The Magnetoencephalographic Signature of Catechol-O-methyltransferase

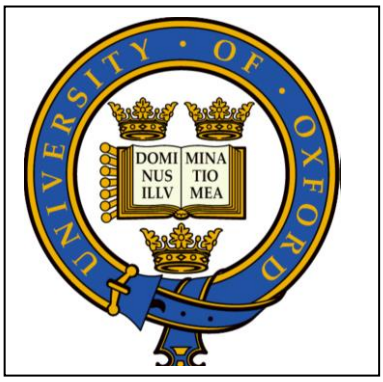
A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Oxford

Sarah Marie Farrell

St John's College

Oxford University

Trinity Term 2012



The Magnetoencephalographic Signature of Catechol-O-methyltransferase

Abstract

Catechol-*O*-methyltransferase (COMT) metabolizes catechols, notably dopamine. The COMT Val^{158}Met polymorphism influences its enzyme activity, and multiple neural correlates of this genotype on dopaminergic phenotypes have been reported, particularly with regards to working memory. COMT activity can also be regulated pharmacologically by COMT inhibitors. The 'inverted-U' relationship between dopamine signalling and cognitive performance predicts that the effects of COMT inhibition will differ according to COMT genotype.

The goal of this thesis was to better understand COMT's impact on brain function and behaviour. Here, 33 subjects homozygous for COMT Val^{158} ('Val homozygotes') and 34 homozygous for COMT Met^{158} ('Met homozygotes') were randomly assigned, double-blind, to a single dose of the brain-penetrant COMT inhibitor tolcapone (200mg) or placebo. They completed the N-back task of working memory, an emotional face processing task, and a gambling task, in a magnetoencephalography (MEG) scanner, allowing both behavioural performance and neural activity to be investigated.

The data presented in this thesis confirm that COMT activity influences performance on, and neural activity during, the N-back task, in a way consistent with the inverted-U model of dopamine function. The effect on risky decision making is novel, and indicates that COMT plays roles in domains beyond working memory, and that such domains may also follow an inverted-U. Neural activity during the faces task and the gambling task also show COMT-modulated differences.

The behavioural results show that the direction of effect of a drug can be influenced by sequence variation in its target gene. They are of translational relevance, since COMT inhibitors are used in the adjunctive treatment of Parkinson's disease and are under evaluation in schizophrenia and other disorders.

The MEG data show that for the three tasks, there are effects of Val^{158}Met genotype, of tolcapone, and their interaction, on neural activity (for example, the P300 during N-back), revealing a complex temporal and spatial pattern which sheds some light on the neural processing underlying these tasks and their previously reported fMRI correlates.

Acknowledgements

"*It was the best of times, it was the worst of times*..."

Charles Dickens, *A Tale of Two Cities*

First and foremost thank you to my supervisors Paul Harrison and Sven Braeutigam, and my supervisor in all but name, Elizabeth Tunbridge. I am truly grateful for all the meetings and words of wisdom. All three of you have been generous with your time, thought-provoking and always kept me in good spirits.

I thank all past and present members of the Harrison lab for their advice, be it scientific or domestic: Mary Walker, Sharon Eastwood and Tracey Lane for general science advice and transferable life skills; Phil Burnet for listening and helping; Jill Betts for making sure I am not the only one laughing at my own jokes; Linda Laatikinen, Helena Cousijns, Helene Savignac and Greg Bristow for fun times in the lab, and Jenni Swettenham and Louisa Coulson for checking up on me.

Thank you to the Wellcome Trust for funding this DPhil as part of a 4-year MSc. and DPhil. studentship. My deepest gratitude to Jonathan Flint for his support and guidance.

Warmest thanks to my friends at college, those from the team, and those back home for sharing and caring. But mainly for making me laugh.

Very special thanks and gratitude go to my favourite people in the entire world. Peter and Nancy Farrell, my outstandingly good-looking and intelligent parents, I am so grateful for your love, support and top quality genes. And to my brother Sean for making me laugh and unknowingly reminding me there are issues; political, ethical and social, to which I should be aware.

"*If you can meet with triumph and disaster. And treat those two imposters just the same…*"

Rudyard Kipling, *If: A Father's Advice to His Son.*

Table of contents

Chapter 1 2

1. Introduction 2

1.1 Dopamine 2

1.2 An inverted-U relationship between prefrontal DA and cognition 3

1.3 Catechol-O-methyltransferase (COMT) 8

1.3.1 COMT isoforms, locations and enzymology 8

1.3.2 COMT Valine^{158}Methionine (Val^{158}Met) polymorphism 10

1.3.3 COMT Val^{158}Met, PFC DA and the inverted-U 12

1.4 Genetic vs. pharmacological manipulation of COMT activity 13

1.4.1 Interaction of tolcapone with genotype 13

1.5 Measuring neural activity 14

1.5.1 fMRI 15

1.5.2 Magnetoencephalography 17

1.5.2.1 COMT and MEG signal 19

1.6 Final comments 24

1.7 Thesis aims, objectives, and outline 24

Chapter 2 28

2. Methods 28

2.1 Recruitment 28

2.2 DNA extraction protocol 30

2.2.1 Calculation of DNA purity and yield 31

2.3 Genotyping using qPCR 31

2.4 Participant confidentiality 32

2.5 Design for the day of the study 32

2.6 Demographics 34

2.6.1 Participant figures 34

2.6.2 Demographic and questionnaire data 35

2.6.3 Questionnaire-based measures and correlations 36

2.7 Statistical analysis of behavioural data 37

2.8 MEG experiments 38

2.8.1 Instrumentation 38

2.8.2 Practical details of MEG procedures 39
2.8.3 Stimuli 40
2.8.4 Approach to analysis 41
2.8.4.1 Pre-processing 42
2.8.4.2 Data epochs 42
2.8.4.3 Statistics 43
2.8.5 Maps 44
2.8.5.1 Global plots 44
2.8.5.2 Probability plots and maps 45
2.8.5.3 Local maps 45
2.8.5.4 Construction of local maps 46
2.8.5.5 Scales 47
Chapter 3 49
3. The effect of the COMT Val^{158}Met genotype on working memory 49
3.1 Introduction 49
3.1.1 Dopamine and working memory 50
3.1.2 COMT Val^{158}Met and working memory studies 51
3.1.3 Tolcapone effects on COMT and working memory 53
3.1.4 Objectives 54
3.2 Methods 55
3.2.1 The N-back task 55
3.2.2 Demographic data 55
3.3 Results 57
3.3.1 Accuracy and reaction time scores as a function of N-back condition 57
3.3.2 Genotype x tolcapone interaction changes depending on N-back load 58
3.3.3 Genotype and tolcapone effect on accuracy scores 58
3.3.4 No correlation between performance and 'happiness' 60
3.3.5 Genotype and tolcapone effects on reaction times 60
3.4 Discussion 62
3.4.1 COMT genotype affects N-back performance 62
3.4.2 COMT genotype x tolcapone interaction on accuracy scores 63
3.4.3 Interpretation of reaction time data 66
3.4.4 Limitations and future studies 67
3.4.5 Conclusion 68

Chapter 4 70
4. Exploring neural correlates of working memory performance using MEG 70
4.1 Introduction 70
4.1.1 The PFC and working memory 70
4.1.2 The effect of COMT on neural substrates of N-back performance 71
4.1.3 'Efficiency' hypotheses in imaging studies 72
4.1.4 Potential time components of interest for working memory and N-back 73
4.1.4.1 COMT modulated P300 74
4.1.5 Summary 75
4.1.6 Objectives 75
4.2 Methods 75
4.2.1 Principles of analysis approach 75
4.2.1.1 Selection Criteria 76
4.2.1.2 Course of analysis 77
4.3 Results 80
4.3.1 Comparison of performance-matched and non-performance-matched data 80
4.3.2 Analysis of time components of interest in performance-matched data 83
4.3.3 Effect of tolcapone on the 275ms time component 85
4.3.4 The effect of genotype on MEG signal during 2-back at 280ms 86
4.3.5 Genotype x tolcapone interaction in the 335ms time component 87
4.4 Discussion 92
4.4.1 The P300 and its modulation by N-back load and COMT 93
4.4.2 COMT-related differences in N-back MEG signal for P300 are shown over both frontal and temporo-parieto-occipital areas 94
4.4.3 A putative inverted-U relationship between MEG signal 'activity' and frontal DA 97
4.4.4 Efficiency 98
4.4.5 Limitations and future studies 100
4.4.6 Conclusion 101
Chapter 5 103
5. The role of COMT in the MEG correlates of emotional face processing 103
5. 1 Introduction 103
5.1.1 What is emotion? 103
5.1.2 A role for DA in emotional processing 103
5.1.3 COMT and emotional processing 105

5.1.4 Face processing 106
5.1.4.1 Temporal components in face processing 107
5.1.5 Objectives 109
5.2 Methods 110
5.2.1 The faces task 110
5.2.2 Characterising time points of interest 111
5.2.3 Displaying the results 114
5.2.4 Demographics 115
5.3 Results 116
5.3.1 Emotional processing over all participant groups 116
5.3.1.1 Attending to cross hairs 116
5.3.1.2 Global plots show clear N170 response 116
5.3.1.3 Time components corresponding to emotional face processing 118
5.3.2 COMT modulation of fearful faces 120
5.3.2.1 Fearful activity maps show different peak activities for different groups 120
5.3.2.2 Characterising the difference in MEG signal at 390ms between placebo and tolcapone groups 121
5.3.2.3 Characterising Val^{158}Met genotype differences in fearful processing at 390ms 124
5.3.2.4 Characterising the genotype x tolcapone interaction at 370ms 126
5.4 Discussion 129
5.4.1 Face-specific response 129
5.4.2 No modulation of the N170 or 250ms components by facial emotional expression 130
5.4.3 The N4-like component for emotional face processing 130
5.4.4 The effect of tolcapone on processing fearful faces at the N4-like component 132
5.4.5 Effect of Val^{158}Met genotype and genotype x tolcapone interactions on fearful faces at the N4-like component 133
5.4.6 Limitations and future studies 135
5.4.7 Conclusion 137
Chapter 6 140
6. The role of COMT in reward processing and decision making 140
6.1 Introduction 140
6.1.1 Dopamine and reward 140
6.1.2 COMT and reward 142
6.1.2.1 Evidence that COMT affects reward 'performance' 142

6.1.2.2 Evidence that COMT affects brain activity during reward processing 144
6.1.2.3 Summary of COMT reward outcome data 146
6.1.3 Objectives 147
6.2 Methods 148
6.2.1 The Gambling Task 148
6.2.2 Analysis of behavioural data 151
6.2.3 MEG analysis 151
6.3 Behavioural results 154
6.3.1 Participants' 'riskiness' (percentage 'high bets') decreases over time. 154
6.3.2 Bets placed were unaffected by prior outcomes 154
6.3.3 Val^{158}Met genotype x tolcapone interaction influences size of bet chosen 154
6.3.4 No COMT effect on reaction time 155
6.3.5 No correlations between gambling performance and other COMT-modulated behavioural measures 155
6.4 MEG results for investigating the neural correlates of reward processing 157
6.4.1 Global maps show neural response to losses is larger than to wins 157
6.4.2 Local plots for the four different loss and win conditions 161
6.4.3 COMT modulation of wins and losses 163
6.4.3.1 Global probability plots show genotype x tolcapone interaction 163
6.4.3.2 Local plots of drug effects on wins and losses at 275ms 165
6.4.3.3 Characterisation of genotype x tolcapone interaction at 285ms 165
6.4.4 No COMT effects on MEG signal in response to magnitude of bet 169
6.5 Discussion 171
6.5.1 Behavioural Discussion 171
6.5.1.1 Risk aversion tendency increases across time 171
6.5.1.2 COMT effect on risk behaviour 172
6.5.2 MEG results discussion 175
6.5.2.1 Losses loom larger than gains, including in the putative MFN 175
6.5.2.2 The effect of COMT genotype on processing outcomes 176
6.5.2.3 COMT genotype x tolcapone interaction and tolcapone main effect modulate MEG signals associated with reward processing 177
6.5.2.4 The effects of COMT over the putative MFN region 179
6.5.2.5 The effects of COMT are widespread 180
6.5.2.6 Possible therapeutic effect of tolcapone 180

6.5.2.7 Limitations and future studies 181
6.5.3 Summary 183
Chapter 7 185
7. Discussion 185
7.1 Summary 185
7.2 Therapeutic implications of the Val^{158}Met genotype x tolcapone interaction 186
7.3 Implications for the use of MEG in studies of COMT and pharmacogenetics 192
7.4 Conclusion 196
Appendices 198
Appendix 1 Questionnaire sheets 198
Appendix 2 Visual analogue scale scores 209
Appendix 3 Befeindlichkeit scores 210
Appendix 4 Pearson correlations between N-back performance and happiness VAS scores. .. 211
References 212

List of Figures

1.1 An inverted-U relationship between DA and PFC performance 4
1.2 A model for the neural mechanism of the inverted-U DA curve 6
1.3 A representation of DA contributions to signal-to-noise ratio 8
1.4 The synthesis and metabolism of DA and noradrenaline 9
1.5 Enzyme activity in the Val^{158}Met polymorphism 10
1.6 Measurable neuromagnetic field 21
1.7 The effect of COMT on the MEG signal 23

2.1 Recruitment protocol 29
2.2 Study design – the scanning day 34
2.3 Visual analogue scales – Happiness 37
2.4 Electa-Neuromag scanner and coil arrangement 39
2.5 Approach to MEG analysis 42
2.6 Probability formula 43
2.7 Area segmentation 46

3.1 The N-back test 56
3.2 Performance as a function of difficulty levels 57
3.3 Interactive effects of COMT genotype and COMT inhibition 59
3.4 Reaction times 61
3.5 Inverted–U schematic 65

4.1 Strategy for MEG analysis of N-back processing 79
4.2 Global plots for performance-matched and non-performance-matched data 82
4.3 Participant group differences in 2-back 84
4.4 Local maps demonstrating differences in activity between placebo-treated and tolcapone-treated groups during performance of the 2-back task at 275ms 89
4.5 Local maps demonstrating genotype effects during 2-back at 280ms 90
4.6 Interaction effects at 335ms during 2-back 91
4.7 Inverted-U schematic for frontal regions at 335ms 98

5.1 Images of the emotional processing task 113
5.2 Strategy for MEG analysis of face processing 114
5.3 The N170 face response 117
5.4 An N4-like component in fearful processing 119
5.5 Global plots showing COMT modulation of fearful face processing 122
5.6 Tolcapone effect on fearful faces at 390ms 123
5.7 The effect of COMT genotype on fearful face processing at 390ms 125
5.8 Local maps exploring genotype x tolcapone interaction effects of processing fearful faces at 370ms 128

6.1 Gambling task 149
6.2 Strategy for MEG analysis of reward processing 153
6.3 Propensity for making 'risky' bets reduces over time 156
6.4 Gambling task behavioural results 156
6.5 Probability plot and activity plot showing differences between losses and wins 159
6.6 Global activity elicited from the four outcome conditions 160
6.7 Local maps for the four outcome conditions 162
6.8 Win-minus-loss contrast separated by the four participant groups 164
6.9 Tolcapone effect on valence (win-minus-loss contrast) 167
6.10 Genotype x tolcapone interaction effects on the win-minus-loss contrast 168
6.11 No COMT effects on magnitude 170
6.12 Inverted-U relationship between DA and risk aversion 173

7.1 Schematics of possible COMT genotype x tolcapone interactions 190

List of Tables

1.1 The dopaminergic pathways of the brain 3

1.2 Factors influencing the MEG signal 19

1.3 Basis for a difference in MEG signal 21

2.1 Demographics of the study cohort 36

3.1 Demographics of subjects used in final N-back sample 56

3.2 General performance by N-back load 58

3.3 Reaction time for N-back task 61

4.1 Demographics of the performance-matched study cohort 77

5.1 Demographics of the subjects 115

6.1 Gambling task reaction times 155

6.2 COMT Val^{158}Met genotype difference in behaviour and evoked responses to valence and magnitude 178

7.1 Summary of findings and their interpretation 187

List of Abbreviations

3-MT- 3-METHOXYTYRAMINE

5-HT- SEROTONIN

5-HTT- SEROTONIN TRANSPORT PROTEIN

ACC- ANTERIOR CINGULATE CORTEX

ADHD- ATTENTION DEFICIT HYPERACTIVITY DISORDER

AMPH- AMPHETAMINE

ANOVA- ANALYSIS OF VARIANCE

ATP- ADENOSINE TRIPOSPHATE

BDI- BECK DEPRESSION INVENTORY

BOLD- BLOOD-OXYGEN-LEVEL-DEPENDENT

cDNA- COMPLEMENTARY DEOXYRIBOSE NUCLEIC ACID

CFS- CEREBROSPINAL FLUID

COMT- CATECHOL-O-METHYLTRANSFERASE

DA- DOPAMINE

DAT- DOPAMINE TRANSPORTER

DHMA- 3,4-DIHYDROXYMANDELLIC ACID

dlPFC- DORSOLATERAL PFC

DOPAC- 3,4-DIHYDROXYPHENYLACETIC ACID

d.p.- DECIMAL PLACE

EEG- ELECTROENCEPHALOGRAPHY

EPSPs- EXCITATORY POSTSYNAPTIC POTENTIAL

ERF- EVOKED RELATED FIELD

FFA-FUSIFORM FACE AREA

fMRI- FUNCTIONAL MAGNETIC RESONANCE IMAGING

fT/cm- FEMTOTESLA PER CENTRIMETRE

GABA- GAMMA-AMINOBUTYRIC ACID

GPCR- G-PROTEIN COUPLED METABOTROPIC RECEPTORS

HPI- HEAD POSITION INDICATOR

HVA- HOMOVANILLIC ACID

Hz-HERTZ

IPSPs- INHIBITORY POSTSYNAPTIC POTENTIALS

IOG- INFERIOR OCCIPITAL GYRUS

IQ- INTELLIGENCE QUOTIENT

L-Dopa- LEVADOPA

LSD- LEAST SIGNIFICANT DIFFERENCE

MB-COMT – MEMBRANE-BOUND CATECHOL-O-METHYLTRANSFERASE

MEG- MAGNETOENCEPHALOGRAPHY

Met-METHIONINE

Mets- REFERRING TO $MET^{158}MET$ HOMOZYGOTES

MFN- MEDIAL FRONTAL NEGATIVITY

Mg- MILLIGRAMS

Ml- MILLILITRES

µl- MICROLITRES

N170- THE COMPONENT 170 MILLISECONDS AFTER FACE PRESENTATION (EEG DATA)

