases, attempts to replicate them have produced contradictory results (Domschke et al. 2004; Kawachi et al. 2004; Ohara et al. 1998). However, this still raises the interesting possibility that COMT Val158Met may influence affective regulation. Could, for example, improved prefrontal efficiency in Met homozygotes be counterbalanced by poorer affective regulation?

Few studies have directly examined the effect of COMT genotype on performance in emotional processing tasks (Defrancesco et al. 2010; Gohier et al. 2014; Gong et al. 2013), but a number have looked at the relationship between Val158Met and brain activation during the processing of emotionally salient stimuli (summarised in table 1.1). A prominent study by Smolka et al. used an fMRI blood oxygen level-dependent (BOLD) assay to examine the influence of COMT genotype on the brain activation elicited by unpleasant, pleasant and neutral affective visual stimuli in a cohort of healthy individuals (Smolka et al. 2005). They observed a significant positive correlation between COMT Met allele loading and brain reactivity in the limbic system, connected regions of the PFC and visuospatial attention system in response to negative emotional stimuli. Furthermore, they calculated that COMT genotype was responsible for up to 38% of the variance in BOLD response to aversive stimuli seen in their cohort.

The effect of COMT genotype was most profound in the ventrolateral PFC (Brodmann area 47), an area of the brain thought to be involved with the processing of negative emotional states (Drevets, 2000; Levesque et al. 2003; Pelletier et al. 2003). Activation of the right amygdala was also found to be considerably more pronounced in those with a greater degree of Met allele loading. The amygdala is

thought to be important for enhancing excitatory inputs from the sensory cortex, whilst also dampening inhibitory input from the prefrontal cortex. Heightened reactivity of the amygdala has been linked to an increase in anxiety-related personality traits and greater vulnerability to the development of mood disorders (Davis et al. 1999; Stein et al. 2007). The amplified response of the amygdala seen in Met carriers could therefore predispose such individuals to abnormalities in affective processing and anxiety.

Another important study linking COMT genotype to affective arousal and regulation is that by Drabant et al. (Drabant et al. 2006). In this study corticolimbic reactivity in healthy individuals during a perceptual task involving fearful and angry faces was assessed using fMRI. The BOLD imaging data showed that COMT Met homozygotes exhibited significantly greater activation in the ventrolateral PFC and right hippocampal formation in response to negative facial expressions than Val homozygotes. Heterozygotes were found to have intermediate levels of activation. Furthermore, a greater degree of functional coupling between prefrontal and limbic regions was also seen in Met/Met individuals, compared with Val carriers. Activity in the hippocampus is thought to play a role in regulating emotional arousal and bringing about states of anxiety (Deacon et al. 2002; McNaughton 1997; Ploghaus et al. 2001; Rusch et al. 2001). Heightened baseline dopamine levels in COMT Met individuals may contribute to an exaggerated hippocampal response to affective stimuli. Such an amplified response could conceivably make Met/Met individuals more sensitive to aversive emotional stimuli.

COMT genotype has also been studied in relation to the affective startle reflex modulation (ASRM) paradigm, an established measure of emotional reactivity (Montag et al. 2008). The startle reflex is an involuntary, defensive response to sudden and intense stimuli involving rapid muscular contraction (Bradley et al. 1990; Blumenthal et al. 2005; Blumenthal & Berg 1986; Grillon & Baas 2003; Grillon 2008). In humans, the startle response is most reliably quantified using electromyography (EMG) to measure amplitude of the eye-blink reflex in response to an acoustic probe, generally consisting of a burst of high-intensity white noise (Anthony 1985; Blumenthal et al. 2005; Grillon & Baas 2003). The magnitude of the startle response can be modulated by accompanying the acoustic probe with presentation of affective stimuli, such as appetitive or aversive images (Bradley et al. 1990). Modulation is dependent on the valence of the accompanying affective stimuli, whereby negatively valenced stimuli potentiate the response and positively valenced ones attenuate it (Bradley et al., 2001; Bradley, Cuthbert and Lang, 1993). ASRM can be used to identify variations in affective processing and abnormalities in startle modulation have been associated with a range of psychiatric phenotypes. For example, an exaggerated startle response to

aversive stimuli has been observed in individuals with anxiety disorders (Kaviani et al. 2004; Larson et al. 2007) as well as those scoring highly on measures of neuroticism (Chan et al. 2007), fearfulness (Cook et al. 1992) and behavioural inhibition (Caseras et al. 2006).

Montag et al. measured the amplitude of startle response to pleasant, unpleasant and neutral picture stimuli in a sample of 96 healthy, female Caucasians. They found that the startle amplitude in response to unpleasant images was considerably greater in COMT Met homozygotes than Val homozygotes. As dopaminergic neurotransmission in the limbic system is thought to strongly influence affect-modulated startle, the authors argued that the difference they observed in startle magnitude resulted from the genotypic difference in dopamine levels. Domschke et al. performed a similar study looking at the relationship between COMT genotype and emotion potentiated startle amplitude, which found no COMT effect (Domschke et al. 2014), although this may reflect the highly environment-specific nature of the polymorphism's effects.

A number of studies linking the Met158 allele to poorer affective regulation have interpreted their findings with reference to the tonic-phasic hypothesis of dopamine regulation in limbic and striatal structures (Grace 1991; Grace 2000). It has been suggested that the low activity Met allele, increases tonic DA transmission thereby diminishing phasic subcortical DA release via activation of inhibitory DA terminal autoreceptors (Bilder et al. 2004). This is associated with an increase in tonic D1 activation and a reduction in D2 stimulation (Durstewitz et al. 2000; Floresco et al. 2003). It is thought that the overall effect of this is an increase in stability of neural networks. Although this could in theory help to sustain working memory representations, which might account for the improved n-back performance seen in Met158 homozygotes (Farrell et al. 2012; Goldberg et al. 2003), it is thought to be at the cost of reduced cognitive flexibility (Bilder et al. 2004). Excessive stability results in reduced flexibility and difficulty updating working memory which could translate into affective perseveration, impaired disengagement and persistence of maladaptive behaviours (Bilder et al. 2004).

Table 1.1: Summary of studies linking COMT Val^{158}Met to emotional processing and selected psychiatric phenotypes

BOLD fMRI studies	
Domschke et al. (2008)	*FMRI was used as an assay of limbic and prefrontal activation in response to a presentation of emotional faces in 20 subjects with panic disorder. Patients with at least one Val158 allele were found to have increased activation of the right amygdala in response to the emotional faces. Furthermore, Val158 allele loading was correlated with greater activation in the*

	orbitofrontal and ventromedial prefrontal cortex in response to fearful, angry and happy faces. The study concluded that their data provided evidence that Val158Met has a role in modulating activation of the amygdala and connected prefrontal regions, in response to emotional faces in individuals with panic disorder. Domschke et al. went on to suggest that COMT genotype may influence an individual's vulnerability toward developing panic disorder, by modulating dopamine levels and thus influencing the processing of anxiety-related cues.
Drabant et al. (2006)	*In this study, the relationship between COMT val158Met genotype and corticolimbic activity during the processing of emotionally salient stimuli was assessed. FMRI was performed in a cohort of 100 healthy individuals of both genders and this found that the degree of Met allele loading was correlated with an increase in reactivity of the ventrolateral PFC and hippocampal formation. Furthermore, the study also found that Met/Met individuals demonstrated increased functional coupling of limbic and prefrontal regions. The degree of coupling between the amygdala and orbitofrontal PFC was inversely correlated with the trait of novelty seeking. The study concluded that these factors may contribute to the increased penetrance of emotional dysregulation seen in Met homozygotes.*
Kempton et al. (2009)	*This study used fMRI to study brain activation in 74 healthy subjects during a facial affect recognition task, in which subjects were asked to identify fearful and neutral faces. The study found that there was no significant main effect of the COMT genotype on brain activation during performance in the task. However, they did find a significant effect of gender on brain activation, with greater activation in the right temporal pole and left amygdala in female participants. Val/Val carriers were found to have greater activation compared to Met/Met carriers, and this was found to be more significant in females. The study concluded that the COMT Val158Met polymorphism modulates gender-related patterns of activation in the limbic system and connected prefrontal regions.*
Opmeer et al. (2013)	*125 participants, made up of individuals with major depressive disorder (MDD) and healthy controls, were scanned using fMRI whilst viewing a presentation of emotional faces. Within the healthy controls, the study found a positive correlation between the Met-allele load and activation of the right inferior frontal gyrus in response to the emotional stimuli. This correlation was not seen in those with MDD. The study concluded that COMT genotype is related to endophenotypes relevant to Major Depressive Disorder.*
Rasch et al. (2010)	*FMRI was used to assess the impact of COMT genotype on signal in the amygdala in response to the viewing of emotional and neutral picture stimuli in 56 healthy subjects. The study found a significant positive correlation between the Met COMT allele and activation of the right amygdala in response to the viewing of unpleasant pictures. The same correlation was not seen when individuals were viewing positive picture stimuli. The Met allele was also associated with increased connectivity between orbitofrontal regions and the right amygdala. The study concluded that genetic variation in the COMT allele could underlie variations in affective processing.*

Smolka et al. (2005)	*FMRI was used to assess the effect of COMT Val/Met genotype on brain activation elicited by unpleasant, pleasant and neutral affective visual stimuli in a cohort of 35 healthy subjects. The study found that brain activation in the limbic system in response to unpleasant visual stimuli was positively correlated with the number of COMT Met^{158} alleles. Furthermore, genotype was found to explain up to 38% of interindividual variance in the FMRI BOLD response elicited by aversive picture stimuli. The study concluded that the augmented activation in limbic and prefrontal regions in response to unpleasant stimuli seen in individuals with a greater number of COMT Met^{158} alleles might contribute to their observed tendency towards negative mood states*
Williams et al. (2010)	*This study examined the influence of COMT Val158Met on subjective ratings of negativity bias and brain activation in response to fearful and happy facial expressions, in a cohort of 46 healthy subjects. The study used an fMRI paradigm involving masked (preconscious) and unmasked (conscious) conditions. Williams et al. found that a greater number of Met alleles was associated with increased brain activation in the amygdala, medial prefrontal regions, brainstem and basal ganglia during the processing of conscious and preconscious facial expressions of fear. This genotypic difference in activation was found to be most apparent in female subjects. The study also found that a greater level of brain activation in Met-allele carriers was associated with greater self-reported negativity bias. Williams et al. concluded that COMT Val158Met-related modulation of brain activation and level of negativity bias could contribute to the emergence of affective disorders.*
Affective Startle Reflex Modulation (ASRM) and Prepulse Inhibition (PPI) Studies	
Comasco et al. (2012)	*This study investigated the effect of COMT Val158met genotype and estradiol levels on startle response towards pleasant and unpleasant images in 73 women who were 38 weeks pregnant. They found that Val homozygotes demonstrated higher startle magnitude than Val/Met individuals. They did not find evidence of affective modulation of the startle response. They concluded that there was a potential oestrogen dose-dependent influence of COMT Val158Met on the magnitude of startle reflex.*
Hermann et al. (2009)	*This study investigated whether COMT Val158Met genotype influences electrophysiological correlates of emotional processing using an early posterior negativity paradigm (EPN) in 8 subjects during the presentation of positively and negatively valenced picture stimuli. They found that Met homozygotes responded with greater sensitivity to aversive stimuli, compared to their Val/Val counterparts.*
Montag et al. (2008)	*96 healthy women, genotyped for COMT Val158Met, were asked to take part in an affective startle reflex modulation (ASRM) paradigm. The study revealed that participants' COMT genotype had a significant influence on their startle reflex modulation by aversive stimuli. Specifically, Met158 homozygotes exhibited a significantly greater startle reflex than their Val158 counterparts. The study concluded that their findings supported prior associations of*

	COMT Met158 with variation in the neural circuitry associated with emotional arousal and regulation and emergence of affective psychopathology.
Roussos et al. (2008)	*Prepulse Inhibition (PPI) was measured in 93 healthy males. The study found that PPI was influenced by COMT genotype. In particular, they identified the Val homozygotes had the lowest PPI and Met homozygotes the highest PPI. They did not however identify a significant difference in the baseline startle amplitude between the Val/Val and Met/Met group.*
Facial Affect Recognition Studies	
Defrancesco et al. (2010)	*88 female students were assessed for their COMT genotype prior to completion of two tasks: the Penn Emotion Acuity Test (PEAT) and the Penn Emotion Recognition Test (ER40). It was found that COMT genotype did not influence the subject's ability to differentiate different intensities of happiness or sadness on the PEAT. Furthermore, COMT genotype had no significant effect on performance in either the ER40 or the PEAT.*
Gohier et al. (2014)	*In their study of 275 healthy subjects, Gohier et al. studied performance in a facial recognition task, which involved identifying emotional facial expressions (fearful, angry, sad or happy) intermixed with neutral faces. They found the Met homozygotes showed a greater bias to perceive neutral facial expressions as being facial expressions of anger compared to Val homozygotes.*
Gong et al. (2013)	*The attentional bias for facial expressions was assessed using a spatial cueing task in 650 college students, genotyped for COMT Val158Met. The study found that Met carriers showed greater engagement bias for negative expressions than Val homozygotes. The study concluded that COMT Val158Met underpinned interindividual differences in attentional bias for negative emotional expressions.*
Weiss et al. (2007)	*This study investigated the relationship between COMT Val158Met genotype and facial emotion recognition and differentiation in 100 healthy individuals. They found that Val/Val carriers were better and faster than Met/Met carriers at recognising negative facial expressions, such as those of anger and sadness. The study concluded that the COMT polymorphism may influence emotion recognition abilities in healthy subjects.*

N.B. Studies listed alphabetically

Although there is a great deal of conflicting data, there remain fairly compelling grounds to believe that COMT Val158Met could influence emotional processing. Nevertheless, the vast majority of studies to date have studied fMRI BOLD signal as a correlate, rather than specifically examining performance data indicating the effects of COMT genotype on overt emotional processing behaviours. Further work in this area is necessary to verify the existence of a link between the polymorphism and

emotional processing in both health and disease and, should a relationship exist, to establish its nature and direction.