NART- NATIONAL ADULT READING TEST

NAT- NORADRENALINE TRANSPORTER

NEH- NEURAL EFFICIENCY HYPOTHESIS

nm- NANOMETRES

NMDA –N-METHYL-D –ASPARTIC ACID

OFC- ORBITOFRONTAL CORTEX

OHBA- OXFORD CENTRE FOR HUMAN BRAIN ACTIVITY

P300- THE COMPONENT 300MS AFTER STIMULUS PRESENTATION (EEG DATA)

PBO- PLACEBO

PCA- PRINCIPAL COMPONENT ANALYSIS

PCR- POLYMERASE CHAIN REACTION

PET- POSITRON EMISSION TOMOGRAPHY

PFC- PREFRONTAL CORTEX

PPI- PREPULSE INHIBITION

qPCR- QUANTITATIVE POLYMERASE CHAIN REACTION

RT- REACTION TIME

S-COMT- SOLUBLE CATECHOL-O-METHYL TRNASFERASE

s.d.- STANDARD DEVIATION

s.f.- SIGNIFICANT FIGURES

SNP- SINGLE NUCLEOTIDE POLYMORPHISM

SQUID- SUPERCONDUCTING QUANTUM INTERFERENCE DEVICE

STAI –SPIELBERGER TRAIT ANXIETY INVENTORY

T1- TIME 1

T2- TIME 2

T3- TIME 3

TMS- TRANSCRANIAL MAGNETIC STIMULATION

TOLC-TOLCAPONE

VAS-VISUAL ANALOGUE SCALES

Val- VALINE

vlPFC- VENTROLATERAL PFC

VMA- VANILLYLMANDELIC ACID

vs.-VERSUS

VSTR-VENTRAL STRIATUM

VTA- VENTRAL TEGMENTAL AREA

WCST- WISCONSIN CARD SORTING TASK

WM- WORKING MEMORY

Y- YEARS

Chapter 1

Introduction

Chapter 1

1. Introduction

1.1 Dopamine

The late 1950s saw the classification of Dopamine (DA) as a distinct bioactive substance in the brain and peripheral tissues (Carlsson, 1958). The four principal DA pathways in the brain are detailed in Table 1.1, and comprise: the nigrostriatal DA pathway, arising from the substantia nigra pars compacta and projecting to the basal ganglia; the mesolimbic pathway, arising from the ventral tegmental area (VTA) and projecting to the limbic striatum (nucleus accumbens) and olfactory tubercle; the mesocortical pathway, arising from the VTA, projecting mainly to the cingulate, entorhinal and medial prefrontal cortices (PFC); and the tuberoinfundibular pathway, from the hypothalamus to the pituitary gland (Lindvall and Bjorklund, 1984). These pathways are involved in motor control, motivation behaviour and cognition, and their dysregulation results in neuropathological or neuropsychological disorders.

The action of DA depends on the type of receptor activated. There are five DA receptor subtypes, all of which are transmembrane G-protein coupled metabotropic receptors (GPCRs). D_1 and D_5 are classed as 'D_1-like' and the rest as 'D_2-like'. D_1-like receptors are positively coupled to the adenylate cyclase pathway[1], whereas D_2-like receptors are negatively coupled to this signalling cascade (Greengard, 2001; Girault and Greengard, 2004). Different receptors have distinct distributions (Farde et al., 1987; Lidow et al., 1991; Gaspar et al., 2011).

[1] Activation leads to conversion of adenosine triposphate to cyclic adenosine monophosphate (ATP to cAMP), activating cyclic AMP-dependent protein kinases to phosphorylate multiple protein substrates by attaching phosphate groups to them; mediating responses in the cell e.g upregulation of transcription. cAMP stops signalling when it is degraded by the enzyme phosphodiesterase. For review see Lachowicz and Sibley, (1997).

Table 1.1 The dopaminergic pathways of the brain

Dopaminergic pathway	Location of origin	Target location	Primary Functions
Nigrostriatal	Substantia nigra pars compacta	Striatum (i.e. caudate nucleus and putamen)	Motor control; some cognitive functions
Mesolimbic	VTA (ventral tegmental area)	Nucleus accumbens, tuberculum olfactorium	Arousal; movement and reward-related behaviours
Tuberoinfundibular	Hypothalamus	Pituitary gland	Control secretion of prolactin and somatotrophin
Mesocortical	Midbrain neurons	Septum, amygdala, prefrontal cortex, brainstem, hippocampus and other limbic areas	Cognition

1.2 An inverted-U relationship between prefrontal DA and cognition

The prefrontal cortex (PFC) densely expresses DA receptors, predominantly D_1 (Lidow, et al., 1991), and receives a large network of ascending inputs from midbrain dopaminergic neurons (Robbins, 2000). Brozoski's seminal paper demonstrated the significant role of DA and other catecholamines in primate cognition (1979). In those studies, working memory (WM) (see Chapter 3 section 3.1 for further details on WM) was impaired in non-human primates after DA (but not serotonin) depletion in the PFC. This observation has been replicated several times (Roberts et al., 1994; Dias et al., 1996; Collins et al., 2000; Crofts et al., 2001). Animal and human studies have since confirmed the requirement for DA in WM (see Chapter 3, section 3.2 for details).

Goldman-Rakic (1995) described the relationship between DA levels in the PFC and PFC function as an inverted-U-shape curve, see also: (Goldman-Rakic, 1995; Goldman-Rakic et al., 2000), shown in Figure 1.1. This was suggested after studies demonstrated that WM (a process which is critically dependent on PFC DA function) is impaired by local infusions of both D_1 antagonists and agonists in the PFC, with a similar pattern shown (albeit to a lesser extent) by D_2 antagonists and agonists; thus, optimal WM performance is seen at intermediate levels of D_1/D_2 stimulation (Sawaguchi and Goldman-Rakic, 1991; Williams et al., 1995; Zahrt et al., 1997).

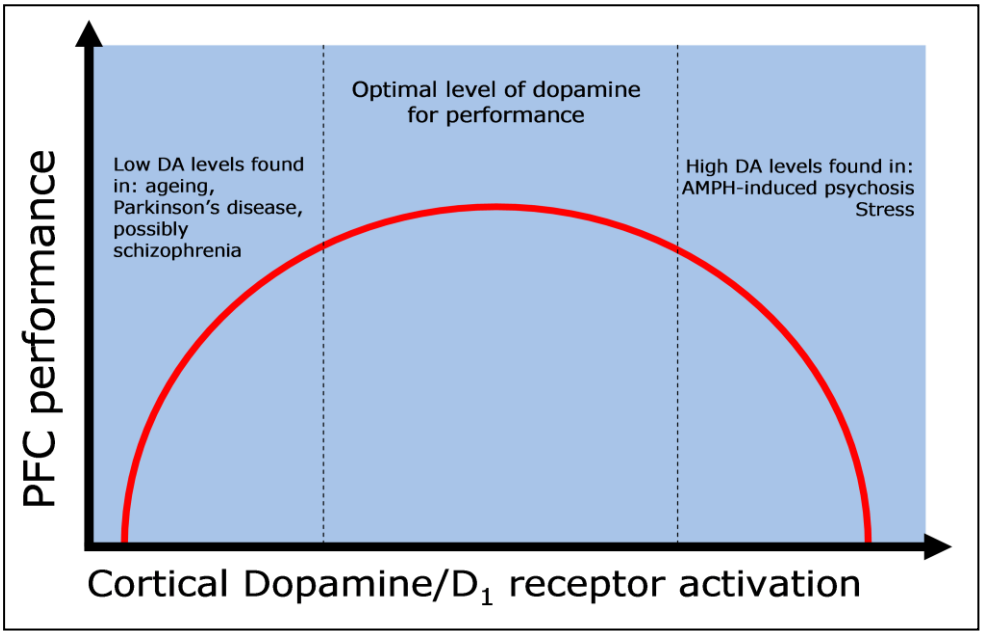


Figure 1.1 An inverted-U relationship between DA and PFC performance. An intermediate level of DA is optimal for WM performance, with both sub- and supra-optimal levels of DA resulting in comparatively worse performance.

Further studies have since confirmed that an excessive increase in DA levels leads to worse PFC performance during WM tasks. For example, D_2 receptor agonists, such as bromocriptine and pergolide administered to healthy young adult humans, reduced performance on WM tasks (Luciana et al., 1992; Kimberg et al., 1997; Kimberg and D'Esposito, 2003). Importantly, this phenomenon is thought to be specifically mediated by excessive stimulation of

the PFC DA system because it was observed in the absence of sedation or changes in sensory motor skills (Luciana et al., 1992).

A number of complementary theories have attempted to explain the inverted-U relationship between PFC DA levels and performance on cognitive tasks (including WM tasks). Goldman-Rakic (1995) proposed that the inverted-U relationship is explained by the action of DA at D_1 receptors. The stimulation of D_1 receptors occur on both pyramidal cells and interneurons, but the effect on pyramidal cells is predominant at low DA levels. Enhanced pyramidal cell firing leads to increased WM performance. However, at some point the D_1 effect on pyramidal cells will plateau and further increase in DA will result mainly in enhancement of interneuron activity, leading to synapses onto pyramidal neurons that inhibit this neuron from firing (Goldman-Rakic et al., 2000). The schematic in Figure 1.2 depicts the three cell types in a circuit along with these three suboptimal, optimal and supraoptimal states. Evidence for this circuitry includes the closer proximity of pyramidal cells to DA release sites than interneurons (Krimer et al., 1997) suggesting initial DA increases affect pyramidal cells and further increases have a greater effect on interneurons. Further evidence is observed along the axon of pyramidal neurons, where diffusion barriers maintain a high concentration of second messengers at associated excitatory synapses, whilst on interneurons, D_1 receptors are located on the dendritic shaft, which allows more diffusion of second messengers and thus a reduced effect (Muller and Connor, 1991; Koch and Zador, 1993).

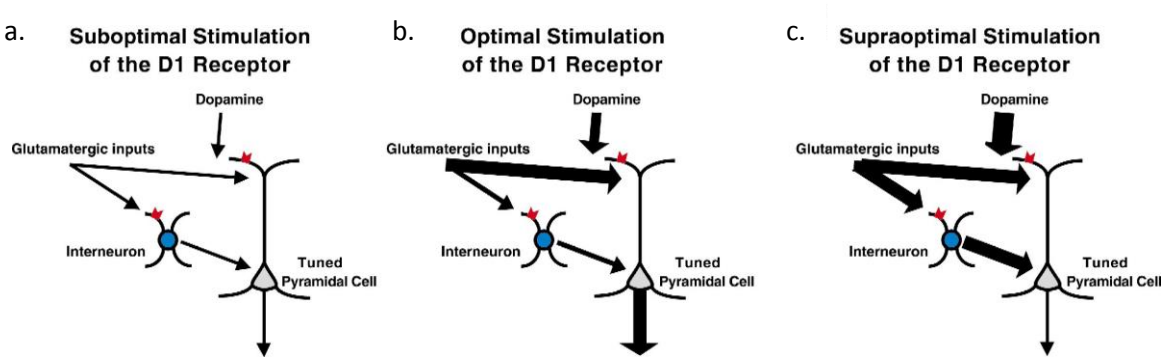


Figure 1.2 A model for the neural mechanism of the inverted-U DA curve. Goldman-Rakic's model for the neural mechanism behind the inverted-U DA curve (taken from Goldman-Rakic et al., 2000) would suggest that D_1 receptor stimulation is at optimal levels in b. where the pyramidal cell is heavily stimulated with glutamatergic afferents compared to inhibitory interneuron stimulation. The diagram in a. represents an under-stimulation from the glutamatergic afferents and c. represents an overstimulation of the inhibitory interneuron. The higher the level of excitatory stimulation to the pyramidal cell, the more likely the pyramidal cell is to fire.

Seamans' (2001) 'dual state' theory takes a different mechanistic approach, and highlights the competing roles of D_1 and D_2 receptors. This is not necessarily mutually exclusive from the above explanation but focuses on the balance between D_1 and D_2 signalling at a network level. Thus, D_1 and D_2 effects are opposing, as demonstrated by the effects of NMDA and GABA-A currents *in vitro* after administering D_1 and D_2 agonists. The two receptors are therefore predicted to have opposite effects on the general excitability of PFC pyramidal neurons (Yang and Seamans, 1996; Gulledge and Jaffe, 1998; Zheng et al., 1999; Seamans et al., 2001; Trantham-Davidson et al., 2004; Tseng and O'Donnell, 2004). The activation of D_1 leads to increases in NMDA currents, which boost recurrent excitation. It also increases $GABA_A$ currents which, the dual state theory argues, has the effect of limiting the set of items encoded in WM by exacerbating the competition between different active groups of neurons. However, this D_1-mediated $GABA_A$ current enhancement, along with reduced glutamate release, also makes it difficult to evoke initial activity. Thus D_1-mediated activity reduces the chances of reaching a threshold of excitability, but increases the ability to stay activated once this is achieved. Therefore, higher D_1 activation from an increase in extrasynaptic DA would increase stability of a particular state of activity, which might aid WM by increasing the robustness of a given WM item

that is encoded by this activity state. The theory thus posits that D_1-predominant conductance makes it difficult to switch between various high activity memory states. In contrast, the role of D_2 activation in this theory is to reduce the general level of neuronal activity and hence reduce the barrier among activity states, allowing the flexible updating of neuronal representations (Durstewitz and Seamans, 2008).

Winterer and colleagues (2004) integrate the 'dual-state' ideas into a network-level theory, focussing on how DA produces optimal signal-to-noise ratio in the PFC resulting in optimal cognitive function (Winterer and Weinberger, 2004). Evidence for the significance of the signal-to-noise ratio includes reports that the amount of frontal background noise is a predictor for poor performance on frontal lobe-dependent cognitive tasks (Winterer et al., 2004b). Single unit recordings of action potentials in the cortex of both rats and non-human primates have shown that the administration of amphetamine (which increases synaptic DA levels), or stimulation of D_1 receptors, reduces the sporadic firing of pyramidal neurons and increases spike rates in selective frequency bands, thereby enhancing the signal-to-noise ratio. These pharmacological effects on single neuron activity are accompanied by improvements in performance on a range of tasks, including choice reaction type[2] as well as oddball tasks[3]. Thus, the increase in the signal-to-noise ratio appears to be mediated by D_1 receptor stimulation working to enhance excitement of target pyramidal cells and increase inhibition of unnecessary surrounding pyramidal cells through activation of the afferent inhibitory interneurons. Thus, the theory draws on ideas from Goldman-Rakic's hypothesis detailed above (Goldman-Rakic et al., 2000).

The relationship between the above and the 'dual-state' theory is qualified in Figure 1.3. A high signal-to-noise ratio is encoded by a predominantly D_1 state, enabling stable encoding of

[2] Participants are expected to respond in a certain way for a certain stimulus e.g. press the right button if a red light is shown and the left button if a yellow light is shown.
[3] Participants respond to a stimulus that is infrequent.

items in WM. A low signal-to-noise ratio is encoded by a predominantly D_2 state, enabling the updating of items in WM.

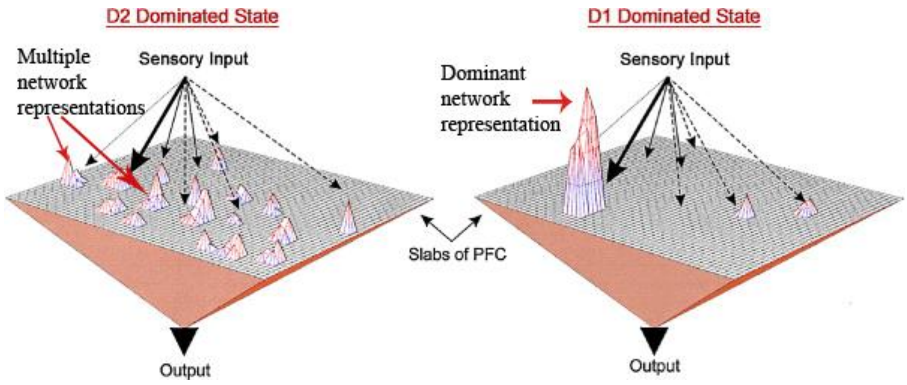


Figure 1.3 A representation of DA contributions to signal-to-noise ratio. The D_1 state (shown on the right) has a high energy barrier required to switch between different network states, resulting in WM representations becoming robust to distraction but less flexible in terms of switching between different activity states (Durstewitz and Seamans, 2008). A D_2 state (shown on the left) involves a reduced energy barrier and concomitant low stability of WM representations (modified from Seamans and Yang, 2004).