1.3 TOLCAPONE

1.3.1 TOLCAPONE IN CLINICAL PRACTICE

Tolcapone (chemical name 3,4-dihydroxy-4′-methyl-5-nitrobenzophenone) is a potent, brain penetrant inhibitor of the COMT enzyme (Dingemanse et al. 1994; Jorga 1998). Tolcapone was originally licensed in 1997, under the brand name Tasmar, and is most commonly prescribed as an adjunct to therapy with levodopa or carbidopa and a dopa decarboxylase inhibitor (DDI) for the treatment of symptomatic Parkinson's disease (PD) (Antonini et al. 2008; Lees et al. 2007). When delivered in conjunction with PD therapies such as L-Dopa, tolcapone prevents conversion of the drug into 3-O-methyldopa (3OMD) by COMT. This increases bioavailability of the active therapy, which can both prolong the dosing interval and prevent troublesome end of dose wearing-off effects, which affect a large number of patients (Jorga 1998; Lees et al. 2007).

Since introduction into clinical practice, the tolerability and safety of tolcapone has received considerable scrutiny (Jorga 1998). Shortly after being licensed, tolcapone was taken off the market in Europe amid concerns that it could cause serious hepatocellular damage. This was prompted after the drug was found to elevate liver transaminases in some patients and three cases of fulminant liver failure were linked to its administration (Playfer, 2008).

In the ensuing period, during which tolcapone was still licenced for use in the US and Canada, no further deaths were attributed to use of the drug. So, after a 7-year hiatus in use, it was decided that tolcapone should be reintroduced into medical practice in Europe with stringent monitoring of liver function in place to safeguard against further cases of liver injury. Use of the drug is now contraindicated in those with liver disease or evidence of impaired liver function. Since these measures have been in place no further cases of liver damage have occurred following tolcapone administration. A recent review of the safety of tolcapone over a 7-year period undertaken by Valeant Pharmaceuticals indicated that only 0.04% of patients suffered from liver injury resulting from use of the drug (Lees et al. 2007; Valeant Pharmaceuticals international 2015). In all cases, liver injury was not advanced enough to produce symptoms of liver disease and was only apparent from the elevated liver transaminases. The most

commonly recorded side effects of the drug are gastrointestinal, such as diarrhoea and vomiting (Antonini et al. 2008).

1.3.2 TOLCAPONE MODE OF ACTION: PHARMACODYNAMICS AND PHARMACOKINETICS

Tolcapone provides reversible inhibition of both peripheral and central COMT, preventing the conversion of dopamine into 3OMD (Jorga 1998). The drug's inhibition is highly specific to COMT, and does not affect any of the other enzymes involved in the catabolism of catecholamines (Tunbridge et al. 2006).

Following oral administration, it takes about an hour for tolcapone to be absorbed and reach peak plasma concentration. The drug is highly protein bound (>99%) with an absolute bioavailability of ≈60%, and an elimination half-life of 1.6 to 3.4hrs (Truong 2009). Erythrocytes have been used as a tool to estimate the extent of COMT inhibition; Studies have demonstrated that tolcapone decreases COMT activity in red blood cells in a dose-dependent manner and a dose of 200mg provides approximately 80% COMT inhibition between 1 and 4 hours (Dingemanse et al. 1994; Jorga 1998; Mannisto & Kaakkola 1999). Following such inhibition, it takes around 18 hours for erythrocyte COMT action to return to baseline levels (Truong 2009).

1.3.3 USING TOLCAPONE TO PROBE COGNITIVE FUNCTION

As mentioned previously, dopamine transporters are relatively scarce in the PFC, making COMT the principal mechanism of dopamine removal from the synaptic cleft (Sesack et al. 1998). COMT inhibition using tolcapone provides a relatively specific means of modulating cortical dopamine signalling, without having a significant impact on levels of noradrenaline (Tunbridge et al. 2004). Another advantage of tolcapone is that it does not particularly alter extracellular dopamine levels in other regions of the brain such as the striatum (Li et al. 1998; Maj et al. 1990), where there is a far higher density of DAT transporters. This means that the drug lacks abuse potential and psychostimulant effects. These properties make tolcapone a valuable research tool to investigate dopamine signalling in the PFC and it has been used in a number of studies to date.

Preclinical studies have demonstrated that a tolcapone-mediated reduction in COMT activity can lead to improvements in cognitive function. In rat models, the drug has been shown to improve performance in a delayed spatial win-shift task (Lapish et al. 2009), augment dopamine release in response to behaviourally salient stimuli (food) (Lapish et al. 2009), improve extradimensional set shifting,

dependent on the medial prefrontal cortex (Troudet et al. 2015; Tunbridge et al. 2004), and enhance spatial working memory in a radial maze task (Liljequist et al. 1997). Tolcapone has also demonstrated potential to enhance cognitive function in humans (Apud et al. 2007; Farrell et al. 2012; Gasparini et al. 1997; Giakoumaki et al. 2008a; Roussos et al. 2009), although, as noted above, there is evidence to suggest that this enhancement may be genotype dependent (Farrell et al. 2012; Giakoumaki et al. 2008b).

1.3.4 TOLCAPONE AND EMOTIONAL PROCESSING

In addition to its cognitive effects, there is also limited evidence to suggest that tolcapone may be able to modulate emotional processing (Farrell et al. 2012; Fava et al. 1999; Moreau et al. 1994). In their 1994 study, Moreau et al. demonstrated that tolcapone administration was able to completely negate the effects of chronic mild stress (CMS)-induced anhedonia, a rat model of depression (Moreau et al. 1994). Furthermore, in a pilot study of individuals with major depressive disorder, tolcapone was found to have a significant antidepressant effect (Fava et al. 1999).

1.4 OUR STUDY

1.4.1 AIMS AND OBJECTIVES

Despite accumulating data suggesting that COMT Val^{158}Met genotype exerts an influence on the processing of emotional stimuli (Drabant et al. 2006; Smolka et al. 2005), a great deal of uncertainty surrounds the existence and nature of such a relationship. The majority of studies to date have examined fMRI BOLD brain activation data, whilst concrete performance data linking COMT Val158Met genotype to emotional processing is scarce. Furthermore, in a number of cases, attempts to replicate findings have failed or even yielded contradictory results (Domschke et al. 2004; Kawachi et al. 2004). For this reason it would be helpful to further examine the link between COMT Val^{158}Met genotype and emotional processing. This will in turn inform the plausibility of associations between Val158Met genotype and a range of psychiatric phenotypes.

The aim of this thesis is to examine the hypothesis that the COMT activity influences affective processing. I have tested this hypothesis in two ways; firstly, by studying whether genetic variation in COMT activity resulting from the Val158met polymorphism influences emotional processing and secondly, by investigating the effects of acute COMT inhibition on the processing of affective stimuli.

In order to do this, I have performed a double-blind, randomised and controlled study measuring performance in a range of emotional processing paradigms, in subjects homozygous for either the Val158 or Met158 allele, randomly assigned to receive either tolcapone or placebo. As well as separately examining whether genotypic variation in COMT activity or acute COMT inhibition influence indices of emotional processing, this study design has also allowed me to investigate whether the two interact.

1.4.2 CHOICE OF METHODOLOGY

I have studied the effect of the COMT Val^{158}Met polymorphism and a 200mg dose of COMT inhibitor tolcapone in a cohort of healthy men, with no psychiatric illness. Our sample size, and basic experimental design, was based on that used by Farrell et al. in their 2012 paper (Farrell et al. 2012).

1.4.2.1 THE EMOTIONAL TEST BATTERY

In order to detect genotypic and tolcapone-mediated variations in attentional bias during the processing of affective stimuli, I used a range of emotional processing paradigms from the Oxford P1Vital Emotional Test Battery (ETB). The ETB is a well-established tool that has been used in numerous studies of antidepressant efficacy. It has been a popular choice as it allows the detection of early changes in cognitive biases that occur immediately after antidepressant administration, well before an individual notices any subjective change in their mood. The ETB should therefore be able to detect baseline genotypic differences in emotional processing bias, as well as any acute alterations in emotional processing that arise from the administration of tolcapone. To our knowledge this is the first time that the ETB has been used to systematically study the effect of COMT genotype on emotional task performance (rather than its neural correlates). Based on the evidence from previous facial recognition tasks (Gohier et al., 2014; Gong et al., 2013) and associations between the COMT Met allele and disorders of affective regulation and anxiety (Mynett-Johnson et al. 1998; Ohara, Nagai, Suzuki & Ochiai, 1998; Woo et al. 2002), I predict that individuals homozygous for the Met COMT allele will demonstrate greater negativity bias in the processing of emotionally salient stimuli than their Val COMT counterparts. Furthermore, I predict that tolcapone administration may interact with COMT genotype, in accordance with an inverted U-shaped curve, as with cognitive functions, making Met carriers perform worse and Val carriers better at emotional processing tasks.

1.4.2.2 STARTLE

In order to study emotional reactivity, I used an ASRM paradigm similar to that used in the Montag et al. study described previously. Although the Montag study found greater startle potentiation in response to aversive stimuli in female Met158 carriers, there have been conflicting results, notably in studies involving male cohorts. In their study, Roussos et al. found no significant genotypic difference in baseline startle amplitude in their sample of 93 healthy males (Roussos et al. 2008). I am seeking to clarify the relationship between COMT Val158Met genotype and magnitude of startle response, and assess whether startle magnitude is modulated by Tolcapone administration. Based on the findings of Montag et al. (Montag et al. 2008) and associations between the COMT Met158 allele and emergence of anxiety disorders (Enoch et al. 2003; Olsson et al. 2005; Woo et al. 2002), I predict that individuals homozygous for the Met158 allele will demonstrate greater startle potentiation in response to aversive picture stimuli.

2. MATERIALS AND METHODS

2.1 STUDY DESIGN

We performed a double-blind, randomised, placebo-controlled, experimental medicine study looking at the effects of COMT Val^{158}Met genotype and a single dose of orally-administered tolcapone on tests of emotional processing (described below) and reward processing (which will be discussed further elsewhere). The single-centre study received ethical approval from the South Central Berkshire Research Ethics Committee (Ref: 14SC0035).

2.2 POWER CALCULATIONS AND PARTICIPANTS

As no directly comparable studies have been performed to date, formal power calculations for this study are not available. However, in this research group's previous study a group size of 15 proved adequate to detect main effects and interactions with 95% power at the P=0.05 level of significance (Farrell et al. 2012). Hence, for my study, a group target of 15 has been chosen, based on this.

In total, sixty-three healthy males aged between 18 and 40 were recruited for the study. Recruitment took place over the period of a year and a half and was carried out by Dr Jessica Scaife and myself. Individuals were recruited via advertisements placed in a local newssheet and on Facebook, posters in colleges and emails circulated via college JCR and MCR mailing lists.