Although the theories described above focus on PFC DA and its role in WM, DA is a key neurotransmitter in many aspects of brain function (some of which will be discussed in the following chapters). There is some evidence for inverted-U relationships in other DA-modulated processes, including some reward-related paradigms such as reversal-learning in response to negative feedback (van der Schaaf et al., 2011) and sensory gating (Millar et al., 2011). However, none of these other processes have been as well-studied as WM. For a review of DA and the inverted-U see Cools and D'esposito (2011). Further discussion on the inverted-U can be found in Chapter 7, section 7.2.

1.3 Catechol-O-methyltransferase (COMT)

1.3.1 COMT isoforms, locations and enzymology

COMT is an enzyme which was first identified and characterized by Axelrod and Tomchick in 1958. It is one of three enzymes (along with monoamine oxidase and aldehyde dehydrogenase)

involved in the degradation of DA to homovanillic acid (HVA) and the degradation of noradrenaline to vanillylmandelic acid (VMA). Figure 1.4 illustrates this pathway.

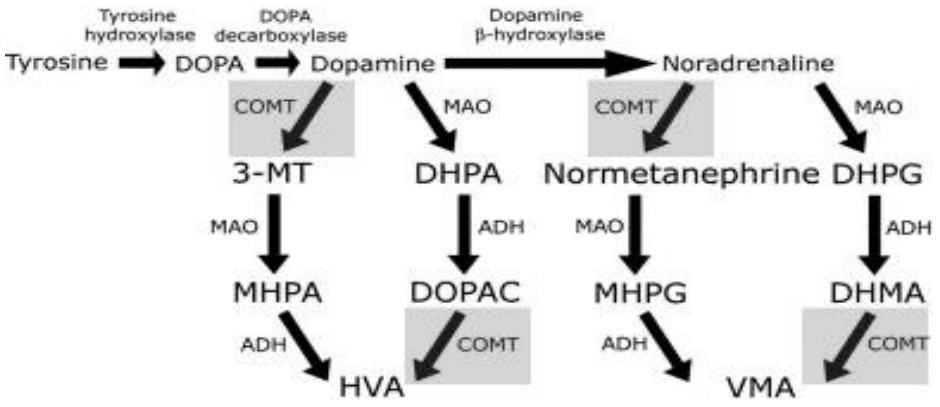


Figure 1.4 The synthesis and metabolism of DA and noradrenaline. COMT is involved in DA metabolism as it converts DA to 3-methoxytyramine (3-MT) and 3,4-dihydroxyphenylacetic acid (DOPAC) to HVA. During noradrenaline breakdown, COMT metabolises noradrenaline to normetanephrine and 3,4-dihydroxymandelic acid (DHMA) to VMA.

The COMT gene is located on chromosome 22q11 (Grossman et al., 1992). It encodes two major transcripts (Lundstrom et al., 1991). The long transcript can be translated into both major isoforms of COMT; the membrane-bound (MB) and the soluble (S) forms. MB-COMT is the predominant form of COMT in the brain and is postulated to play an important role in modulating cortical DA signalling (Matsumoto et al., 2003; Meyer-Lindenberg et al., 2005; Papaleo et al., 2008). MB-COMT has a higher affinity for DA than S-COMT, and is more important for inactivation of DA in the brain (Lotta et al., 1995).

It has previously been proposed that these different forms of the enzyme might be localized differently. S-COMT could be localized to glia and MB-COMT to neurons (Roth, 1992). Ultrastructurally, several studies suggest MB-COMT is present on the plasma membrane and therefore potentially protrude into the synapse (Raxworthy et al., 1982; Kastner et al., 1994). However, other studies indicate COMT is exclusive to intracellular membranes, including the rough endoplasmic reticulum (Ulmanen et al., 1997), implying that DA would have to be

transported from outside the cell for it to be metabolized by COMT (Myohanen et al., 2010). A more recent study of rat cortical neurons reconciles previous investigations, concluding that MB-COMT is located within the cell body, axons, and dendrites, but protrudes outwards. The study analysed the orientation of MB-COMT with computer simulation, flow cytometry and a cell surface enzyme assay to reveal that the C-terminal catalytic domain of MB-COMT is in the extracellular space, suggesting MB-COMT can inactivate synaptic and extrasynaptic DA on the surface of presynaptic and postsynaptic neurons (Chen et al., 2011).

1.3.2 COMT Valine^{158}Methionine (Val^{158}Met) polymorphism

The Val^{158}Met polymorphism (Lachman et al., 1996; Chen et al., 2004) is a common functional polymorphism at codon 158 in MB-COMT (or codon 108 in S-COMT). The alleles are co-dominant, and Met homozygous subjects have significantly lower activity than Val homozygous subjects; in the brain, the difference is about 40% (Chen et al., 2004), see Figure 1.5. Europeans have nearly equal frequencies of the two alleles, although the frequencies of the two alleles vary markedly in other populations (Palmatier et al., 1999).

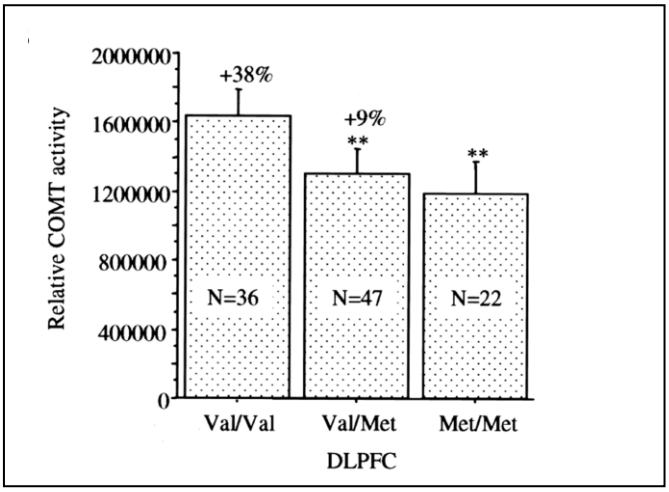


Figure 1.5 Enzyme activity in the Val^{158}Met polymorphism. Val homozygous subjects have higher enzyme activity in the PFC than Met homozygous subjects (taken from Chen et al., 2004).

Lowering COMT activity (manipulated either genetically or pharmacologically) has been shown to increase DA transmission in the rodent PFC (Karoum et al., 1994; Gogos et al., 1998; Tunbridge et al., 2004; Yavich et al., 2007; Papaleo et al., 2008). Although it has never been shown directly that the Val^{158}Met polymorphism modulates cortical DA levels in humans (as this is not technically possible), PET data are consistent with this hypothesis. That is, D_1 receptor availability in the PFC has been shown to be greater in Val, compared to Met homozygous subjects (Slifstein, 2008). Given that previous studies have shown lower levels of cortical DA to be associated with higher D_1 receptor availability (Abi-Dargham et al., 2002), it is reasonable to suggest that Val homozygous subjects have lower cortical DA levels. Thus, it is plausible that the Val^{158}Met polymorphism influences PFC DA levels. Figure 1.4 suggests that COMT activity will also affect noradrenaline metabolism. However, evidence that the role of COMT is significant for regulating central noradrenaline levels is lacking. Notably, all the animal studies that demonstrated effects of COMT on DA function have failed to find any effect on noradrenaline (Gogos et al., 1998; Tunbridge et al., 2004). Thus the impact of COMT is discussed throughout this thesis only in terms of effects on DA, although I cannot rule out a noradrenergic basis for some of the effects.

Although the Val^{158}Met polymorphism is the major genetic determinant of prefrontal COMT activity (Chen et al., 2004), other polymorphisms within the gene also affect its activity. Most notably, a haplotype within the COMT gene, which includes the Val^{158}Met polymorphism, has a greater influence on enzyme activity than the Val^{158}Met polymorphism alone (Nackley et al., 2009). However, the Val^{158}Met polymorphism remains the most widely studied polymorphism, and the single locus that explains the greatest variance in enzyme activity, and is therefore the focus of this study.

1.3.3 COMT Val^{158}Met, PFC DA and the inverted-U

Since COMT modulates PFC DA, and PFC DA is crucial for normal cognitive function, it seems likely that COMT influences cognitive processing. The first study to address this question was the landmark paper by Egan and colleagues, examining the relationship between Val^{158}Met and the Wisconsin Card Sorting Task[4] (WCST) of executive cognition, and neurally using an N-back WM task in an fMRI scanner. Performance on the WCST was better in Met homozygous subjects than Val homozygous subjects, with heterozygotes scoring intermediate levels; where the allele explained 4% of the difference in scores (Egan et al., 2001). Subsequent behavioural studies pertaining to COMT and WM have had mixed results, and will be discussed in more detail in Chapter 3, section 3.1.2.

Egan also found a Val^{158}Met effect on the blood-oxygen level dependent (BOLD) response (a measure of fMRI activity) during the N-back task. Activity in dorsolateral prefrontal and cingulate areas was lower in Met homozygous subjects than Val homozygous subjects. As before, the heterozygous subjects showed the intermediate phenotype. This led to the interpretation of more efficient processing in those with the Met allele (Egan et al., 2001). Section 1.5 discusses BOLD response and 'efficiency'. Since this initial demonstration by Egan, there have been numerous other similar studies, and the association between Val^{158}Met and PFC activity (as measured by BOLD response) has been consistently replicated, and is significant by meta-analysis (Mier et al., 2010).

The fMRI studies of drug manipulation of DA in subjects' genotyped for COMT Val^{158}Met have demonstrated inverted-U features. The high level of BOLD response in the PFC of Val homozygous subjects was attenuated when DA levels were increased with amphetamine during the N-back WM task (Mattay et al., 2003) or with the COMT-inhibitor tolcapone during a verbal

[4] In this task, cards of different colours, shapes and number of objects appear. Participants have to match cards depending on a rule (e.g. 'match by colour'), which changes throughout the task.

episodic memory task (Apud et al., 2007; Roussos et al., 2009), where fMRI activity became more 'Met-like'. In contrast, tolcapone administration to the Met homozygous group decreased PFC activity in line with that expected from the inverted-U hypothesis.

1.4 Genetic vs. pharmacological manipulation of COMT activity

This thesis will examine the separate and interactive effects of genetic vs. pharmacological manipulation of COMT activity. I will use the Val^{158}Met polymorphism to examine the effect of genetically-encoded differences in enzyme activity levels of COMT. In addition, I will study the effects of tolcapone, a COMT inhibitor which penetrates the blood-brain barrier (Ceravolo et al., 2002; Da Prada et al., 1991). Relevant to my work, a single oral dose of 200mg tolcapone in humans causes a 70-80% inhibition of blood COMT-activity after 1.8hrs and lasts for 1-4hours, with an elimination half-life of 2.1hrs (Dingemanse et al., 1995).

Tolcapone is used in Parkinson's disease as an adjunct to levodopa, its use limited by hepatotoxicity (see Chapter 7, section 7.2). Effects of tolcapone on WM have never been tested in clinical trials, although Gaspirini and colleagues found that chronic tolcapone treatment for six months improved the performance of eight patients with advanced Parkinson's disease on tests of attention, memory, and executive function (Gasparini et al., 1997). However, tolcapone has been used experimentally, as will be introduced in Chapter 3, section 3.1.3.

1.4.1 Interaction of tolcapone with genotype

The effect of tolcapone may differ between Val and Met homozygous subjects both behaviourally and in terms of BOLD response activity. This has been demonstrated in three studies (Apud et al., 2007; Giakoumaki et al., 2008; Roussos et al., 2009), all of which have found

some evidence of effect on either performance or activity as measured by fMRI (see section 3.1.3).

Notably, whilst both the Val^{158}Met polymorphism and tolcapone administration impact on COMT activity, their impact on brain function is not necessarily expected to be identical. Specifically, in contrast to acute COMT inhibition, genetically-encoded differences in COMT activity are presumably present throughout the lifespan. This raises the possibility that some of the effects of the Val^{158}Met polymorphism may have arisen through neurodevelopmental adaptation, which may contrast with the direct impact of transient COMT inhibition in the adult brain by tolcapone.

A single, oral administration of tolcapone provides an effective and feasible pharmacological tool to study the consequences of short-term COMT inhibition, complementing the genetic dissection (and presumably 'chronic' differences in COMT activity) provided by the Val^{158}Met polymorphism. Moreover, as outlined above, there are some data suggesting an interaction between tolcapone administration and genotype (Apud et al., 2006; Giakoumaki et al., 2008), akin to the amphetamine study mentioned earlier (Mattay et al., 2003). This concept will be explored further in the current research.

1.5 Measuring neural activity

There are no studies that use MEG to explore COMT. However, many fMRI studies are present among the COMT literature, therefore to contextualise the findings of this thesis, comparisons will be made between data from these two fields, thus requiring an understanding of the differences between them. Therefore, although this study does not contain fMRI analysis, I

present an explanation of the BOLD response and the concept of efficiency in fMRI studies, as well as a guide to how MEG Is measured.

1.5.1 fMRI

The BOLD response

The BOLD response is used in fMRI as an indirect measure of neural activity. It is brought about by transient changes in oxygenated haemoglobin. The changes result from a switch in the proportion of deoxyhaemoglobin (which is paramagnetic) and oxygenated haemoglobin (which is not paramagnetic) thus causing alterations in the magnetic resonance signal. A signal increase stems from an increase in cerebral blood flow overcompensating for the decrease in oxygen saturation (Fox and Raichle, 1986; Fox et al., 1988). The BOLD response is of the order of seconds, beginning roughly two seconds after a sensory or motor act (Kwong et al., 1992), rising to a plateau 6-9 seconds after stimulus onset, before returning to baseline. Optogenetic techniques in rats have been used to measure BOLD correlates of neuronal inhibition and excitation (Lee et al., 2010). The BOLD responses were shown to correlate positively with excitatory post-synaptic potentials (EPSPs) and negatively with inhibitory postsynaptic potentials (IPSPs), and a number of studies show a linear relationship between the two (Mathiesen et al., 1998; Brinker et al., 1999; Rees et al., 2000). For a review on the BOLD signal and its physiological basis, see Logothetis and Wandell (Logothetis and Wandell, 2004).

The neural efficiency hypothesis

The Neural Efficiency Hypothesis (NEH) was first expounded by Haier et al. (1998) in a PET study. Eight participants demonstrated an inverse relationship between brain glucose metabolism and performance of Raven's Advanced Progressive Matrices, a test for intelligence (Haier et al., 1988). Many fMRI studies have since produced evidence in line with the NEH, particularly in relation to cognition (Reichle et al., 2000; Rypma et al., 2002; Weissman et al., 2002; Knauff et al., 2003; Ramsey et al., 2004; Prat et al., 2007). These studies all suggested a

negative relationship between intelligence and brain activation, namely the larger the BOLD response, the more 'inefficient' the cognition. For an exhaustive list of these studies see Neubauer and Fink (2009).

The NEH, however, has been contested, with a body of studies providing contradictory evidence, which shows greater activity relating to greater cognitive ability. These studies typically involved long term memory tasks (Klimesch et al., 1996; Klimesch et al., 1997; Klimesch et al., 1999; Grabner et al., 2003; Klimesch et al., 2006). For example, a study by Haier et al. (2003) found brighter individuals (as judged by higher scores in non-reasoning tasks of intelligence) exhibited higher activation, especially in areas outside frontal regions (Haier et al., 2003). Other studies using tasks that previously yielded evidence in favour of NEH (see Vitouch et al., 1997), reported no relationship between brain activation and ability (Lamm et al., 2005).

Some studies reconcile these differences, reporting that NEH may only be valid for certain demographics, certain tasks, task difficulty, and certain parts of the brain. For full review see Table 2 in Neubauer and Fink (2009). The latter study concludes that NEH validity may be restricted to, or at least particularly important for, frontal regions of the brain for low to moderate difficulty tasks or high difficulty tasks when enough practice occurred for strategies to develop.

COMT and neural efficiency

COMT is likely to affect neural efficiency given that DA levels have been shown to affect the BOLD response in the PFC. By depleting and replenishing DA in patients with Parkinson's disease, Mattay and colleagues provided evidence that cortical regions in WM tasks exhibit greater activation during the hypodopaminergic state, in line with the NEH (Mattay et al., 2002).

As with NEH in general, some COMT studies support the hypothesis, others do not. As mentioned previously (section 1.3.3), several studies have suggested that Met homozygous subjects (deemed to be the higher cognitive performers) are associated with decreased neural activation (Mier et al., 2010). Thus, Val^{158}Met affects BOLD in PFC during working memory, robustly, therefore supporting NEH. However, data in other regions and tasks are less clear. One study illustrates further complexities demonstrating that Met homozygous individuals (who perform higher in the Multi-source interference task) have lower activity in some frontal areas, but higher activity in others, ostensibly demonstrating both greater efficiency and greater recruitment capacity in separate regions during the task (Green et al., 2012). Interestingly, treatments that transiently increase DA availability in PFC also appear to lead to lower BOLD responses and are associated with intelligence (Meyer-Lindenberg et al., 2005; Apud et al., 2007).

The studies that appear to contradict the NEH specifically relating to Val^{158}Met comparisons, report higher activation in Met homozygous subjects. This includes BOLD response measures during a response inhibition task (Congdon et al., 2009), a visual oddball task (Winterer et al., 2006) and an N-back task when focussing on areas outside of the frontal regions (Stokes et al., 2011). Nevertheless, there is a broad consensus that, at least in dlPFC during certain WM tasks, there is a greater 'efficiency' (i.e. lower BOLD response) in Met than Val homozygous subjects; for reviews see Heinz and Smolka (2006) and Dickinson and Elvevag (2009). As such, one of the questions to be addressed here (especially in Chapter 4) is the extent to which MEG detects any plausible correlate of this 'efficiency' finding.

1.5.2 Magnetoencephalography

This thesis applies magnetoencephalography (MEG). MEG is a non-invasive technique that allows the detection of minute magnetic fields outside the skull associated with neural activity. This is a relatively new technology opening another window into brain dynamics on a

millisecond scale previously accessible only through electroencephalography (EEG) and related techniques (Hari and Lounasmaa, 1989; Hämäläinen et al., 1993; Vrba and Robinson, 2001). Unlike imaging methods such as MRI and PET which measure brain activity indirectly via the haemodynamic response and metabolic effects (Parra et al., 2004), MEG signals are assumed to directly reflect neural activity.

In the neuron's resting state, a small voltage of about 70mV is maintained across the cell membrane, with the inside of the cell being negatively charged. Information is coded by changes in this membrane potential, and certain changes, at suitable locations, can cause action potentials. Associated with action potentials are ionic currents that give rise to time-varying magnetic fields. These fields fluctuate with a time scale of approximately 1ms and a field strength decreasing rapidly with distance from source as $1/d^3$ (where d is distance). When an action potential reaches a synapse, a neurotransmitter is released crossing the synaptic cleft, giving rise to a postsynaptic potential, which is accompanied by a current flow. As a consequence, fluctuating magnetic fields are generated at the neural membrane with a typical time scale of about 10 to 100ms and a strength decreasing with distance from source as $1/d^2$.

The postsynaptic potentials (EPSPs more than IPSPs and calcium currents) are thought to be more influential in the MEG signal than action potentials (Buzsaki et al., 2012). This general consensus is agreed for two main reasons:

1. The strength to distance relationship is more favourable for postsynaptic potentials than action potentials ($1/d^2$ vs $1/d^3$).
2. The longer time scale (10ms and longer compared to 1ms) facilitates a coherence, or summation over thousands of neurons needed for a signal of detectable strength.

Pyramidal neurons in the cortex are thought to provide the biggest contribution to the MEG signal, as they are arranged in palisades with apical dendrites aligned perpendicular to the skull (da Silva, 2010), whereby the geometry of the cell's apical dendrites supports a spatial summation of postsynaptic currents orthogonal to the cortical surface. Note that this necessary perpendicular alignment only occurs in the walls of the sulci, as opposed to the top of the cortical gyri, where the pyramidal cells are parallel to the skull and thus do not create an 'open field' (see Figure 1.6). For reviews see da Silva (2010) and Buzsáki et al. (2012).

1.5.2.1 COMT and MEG signal

In summary, the MEG signal depends on a multitude of factors including the location and depth of the source, orientation of neurons activated, synchrony of population neural activity among pyramidal neurons and apical dendrites (it takes approximately 50 000 in a 0.6mm^2 for coils to pick up a signal), density of the neurons, and synaptic activity generated at the dendrites. These are listed in Table 1.2.

Table 1.2 Factors influencing the MEG signal

Cause	**Cause type**	**Notes**
Location of neurons	anatomical	
Depth of neurons	anatomical	
Orientation of neurons	anatomical	Alignment perpendicular to skull
Density of neurons	anatomical	
Synchronous activity	functional	Greater than 50 000 in 0.6mm^2
EPSPs and IPSPs	functional	EPSPs>IPSPs
Ca^+ currents	functional	

Factors influencing the MEG signal are classified as 'anatomical' and 'functional' based causes. The biggest influence on MEG signal is the synchronous activity of pyramidal neurons in alignments perpendicularly orientated to the skull, and the postsynaptic potentials made up of EPSPs and IPSPS, the former of which is thought to have the predominant effect.

In the context of this somewhat limited level of understanding of the information generated by MEG, I will assume that a difference in MEG signal or 'activity' corresponds to a difference in synchrony of firing among groups of pyramidal neurons. In turn, I assume that this

change in the synchrony arises from a change in EPSPs (although changes in IPSPs and changes in calcium currents could also contribute). However, if this is what MEG is measuring, then how might COMT activity affect this? We can explore this question in terms of the factors mentioned in Table 1.2, or the circuitry that might be affected by DA and COMT. I will now take these in turn.

Changes in DA levels by differential COMT activity may alter the average synchronous activity that gives rise to the MEG signal. Table 1.3 summarises how this might take place and suggests whether signals could be modified by COMT genotype or tolcapone administration.

Differences in MEG signal could result from 'anatomical' causes (e.g. cell density, orientation and dendritic diameter), or a 'functional' cause (e.g. a difference in number, signalling and size of EPSPs and IPSPs). The anatomical causes are unlikely to be influenced by COMT, but it is possible that Val and Met homozygous subjects might have different pyramidal neuron packing, placements etc., as a correlate of different amounts of DA/COMT activity, during brain development and maturation. To my knowledge there are no such data on this, although some research claims that the COMT Val^{158}Met polymorphism is associated with differences in white matter architecture (Li et al., 2009). Genotype differences in MEG signal are more likely to be due to a 'functional' cause, and differences due to acute drug administration can only plausibly be explained in this way. Thus, for this discussion, I will assume that MEG signal differences caused by both genotype and tolcapone are predominantly mediated by the 'functional' factors. Therefore, higher signal is taken to be a result of more synchronous activity of the pyramidal neurons under the MEG gradiometers (see Chapter 2, section 2.8.1 for MEG technical details). This in turn could reflect either a greater synchrony of the same population of neurons, or a greater proportion of neurons firing synchronously, or possibly larger post synaptic potentials.

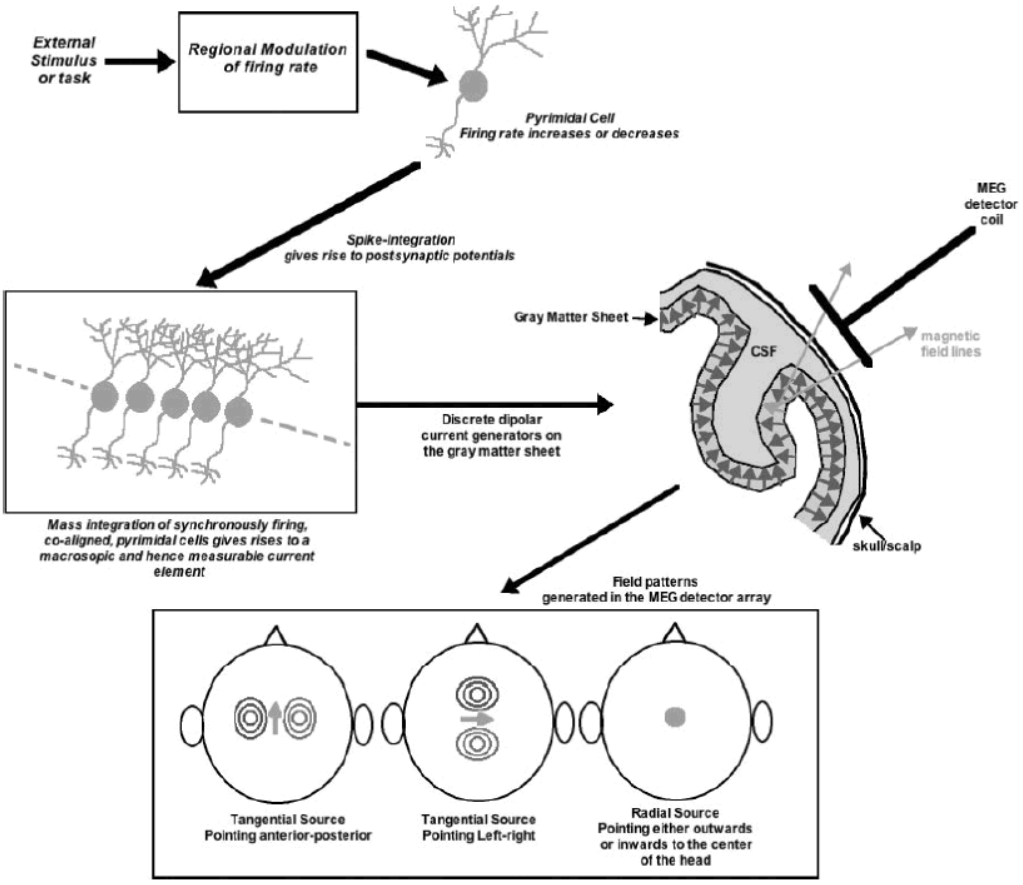


Figure 1.6 Measurable neuromagnetic fields. This schematic demonstrates how a detectable neuromagnetic field may be generated. Neurons are required to be based in the walls of the sulci, perpendicular to the scalp. These neurons must be groups of synchronously firing aligned cells, such as the pyramidal cells. Note that for a radial source, no field is measured. (Take from Singh in Senior et al., 2006)

Table 1.3 Basis for a difference in MEG signal

Cause	Cause type	Affected by tolcapone	Affected by Val^{158}Met
Difference in location of neurons	anatomical	impossible	possible
Change in depth of neurons	anatomical	impossible	possible
Difference in orientation of neurons	anatomical	impossible	possible
Changes in density of neurons	anatomical	impossible	possible
Changes in synchronous activity[5]	functional	likely	likely

Factors that may contribute to differences in the MEG signal are listed. They are classified as 'anatomical' and 'functional' based causes. The 'anatomical' causes may only be caused by the genotype differences, and not by transient tolcapone changes. Both tolcapone and genotype are likely to act via the 'activity' differences, most likely the magnitude of synchronous activity of pyramidal neurons in alignments perpendicularly orientated to the skull and the postsynaptic potentials.

[5] As mentioned, this could reflect changes in EPSPs, IPSPs and calcium currents.

Switching focus to the circuitry underlying COMT modulation of pyramidal activity, if MEG signals arise primarily from pyramidal (glutamatergic) neurons, how can they be affected by dopaminergic factors (whether this is mediated by genotype or drug)? Clearly, such effects must be indirect. DA is a neuromodulator, and intrinsically it is neither excitatory nor inhibitory. Its net effect depends on which receptors are activated, and upon which neurons they reside. The diagram below (Figure 1.7) enables us to think about how changes in COMT activity, and therefore changes in extrasynaptic DA levels, can affect the dendritic synaptic activity or EPSPs generated in pyramidal neurons. I assume the DA receptors are largely D_1-like receptors, as this is how previous studies consider pyramidal-interneuron-glutamatergic circuitry (Goldman-Rakic et al., 2000), because these are the most abundant DA receptor in the cortex (Lidow et al., 1991; Hall et al., 1994; Hurd et al., 2001). Therefore I assume primarily excitatory effects of DA.

Essentially, the net effect of DA upon pyramidal neuron activity will be a reflection of dopaminergic actions at different synapses. If DA mainly acts via (1) (see Figure 1.7), the effect will be mainly excitatory and therefore lead to an increase in MEG signal; whilst DA taking effect at (2), will lead to a decrease in MEG signal. The net effect on MEG signal will be the balance of these two effects. The COMT effects may differ from one brain area to another in terms of size of the net effect on the local population of pyramidal neurons. This could be either due to the activities of synapses (1) and (2) (see Figure 1.7) differing, or due to differential expression of DA receptors (e.g. $D_1 > D_2$ or $D_1 >> D_2$), and/or because of the differential contributions of COMT between areas. The overall size of effect of pyramidal neurons depends on the relative excitatory and inhibitory effects at (1) and (2b) respectively (Figure 1.7), which in turn depends on the amount and type of receptors on the pyramidal cell and interneurons, and also the differences in levels of COMT activity between the two groups of interest. The 'net effect' will reflect how many populations of neurons are aligned and firing synchronously, in the correct orientation and in a way for the scanner to detect related EPSPs. The putative DA-blood vessel synapse at (3) is

included to suggest one way changes in DA could potentially affect the BOLD response, though there is no evidence to suggest presence of COMT around this area.

The same factors which explain how differences in MEG signal can arise, can also explain how significant effects (e.g. of drug) could go undetected by MEG. For example, a 'false negative' could occur if the pyramidal cell population affected by the drug is not aligned with the necessary orientation to the scalp to be detected by MEG. Or, the drug could produce opposing effects in synchrony in subpopulations of pyramidal neurons at a given location, such that they cancel each other out in terms of MEG signal. Thus, an absence of a difference in MEG signal is not synonymous with an absence of functional effect (of drug or genotype) at that location and/or time point.

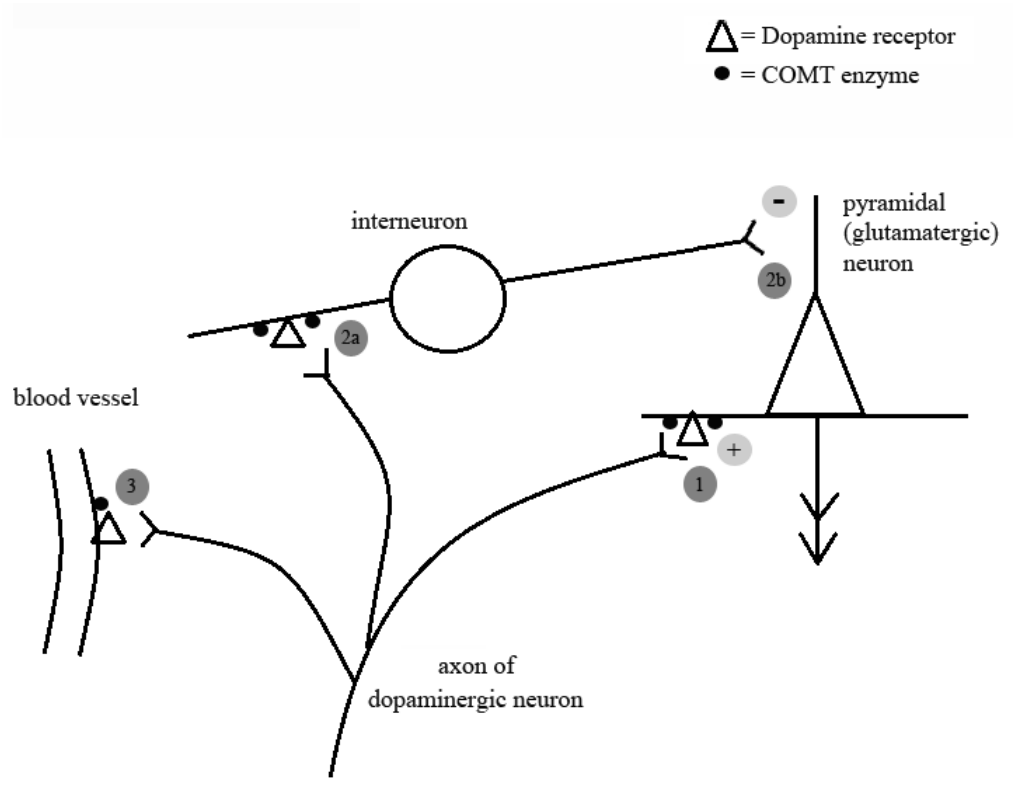


Figure 1.7 The effect of COMT on the MEG signal. A schematic illustrating the avenues by which COMT (via alterations in DA) could cause changes in the firing of a pyramidal neuron. Although one neuron is shown, it is the population of many thousands of adjacent neurons that is of relevance. (1) represents the DA-pyramidal neuron synapse, (2a) represents the DA-interneuron synapse, (2b) represents the interneuron-pyramidal neuron synapse and (3) represents the DA-blood vessel synapse.

1.6 Final comments

The information above provides a selective overview of the links between DA, PFC function and cognition, and how these might be impacted by COMT. Other issues will be discussed later in the thesis, where relevant, including phenotypes other than WM and other cortical regions. Firstly, the introduction above focuses exclusively on DA's role in the PFC, because of its central role in cognitive function and the known impact of COMT on DA levels in this region. However, many other brain regions are obviously important for these processes, and many of these are likely to be modulated by COMT. Specific examples are discussed in the following chapters, where relevant. Finally, the introduction focuses primarily on WM as a PFC DA-dependent phenotype. The reason for this was that the PFC is the region where most of the evidence for the inverted-U was obtained from studies of WM (especially spatial WM). In fact, the importance of COMT for other cognitive processes (specifically emotional processing and reward processing) forms a major component of this thesis. These tasks (or tasks very similar to those chosen) have all been demonstrated to be affected by DA or, more specifically, COMT. More information specific to these tasks are found in the introductory sections of their relevant chapters (3, 4, 5 and 6).