2.3 GENOTYPING THE VAL^{158}MET POLYMORPHISM

2.3.1 BUCCAL SWABBING

In order to provide DNA samples, men interested in participating were sent buccal swab kits. The kits consisted of two 2ml Eppendorf tubes (Sarstedt, UK) each containing a buccal swab brush (MasterAmp Buccal Brush, Epicentre) and clear instructions explaining how to collect the buccal cell samples (see Appendix). Participants were asked to slowly and firmly brush the inner surface of their cheek from top to bottom with the swab 15 times. Following collection of the sample, participants were instructed to return the buccal brush directly to the Eppendorf tube. The two samples were then placed into a labelled bag and sent back to the Department of Psychiatry where, upon arrival, they were stored in a freezer at -20°C until DNA extraction.

2.3.2 EXTRACTION OF GENOMIC DNA FROM BUCCAL SWABS

ChargeSwitch®gDNA Buccal Cell Kits were used to isolate genomic DNA from the samples according to the manufacturer's instructions (Invitrogen, Waltham, USA) by research technician, Li Chen. Briefly, ChargeSwitch® Lysis Buffer was mixed with Proteinase K to prepare a Lysis mix that was incubated with each sample at 37°C for 20 minutes. The digested supernatant was transferred to a new tube and a Purification Mix was added, consisting of ChargeSwitch® magnetic beads and ChargeSwitch® purification buffer. This was incubated at room temperature for 1 minute in order to allow the DNA to bind to the ChargeSwitch® magnetic beads. The sample was placed into a MagnaRack™ (Thermofisher, MA, USA) for approximately 1 minute, to allow the beads to clump together, forming a tight pellet. Next, the supernatant was then discarded, leaving a pellet, and the DNA was washed using ChargeSwitch® Wash Buffer. The beads were briefly resuspended in solution with the washing buffer and once a pellet of beads had reformed, the supernatant was removed and discarded as before. The washing process was repeated and then the sample was removed from the MagnaRack™.

Next, the DNA was eluted by pipetting up and down with ChargeSwitch® Elution buffer and incubating for a minute. The sample was returned to the MagnaRack™ and, once a pellet of the magnetic beads had formed, the supernatant containing extracted DNA was transferred to a sterile microcentrifuge tube. The purified DNA sample was returned to a freezer for storage at -20°C.

2.3.3 RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) GENOTYPING ASSAY

REACTION CONDITIONS

PCR was carried out using PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Amersham, England) according to the manufacturer's instructions. 7.5µl genomic DNA was added to each well along with 1.25µl of each primer (Forward: 5' ACT GTG GCT ACT CAG CTG TG 3'; Reverse: 5' CCT TTT TCC AGG TCT GAC AA 3') and 15µl molecular biology grade water, making a total reaction volume of 25µl. Tubes were vortexed gently and then centrifuged before being placed in the thermal cycler (Veriti® Thermal Cycler, Applied Biosystems, USA). PCR reactions consisted of 35 cycles, each of which involved three stages: denaturing (94°C for 35 seconds) and annealing (53°C for 30 seconds) followed by extension (72°C for 60 seconds).

ENZYMATIC DIGESTION

The PCR product was digested by adding 1µl Hsp92II restriction enzyme (Promega) with 0.35µl acetylated bovine serum antigen (BSA; Promega), 0.15µl molecular grade water and 3.5µl of the recommended buffer

(also Promega) to the PCR product. Samples were left to digest at 37°C for 12 hours before being separated by agarose gel electrophoresis.

AGAROSE GEL ELECTROPHORESIS

I prepared agarose gels by boiling 3% w/v Agarose in 1x Tris-acetate-EDTA (TAE) buffer containing 0.1 µg/ml ethidium bromide. Samples were prepared by adding gel loading dye (purple, no SDS; New England Biolabs) and then loaded into the gel alongside a DNA ladder (100bp ladder, New England Biolabs). Bands were visualised and photographed under ultraviolet light, using the AlphaImager 3400 system (Protein Simple, California, USA).

Digestion of the Val^{158} allele with Hsp92II produces an 114bp fragment in addition to 27 and 28bp fragments (which can't be detected using this particular assay). On the other hand, as the Met^{158} allele has a different restriction site, digestion of this allele produces a shorter 96bp fragment, along with smaller 18, 27 and 28bp fragments (that are, again, undetectable). The presence of these 96bp and 114bp bands on the agarose gel allows identification of the individual's genotype. All samples were run in duplicate.

2.4 SCREENING FOR PSYCHIATRIC CONDITIONS

As we were performing a study in healthy individuals, men identified as being homozygous for the COMT Val or Met polymorphism were invited to do a 15-minute telephone screening interview in order to exclude those with a history of psychiatric or neurological disorders and those taking psychotropic medications.

The interview primarily consisted of questions from an approved mental health screening questionnaire (Structured Clinical Interview for DSM-IV Axis 1 Disorders). This was so that individuals who met the criteria for any current or past axis 1 disorder could be identified. In addition, individuals were asked about nicotine usage and any smoking habits, as participants were required to be non-smokers. All participants denied the use of illicit substances. Due to the small risk of hepatotoxicity accompanying tolcapone use, participants were also questioned about their alcohol intake (those with intake >30 units/week were excluded) and whether they had any history of liver disease.

If an individual did not meet any of the exclusion criteria, I obtained permission to contact their GP. GPs were asked to check the participant's medical records to provide confirmation that they did not

meet any of the exclusion criteria and that there were no medical reasons why it would be unsafe for them to take part in the study. From the initial group of 420 males, aged between 18-40, that were genotyped and screened, we identified 63 eligible participants who formed two groups based on their COMT genotype (Met/Met group, n=28;Val/Val group, n=35).

2.5 RANDOMISATION AND BLINDING

Within each genotype group, subjects were randomised to receive either 200mg tolcapone or placebo sugar pills. Matching capsules were prepared and, as the study was double blind, both the subject and I were blind to both genotype and drug allocation.

2.6 NEUROPSYCHOLOGICAL TESTING

All participants were invited to come in for testing in the morning at 9.15am. Testing was performed by myself or Dr Jessica Scaife, prior to me joining the research group in June 2015. Upon arrival at the Psychiatry department, we reminded participants of the study details and their right to withdraw before asking them to reconfirm their consent. Following this, participants were asked to complete the National Adult Reading Test (NART), as a measure of verbal IQ, along with the State-Trait Anxiety Inventory (STAI) (Spielberger et al. 1983). The STAI includes 20 items, such as 'I am worried' and 'I feel calm', to assess state anxiety (STAI-S), a transitory condition associated with subjective feelings of apprehension or tension, generally accompanied by intensified activity of the autonomic nervous system. A further 20 items, such as 'I worry too much over something that doesn't really matter' and 'I am a steady person', are used to measure trait anxiety (STAI-T), which can be defined as the enduring tendency of an individual to respond to perceived threats in the environment with anxiety (Spielberger et al. 1983). Each item is rated on a four-point scale, with higher scores indicating greater anxiety. After participants had completed these initial paper measures, they were given tolcapone tablets (200mg by mouth) or matching placebo capsules, prepared by a pharmacy. Tolcapone is rapidly absorbed after oral administration, producing 70-80% peripheral blood COMT inhibition between 1 and 4 hours at the dose given (Dingemanse et al., 1994). For this reason, we started to test 90 minutes after tablet administration and all testing was completed within 4 hours of tablet administration.

2.6.1 SUBJECTIVE RATING SCALES

Participants were asked to rate their alertness, drowsiness, anxiety, sadness, happiness and nausea using Visual Analogue Scales (VAS). A VAS consists of a straight line on which, at either end, the extreme boundaries of a sensation or feeling are labelled (see appendix). In our case, the scale was from 0, sensation completely absent, to 10, representing the sensation present at the greatest intensity imaginable. Participants were asked to respond by drawing a mark through the line at the point that most accurately reflected their experience of the sensation in question at that moment.

Alongside the VAS, participants were also asked to complete Profile of Mood States (POMS) questionnaires (McNair and Lorr, 1971). The POMS questionnaire is made up of 65 items, rated on a 5-point scale (see appendix). Each item is a different adjective that can be used to describe a feeling or mood (for example tense, energetic and relaxed). Participants were told to rate each item according to their feelings at that moment using a scale from 1 to 5 as follows; 1 – not at all, 2 – a little, 3 – moderately, 4 – quite a bit, 5 - extremely. The questionnaire produces ratings on 6 mood sub-scales (anger, confusion, depression, fatigue, tension and vigour), which combine to give a total mood disturbance score (TMD).

VAS and POMS psychometric rating scales were given to participants at two time points during the test day; upon arrival in the psychiatry department and then at 4 hours following tablet administration. The change in POMS and VAS scores was calculated by subtracting the baseline score from the score taken 4 hours following administration of tolcapone or placebo.

2.6.2 TESTS OF REWARD PROCESSING

Prior to completion of the P1Vital Emotional test battery, described below, participants were asked to complete three reward processing tasks, which will be written up fully elsewhere. Briefly, in the first task, described in full by Behrens et al., participants played a game in which they were asked to choose between blue and green rectangles, which were each associated with a different number of points (between 0 and 100). In each trial, participants had to choose a coloured rectangle, based on successes in previous trials and the rectangles' point values. If they chose the correct rectangle, they were rewarded with the number of points associated with it, and the aim of the game was to accumulate points in order to reach a specific target (Behrens et al, 2007). In the second task, described in full by elsewhere, participants had to make a series of choices between a certain win or loss of money and a 50% probability of winning or losing an amount of money.

Finally, participants were asked to complete a monetary decision making task in which they had to decide whether to gamble a high or low amount. Specifically, participants were given £10 to gamble with and asked to choose between making 5 pence and 25 pence gambles by selecting one of two grey boxes labelled with either "5"or "25" (Gehring and Willoughby, 2002). In total, these three tasks took 1 hour to complete, after which my participants performed the ETB and emotion potentiated startle paradigms.

2.6.3 EMOTIONAL TEST BATTERY

2.6.3.1 FACIAL EXPRESSION RECOGNITION TASK (FERT)

Subjects were asked to look at facial stimuli presented on a computer screen and identify their emotional expression. Facial stimuli were taken from the Pictures of Affect series (Ekman and Friesen, 1976) and featured one of 6 basic emotions (happiness, sadness, surprise, anger, fear or neutral). Faces had been morphed between a 100%, full emotion image and a 0%, neutral image in 10% steps, giving a total of 10 different intensities for each face. Four examples of each emotion were given at each intensity using the faces of 10 different individuals, meaning that a total of 250 facial stimuli were presented to the participant. Each face was shown for 500ms and faces were presented on the computer screen in a random order, interspersed with blank screens.

Participants were told to respond to each face as quickly and as accurately as they could, by pressing a correspondingly labelled key on the keyboard. The accuracy of their choices, number of faces misclassified and reaction times were measured for this task.

2.6.3.2 EMOTIONAL CATEGORISATION TASK (ECAT) AND EMOTIONAL RECALL TASK (ERT)

Participants were shown personality characteristic words and asked to respond by indicating whether they would 'like' or 'dislike' to be described with each characteristic. The task consisted of 60 characteristic words which were matched for length and selected to be extremely likeable (e.g. cheerful, neat, generous) or dislikeable (e.g. pompous, rude, selfish). Each word was shown on the screen for 500ms and participants were requested to respond as quickly and accurately as possible by pressing either the 'like' or 'dislike' button on a labelled keyboard. Their classifications and the reaction times for words correctly classified were measured.

Approximately 15 minutes after the completion of the emotional categorisation task, participants were asked to recall as many of the personality characteristic words from ECAT as possible. They were

given a two-minute time window in which to write down all the words they could remember. The number of correct and incorrect positive and negative words recalled was recorded.

2.6.3.3 FACES DOT-PROBE TASK

For each trial, participants were asked to fix their gaze on a cross shown in the centre of the screen. After a short time, the fixation cross disappeared and was replaced by a pair of faces presented vertically at the top and the bottom of the screen. Soon after, these faces disappear and were themselves replaced by the cue (a pair of dots) appearing in either the top or the bottom half of the screen. Participants were asked to respond by indicating whether the dots were horizontally (. .) or vertically (:) aligned by pressing the appropriate keys on a labelled keyboard as quickly and accurately as possible.