1.7 Thesis aims, objectives, and outline

The broad aim of this study was to better understand the role of COMT on aspects of brain function and behaviour, and more particularly to explore how variation in COMT activity impacts upon MEG signal during three tasks (as well as upon the performance of these tasks where relevant). MEG was utilised to complement the many prior studies of COMT that have used fMRI, given the differing characteristics and strengths of the two imaging modalities described above; the fMRI findings were also used to guide the MEG analyses. COMT variation was determined genetically, selecting subjects homozygous for the functional Val^{158}Met

polymorphism, and pharmacologically using the COMT inhibitor tolcapone. The experimental design allowed the study of main effects of genotype and drug, and their interactions, which in turn allowed the inverted-U hypothesis of DA function to be investigated.

Three tasks were selected to cover different aspects of cognition and behaviour known or suspected to be influenced by COMT:

1. N-back task – This task measures continual processing of WM. As described above, a COMT effect on BOLD response during the N-back has previously been established in performance-matched groups, although studies of the impact of COMT on N-back performance itself have produced mixed results. This task is explained further in Chapter 3, section 3.2.1.
2. Emotional face processing- This task measures brain activity in response to passively viewing fearful, happy, or neutral faces. Again, a COMT genotype effect has been established in similar tasks in fMRI studies, as discussed in Chapter 5, section 5.1.3.
3. Gambling- This task (Gehring and Willoughby, 2002) involves participants choosing to place a large or small bet. This task allowed me to look at how COMT influences the choice between large and small bets (relatively unexplored) and how loss and win outcomes are processed in terms of MEG signal. There is evidence from EEG and fMRI studies that suggest COMT genotypes differ in this comparison of win and loss processing. This task is explained further in Chapter 6, section 6.2.1.

To achieve these goals, a cohort of healthy male Met or Val homozygous volunteers was recruited for this study, with half in each genotype group randomised to tolcapone and half to placebo in a double-blind between-subjects design. The subjects undertook the three tasks within the MEG scanner. **Chapter 2** describes the core procedures and methods involved in the

study and its analysis, and presents the demographics of the study cohort. **Chapter 3** describes the behavioural results of the N-back study, and shows that performance is affected by a qualitative interaction between genotype and tolcapone. The MEG results for the N-back task are presented in **Chapter 4**, with a focus on the difference between 2-back and 0-back activations. It shows that, amongst the subgroup of subjects matched for performance, there are main effects of drug and genotype, and interactions between them, on several components of the MEG signals. In particular, the analysis focuses on the P300 component because of its known role in WM. **Chapter 5** explores the impact of COMT on MEG signals during emotional face processing. Firstly, it looks at the well-known N170 face-processing response and the effects of emotion at this time component. Secondly, it moves on to time components shown to demonstrate significant COMT effects. Thus, the focus becomes the N4-like time component in the fearful-neutral contrast. MEG signal generated by fearful vs. neutral faces at 400ms post-stimulus is significantly modulated by genotype, drug, and the interaction between them. **Chapter 6** presents the behavioural and MEG data from the gambling task. I first demonstrate genotype, drug and interactive effects on gambling choices (risky or risk-averse). Secondly, MEG activity elicited upon win or loss outcome is assessed with respect to COMT, focussing on the 250-300ms time component, given robust effects of wins vs. losses on this component in the previous literature. Thirdly, the impact of COMT on MEG activity during win vs. loss outcome is presented, and is shown to complement previous EEG data. Further, I demonstrate the impact of COMT inhibition, and its interaction with genotype on processing win and loss outcomes. Finally, **Chapter 7** summarises the main findings before briefly discussing the broader implications, and the complexities, of the data**.**

Chapter 2

Methods

Chapter 2

2. Methods

This chapter describes the core procedures and methods used in the current investigation including: subject recruitment, DNA extraction and genotyping, MEG data acquisition and analysis, and the demographic characteristics of the final cohort tested. Specific details of additional methods used (e.g. cognitive tasks) are presented in subsequent chapters.

The study described in this thesis was a double-blind, randomized, placebo-controlled (lactose pills), single-dose study using orally-administered tolcapone (200mg), given to healthy male volunteers selected on the basis of homozygosity for each allele of the Val^{158}Met polymorphism. This study was approved by Oxfordshire Research Ethics Committee (B 09/H0605/69).

In the MEG scanner, subjects completed three tasks relating to WM (the N-back task, lasting approximately 15 minutes), emotional processing (using images of faces, lasting approximately 20 minutes) and a gambling task (lasting approximately 30 minutes). Each task will be explained in their relevant chapters. These domains were selected based on previous studies suggesting a role for COMT (see Chapter 1). The MEG scanner affords a fine temporal resolution and allows the study of neural correlates of these three tasks and assessment of how COMT alters them via both genotype and tolcapone administration.

2.1 Recruitment

The target was to recruit and scan approximately 60 subjects (around 15 in each of the four groups: Val^{158}Val tolcapone, Val^{158}Val placebo, Met^{158}Met tolcapone, Met^{158}Met placebo). No females took part in this study. Male-only recruitment was decided upon for two reasons. First, to

escape confounding factors relating to sexual dimorphisms in COMT's function (Harrison and Tunbridge, 2007). Second, to avoid ethical and logistical problems associated with administering tolcapone to women of child-bearing age e.g. interactions with the menstrual cycle or the contraceptive pill, and the requirement for pregnancy testing.

Individuals were recruited over an 18 month period. The study was advertised on Facebook, and via posters and leaflets placed in University buildings. Emails were sent to College mailing lists through the appropriate governing JCR/MCR Presidents. All subjects gave informed consent and provided two buccal swabs. In order to confirm there were no medical contraindications to tolcapone (e.g. history of liver disease), GP consent was collected for each participant homozygous for either the Val or Met allele. Figure 2.1 shows a flow chart depicting key stages in the recruitment protocol.

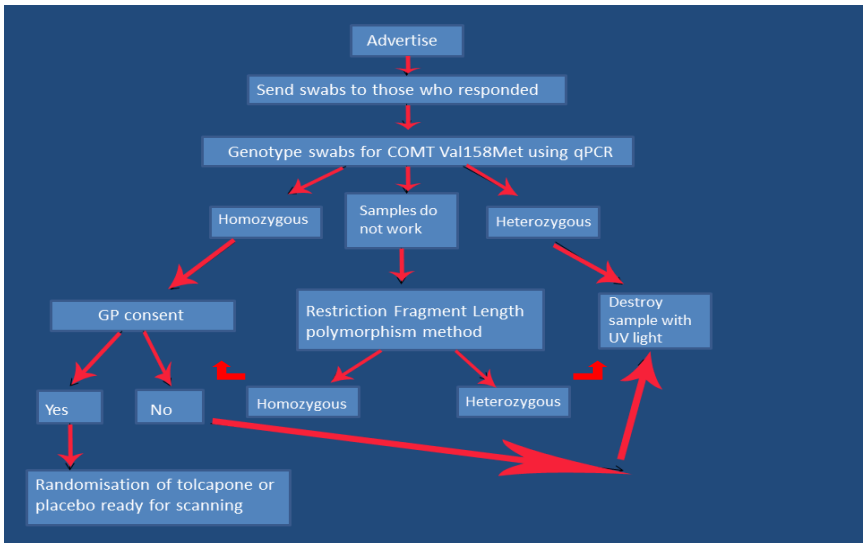


Figure 2.1 Recruitment protocol. The sequence of events necessary to collect participants ready for the scanning day.

On the day of testing, participants completed a set of questionnaires relating to mood, personality and verbal IQ (for more information see section 2.6.3). Within each genotype group, half were randomised to receive the COMT inhibitor, and half the placebo. Randomisation was organised by PJH, whilst I was blind to genotype and drug condition and remained so until data

acquisition was complete. Tolcapone was supplied by the pharmacy of the Oxfordshire and Buckinghamshire Mental Healthcare NHS Trust. The tolcapone was then encapsulated, to make it indistinguishable from the placebo capsules, by a Pharmacy approved to do so under the Good Manufacturing Practice (Pharmacy at Guy's and St Thomas' NHS Foundation Trust).

Participants were sent envelopes with two 1.5ml eppendorf tubes containing a cytology brush and a set of instructions. For maximal transfer of cells per brushing, participants were requested to slowly brush the entire inner surface of their cheek from the upper gum-cheek 'gutter' to the lower gum-cheek 'gutter' approximately 20 times. Swabs were returned to the Department of Psychiatry by post and stored at -20^{o}C as soon as they were received at the Neuroscience Building until DNA extraction.

2.2 DNA extraction protocol

The DNA was extracted and purified from cheek swabs using a ChargeSwitch genomic DNA Buccal Cell kit (Qiagen) and following manufacturer's recommendations. Briefly, the buccal cell sample was transferred to a sterile microcentrifuge tube containing 1ml of a solution of proteinase K, and incubated at 37^{o}C for 20 minutes.

To bind the DNA, the digested supernatant was first transferred to a new, sterile microcentrifuge tube. One hundred and forty microlitres of a purification mix containing magnetic beads was added to the sample, and pipetted up and down (5 times) to mix. A one minute incubation period allowed the magnetic beads to bind to the DNA. The samples were then placed in the 'Magnarack' for one minute, to pellet the magnetic beads and attached DNA. The supernatant was then pipetted out without removing the tubes from the Magnarack. A wash buffer (1ml) was then added to each sample to briefly resuspend the beads in solution. After one

minute, the magnetic bead pellet re-formed and the supernatant was again removed (and discarded). This step was then repeated once more prior to DNA elution.

To elute the DNA the tubes containing the pelleted magnetic beads were removed from the MagnaRack, and 150µl of ChargeSwitch Elution Buffer (pH 8.5) was added to the tubes and pipetted up and down gently 10 times to resuspend the magnetic beads. After one minute the samples were placed in the MagnaRack for one minute to give the beads time to form a tight pellet. The supernatant was then removed and transferred to a sterile microcentrifuge tube, as it contained the DNA. At this point all magnetic beads were discarded.

2.2.1 Calculation of DNA purity and yield

Calculation of DNA concentration was performed to assess the DNA yield from each extraction performed. Nucleic acid levels were measured by dual absorption spectrophotometry at wavelengths of 260nm and 280nm. The extent to which light with a wavelength of 260nm is absorbed by a sample is a proxy measure of its nucleic acid content (50 absorbance units represent 1mg ml^{-1} of DNA). The ratio of the absorbance at 260nm and 280nm (≥1.8) is used as an indicator of sample purity i.e. the sample does not contain significant levels of protein relative to nucleic acid levels. Repeated calibration of the spectrophotometer with DNA standards and water 'blanks' was required to maintain measurement accuracy. The purified DNA was stored at -20^{o}C and freeze-thawing was minimised.

2.3 Genotyping using qPCR

Samples with low yield were concentrated using the QIAAmp DNA Micro Kit (Qiagen, UK). All samples were genotyped using a Taqman SNP Genotyping Assay (C_25746809_50; Applied

Biosystems, UK) according to manufacturer's recommendations, and using a 7900HT Fast Real-Time Polymerase chain reaction (PCR) system (Applied Biosystems, UK).

All samples were processed twice and if this did not produce a consistent result, the second cheek swab was taken (32 cases). Samples that could not be genotyped after this (23 cases) were processed using a Restriction Fragment Length Polymorphism method described by Tunbridge et al. (2004).

2.4 Participant confidentiality

The participants' anonymity (and the blind of the study) was ensured as follows. Each participant was assigned an ID number once they had consented to take part. Apart from a master file held by PJH, all documentation, including screening and scan data was identified only by the participant's ID number. All documents were stored on a password protected computer, on a secure server, and only accessible by study staff and authorised personnel. The study complied with the Data Protection Act (1998).

2.5 Design for the day of the study

After acquiring GP consent, participants attended the Oxford Centre for Human Brain Activity (OHBA). They arrived two hours prior to scanning and were given tolcapone or placebo (2x100mg tolcapone capsules, or two matched placebo capsules). During the two hour period before scanning they were asked to fill out two sets of questionnaires pertaining to mood, personality and health, with a third set completed after the scan. Practical details of attaching coils and acclimatising participants to the scanner can be found in section 2.8.2.

The following questionnaires were completed by participants (see Appendix 1 for examples of each questionnaire set):

1. Participants were first asked a series of questions about age, smoking and drinking habits.
2. National Adult Reading Test (NART). This is a measure of premorbid (verbal) intelligence (Nelson, 1982). Patients were asked to read aloud words off a list and scored higher the more words they pronounced correctly.
3. Beck Depression Inventory (BDI). This is a 21-question multiple-choice self-report inventory to measure the severity of depression (Beck et al., 1961). Participants were asked to rate how they have been feeling in the past week, including the day of the study, on a range of depressive and related symptoms. They were asked to circle the statement which most captured their subjective state, from a series of four options for each question.
4. Spielberger State Anxiety Inventory (Spielberger et al., 1983). Participants were asked to rate the extent to which they agreed with statements on how they felt at that particular moment, in order to measure intensity of anxiety experienced at the time of assessment.
5. Spielberger Trait Anxiety Inventory (Spielberger et al., 1983). Participants were asked to rate the extent to which they agreed with a list of statements, in order to measure the general tendency for experiencing anxiety.
6. Visual Analogue Scales (VAS). Participants were shown horizontal lines pertaining to the seven categories written to the side of the line: 'Alertness', 'Drowsiness', 'Anxiety', 'Happiness', 'Nausea' and 'Sadness'. Participants were asked to place a vertical line at the point which best represented how they felt at that moment.
7. Befeindlichkeit Scale. This is a well-being scale (Abele-Brehm and Brehm, 1986). Befeindlichkeit translates from German as 'how-you-are-ness'. Thought to be a measure of energy levels, participants were presented with pairs of words and asked to cross the box of the word they felt was most relevant for them in their present feeling state.

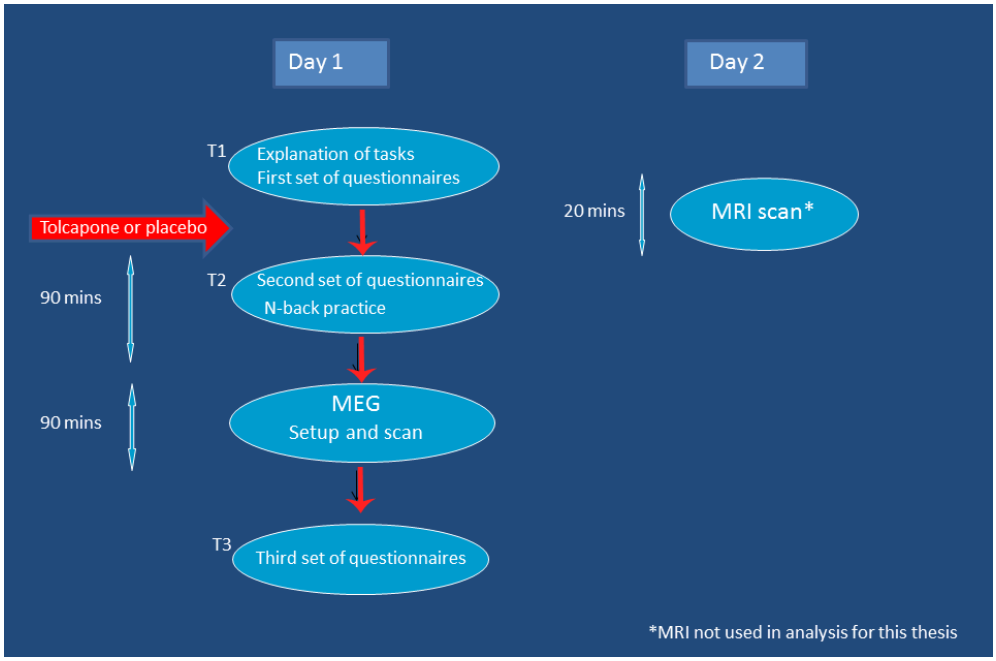


Figure 2.2 Study design- the scanning day. The subject first completed a set of questionnaires (T1), and was then given a pill. After approximately one hour, a second set of questionnaires was filled out (T2). Around 90 minutes after taking the pill, the subject was prepared to enter the scanner. This included sitting in the scanner to check for excessive noise levels on the participant, practicing the N-back, attaching coils and measuring the head shape. The actual scan time was approximately one hour. A third set of questionnaires was completed once the subject left the scanner (T3). The subject was also required to undergo a structural MRI scan within three months of participating in the study, which will be used during source analysis performed outside the remit of this thesis.

2.6 Demographics

The Val homozygous subjects who were given tolcapone are referred to as the 'Val tolcapone' group, the Val homozygous subjects who were given placebo as the 'Val placebo' group, the Met homozygous subjects who were given tolcapone as the 'Met tolcapone' group, and the Met homozygous subjects who were given placebo as the 'Met placebo' group. For simplicity, homozygous subjects are sometimes referred to as Met or Val 'homozygotes'.