Pairs of photographs of individuals with different expressions were obtained from the Pictures of Affect series (Ekman and Friesen, 1976). The faces were shown in one of three different pairings:

- ***NEUTRAL-NEUTRAL*** – two neutral faces
- ***FEARFUL-NEUTRAL*** – one fearful face and one neutral face
- ***HAPPY-NEUTRAL*** – one happy face and one neutral face

Trials were either congruent, where the probe appeared in the same location as emotional face, or incongruent, where the probe appears in the opposite position to the emotional face. Furthermore, emotional faces (i.e. fearful or happy) occupied the top of the screen and bottom of the screen with equal frequency. There were also two different stimulus presentation intervals: Short and Long. The short presentation interval, in which faces were presented very briefly for 100ms and then replaced by a jumbled face mask, is used to measure subliminal vigilance to the stimulus in question. The long presentation interval, in which faces were presented for 1000ms, measures supraliminal vigilance.

The rationale behind the task is that participants will be faster to respond to a probe if shown in the same location as the face to which they were attending. For example, if the participant was attending to a fearful face in the bottom half of the screen, they would be relatively faster at responding to a dot-probe also shown in the bottom half of the screen than to one shown in the top half of the screen. During this task, reaction time is measured and used to assess attention to positive versus negative stimuli. Attentional vigilance scores can be calculated by subtracting the mean reaction time from congruent trials

with that from incongruent trials. Positive scores indicate attentional vigilance towards emotional stimuli, whilst negative scores suggest an attentional bias away from emotional stimuli.

2.6.4 EMOTION POTENTIATED STARTLE

Participants were asked sit in front of a computer screen and passively view a presentation of images, whilst bursts of white noise were delivered periodically via headphones. Picture stimuli were obtained from the International Affective Picture System (IAPS) and chosen to elicit positive, negative or neutral emotions (Lang, Bradley and Cuthbert, 1997). Each image was presented for a period of 13 seconds, with an inter-trial interval of between 11 and 15 seconds. Images were presented in three blocks, each of which lasted for approximately 15 minutes and contained 21 images, 7 of each category, interspersed with blank screens in a fixed, random order.

As they were watching the presentation of picture stimuli, participants had the magnitude of their eye blink startle reflex in response to acoustic probes measured using electromyography (EMG) (EMG Startle Response System, San Diego Instruments, INC, USA). Firstly, the skin around the eye was prepared by wiping with a gauze pad soaked in alcohol to remove oil and dead skin that could impede the flow of current. Following this, electrodes coated in electrode gel were attached to the surface of the skin using adhesive collars. In total, three small Ag/AgCl electrodes were placed around the subject's right eye, overlying the Orbicularis Oculi muscles in order to measure action potentials generated within the muscle (see figure 2.1).

95dB bursts of white noise, delivered binaurally using headphones, were used as acoustic probes. Each probe had an almost instantaneous rise time and lasted for 50ms. They were delivered at 1.5, 4.5 or 7.5s after presentation of the picture stimuli. In order to reduce predictability or expectation of the probe, two trials of each valence were not accompanied by a startle probe. This meant that out of the 21 images in each block, only 15 contained a startle probe (5 of each picture category). On the same grounds, a further three acoustic probes were delivered during the inter-trial interval in each block.

Prior to beginning the presentation, participants viewed a shorter introductory presentation, consisting of nine neutral images accompanied by nine startle probes. This was in order to familiarize participants with the startle procedure and habituate them to the acoustic probes used.

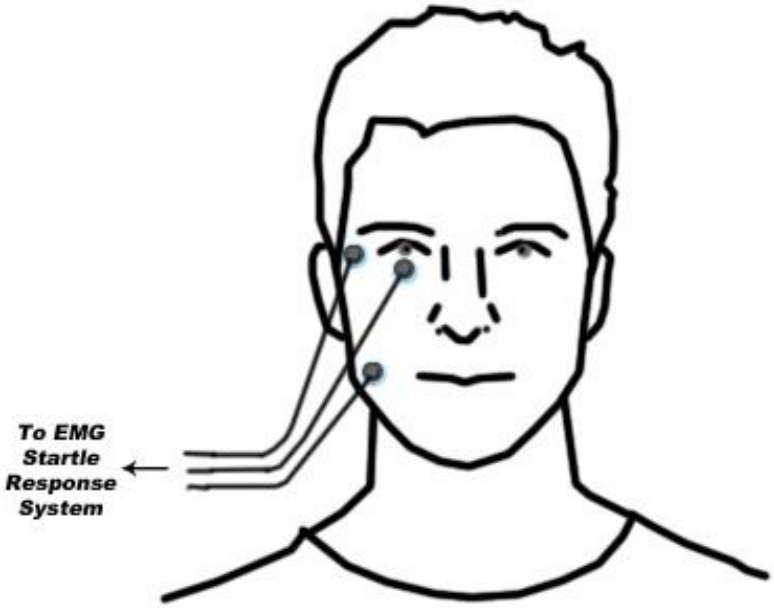


Figure 2.1: *Diagram showing the placement of EMG electrodes overlying the Orbicularis Oculi muscles used in my study. One electrode is placed below the lower eyelid, in line with the pupil in forward gaze. In the same manner, a second electrode is placed at the corner of the subject's eye. The final electrode, acting as a signal ground electrode, is placed on the mastoid, as this is considered to be an electrically inactive site (Blumenthal et al., 2005)*

I analysed the startle data with Dr Jessica Scaife, using validated rules (Blumenthal et al. 2005; Grillon & Baas, 2003) to identify Vmax, calculate startle amplitude and exclude data in which there was too much noise on the EMG trace, in order to minimise subjectivity. Specifically, data were pre-processed using the following scoring parameters; baseline: 20ms, analysis start: 0ms, end analysis: 80ms, response window: 8, rolling average: 2, filter order: 0, pass frequency: 11, response criterion: 2. Startle latency was measured as the time elapsed before the first point on the EMG trace that was greater than three times the mean of the baseline EMG trace (Grillon and Davis, 1995) and the response peak, used to calculate startle amplitude, was defined as the maximum value on the EMG trace within the our specified time window of 0-80ms (Grillon and Davis, 1995). Where multiple peaks occurred, the maximum value was recorded as the response peak, unless the EMG trace had returned to baseline for long enough that the later peak was clearly not a component of the stimulus-elicited response. Trials were excluded if the baseline was greater than one third of the maximum peak (Vmax) or if the amount of noise made it difficult to detect a response peak.

2.7 STATISTICAL ANALYSIS

Data were analysed using IBM SPSS 24 for Windows software (USA). Subjective rating scale scores were analysed with repeated measures analysis of variance (ANOVA) with COMT genotype (Met/Met v. Val/Val) and drug (placebo v. tolcapone) as the between-subjects factors.

Emotional processing paradigms (FERT, ECAT, EREC, Dot-probe and were analysed, with guidance from Professor Catherine Harmer and Dr Liliana Capitao, using split-plot analysis of variance (ANOVA) with COMT genotype (Met/Met *v.* Val/Val) and drug (placebo *v.* tolcapone) as the between-subjects factors and emotion/valence as the within-subjects factor. For the faces dot-probe task, duration of stimulus presentation (short *v.* long) was added as a further within-subjects factor.

As this was an experimental medicine study, a *p* value below 0.05 was taken to denote statistical significance and marginal differences with a p value below 0.10 have also been reported.

3. RESULTS

3.1 SAMPLE DEMOGRAPHICS

In total, 63 healthy male Val^{158}Met homozygotes completed the study, of whom 28 were Met158 homozygotes and 35 were Val158 homozygotes. The groups were well matched, with no significant differences in terms of age, IQ, trait anxiety or level of education (see Table 3.1). Subjects were aged between 18 and 35 and IQ, estimated using the National Adult Reading Test (NART), ranged between 99 and 126, with the mean IQ of the cohort being 115 (±6). The overwhelming majority of subjects were not able to correctly identify whether they had been given tolcapone or placebo (38 of 65 guessed incorrectly), and only 6 participants of the 27 who guessed correctly thought that they were given tolcapone, indicating that the blind was maintained.

Table 3.1: *Baseline Characteristics*

	Met-COMT Placebo	Val-COMT Placebo	Met-COMT Tolcapone	Val-COMT Tolcapone	Total	One-way ANOVA (p)
Number	14	18	14	17	63	...
Ethnicity	13C, 1EA,	15C, 2SA, 1EA	14C	14C, 1EA, 1H	...	...
Handedness	12R, 2L	17R, 1L	12R, 2L	16R, 1L	57R, 6L	...
Age (Years)	23.8 (4.5)	24.6 (5.3)	24.8 (4.7)	23.4 (5.6)	24.1 (5.0)	.86
Years in Education	17.9 (2.1)	17.2 (2.5)	17.3 (3.2)	16.3 (2.5)	17 (2.6)	.51
IQ: NART	118 (5)	116 (6)	114 (6)	113 (7)	115 (6)	.20
Trait Anxiety: STAI-T	30 (7)	31 (7)	33 (8)	34 (7)	32 (7)	.36

Data are given as mean (standard deviation) unless indicated. COMT, Catechol-O-methyltransferase; ANOVA, Analysis of Variance; NART, National Adult Reading Test; STAI-T, Spielberg State Trait Anxiety Inventory – Trait; C, Caucasian; EA, East Asian; SA, South Asian; H, Hispanic

The ETB data of one subject were removed from the dataset in the Met-COMT tolcapone group as he was not ETB naïve. A second subject's data from the Val-COMT tolcapone group could not be included in the facial expression recognition task due to a technical error. This meant that a total of 61 participants completed the FERT, with final sample sizes as follows: 14 Met-COMT placebo, 18 Val-COMT placebo, 13

Met-COMT tolcapone and 16 Val-COMT tolcapone. Demographics for those who completed the FERT, and the rest of the ETB were virtually identical to those shown above for the entire cohort.

3.2 SUBJECTIVE STATE

Initial subjective state measures, prior to the administration of placebo or tolcapone, including the total mood disturbance and the 6 composite factors of the POMS scale, state anxiety (STAI-S) and visual analogue scale ratings of happiness, sadness, alertness, drowsiness, anxiety and nausea, were uninfluenced by COMT (all F's <1.00; Table 3.2). Although there was no baseline difference between the groups in POMS total mood disturbance $F(3, 58)=2.63$, $P=.06$), the composite measure of scores from each of the six POMS sub-scales (anger-hostility, confusion-bewilderment, depression-dejection, fatigue-inertia, tension-anxiety and vigour-activity), I went on to perform exploratory analysis to see whether there were any baseline differences in any of the POMS sub scales. This analysis identified a genotype-independent baseline difference in the POMS subjective ratings of tension-anxiety, which was higher in both groups that would go on to receive the dose of tolcapone ($F(3, 58)=4.34$, $P=.01$).

I also analysed the change in POMS scores between baseline and 4 hours. As I had no a priori hypothesis regarding the effect of genotype or tolcapone on specific POMS sub-scales, I began by looking to see whether there was any effect on total mood disturbance, the composite measure. I identified no main effects of drug ($F(1, 57)=.98$, $P=.33$) or genotype ($F(1, 57)=.10$, $P=.75$) nor any interactive effects of the two ($F(1, 57)=.002$, $P=.97$). I then went on to perform an exploratory analysis to see if there was an effect of genotype or drug on any of the POMS sub scales. There was no effect of drug or genotype, nor any interactive effects of the two on change in POMS scores at 4 hours (F's<2.64, P's>.11). In addition, there was no effect of drug x POMS factor, genotype x POMS factor or genotype x drug x POMS factor (F's<.59, P's>.64).

There was no significant main effect of drug or genotype, nor an interaction between them, on VAS subjective ratings of mood at 4 hours post drug administration (all F's<1.00, all P's>.11)(see Table 3.2).