2.6.1 Participant figures

327 samples were genotyped. Homozygotes were identified and confirmed. 102 men were homozygous (35 Val homozygotes; 67 Met homozygotes), the rest were either heterozygous

or unconfirmed. 72 men were scanned. Of these, 67 completed the study (33 Val homozygotes, 34 Met homozygotes) and were included here (Table 2.1). Five subjects were excluded for various reasons: one had an excessive alcohol intake (>30 Units per week), one had a high BDI score (>22), one was a heterozygote scanned by mistake, and two were omitted due to technical problems during the scan. Not all of the 67 subjects completed each task during the study; actual sample sizes and demographics are reported in the relevant chapter if they differ from that shown in Table 2.1.

It is noteworthy that the proportion of Met to Val homozygotes identified here (approximately 2:1) deviate somewhat from the proportion (1.25:1) expected in a primarily Caucasian population (Palmatier et al., 1999). It is possible that an excess of the Met allele may arise from the majority of participants stemming from the student population of Oxford University, where strict selection based, at least partially, on cognitive ability takes place. The putative Met advantage on WM (see Chapter 3) may advantage these allele bearers.

The blind was maintained, with 59 of the 67 subjects thinking that they had received placebo, including 30 of the 33 who in fact had tolcapone.

2.6.2 Demographic and questionnaire data

The final four groups used for the study showed no significant differences between them (see section 2.7) for all demographic variables shown in Table 2.1.

Table 2.1 Demographics of the study cohort

	Met placebo	Val placebo	Met tolcapone	Val tolcapone
Number	18	16	16	17
Ethnicity[a]	17C, 1I	12C, 2I, 1Ch, 1A	16C	14C, 2I, 1Ch
Age (y)	22.6 (3.2)	24.0 (5.1)	24.4 (9.0)	23.9 (3.9)
NART	118 (4)	117 (5)	115 (5)	116 (6)
Alcohol units/week	10 (7)	8 (5)	7 (5)	8 (8)
Cigarettes/day	0 (0)	0.4 (1.3)	0.4 (1.5)	0.2 (0.7)
BDI	3.9 (5.3)	3.5 (3.3)	3.7 (3.7)	3.1 (2.9)
STAI	32 (8)	39 (7)	33 (10)	37 (8)

Values are mean (s.d.).
NART: National Adult Reading Test. BDI: Beck depression inventory. STAI: Spielberger Trait Anxiety Inventory.
[a] C: Caucasian; I: Indian; Ch: Chinese; A: African Caribbean.

2.6.3 Questionnaire-based measures and correlations

Visual-analogue scales for alertness, drowsiness, anxiety, happiness, nausea and sadness were recorded at three times during the study, referred to as T1, T2 and T3 (see Figure 2.2). On these VAS ratings, Met homozygous subjects rated themselves happier than Val homozygous subjects overall (7.2+/- 0.3 vs. 6.3+/- 0.3, $F_{(1,61)}$ =5.58, p=0.021), including at baseline i.e. ratings taken prior to taking the pill (7.1 +/- vs 6.0+/- 0.3.p=0.013). Happiness also showed a genotype x tolcapone x time interaction [$F_{(1,61)=}$5.2, p=0.026], due to an increase in ratings with time in the Val tolcapone group (p=0.015, comparing time points 1 and 3) which was absent in other groups; see Figure 2.3. None of the other VAS ratings were affected by genotype or drug, nor did the groups differ in their depression and anxiety ratings. For a full set of VAS scores see Appendix 2.

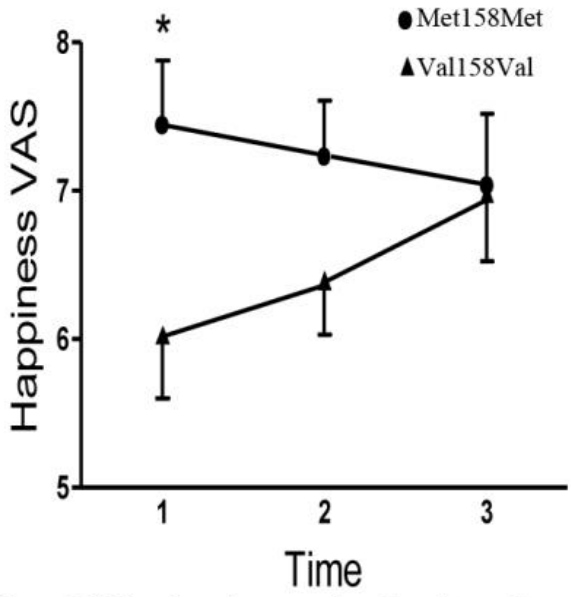


Figure 2.3 Visual analogue scales- Happiness. Scores are recorded three times during the study. Happiness scales showed significant difference between genotypes (whilst on tolcapone) during the first questionnaire set.

Befeindlichkeit questionnaires, with three sets of scores for personality, mood and overall (sum of the previous two scores) were also completed three times during the study day. There were no significant differences found between T1, T2 and T3, nor were there any significant drug, genotype, or interaction effects on the Befeindlichkeit scores (see Appendix 3).

2.7 Statistical analysis of behavioural data

Statistical analyses of the behavioural data (i.e. N-back task and gambling task) were carried out in SPSS for Windows 17, as described in the relevant chapters. In each case, the approach was to use an Analysis of Variance (ANOVA) to identify main effects of tolcapone, genotype, or their interaction, followed by post-hoc comparisons (using Least Significant difference) to follow up significant results. Correlations between variables were explored using Pearson's coefficient. Significance was set at p=0.05.

2.8 MEG experiments

A brief introduction to MEG and its signal basis was given in the previous chapter. Here, I give details of the instrumentation, describe the practicalities of how it was used in the present study, and outline the principles of the analytical approach adopted.

2.8.1 Instrumentation

The magnetic field strength produced by the human brain is extremely small, typically one part in one thousand million of the Earth's magnetic field. To date, the only instrument with the required sensitivity is the Superconducting Quantum Interference Device (SQUID), which operates at the cryogenic temperature of liquid helium (about -269C°), where superconductivity facilitates the detection of brain magnetic fields via an even smaller natural entity known as the flux quantum. Typically, a SQUID device is coupled to a so-called 'pick-up' coil which defines the spatial sensitivity of the individual MEG detector. Loosely speaking, SQUID is comparable to a modern digital camera which can yield different images depending on the lens (pick-up coil) used, e.g. telephoto or wide angle. That is to say, the pick-up coil can define whether the focus is on a particular area or a wider field.

The VectorView™ (Elekta-Neuromag) whole-head scanner at OHBA was used for this thesis. The system features a total of 306 detectors at 102 locations. At each location there are three pick-up coils (and 3 SQUIDS): two gradiometers and one magnetometer, the latter of which is a square loop placed around two figure-of-eight shaped gradiometers, which themselves sit perpendicular to each other (Figure 2.4c).

a. b. c.

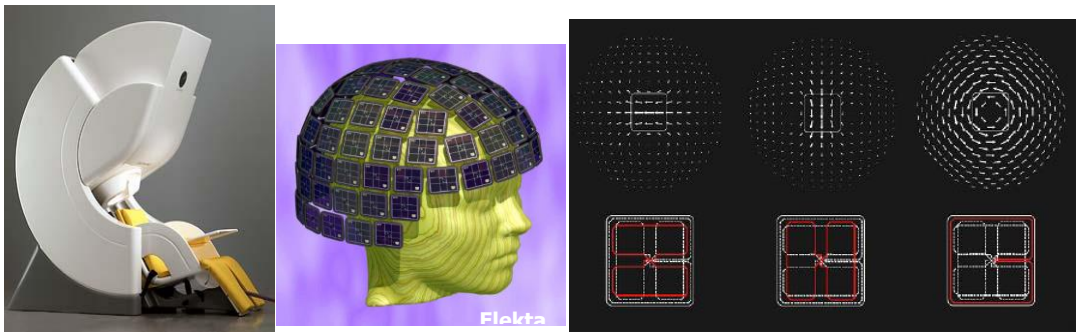


Figure 2.4 Elekta-Neuromag scanner and coil arrangement. These images show a. the MEG scanner, b. placement of coil sets around the skull and c. the arrangement of coils, those highlighted in red produce the field shown directly above (images from Elekta-Neuromag).

Note that each gradiometer will give a zero reading if a magnetic field is exactly the same in the two loops constituting that gradiometer, implying sensitivity to the spatial variation of magnetic fields. The further away a source is from the gradiometer, the more homogenous the magnetic field becomes indicating a suppression of distant fields. In contrast, the magnetometers are sensitive to near and far sources. In order to avoid interferences caused by external electromagnetic fields, in particular in the case of magnetometers, the MEG scanner is housed within a purpose-built magnetically shielded room.

2.8.2 Practical details of MEG procedures

Before performing the tasks, participants spent a few minutes in the scanner whilst I checked that their background electromagnetic levels (noise) were not unusually high, which may have indicated that the participant was still wearing jewellery or had certain dental work that may need to be taken into account during analysis. The participant was then asked to step outside the scanner ready for the attachment of coils.

Four head position indicator (HPI) coils were affixed symmetrically to the head of each participant, two on the forehead and two behind the ears. The position of the coils was then

registered with the computer, along with the nasion and lateral preauricular areas and up to twenty-five other random points around the head. Each of the HPI coils transmits at a different frequency, and thus they were able to provide a unique spatial location. The computer established the HPI coil locations prior to the task using three-dimensional digitizers that assimilate the coils and estimate the position of the participants' head relative to the MEG sensors during the task.

During the scan, an electro-oculogram and electrocardiogram were used to monitor eye movement and heart beat respectively, and were attached near to the participants left eye and on both wrists respectively. This was used to account for signal artefacts related to eye blinks and any other major movements.

2.8.3 Stimuli

The individual tasks are detailed in the appropriate chapter, but here I will provide the general aspects of stimulus presentation. 'Presentation', is a stimulus delivery and presentation control package for neuroscience (http://www.neurobs.com/ last accessed August 2012). Visual stimuli included a mix of pre-prepared and generated images for the tasks. Images were back-projected on a translucent screen, approximately 1.2 metres from the eye with a visual angle of <4 degrees. For the synchronization-trigger, each stimulus and sequence of stimuli of interest was accompanied by a unique trigger pulse and sent to the MEG acquisition for synchronization. The feedback generated from this was stored on a central server ready for analysis. Delays in the system, of approximately 30ms, are common and are measured with a photodiode. These were taken into account in the analysis in order to have a one-to-one correspondence with onset and time.

The sampling rate used was 1000Hz, with a pre-digitization band pass: 0.03-330Hz. The sampling rate is the speed at which a signal is digitised. A value is taken 1000 times a second (or one sample every millisecond). If a signal changes too rapidly compared to the sampling rate, the resultant sequence of numbers is not a faithful representation of the original signal. Therefore, to avoid this, a pre-digitisation (sometimes called an 'anti-alias') filter is used to restrict the signal before being sampled.

2.8.4 Approach to analysis

MEG analyses are inherently complex, and the application of MEG to the study of WM, emotional processing and reward processing is in its infancy. Indeed, when this study began, there were no such reports in the literature, no MEG studies on the scale of this one, and no consensus as to the approaches to be taken to data analysis. Reflecting these issues, the analyses used in this thesis are inevitably preliminary, and focus on specific components and epochs of the MEG data rather than attempting a comprehensive evaluation. The rapid developments in MEG analysis, and the options for further analysis of the data, are discussed in Chapter 7, section 7.3.

All results shown in this thesis are limited to information taken from gradiometer data, which focuses on closer sources picked up by the two orthogonal figure of eight coils as opposed to the larger magnetometer coil which potentially picks up deeper sources.

The basic approach follows the 'In-House' methods that were available at the time within OHBA. Technical issues encountered during the development of these analyses meant that I analysed part of the data manually as opposed to using scripts. For general analysis protocol see Figure 2.5.

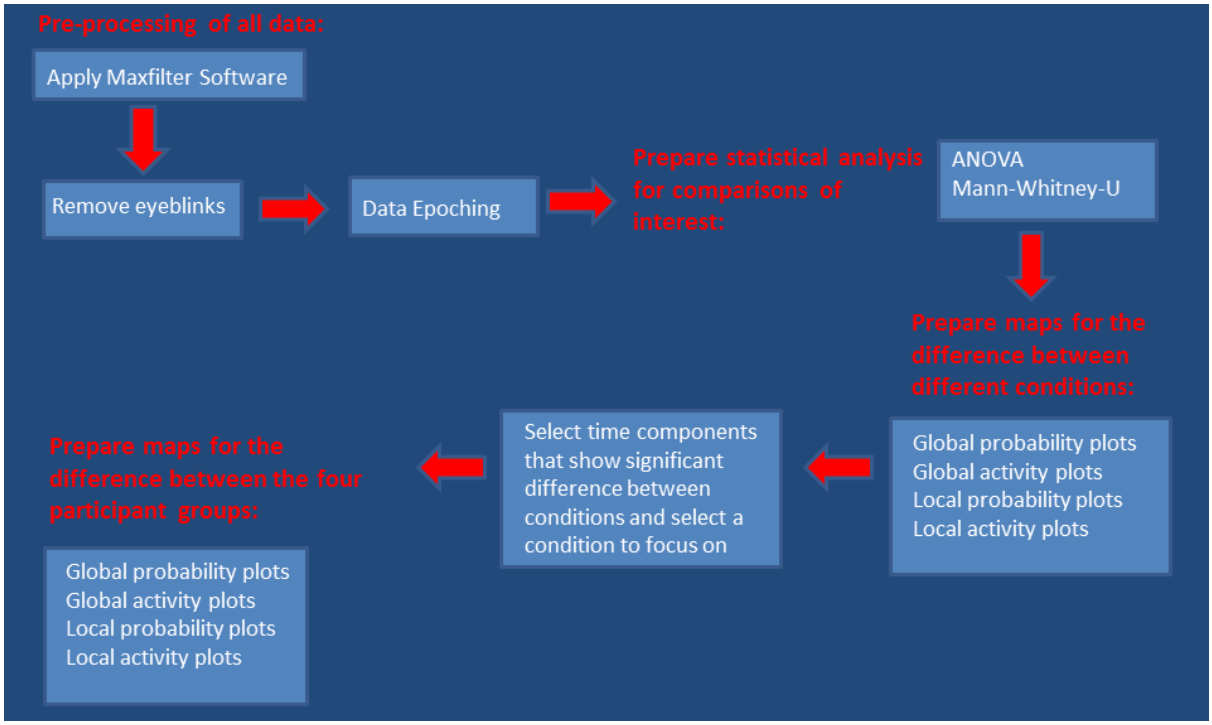


Figure 2.5 Approach to MEG analysis. This flowchart follows the general protocol for processing MEG data.

2.8.4.1 Pre-processing

Data were filtered using Eleka Neuromag Maxfilter 2.1. Maxfilter software applies a spatial filter to the data to reduce magnetic interference and artefacts from signals arising outside of the head. The electro-oculogram (EOG) was used to identify eye blinks, which were subtracted from the continuous data using a method based on principal component analysis (PCA). Subsequently, the data were inspected for remaining high amplitude and other artefacts that were not detected by MaxFilter. The affected epochs were rejected before further analysis.

2.8.4.2 Data epochs

Event related fields (ERF; also called Evoked responses), that is, brain activity following the presentation of a stimulus, were averaged separately for each participant and for each condition of interest. The average signal between 300ms before, and at the onset of, the stimulus (0ms) served as the baseline. A latency range from 0ms to up to 2000ms after stimulus onset was

chosen for analysis and cut down to 600ms after the stimulus at the stage of producing graphical representation of the data. The evoked responses were filtered between 1 and 30Hz.

2.8.4.3 Statistics

Statistical differences in ERFs between the conditions of interest across participants were analysed employing a time-dependent measure *P(t)* the details of which have been fully specified elsewhere (Braeutigam et al., 2004).

$$\mathrm{P}(t) = \text{probability}\left(\chi^2\right) \qquad \left(\chi^2\right) = -2\sum_{i=1}^{N} \ln\left[f_i(t)\right]$$

Figure 2.6 Probability formula. This formula is used for calculating statistical differences using a time-dependent, chi-squared test measure.

N denotes the number of channels. For each channel, $f_i(t)$ denotes a non-parametric statistical test applied to evoked fields at each time point. Then, P(t) describes significant differences across all channels. This test corrects for multiple comparisons at a time point, where that time point may be significant in more than one channel. For each significant interval [P(t)<0.01], the spatial distribution of the significant difference was given by f_i. Correspondence between the spatial distribution of the significant difference and the spatial distribution of ERFs (using grand-average power maps made from the root-mean-square of the two gradiometers in a pair) was explored.

A non-parametric test (*f)* was used in the formula above. Dependent on the task and statistical question in mind, f was chosen to be a rank ANOVA or Mann-Whitney-U test. These tests do not rely upon matched samples or a normal distribution of brain signals across participants.

This method is conceptually similar to common event-related field techniques of comparing evoked peak amplitudes across subjects and conditions as available in FieldTrip using the ft_timelockstatistics function. See also Seeck et al. (1997) for a precursor to the methods used by the 'In-House' methods in this thesis. The measure does not require the prior identification of peaks in the waveform and takes into account the data from all detectors. For the purpose of this thesis, only gradiometer channels are considered as their focal property facilitates estimation of neural activity directly from the signals observed over a given region. In other words, the outputs of each pair of gradiometers are most sensitive to tangential current flow in the region directly below the detectors. The local root-mean-square signal summed over the two readings is a measure of current strength.