Table 3.2: *POMS and VAS Subjective State Changes.*

	Met COMT Placebo	Val COMT Placebo	Met COMT Tolcapone	Val COMT Tolcapone	One-way ANOVA (p)
POMS					

Anger-hostility					
Baseline	1.29 (1.44)	2.44 (3.33)	2.85 (4.74)	2.12 (2.33)	*.60*
POMS change	-.36 (2.41)	-.06 (2.92)	.36 (3.73)	.35 (2.21)	*.88*
Confusion-bewilderment					
Baseline	3.71 (1.68)	3.61 (2.20)	5.15 (3.02)	5.35 (2.67)	*.09*
POMS change	.57 (1.79)	.61 (2.25)	1.79 (3.64)	1.35 (3.32)	*.58*
Depression-dejection					
Baseline	0.71 (0.99)	1.83 (2.83)	2.85 (4.45)	3.71 (5.63)	*.19*
POMS change	.07 (1.64)	-.33 (2.93)	2.00 (4.85)	.06 (3.29)	*.24*
Fatigue-inertia					
Baseline	3.93 (3.32)	3.72 (3.10)	4.77 (3.94)	5.88 (5.44)	*.41*
POMS change	.93 (2.87)	1.00 (3.79)	2.79 (5.37)	.824 (3.86)	*.51*
Tension-anxiety					
Baseline	2.50 (2.53)	2.17 (2.07)	5.62 (4.86)	4.71 (2.82)	*.01*
POMS change	1.14 (2.71)	1.67 (3.18)	1.21 (4.14)	.41 (4.43)	*.79*
Vigour-activity					
Baseline	18.46 (4.50)	17.33 (5.71)	16.54 (4.43)	15.24 (3.83)	*.31*
POMS change	-4.36 (3.79)	-2.44 (3.84)	-2.21 (4.44)	-3.88 (4.03)	*.38*
Total Mood Disturbance (TMD)					
Baseline	-6.21 (7.40)	-3.56 (12.25)	4.85 (21.55)	6.53 (16.44)	*.06*
POMS change	6.71 (7.57)	5.33 (11.34)	10.21 (18.45)	6.88 (13.72)	*.78*
VAS					
Alertness					
Baseline	63.43 (13.90)	68.00 (19.06)	63.50 (17.50)	60.24 (18.2)	*.67*
4 Hours	61.21 (16.34)	61.06 (18.45)	54.36 (21.58)	52.76 (20.09)	*.47*
Drowsiness					
Baseline	32.29 (17.06)	28.94 (21.80)	16.71 (10.60)	29.29 (27.06)	*.20*
4 Hours	32.86 (24.18)	24.89 (15.38)	37.64 (23.01)	31.71 (27.21)	*.46*
Anxiety					
Baseline	11.86 (11.16)	15.83 (17.80)	18.64 (19.32)	10.18 (8.47)	*.39*
4 Hours	6.36 (10.14)	12.22 (19.15)	14.43 (17.03)	8.06 (12.11)	*.46*
Happiness					
Baseline	68.57 (13.29)	65.67 (21.13)	68.50 (16.00)	64.00 (17.67)	*.85*
4 Hours	66.07 (17.80)	63.28 (15.13)	62.50 (24.62)	62.12 (26.67)	*.96*
Nausea					
Baseline	3.43 (8.83)	3.00 (3.77)	3.86 (5.39)	5.24 (9.20)	*.81*
4 Hours	1.21 (4.00)	7.83 (12.59)	5.79 (8.34)	3.94 (6.90)	*.20*
Sadness					
Baseline	3.43 (5.02)	6.39 (8.60)	10.14 (10.60)	13.71 (19.16)	*.11*

4 Hours	5.79 (7.80)	11.39 (19.34)	14.29 (13.61)	12.47 (17.19)	*.52*

Data are given as mean (standard deviation) unless indicated. POMS, Profile of Mood States; VAS, Visual Analogue Scales. POMS change = baseline POMS rating – 4 hour POMS rating.

3.3 FACIAL EXPRESSION RECOGNITION

Accuracy: As we did not have any particular a priori hypotheses regarding the effect of COMT genotype or tolcapone on recognition of particular facial expressions, I first performed an analysis to see if there were any overall effects on accuracy at identifying facial expressions, using baseline POMS tension-anxiety as a covariate. I found no significant main effect of drug or genotype, nor an interaction of the two on overall accuracy at identifying facial expressions of any emotion (F's<.69, P's>.41). Next, I analysed to see if there were effects of drug or genotype on accuracy at identifying grouped positive and negative expressions, using POMS tension-anxiety as a covariate (figure 3.1). I found a significant effect of valence whereby individuals across all groups were more accurate at identifying positive (happy or surprised) than negative (fearful, disgusted, sad or angry) facial expressions ($F(1, 55)=59.30$, $P=2.69x10^{-10}$). However, there were no significant main effects of drug or genotype on accuracy of identifying positive versus negative expressions, nor were there any interactions involving drug or genotype (F's<.36, P's>.55). There were no interactive effects of valence with genotype, drug and genotype x drug (F's<2.52, P's>.12).

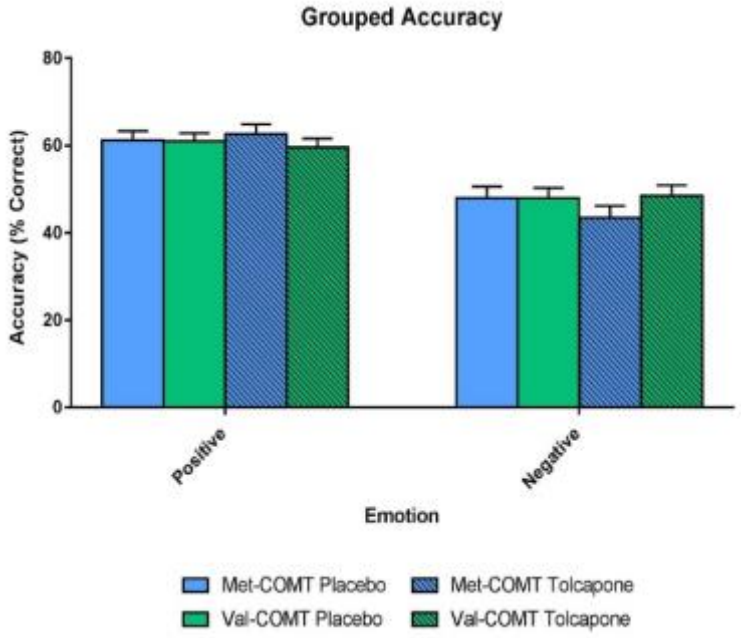


Figure 3.1: *Effect of COMT genotype and tolcapone administration on the accuracy of recognition of grouped positive (happy and surprise) and negative (fear, disgust, sadness and anger) facial expressions. Values are adjusted means, with vertical bands representing standard error values. N=61*

I performed an exploratory, ungrouped analysis to see if there were any effects of COMT genotype or tolcapone on accuracy at identifying specific facial expressions of anger, disgust, fear, happiness, sadness, surprise and neutral expressions, using baseline POMS tension-anxiety as a covariate. There was a significant main effect of emotion ($F(6, 330)=13.41$, $P=7.59 \times 10^{-11}$) driven by more accurate recognition of happy and neutral facial expressions across the groups. However, the analysis identified no significant main effect of drug ($F(1,55)=.84$, $P=.36$) or genotype ($F(1,55)=1.16$, $P=.29$) on accuracy at identifying facial expressions (see Figure 3.1), nor an interaction between them ($F(1, 55)=.92$, $P=.34$). Furthermore, there were no interactive effects of emotion with genotype, drug and genotype x drug ($F's<1.21$, $P's>.31$). Baseline POMS tension-anxiety was not a significant covariate in any of my analyses of FERT accuracy ($F's<1.27$, $P's>.29$).

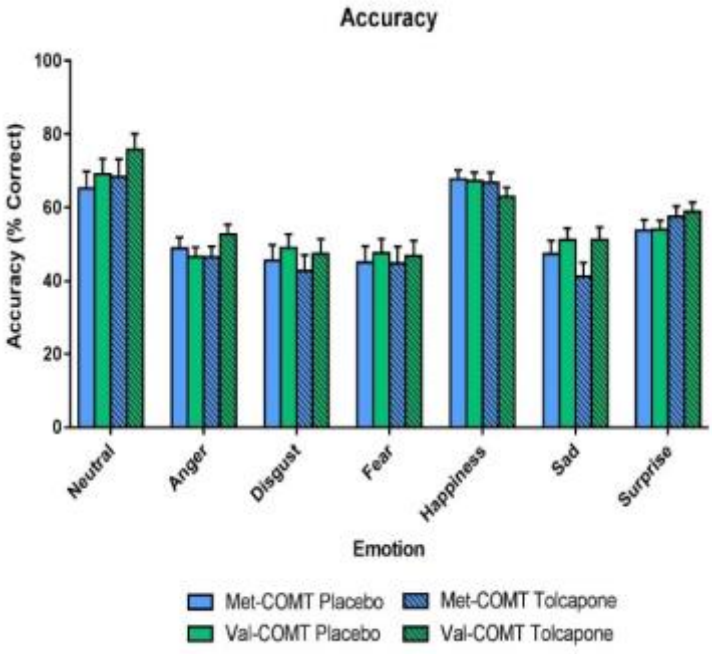


Figure 3.2: *Effect of COMT genotype and tolcapone on the accuracy of recognition of angry, disgusted, fearful, happy, sad, surprised and neutral faces. Values represent the percentage of correct responses over the range of different intensity levels of each emotion. Values are adjusted means, with vertical bars representing the standard error*. N=61

Reaction Time: There were no main effects of drug, genotype nor their interaction on reaction time to identify facial expressions (F's<1.27, P's>.26)(Figure 3.3). There was a significant effect of emotion ($F(6, 330)=5.81$, $P=.85x10^{-4}$), whereby individuals were slowest at identifying facial expressions of fear compared to other emotions (P's<.04 x 10^{-2}) and fastest at identifying neutral and happy facial expressions (P's<0.02). There were no interactive effects of emotion and drug ($F(6, 330)=.1.16$, P=.33) but there was a trend level interactive effect of emotion and genotype ($F(6, 330)=2.07$, P=.06). Follow up analyses revealed that this trend level effect of emotion x genotype was driven by significantly slower identification of facial expressions of disgust by Met homozygotes than Val homozygotes ($F(1, 55)=5.33$, P=.03). This finding should be treated with caution, given the absence of a significant emotion x genotype interaction. Baseline POMS tension-anxiety was again included as a covariate in analysis of FERT reaction time ($F(1, 55)=.04$, P=.84).

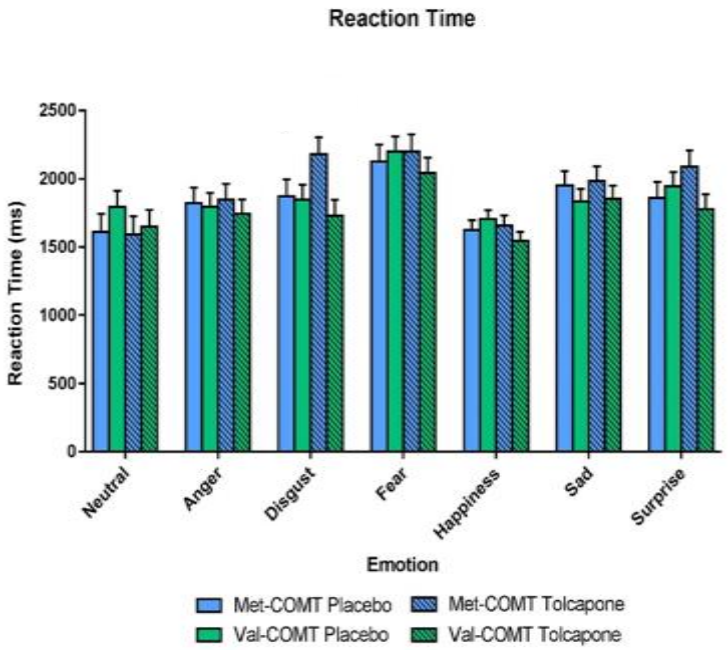
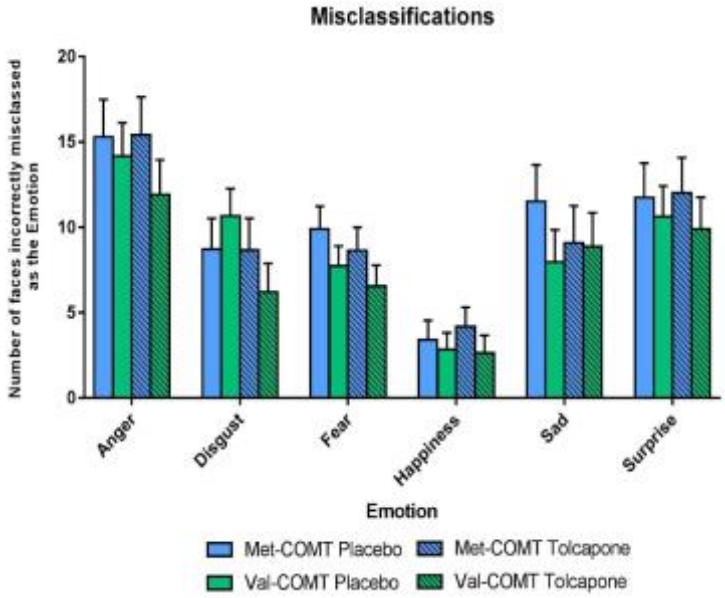


Figure 3.3: *The effect of COMT genotype and tolcapone administration on time taken to identify facial expressions of anger, disgust, fear, happiness, sadness, surprise or neutral. Values are mean reaction times with standard error values indicated by vertical* bars.

Misclassifications: I identified no main effect of drug ($F(1,55)=.02$ P=.90), genotype ($F(1, 55)=1.56$, P=.22), nor their interaction ($F(1,55)=.02$ P=.96), on the number of faces misclassified. There were also no interactions of drug or genotype with emotion identity (F's<.59, P's>.74). There was a significant effect of emotion ($F(6, 330)=115.73$, $P=.29 x10^{-58}$), whereby faces were more frequently misclassified as being expressions of anger or surprise (P's<0.04) and less frequently misclassified as being facial expressions of happiness (P's<$.70x10^{-7}$).Baseline POMS tension-anxiety was included as a covariate ($F(1,55)=.67$, P=.42).

Figure 3.4: *The effect of COMT genotype and tolcapone administration on number of faces misclassified as anger, disgust, fear, happiness, sadness or surprise. Values shown are the mean number of faces misclassified as each emotion with standard error values indicated by vertical bars*



3.4 EMOTIONAL CATEGORIZATION AND EMOTIONAL RECALL

ECAT: there were no effects of valence, genotype, drug or genotype x drug on subjects' ability to correctly classify self-referential words as being positively or negatively valenced (all F's<.74, P's>.39). There was a significant effect of valence x drug x genotype (F(1,57)=6.30, P=.02), and follow up analysis identified that this was driven by correct classification of more positive than negative self-referrent words in Val homozygotes in the placebo group (F(1, 57)=3.88, P=.05)(see table 3.3).

No main effect of genotype or drug, nor their interaction, were found on the reaction time taken to classify words as being either positive or negative (F's<1.63, P's>.21). There was an effect of valence on the time taken for subjects to classify self-referrent words, whereby individuals were faster at classifying positive words than negative words (F(1, 57)=6.42, P=.01). In addition, a trend effect of valence x drug x genotype was observed (F(1,57)=3.03, P=.09). Follow up analyses identified that this interaction was driven by faster classification of positive words in Val homozygotes given placebo compared to Val homozygotes given tolcapone (F(1, 57)=.25, P=.12) (see table 3.3).

Table 3.3: *Number of positive and negative words correctly classified in the ECAT, and the reaction times to classify them*

	Met-COMT Placebo	Val-COMT Placebo	Met-COMT Tolcapone	Val-COMT Tolcapone	One-way ANOVA (p)
Positive words correctly classified	28 (1.38)	29 (1.80)	28 (1.39)	28 (1.11)	.51
Negative words correctly classified	28 (1.58)	28 (1.59)	28 (1.88)	29 (.97)	.32
Reaction time to classify positive words (seconds)	1.07 (.16)	1.02 (.24)	1.09 (.22)	1.13 (.18)	.45
Reaction time to classify negative words (seconds)	1.10 (.17)	1.13 (.23)	1.20 (.26)	1.21 (.23)	.65

Data are given as mean (standard deviation) unless indicated. COMT, Catechol-O-methyltransferase; ANOVA, Analysis of Variance

EREC: There was no main effect of genotype ($F(1, 57)=.97$, $P=.33$) or drug ($F(1, 57)=.23$, $P=.63$), nor any interactive effect of the two ($F(1, 57)=.20$, $P=.66$), on the correct recall of positive or negatively valenced words in the ECAT (see fig. 3.5a). There were no significant interactions of drug or genotype and valence ($F's<1.93$, $P's>.17$), but there was a significant effect of valence, whereby more positive words were correctly remembered than negative words ($F(1, 57)=6.55$, $P=.01$). Exploratory post-hoc tests revealed a trend for greater recall of positive words by Met homozygotes than Val homozygotes in both the placebo and tolcapone groups (simple main effects analysis: $F(1, 57)=2.96$, $P=.09$).

Similarly, there was no main effect of drug, genotype, valence or genotype x drug on positive or negative words falsely recalled in the ECAT (all F's<1) (see fig. 3.5b). There was a trend level effect of valence x drug x genotype (F(1, 57)=3.16, P=.08), which follow-up analysis revealed was driven by a trend towards greater false recall of positive than negative words by Met homozygotes in the placebo group compared to the Met-tolcapone group (F(1, 57)=3.90, P=.05).

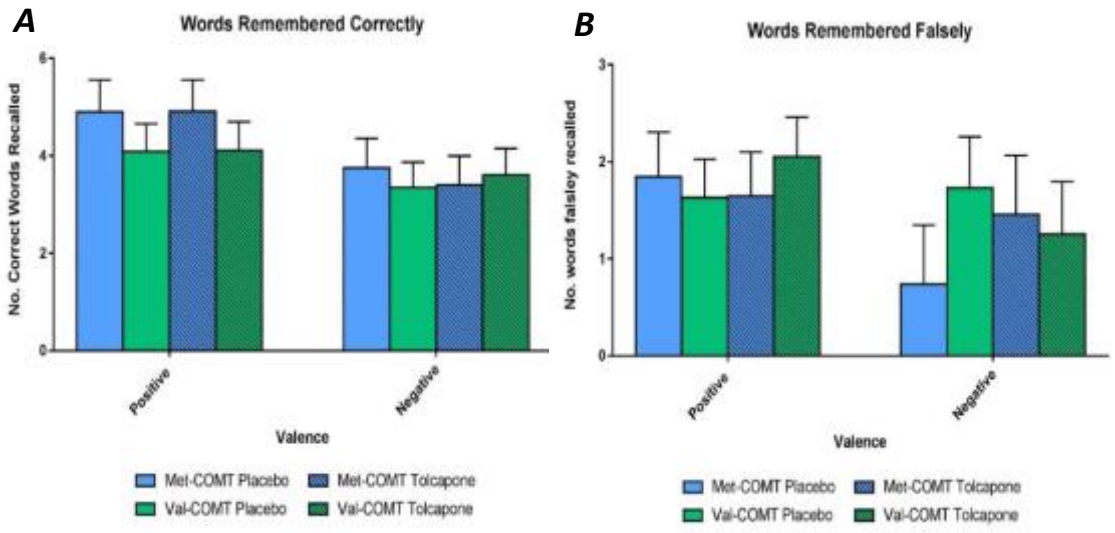


Figure 3.5: ***A)*** *The effect of COMT genotype and tolcapone administration on the number of positive and negative words from the emotional categorisation task correctly recalled* ***B)*** *the number of positive and negative words falsely recalled. All values are means and standard errors are indicated by vertical bars.*

3.5 ATTENTIONAL DOT-PROBE TASK

In order to reduce the impact of outliers, trials with response times greater than 1200ms or less than 200ms, and those which were 3 standard deviations above or below the mean reaction time (calculated after the first cropping of data), were excluded from the analysis (Browning et al., 2012). Using these criteria, 618 trials were excluded from a total of 12,096 trials, amounting to 5.11% of trials. There was no significant difference in the frequency with which trials of a particular condition were excluded, nor was there a significant difference in the number of trials excluded between the different groups (all F's<1). Attentional vigilance scores were then calculated by subtracting mean reaction time values for congruent trials (where the dot probe was in the same location as the stimulus) from mean reaction time values from incongruent trials (where the dot probe was in the opposite location to the stimulus). Hence, positive

values indicate attentional vigilance towards a stimulus, negative scores indicate vigilance away from a stimulus and zero scores indicate that there is no bias in vigilance.

Data was analysed using a 2 x 2 x 2 x 2 ANOVA with stimulus valence (happy v. fearful) and duration of stimulus presentation (short v. long) as the within-subjects factors, genotype and drug as the between-subjects factors and baseline POMS tension-anxiety as a covariate. This showed that there was no main effect of drug or genotype, nor any interactive effects of the two, on attentional vigilance toward happy and fearful emotional faces (F's<1.31, P's>.26) (see figure 3.6). There was a significant effect of valence, whereby subjects were generally vigilant towards fearful stimuli and vigilant away from happy faces ($F(1, 56)=9.40$, $P=.003$). Furthermore, there was a trend effect of valence x duration x genotype ($F(1, 56)=3.29$, $P=.08$). Follow up analysis indicated that this trend effect was driven by increased vigilance away from happy faces presented for a long period (unmasked) in Met homozygotes compared to Val homozygotes ($f(1,56)=6.36$, $P=.01$). The trend was also driven by significantly increased vigilance towards fearful faces presented for a short time than happy faces presented for a short time in Val homozygotes (($F(1,56)=5.84$, $P=.02$) and significantly increased vigilance towards fearful faces than happy faces presented for a long time in Met homozygotes ($F(1, 56)=6.40$, $P=.01$). However, in the absence of a significant main effect, these results should be considered exploratory.

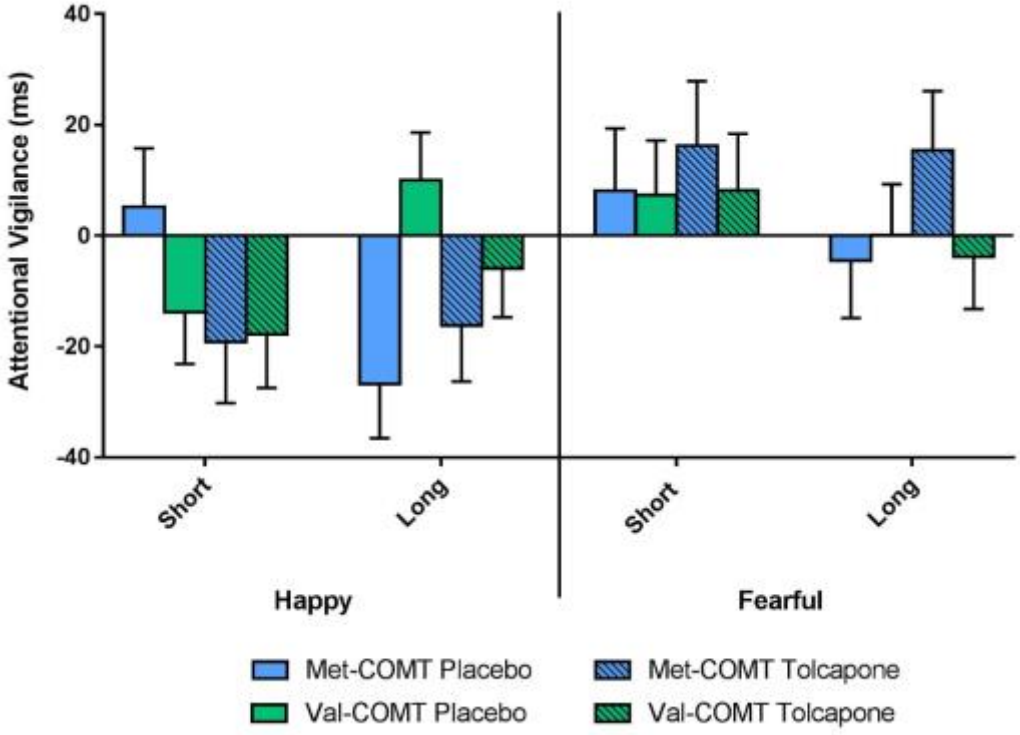


Figure 3.6: *The effect of COMT genotype and tolcapone on Attentional dot probe. The figure shows attentional vigilance scores (mean reaction time for incongruent trials (ms) – mean reaction time for congruent trials (ms)), where a positive attentional vigilance score indicates vigilance towards a stimulus, a negative score indicates vigilance away from a stimulus and a zero score indicates no bias in vigilance. We tested using stimuli of two valences (happy and fearful) and the stimuli were shown for either a short or long period.*

3.6 AFFECTIVE STARTLE REFLEX MODULATION

Of the 63 participants who completed our ASRM paradigm, the data of 21 participants were removed due to excessive noise on the EMG trace, leaving the data from 42 Val^{158}Met homozygotes to be included in our analyses. Final sample sizes were as follows: 10 Met-COMT placebo, 13 Val-COMT placebo, 9 Met-COMT tolcapone, 10 Val-COMT tolcapone. The groups remained well matched in terms of age, years in education, IQ and trait anxiety (STAI-T) score, with no significant differences identified between them (see table 3.4).