To assess differences in peak latency between groups, data was extracted from the 'In-House' software and imported into Matlab, where codes were used to find the maximum peak and specific latencies between given time points of focus for each individual. The data were then imported into SPSS, where ANOVAs were run on the average time for the peak of interest.

2.8.5 Maps

2.8.5.1 Global plots

Global plots are based on all gradiometer channels, and can be calculated for individual evoked responses or for grand-mean responses (group averages). The plots represent signal power, assumed to be a direct correlate of neuronal activity, over the whole brain (all gradiometer coils added together). When discussing these figures I will explore comparisons of power strengths in terms of 'activity', with activity referring to local or global power signal observed over a region; this can be interpreted as a measure of the strength of neural generators.

2.8.5.2 Probability plots and maps

Two types of images reflecting probabilities are shown, i. probability plots relating to the activity shown in global plots, and ii. probability maps relating to the activity shown in local maps. Probability maps are based on the statistical tests f_i, as described above (see section 2.8.4.3), combined into a single probability value for each gradiometer pair. The Y axis represents the negative logarithm of the p-value. In the probability plots, the 0.01 value is marked for reference on this axis, selected to be more stringent than the p=0.05 adopted in studies using similar methods. The individual peaks of interest (i.e. those selected for further analysis based on $p<0.01$) are labelled with their specific significance value. Section 2.8.4.3 explains how the statistics were corrected for multiple channels; the selection of specific time regions of interest for each individual chapter avoids possible issues regarding correction for multiple time points. However many of the comparisons are extremely significant and thus likely to withstand further, more stringent levels of correction, for example $p=3.7\times10^{-20}$ in Figure 4.3a., $p=2.3\times10^{-14}$ in Figure 5.5c., and $p=3.2\times10^{-59}$ for Figure 6.6. In the probability maps, a scale bar is provided (and $p<0.05$ is taken to be significant). Further aspects of analysis strategy and choices were specific to each task, and are described in the relevant chapter, and first described for N-back in Chapter 4 section 4.2.1.2.

2.8.5.3 Local maps

Local maps were generated to give a general idea of regions of interest for the different tasks and to explore local differences between drug and genotype groups. They are based on gradiometer channel pairs, and can be calculated for individual evoked responses or for grand-mean responses. Localities will be referred to as either frontal, vertex, parietal, temporal or occipital as is shown in Figure 2.7 below. This terminology is based on the spatial positioning of channel groups defined by the manufacturer (see hardware manual, Elekta Neuromag, NM 20125A-G).

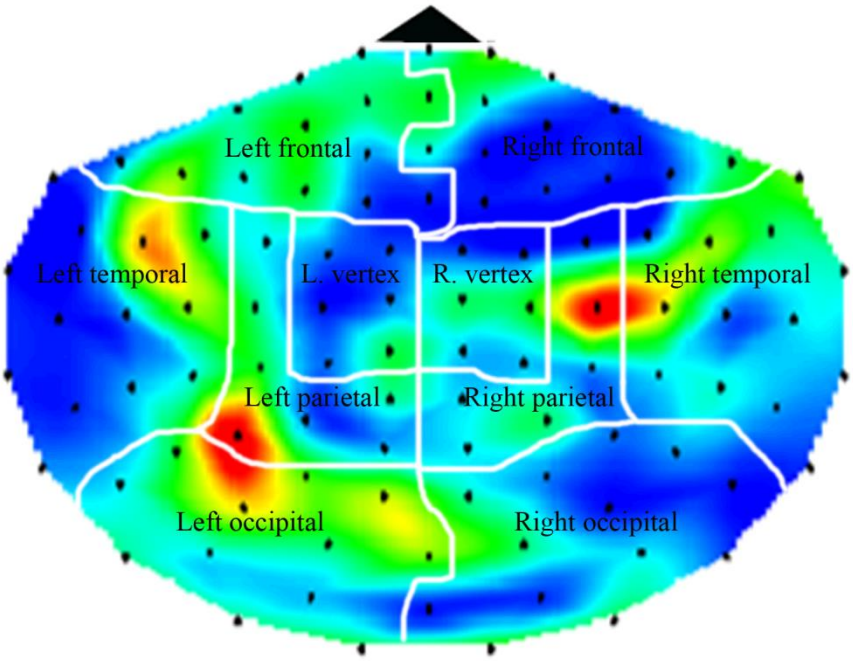


Figure 2.7 Area segmentation. This figure shows how areas are categorized into frontal, temporal, parietal, vertex and occipital areas.

Proportions of these local maps vary slightly between figures, i.e. some may look wider for a given height. This was rectified as much as possible with other analysis or presentation programs once the MEG_plotter program provided initial maps. The dimensions (height and width) of the maps depend on the how the maps were displayed on the MEG_plotter screen. Hence, local maps may appear rounder if they were initially explored as part of a larger group of local maps. All maps are positioned so the top is the forebrain (with a black triangle representing the nose) and the bottom represents the occipital areas at the back of the head.

2.8.5.4 Construction of local maps

The local maps were constructed from signals averaged over two sets of gradiometers at a given time, as opposed to all gradiometer sets at once, which would give a whole head activity representation. This was not a cluster analysis, and so maps demonstrating areas of significant

difference between the groups were not weighted by the presence of significant difference in a nearby region.

Initial inspections of local data involved taking a time of interest (as found above), displaying the local probability plot from the relevant ANOVA calculations, along with an activity plot for the same time frame for each individual group of interest. The areas of significance ($p<0.05$) from all the local probability plots were first identified. This was followed by an examination of activity plots for the relevant participant group within these key areas.

2.8.5.5 Scales

Local probability plots are always shown with a scale from 0 to 2.1, representing the negative logarithm of the p value, with dark red areas showing significance of $p<0.01$. Local activity maps shown in the same figure are scaled to the same femtotesla per centimetre (fT/cm) values. All values are expressed to the nearest millisecond or 2 significant figures (s.f.) for p values and power values. Significance values are always given for the specific area that shows the greatest difference between the conditions under statistical analysis.

Chapter 3

The effect of COMT Val^{158}Met genotype on working memory

CHAPTER 3

3. The effect of the COMT Val^{158}Met genotype on working memory

3.1 Introduction

The term working memory (WM) was coined by Miller, Galanter and Pribram in 1960 in their book 'Plans and the Structure of Behaviour', and was subsequently used by cognitive psychologists exploring the components of human memory (Atkinson and Shiffrin, 1968; Olton and Papas, 1979; Baddeley, 2010). More recently, WM has been described as 'the ability to retain goal-relevant information made recently available to make predictions, and to integrate it with current sensory input to guide behavioural decisions' (Durstewitz and Seamans, 2008). This skill is utilised when performing complex tasks such as reading, comprehension and learning (Baddeley, 2010). These processes critically involve the PFC, particularly its medial and dorso-lateral areas. The main components of WM were initially proposed to comprise of two 'slave systems' named the 'phonological loop' and the 'visuospatial sketchpad', and a central executive system acting in a supervisory capacity, to direct attention to relevant information (Baddeley, 1974). The phonological loop has a short term memory store that decays rapidly and an articulatory rehearsal component. The visuospatial sketchpad reputedly involves temporary storage and manipulation of spatial and visual information. In 2000, an 'episodic buffer' was added as a third slave system of the WM schema (Baddeley, 2000) and was described as important for linking information across the previously described aspects of WM (such as visual and spatial elements) and for temporal sequencing. This component is assumed to have links to both semantic memory and long-term memory (Baddeley, 2010).

The study of WM is crucial to understand the pathological processes underlying cognitive deficits, including those in neuropsychiatric disorders, and to provide the necessary information to assist in the development of ameliorative strategies. Impairment of WM is a key feature of

disorders such as schizophrenia (Vaz-Serra et al., 2010), Alzheimer's dementia (Owen, 2004), Parkinson's disease (Koerts et al., 2009; Kehagia et al., 2010) and attention deficit hyperactivity disorder (Fassbender et al., 2011; Palladino and Ferrari, 2011).

Many investigations have used the 'N-back task', to evaluate the integrity of WM. It exists in many forms but essentially, it requires subjects to remember and constantly update sequential letters or numbers. The version used here is described in Chapter 3, section 3.3.1. The task parametrically varies WM load, which corresponds to task difficulty, while keeping other task conditions constant. In healthy participants, increased WM load is associated with poorer performance (Braver et al., 1997; Manoach et al., 1997; Ragland et al., 2002): details are discussed below. In schizophrenia, although the inverse correlation between memory load and performance still exists, patients perform worse than matched controls, and often cannot complete the task with the maximum memory load (Perlstein et al., 2001). The N-back task has not only been widely used to assess WM in several pathological states but, as discussed below, to study effects of genotype on WM. It has also been extensively used in fMRI studies to assess the neural correlates of WM. For these reasons, the N-back task was chosen for this study.

3.1.1 Dopamine and working memory

Brain dopamine (DA) is heavily implicated in executive and cognitive processes, including WM. Evidence to support this includes the following:

1. Numerous animal studies demonstrate that the pharmacological manipulation of D_1 receptors in the frontal cortex modulates WM (Sawaguchi and Goldman-Rakic, 1991; Williams and Goldman-Rakic, 1995; Zahrt et al., 1997; Muly et al., 1998).

2. Schizophrenia patients perform poorly in WM tests. This is interpreted to be related to their lower DA turnover, as measured by cerebrospinal fluid concentration of the DA metabolite HVA (Weinberger et al., 1988).
3. Abi-Dargham et al. (2002) observed increased PFC DA D_1 receptor availability in schizophrenia patients, which strongly predicted WM performance and was interpreted as a reflection of diminished DA innervation.
4. Subjects with low WM capacity have significantly lower DA synthesis capacity in the striatum compared to subjects with high WM capacity, as revealed by positron emission tomography (PET) using with the radiotracer 6-(18f) fluoro-L-m-tyrosine (Cools et al., 2008; Cools and D'Esposito, 2011).
5. Impaired WM in Parkinson's disease patients is likely to arise from the low levels of DA throughout the brain (Beato et al., 2008).

These studies allow the conclusion that WM performance is critically dependent on DA neurotransmission in the PFC. For more information of how DA operates in the PFC see Chapter 1 including section 1.2, which expounds the inverted-U relationship between prefrontal DA and cognition. There are also numerous reviews on this subject (Grace, 1995; Goldman-Rakic et al., 2000; Winterer and Weinberger, 2003; Seamans and Yang, 2004).

3.1.2 COMT Val^{158}Met and working memory studies

Reports on how the Val^{158}Met polymorphism affects WM tasks are mixed (see Chapter 1, section 1.3.3). The detrimental effects of the Val allele on cognitive tasks such as N-back (Goldberg et al., 2003; Bertolino et al., 2004) and the Wisconsin Card Sorting Task performance (Joober et al., 2002; Malhotra et al., 2002; Rosa et al., 2004; Barnett et al., 2007) have been replicated in several studies. This section focuses on the N-back studies.

Goldberg and colleagues demonstrated that Val homozygous subjects scored lower than Met homozygous subjects in moderate difficulty conditions in the N-back, thus suggesting the COMT genotype influenced the executive processes involved in WM (Goldberg et al., 2003). This effect was assumed to be specific to WM as genotype did not influence the Continuous Performance Test, which involves the recruitment of sub-processes related to attention, load and delay. The interaction between genotype and performance in the N-back task was replicated in a larger sample (Diaz-Asper et al., 2008).

Despite this and some other studies reporting worse WM performance (outside of N-back) in Val than Met homozygous subjects, the literature overall is not consistent (Goldberg et al., 2003). For example, although Bruder and colleagues found a Val^{158}Met genotype effect on a different WM task (Letter Number Sequencing) this did not hold for all tasks in the study, including the N-back (Bruder et al., 2005; Barnett et al., 2007). In addition, Barnett and colleagues' undertook a meta-analysis of studies investigating the COMT Val^{158}Met variant and WM performance (Barnett et al., 2008). The study found no overall association, although there is some controversy over the utility of the meta-analytical approach for combining non-standardised neuropsychological data of this type (Goldman, 2009). More recently Blanchard et al. (2011) also failed to find a Val^{158}Met effect on N-back performance.

In spite of these conflicting results, the balance of evidence arguably supports the hypothesis that the high activity Val allele is associated with poorer WM performance, compared with the Met allele, at least under some conditions. This hypothesis is also indirectly supported by studies showing that the COMT knockout mouse shows superior WM compared to its wild-type littermate controls (Papaleo et al., 2008).

The inconsistencies that occur in the N-back results may be explained by other factors influencing where a given Val^{158}Met individual may lie on the inverted-U curve. This could include drug effects, effects of other genotypes or environmental factors. Recently, for example, it has been demonstrated that the Val allele confers an advantage in WM performance under stressful conditions (Buckert et al., 2012), as predicted by the inverted-U schematic shown in Figure 1.1. The following section explores this a little further, focussing on drug modulation.

3.1.3 Tolcapone effects on COMT and working memory

The inverted-U model of DA (described in Chapter 1, section 1.2) predicts that COMT inhibition should have differential effects on WM performance depending on Val^{158}Met genotype. COMT inhibition increases prefrontal DA (Tunbridge et al., 2004) thus moving individuals rightwards on the curve. The effect of being moved rightwards will be affected by the starting position i.e. Val homozygous subjects, with their higher COMT activity, sit to the left of Met homozygous subjects. A given degree of COMT inhibition could thus move one group closer to the optimum whilst moving another beyond this peak.

Prior data provide some support for this predicted interaction. Apud and colleagues (2007) used repeated tolcapone administration (200mg three times a day for a week) in a within-subject crossover design. They did not identify any main effects or interactions on N-back accuracy, but did find an interaction on intradimensional set-shifting, such that tolcapone improved Val homozygous subjects but impaired Met homozygous subjects. They also observed faster reaction times (RT), in particular for the more demanding conditions, and reduced dorso-lateral PFC activity after tolcapone, compared with placebo. Similar results in the N-back task were found by Roussos and colleagues (2009) using a single 200mg oral capsule of tolcapone (or

placebo) over two weekly sessions. In addition to these cognitive tasks, prepulse inhibition[6] (PPI) scores have indicated that, on placebo, Val homozygotes had lower PPI than Met homozygotes, but tolcapone increased these levels in Val homozygous subjects. A trend effect of tolcapone decreasing PPI in Met homozygous subjects was also recorded (Roussos et al., 2009). Giakoumaki and coworkers used a single dose of tolcapone (200mg) in a within-subjects crossover design to explore the tolcapone effect on WM. They failed to find a genotype x tolcapone interaction for N-back accuracy, but did find a genotype x tolcapone interaction for N-back RT, which was improved by tolcapone in Val- but not Met-homozygous subjects (Giakoumaki et al., 2008). They also found a trend-level effect on another test of WM, letter-number sequencing, with tolcapone again selectively improving Val homozygous subjects.

Thus, to date, specific tests of the hypothesis that Val^{158}Met genotype will affect WM response to COMT inhibition are inconclusive, although notably COMT Val^{158}Met does modulate the cognitive and behavioural effects of some other dopaminergic drugs including; amphetamine, as demonstrated in the WCST with Val homozygous subjects improving and Met homozygous subjects deteriorating after amphetamine (Mattay et al., 2003); antipsychotics, where schizophrenia patients with the Met allele improved in a series of cognitive tests (Weickert et al., 2004); and the clinical response to methylphenidate, where severity of attention deficit hyperactivity disorder symptoms reduced significantly more in Val- compared to Met homozygous subjects (Kereszturi et al., 2008).

3.1.4 Objectives

The aim of this chapter was to investigate how COMT Val^{158}Met and the pharmacological manipulation of COMT activity affect WM performance. This was achieved using the N-back in

[6] When a preceding stimulus attenuates a startle response, measured by muscle responses.

healthy male volunteers selected to be homozygous for Val or Met, with subjects in each genotype group randomly assigned to tolcapone or placebo. Significant effects were not necessarily expected given the mixed literature, but it was hypothesized that if effects occurred, they would be consistent with the following:

i. Met homozygotes will score higher (% accuracy) than their Val counterparts, particularly in the 2-back condition.
ii. Tolcapone will affect scores in a manner consistent with the inverted-U theory of DA and WM i.e Val homozygotes will increase, and Met homozygotes decrease, their accuracy score.

3.2 Methods

For information on recruitment, genotyping and analysis see Chapter 2, section 2.1.

3.2.1 The N-back task

In the version of the task used in this thesis, participants are required to compare a current item to a previously presented item, N items ago (e.g. two items back) that is maintained in the WM. In the version of the task used here, subjects respond to digits (1-4) that were presented 0 (current), 1, 2 or 3 positions back in a sequence of numerals (Egan et al., 2001). The design is a block design. Each block of 0-,1-,2- and 3- back run after each other and this is repeated six times in total. Within each block a number is displayed every 1800ms. Each number is held on the screen for 160ms, followed by a wait showing just the blank circles in the rectangular box. The task takes approximately 15-20 minutes to complete, depending on how long the participant takes to press a button to signal that they are ready to start a new block.