Table 3.4: *Baseline characteristics of participants in the acoustic startle reflex modulation paradigm*

	Met-COMT Placebo	Val-COMT Placebo	Met-COMT Tolcapone	Val-COMT Tolcapone	Total	One-way ANOVA (p)
Number	10	13	9	10	42	...
Ethnicity	9C, 1EA	11C, 1SA, 1EA	9C	9C, 1H	...	...
Handedness	8R, 2L	12R, 1L	8R, 1L	9R, 1L	37R, 5L	...
Age (Years)	24.8 (4.8)	25.0 (5.1)	24.3 (4.6)	23.7 (6.3)	24.5 (5.1)	.93
Years in Education	18.5 (1.4)	17.5 (2.8)	17.0 (3.4)	15.6 (2.1)	17.2 (2.6)	.31
IQ: NART	118 (5)	116 (6)	114 (6)	113 (7)	115 (6)	.26
Trait Anxiety: STAI-T	31 (7)	30 (5)	36 (8)	35 (7)	33 (7)	.10

Data are given as mean (standard deviation) unless indicated. COMT, Catechol-O-methyltransferase; ANOVA, Analysis of Variance; NART, National Adult Reading Test; STAI-T, Spielberg State Trait Anxiety Inventory – Trait; C, Caucasian; EA, East Asian; SA, South Asian; H, Hispanic

Simple main effects analysis showed that there was no main effect of valence on startle amplitude (see figure 3.7).

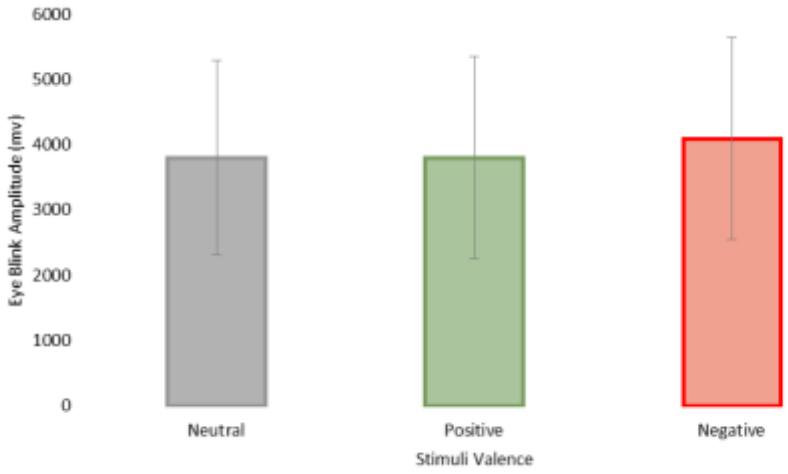


Figure 3.7: *The effect of stimuli valence on amplitude of startle responses. Values are mean values for raw amplitude (mv) with vertical error bars representing the standard error values. In our affective startle reflex modulation paradigm, subjects were shown neutral, positive and negative picture stimuli, as described in section 2.6.4.*

I went on to perform an ANOVA, with stimulus valence (positive, negative or neutral) as the within-subjects factor and genotype and drug as the between-subjects factors. However, given the lack of valence effect, which certainly draws into question the assay sensitivity of our startle paradigm, I interpret this with caution. The analysis demonstrated that there was no effect of genotype or genotype x drug on the amplitude of startle response (F's<.21, P's>.65). The ANOVA did identify a trend effect of drug (F(1,38)=3.93, P=0.06), which follow up analysis revealed was driven by increased startle magnitude in those given 200mg tolcapone (see figure 3.8, below).

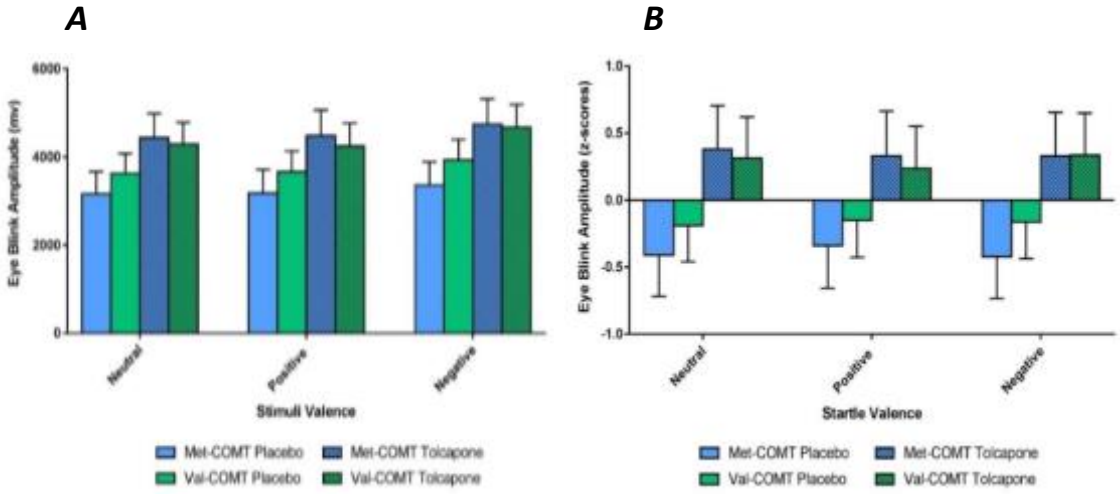


Figure 3.8: *The effect of COMT genotype and tolcapone administration on emotion-potentiated startle responses. Values are mean values for raw amplitude (mv)(**A**) or z-transformed scores (**B**) for eye-blink responses. Subjects were shown neutral, positive and negative picture stimuli. Values are means with vertical bars indicating standard error values.*

4. DISCUSSION

In the present study, I investigated the hypothesis that COMT activity might influence emotional processing in healthy male Val158Met homozygotes at baseline (the placebo condition) and following a 200mg dose of brain penetrant COMT inhibitor, tolcapone. To investigate this, I used a battery of neuropsychological tasks shown to be sensitive to positive and negative biases in the processing of affective stimuli (Chan, Goodwin, & Harmer, 2007; Harmer et al. 2011) and providing a physiological measure of emotional reactivity (Montag et al. 2008).

4.1 NO EFFECT OF COMT VAL^{158}MET ON MEASURES OF EMOTIONAL PROCESSING

I examined the hypothesis that variation in COMT activity arising from the Val158Met polymorphism influences the processing of affective stimuli using the Oxford P1Vital Emotional Test Battery. My findings indicated that COMT genotype does not impact behavioural measures of emotional processing. Specifically, Val158Met genotype had no significant effect on the accuracy and speed of facial expression recognition, nor assessment of word valence, the recall of positive or negatively valenced words or attentional vigilance towards happy or fearful faces.

In the absence of any main effects, I performed a number of exploratory post-hoc tests. These revealed that Met homozygotes may be slower than Val homozygotes at identifying facial expressions of disgust. Additionally, in the attentional dot-probe task, Met homozygotes were more vigilant away from happy faces, presented for a long duration, than Val homozygotes. These results should, however, be treated with caution as they are likely to be chance findings.

Only a small number of studies have specifically looked at the influence of COMT Val^{158}Met on ability to differentiate facial expressions, and existing results are inconsistent (Defrancesco et al. 2010; Gohier et al. 2014; Kempton et al. 2009; Weiss et al. 2007). My findings that the Val^{158}Met polymorphism had no effect on accuracy and speed with which positive and negative facial expressions could be identified are consistent with those of Kempton et al. (2009). In their study of 74 healthy adults, they identified no effect of COMT genotype on performance in a facial recognition task, in which participants had to differentiate fearful and neutral faces (Kempton et al. 2009). This finding was also echoed by Defrancesco et al.'s study, which found no statistically significant effect of COMT genotype on recognition of emotional faces in a sample of 88 female students, assessed using the Penn Emotion Acuity Test (PEAT) (Defrancesco et al. 2010). On the other hand, a study by Weiss et al. found that Val homozygosity was

associated with quicker recognition of negative facial expressions, in a cohort of 100 healthy males and females (Weiss et al. 2007). These inconsistencies may reflect differences in the facial recognition tasks used, such as the number and intensity of emotions that participants were asked to identify. It would certainly be interesting to investigate the relationship between COMT genotype and facial expression recognition further, perhaps repeating the ETB FERT task with a larger sample size.

My findings in the emotional categorisation, emotional recall and faces dot-probe task suggested that COMT Val158Met genotype is not associated with attentional bias in the processing of emotionally salient stimuli. There has been little research focusing on the role of COMT in modulating attentional bias. My results are somewhat at odds with previous findings (Gong et al, 2013; Lonsdorf et al. 2014). In their study of 650 college students, Gong et al. found that Val158Met genotype influenced attentional capture to negative emotional stimuli. Specifically, Met carriers were more liable to be attracted to negative emotional expressions than Val homozygotes. Similarly, Lonsdorf noted an effect of COMT Val158Met genotype on attentional bias using a visual-search paradigm (VSP) involving pictures of angry and happy faces of both genders. Only Val/Val carriers exhibited greater bias towards happy female faces in the paradigm. In light of these inconsistencies, continued study of the effects of Val158Met on performance in emotional processing tasks would be worthwhile.

One reason why genotypic variation in COMT activity may not influence emotional processing is that the enzyme plays only a small role in much of the neural circuitry underlying emotionality. Due to the abundance of dopamine transporters in most regions of the brain, COMT has a relatively small part in DA removal from the synapse in subcortical regions. How might this be squared with the fMRI BOLD data indicating differential Val158met brain activation in limbic circuitry?

Though there are some inconsistencies (Bishop et al. 2006; Domschke et al. 2008; Drabant et al. 2006), evidence from fMRI imaging studies largely suggests that COMT Val158Met is associated with differences in brain activity during the processing of affective stimuli (Rasch et al. 2010; Smolka et al. 2006; Smolka et al. 2007; Williams et al. 2010). Furthermore, a number of studies have observed differential activation in limbic structures, such as the amygdala and hippocampal formation (Domschke et al. 2008; Drabant et al, 2006; Rasch et al. 2010). Notably, Meyer-Lindenberg and others argue that measurement of quantitative biological traits, such as BOLD response, is considerably more sensitive at detecting the subtle effects of genetic variants than studies looking at overt behaviour (Meyer-Lindenberg & Weinberger,

2006; Rasch et al. 2010), owing to the fact that they are significantly closer to the variant's direct physiological effects. Despite COMT's lesser role in limbic DA metabolism, genotypic variation in the enzyme's activity may still result in measurable differences in BOLD signal, especially if changes in PFC DA signalling have 'downstream' effects in other brain regions (Akil et al. 2003; Meyer-Lindenberg et al. 2005. However, these differences may not be great enough to reach the level of overt behaviour. Additionally, differences in brain activation during the processing of affective stimuli do not necessarily correlate to 'better' or 'worse' emotional processing ability.

The most likely explanation for my negative findings is that variation in corticolimbic DA signalling resulting from COMT Val158Met has no significant behavioural manifestation, with regards to affective processing. Nevertheless, it is still worth considering that limitations in the experimental set up may have resulted in false negative effects. For example, whilst the ETB is a validated tool to test antidepressant efficacy and the predicted effects of valence in each of our tasks suggest assay sensitivity, this is to my knowledge, the first time it has been used to probe the effects of the COMT Val158Met polymorphism. The paradigms involved, could simply not be sensitive enough to detect the minute variations in affective processing that may arise from the genetic variant in an experimental medicine study of this size. Furthermore, even in our relatively genetically homogeneous sample, the noise resulting from the influence of other genetic variants on emotional processing, including other functional SNPs in COMT (Gothelf et al. 2014; Nackley et al. 2007), may obscure the 'pure' effects of COMT Val158Met.

Another point of note is that divergent results from the cognitive studies of COMT Val^{158}Met suggest that the polymorphism's effects may only become apparent in highly-specific environmental conditions. For example, studies looking at the effect of COMT genotype on performance in the N-back task of working memory have produced conflicting results. Whilst a number of studies have found that Met COMT homozygotes outperform their Val COMT counterparts (Barnett, 2008; Farrell, 2012; Goldberg, 2003), other studies have not identified any effect of COMT genotype (Blanchard, 2011; Diaz-Asper, 2008). Farrell et al. proposed that this could reflect the inconsistent nature of the N-back tasks used in different studies. In the study by Farrell et al., the N-back paradigm used required rapid updating and considerable interference management, making it a relatively challenging version of the task, as indicated by their performance data. It is possible that differences in N-back performance arising from the Val^{158}Met polymorphism are only evident in tasks placing greater demands on working memory and that the lack of effect observed by other studies resulted from the use of relatively easier tasks. Although different neural

circuitry is required for the processing of affective stimuli, there may be a similar pattern of effects, with genotypic differences only becoming apparent in the processing of highly specific, perhaps even more 'challenging', stimuli.

4.2 FURTHER WORK IS NEEDED TO PROBE THE INFLUENCE OF COMT VAL158MET ON PHYSIOLOGICAL MEASURES OF EMOTIONAL REACTIVITY

Another aim of my study was to investigate whether variation in COMT activity arising from the Val158Met polymorphism influences emotional reactivity towards positive, negative and neutral picture stimuli, using an affective startle modulation (ASRM) paradigm. I found no evidence that COMT Val158Met genotype influences the amplitude of startle response to positive, negative or neutral picture stimuli. However, a lack of overall valence effect called into question the assay sensitivity of our ASRM paradigm, and casts doubt on these findings. Further study to elucidate the role of COMT Val158Met on emotional reactivity, as measured using the ASRM paradigm, is certainly warranted.

Few studies have examined the role of COMT Val158Met in modulating startle response. Consistent with my findings, Roussos et al. (2008), who also investigated startle amplitude in a cohort of healthy males, found that, although COMT genotype modulated prepulse inhibition (PPI), there was no difference in baseline startle amplitude between Val homozygotes and Met homozygotes. Similarly, Giakoumaki et al (2008), also found no difference in startle amplitude between Val/Val and Met/Met groups in the placebo condition in their study of 24 healthy males. In contrast, studies involving female subjects have identified COMT genotypic effects on startle magnitude. For example, Montag et al. (2008), who studied the effect of Val158Met in healthy women with low estradiol levels, found that Met158 homozygotes exhibited markedly greater startle reflex compared with Val158 carriers. Comasco et al. (2012), on the other hand, found Val homozygosity to be associated with greater startle amplitude in their study of women at 38 weeks pregnant. It is possible that these inconsistencies reflect a sexually-dimorphic effect of COMT (Harrison & Tunbridge, 2008).

4.3 TOLCAPONE MAY HAVE SUBTLE INFLUENCES ON PHYSIOLOGICAL MEASURES OF EMOTIONAL REACTIVITY

I also examined the hypothesis that variation in COMT activity arising from acute COMT inhibition influences emotional reactivity and the processing of affective stimuli. I found that 200mg tolcapone had no effect on the accuracy or speed of facial expression recognition or classification of self-referent words.

Furthermore, the drug did not affect the recall of positive and negatively valenced words or influence attentional vigilance towards happy or fearful faces.

As metabolism by COMT is the major pathway of DA removal from the synapse in the PFC (see section 1.2.1), tolcapone is likely to have its most pronounced effects on PFC-dependent cognitive functions, including executive function and working memory. Hence, these findings may reflect the lesser extent to which the drug's modulation of COMT activity alters DA signalling in other brain regions, such as those involved in the processing of emotionally salient stimuli.

Whilst no effect of tolcapone was observed on performance data from the emotional processing tasks used here, administration of the drug was associated with a trend level, genotype-independent increase in the amplitude of startle response towards positive, negative and neutral picture stimuli. This is likely to result from heightened activation of central dopaminergic and noradrenergic systems, which is thought to increase the magnitude of startle response in rodents and humans (Davis, 1985; Morgan et al. 1993). A study by Giakoumaki et al (2008) involving administration of a single 200mg dose of tolcapone or placebo, found a trend level increase in startle amplitude following tolcapone administration in the case of Met homozygotes but not in Val homozygotes. They took this finding as reflecting a dysphoric mood induced by tolcapone in Met158 homozygotes, citing evidence to suggest an association between Met homozygosity and negative mood states. It is important to remember when interpreting our findings that the lack of a main effect of valence does draw into question the assay sensitivity of our startle paradigm and, as such, additional research into the interactive effects of tolcapone and COMT genotype on modulating emotional arousal and startle magnitude is required.

4.4 STRENGTHS AND LIMITATIONS

There were a few limitations to my study, which should be considered when interpreting its findings. Firstly, the study cohort was relatively homogeneous, generally consisting of young, healthy, well-educated Caucasian men, with above-average IQ. This sample homogeneity would have been somewhat advantageous, in the sense that it may have helped to minimise noise resulting from between-subjects differences. However, the uniformity of the sample may limit the generalisability of our findings in more diverse populations.

Most notably, we only studied the effect of COMT Val158Met on emotional processing in males, which may diminish the applicability of our findings to females. This decision was made on the basis of

avoiding effects of COMT sexual dimorphism, as oestrogen is known to be a transcriptional regulator of the COMT gene (Harrison & Tunbridge 2008). Pragmatically, it would have been extremely challenging to control for hormonal status in female participants, and additionally we would have had to ensure female participants were not pregnant before giving them a drug associated with severe hepatocellular toxicity. The generalisability of our findings may also be somewhat reduced by our inclusion of only those aged between 18 and 40. Epigenetic factors, including age, nutrition and stressful life events, influence methylation of the COMT gene and thus have a bearing on COMT activity (Nohesara et al. 2011). Thus, our results may have differed had we allowed older individuals to participate, due to epigenetic changes associated with ageing.

Whilst adequate screening measures were taken to ensure that our cohort of participants were healthy, non-smoking individuals, it is difficult to guarantee that this was the case. It is possible that there was undiagnosed psychopathology, or psychopathology present at a subclinical level, within our study group and this may have influenced our findings. Furthermore, smoking abstinence and abstinence from drugs was assessed by self-report only. Ideally we would have performed drug screens to rule out the possibility of non-disclosure influencing our results. Additionally, despite the use of baseline measures, which indicated that our four groups were generally well matched, there was a baseline difference in POMS tension-anxiety scores, which were higher in the two groups that would go on to receive tolcapone. I have included this as a covariate in the analyses, where I feel it is appropriate (see section 3), to try and minimise any confounding effect of this variable. Other confounding variables, such as level of satiety or fatigue, may have also have influenced individual performance in our emotional processing paradigms (Thomas & Dourish, 2016).

Finally, due to time constraints limiting recruitment into my study, the sample size may not have been sufficiently large to detect subtle genotypic differences in emotional processing, particularly in the ASRM paradigm in which data from a number of participants had to be excluded. Our sample size was based on that used in the study by Farrell et al. study, which did identify COMT genotypic differences, albeit mainly relating to cognitive effects of Val158Met genotype, rather than effects on emotional processing per se (Farrell et al. 2012). It is possible that effects may become more apparent in larger samples.

4.5 CONCLUSIONS

The current study found little evidence to support the hypothesis that COMT activity influences ability to process affective stimuli. Neither variation in COMT activity arising from the Val158met polymorphism nor acute COMT inhibition, using a 200mg dose of tolcapone, were associated with striking differences in performance data from emotional processing tasks. This study is the first to examine the influence of COMT Val^{158}Met on emotional processing using the emotional test battery and has given insight into possible methodological limitations of using the ETB as a tool to probe the effects of the genetic variant. These results may help further our understanding of COMT Val^{158}Met's role in underlying normal variation in emotional processing and contributing to penetrance and severity of psychiatric disorders.

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Profile of Mood States

Subject number:

Date:

Instructions: Below is a list of words that describe feelings people have. Please read each one carefully. Then circle ONE of the answers to the right of that word which describes HOW YOU FEEL RIGHT NOW.

	Not at all	A little	Moderately	Quite a bit	Extremely
	1	**2**	**3**	**4**	**5**
Friendly	1	2	3	4	5
Tense	1	2	3	4	5
Angry	1	2	3	4	5
Worn Out	1	2	3	4	5
Unhappy	1	2	3	4	5
Clear headed	1	2	3	4	5
Lively	1	2	3	4	5
Confused	1	2	3	4	5
Sorry for things done	1	2	3	4	5
Shaky	1	2	3	4	5
Listless	1	2	3	4	5
Peeved	1	2	3	4	5
Considerate	1	2	3	4	5
Sad	1	2	3	4	5
Active	1	2	3	4	5
On edge	1	2	3	4	5
Grouchy	1	2	3	4	5
Blue	1	2	3	4	5
Energetic	1	2	3	4	5
Panicky	1	2	3	4	5
Hopeless	1	2	3	4	5
Relaxed	1	2	3	4	5

Unworthy	1	2	3	4	5
Spiteful	1	2	3	4	5
Sympathetic	1	2	3	4	5
Uneasy	1	2	3	4	5
Restless	1	2	3	4	5
Unable to concentrate	1	2	3	4	5
Fatigued	1	2	3	4	5
Helpful	1	2	3	4	5
Annoyed	1	2	3	4	5
Discouraged	1	2	3	4	5
Resentful	1	2	3	4	5
Nervous	1	2	3	4	5
Lonely	1	2	3	4	5
Miserable	1	2	3	4	5
Muddled	1	2	3	4	5
Cheerful	1	2	3	4	5
Bitter	1	2	3	4	5
Exhausted	1	2	3	4	5
Anxious	1	2	3	4	5
Ready to fight	1	2	3	4	5
Good-natured	1	2	3	4	5
Gloomy	1	2	3	4	5
Desperate	1	2	3	4	5
Sluggish	1	2	3	4	5
Rebellious	1	2	3	4	5
Helpless	1	2	3	4	5
Weary	1	2	3	4	5
Bewildered	1	2	3	4	5
Alert	1	2	3	4	5
Deceived	1	2	3	4	5
Furious	1	2	3	4	5

Efficacious	1	2	3	4	5
Trusting	1	2	3	4	5
Full of pep	1	2	3	4	5
Bad-tempered	1	2	3	4	5
Worthless	1	2	3	4	5
Forgetful	1	2	3	4	5
Carefree	1	2	3	4	5
Terrified	1	2	3	4	5
Guilty	1	2	3	4	5
Vigorous	1	2	3	4	5
Uncertain about things	1	2	3	4	5
Bushed	1	2	3	4	5

State Questionnaire

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel ***right now,*** that is, ***at this moment in time.*** There are no right and wrong answers. Do not spend too much time on each statement but give the answer which seems to describe your present feelings best.

	Not at all	Somewhat	Moderately	Very much
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over Possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

Trait Questionnaire

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you GENERALLY feel. There are no right and wrong answers. Do not spend too much time on each statement but give the answer which seems to describe you best.

	Not at all	Somewhat	Moderately	Very much
1. I feel pleasant	1	2	3	4
2. I feel nervous and restless	1	2	3	4
3. I feel satisfied with myself	1	2	3	4
4. I wish I could be as happy as others seem to be	1	2	3	4
5. I feel like a failure	1	2	3	4
6. I feel rested	1	2	3	4
7. I am 'cool, calm and collected'	1	2	3	4
8. I feel that the difficulties are piling up so that I cannot overcome them	1	2	3	4
9. I worry too much over something that doesn't really matter	1	2	3	4
10. I am happy	1	2	3	4
11. I have disturbing thoughts	1	2	3	4
12. I lack self-confidence	1	2	3	4
13. I feel secure	1	2	3	4
14. I make decisions easily	1	2	3	4
15. I feel inadequate	1	2	3	4
16. I am content	1	2	3	4
17. Some unimportant thoughts run through my mind and bother me	1	2	3	4
18. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
19. I am a steady person	1	2	3	4
20. I get in a state of tension or turmoil as I think over recent				

concerns and interests	1	2	3	4

Visual Analogue Scales

Please place a vertical line at the point on the horizontal line which most represents how you feel at the moment (e.g.: —┼—). **DON'T** spend a long time thinking about each one. Each scale runs from **0** to **10**, with **10** being the **MOST YOU COULD EVER IMAGINE** and **0** being **COMPLETELY ABSENT.**

Alertness: **0** _____ **10**

Drowsiness: **0** _____ **10**

Anxiety: **0** _____ **10**

Happiness: **0** _____ **10**

Nausea: **0** _____ **10**

Sadness: **0** _____ **10**