3.2.2 Demographic data

Participants who scored less than 57% on the 0-back (more than two standard deviations

below the mean) were excluded, under the assumption that they were not attending to task. The demographic figures are displayed in Table 3.1.

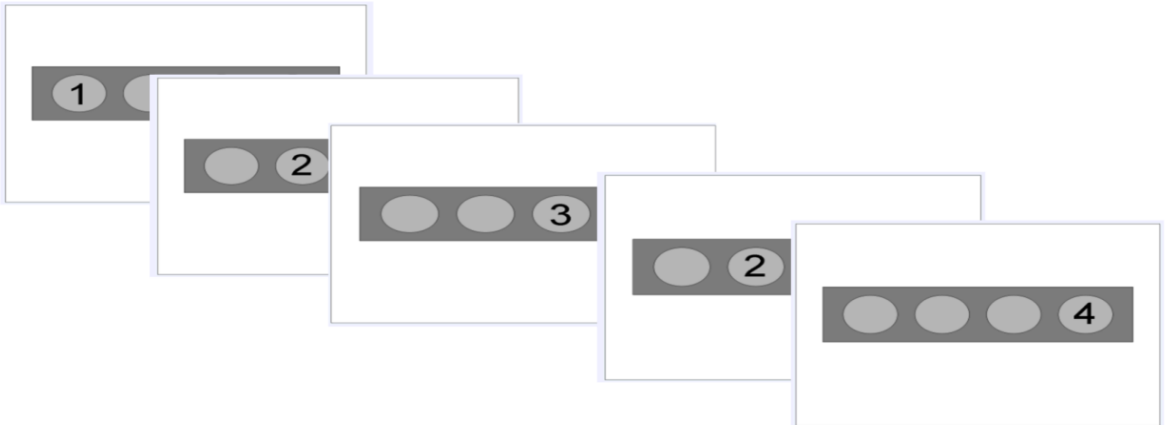


Figure 3.1 The N-back task. This figure shows images that appear every 1.8 seconds. A series of numbers appear on the screen and the participant has to remember the previous few numbers until they have tapped out the sequence on the button box.

Table 3.1: Demographics of subjects used in final N-back sample

	Met placebo	Val placebo	Met tolcapone	Val tolcapone
Number	15	15	16	14
Ethnicity[a]	14C, 1I	11C, 2I, 1Ch, 1A	16C	11C, 2I, 1Ch
Age (y)	23 (3.4)	24.1 (5.3)	24.4 (9.0)	24.1 (3.6)
NART	118 (4)	117 (5)	115 (5)	116 (6)
Alcohol units/week	11 (8)	8 (5)	7 (5)	9 (9)
Cigarettes/day	0 (0)	0.5 (1.4)	0.4 (1.5)	0.2 (0.8)
BDI	3.7 (5.5)	3.7 (3.3)	3.7 (3.7)	3.4 (3.1)
STAI	29 (7)	33 (8)	33 (10)	31 (5)

Values are mean (s.d.).
NART: National Adult Reading Test. BDI: Beck depression inventory. STAI: Spielberger Trait Anxiety Inventory.
[a]C: Caucasian; I: Indian; Ch: Chinese; A: African Caribbean.

3.3 Results

This section firstly looks at the results across all participants together. It then moves on to report how drug and genotype affect N-back performance.

3.3.1 Accuracy and reaction time scores as a function of N-back condition

Accuracy scores over the whole group differed between WM loads [$F_{(2.3,100.1)}$=179.2,p=3.9x10^{-36}] showing a decrease as N-back load increased, ranging from a score of 91.5% in 0-back, to 49.2% in 3-back (see Table 3.2 and Figure 3.2). According to post-hoc analyses, scores differ between all groups (all p<0.01). Similarly, RT across the whole group differ between WM loads [$F_{(2.1,84.6)}$=33.6,p=7.8x10^{-12}] showing an increase in time as N-back load increased. Also, RTs differ between all groups (all P<0.05) except the 2-back vs. 3-back comparison.

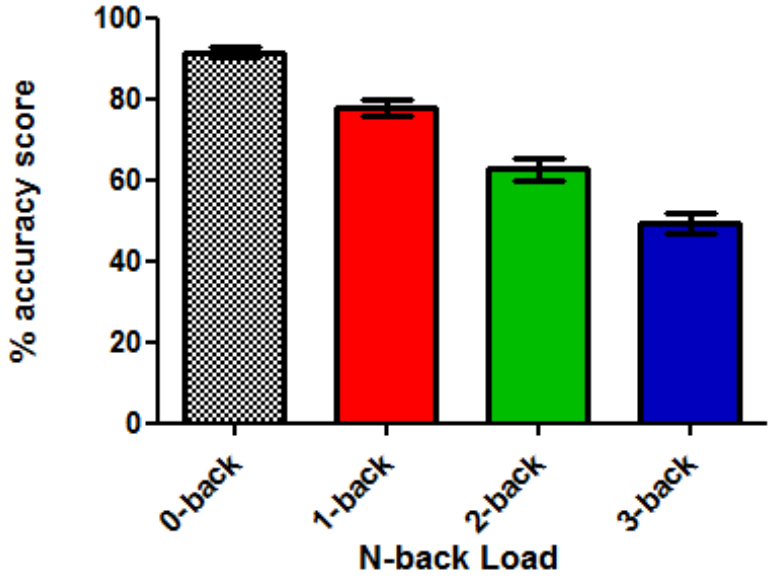


Figure 3.2 Performace as a function of difficulty levels. This figure shows accuracy scores over increasingly difficult N-back conditions. Performance decreases with increase in task difficulty.

Table 3.2: General performance by N-back load

	0-back	1-back	2-back	3-back
Number of participants	60	60	60	60
Mean % score	91.6 (8.4)	78.0 (16.4)	62.7 (20.9)	49.2 (18.7)
Mean RT (ms)	515 (108)	578 (192)	709 (252)	743 (277)

3.3.2 Genotype x tolcapone interaction changes depending on N-back load

There was a genotype x tolcapone x difficulty interaction from 0-back to 3-back [$F_{(2.3,130.3)}$=3.9,p=0.018].

3.3.3 Genotype and tolcapone effect on accuracy scores

Given the three-way interaction, we explored genotype x tolcapone interactions separately for each back condition. There was a genotype x tolcapone interaction for all conditions (see Figure 3.3). This genotype x tolcapone result was most striking for the 2-back condition (Figure 3.3c). On placebo, Met homozygous subjects out-performed Val homzoygous subjects (p=0.006), but in the tolcapone group this difference was reversed such that Val homozygous subjects out-performed Met homozygous subjects (p=0.022); tolcapone significantly impaired performance in Met homozygous subjects (p=0.019) but improved it in Val homozygous subjects (p=0.007). Results were similar, but less significant, for 0-back (Figure 3.3a), 1-back (Figure 3.3b), and 3-back (Figure 3.3d). The genotype x tolcapone interaction accounted for 19% of the overall N-back performance, and for 13.5%, 9%, 15.5% and 9% of the variance in 0,1,2 and 3-back performance respectively (adjusted R^2). A post hoc power calculation revealed that the

sample sizes used in the 0, 1, 2 and 3-back tasks, generated observed powers of 0.724, 0.806, 0.952 and 0.795, respectively.

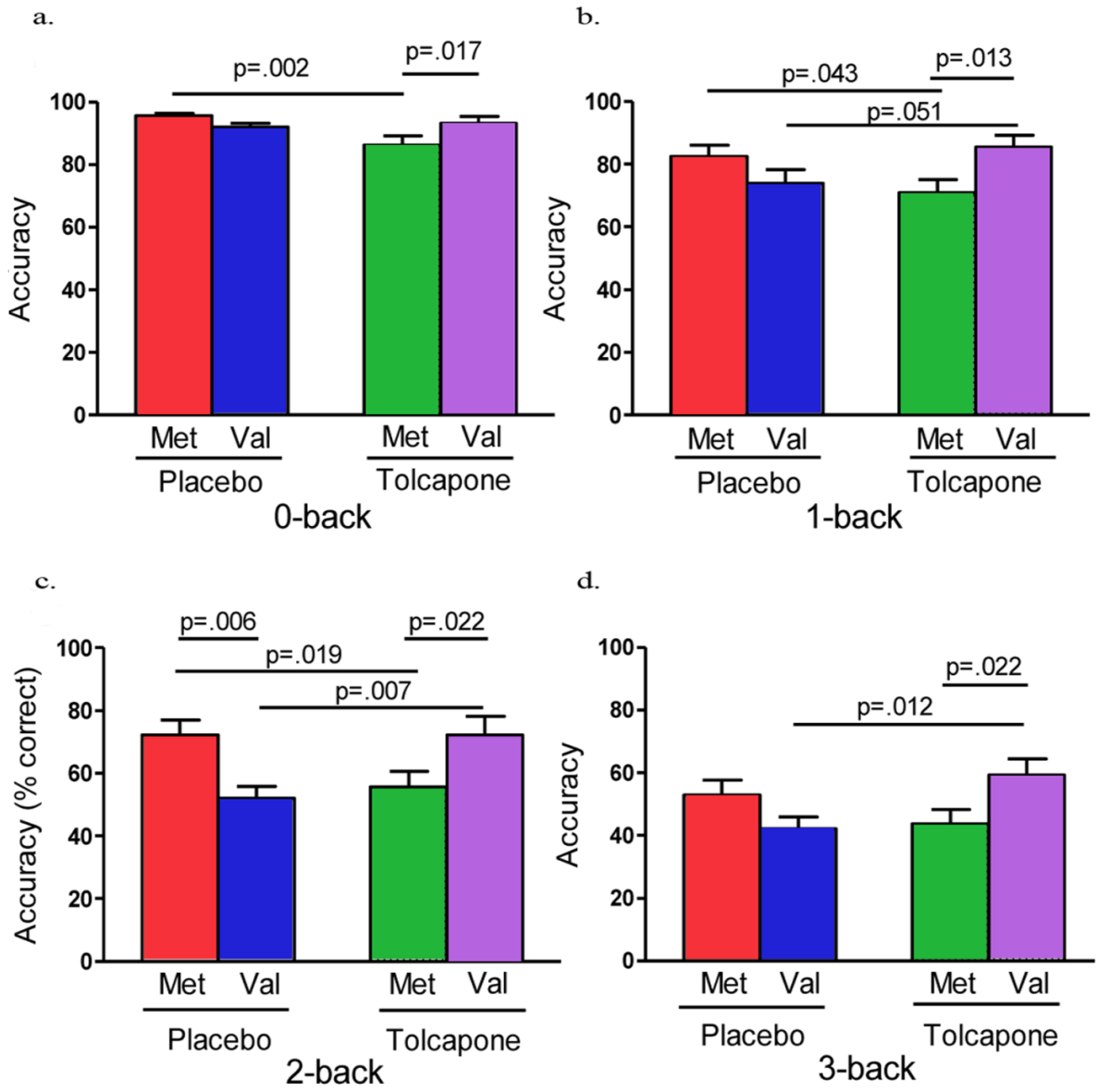


Figure 3.3 Interactive effects of COMT genotype and COMT inhibition.
a. 0-back. Main effect of drug [$F_{(1,56)}$=4.14, p=0.047] and a genotype x tolcapone interaction [$F_{(1,56)}$=6.75, p=0.012]
b. 1-back. Genotype x tolcapone interaction [$F_{(1,56)}$=9.26, p=0.006]. Tolcapone impairs performance in Met homozygous subjects and, as a trend, improves it in Val homozygous subjects.
c. 2-back. Genotype x tolcapone interaction [$F_{(1,56)}$=13.62, p=0.001]. Met homozygous subjects perform better than Val homozygous subjects on placebo. Tolcapone reverses this difference, impairing Met homozygous subjects and enhancing Val homozygous subjects.
d. 3-back. Genotype x tolcapone interaction [$F_{(1,56)}$=8.03, p=0.006]. Tolcapone improves performance in Val homozygous subjects.

As a significant genotype x tolcapone interaction was found in the 0-back condition, in which there is no WM load, a supplementary analysis was performed. For each subject, 1-, 2-, and 3-back performance was expressed as a percentage of 0-back performance and entered into a repeated-measures ANOVA with three levels of difficulty. This showed a significant genotype x tolcapone interaction [$F_{(1,60)}$=9.2; p=0.004], with Met homozygous subjects outperforming Val homozygous subjects in the placebo groups (p=0.024) and Val homozygous subjects tending to outperform Met homozygous subjects in the tolcapone groups (p=0.053). Thus, a significant interactive effect of drug and genotype on WM remained when the 0-back result was used as a control.

3.3.4 No correlation between performance and 'happiness'

Since we found genotype x tolcapone interactions on N-back performance and the happiness VAS rating (see Chapter 2, section 2.6.3), we investigated whether these variables correlated with each other. We found no significant correlations (-0.15<R<0.14; all p>0.05, see Appendix 4). Neither did inclusion of happiness VAS score as a covariate affect the N-back results (data not shown).

3.3.5 Genotype and tolcapone effects on reaction times

RTs for each N-back condition are shown in Table 3.3 and Figure 3.4, separating the data into the four participant groups. There was a trend-level RT x genotype effect [$F_{(1.9,98.7)}$=2.8,p=0.070], with Met homozygous subjects reacting faster than Val homozygous subjects (p=0.049). Tolcapone decreased RT in Val homozygous (p=0.046) but not in Met homozygous (p=0.61) subjects. On placebo, Met homozygous subjects reacted faster than Val

homozygous subjects on the 2-back (p=0.046) and 3-back (p=0.039); no genotype differences in RT were seen for subjects on tolcapone.

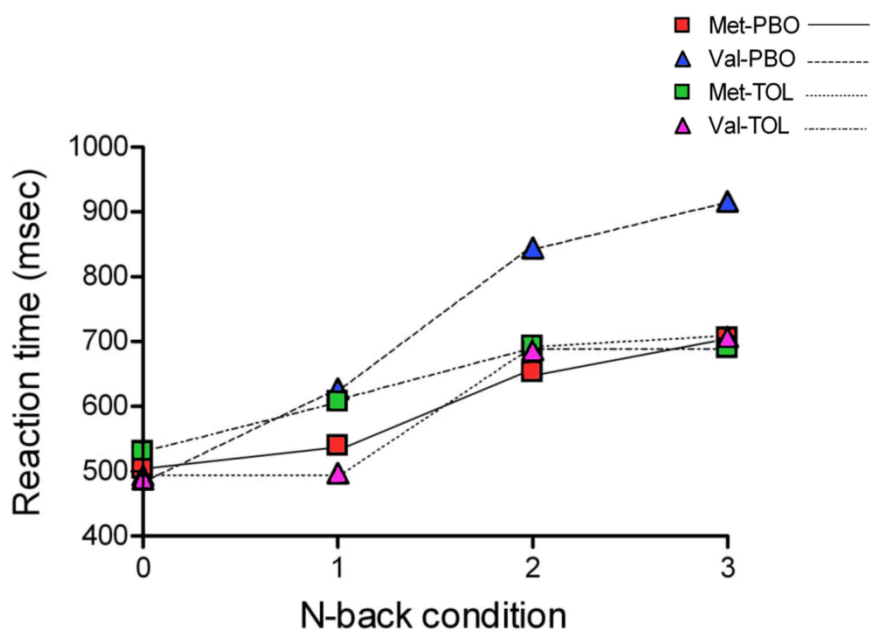


Figure 3.4 Reaction times. Times are plotted for individual N-back conditions and separated by drug and genotype groups. The Val placebo group were slower than the other groups in both 2-back and 3-back conditions

Table 3.3 Reaction times for N-back task

	Met placebo	**Val placebo**	**Met tolcapone**	**Val tolcapone**
N-back task				
0-back RT (ms)	504 (99)	494 (68)	531 (104)	488 (65)
1-back RT (ms)	540 (199)	627 (200)	608 (200)	497 (172)
2-back RT (ms)	653 (237)	844 (256)	693 (233)	687 (282)
3-back RT (ms)	706 (290)	916 (269)	690 (255)	707 (245)

Values are mean (s.d.). RT: reaction time.

3.4 Discussion

This chapter investigated behavioural performance among Val and Met homozygous subjects during a WM paradigm, the N-back task. Half of each genotype group took a 200mg tolcapone capsule, the other half a matching placebo capsule (see Chapter 2, section 2.1 for more details on recruitment and section 2.5 for the study design). The study had two main findings. Firstly, on placebo, Met homozygous subjects outperformed Val homozygous subjects on the 2-back task. Secondly, and more notably, genotype interacted with COMT inhibition by tolcapone to affect WM performance. Compared to those given placebo, tolcapone improved WM in Val homozygous subjects but impaired it in Met homozygous subjects. These interactions were qualitative and robust, and the drug effect was sufficient to reverse the baseline genotype differences.

3.4.1 COMT genotype affects N-back performance

My findings that, on placebo, Met homozygous subjects performed better than Val homozygous subjects during the 2-back condition replicates the result of one large study (Goldberg et al., 2003; Diaz-Asper et al., 2008) but many other studies (Goldberg et al., 2003), and a meta-analysis (Barnett et al., 2008), have been negative.

The discrepant results between studies are likely to reflect, in part, the nature of the N-back task used. The task used here is continuous and therefore has high updating and interference management demands, and is thought to be more dependent on COMT (Callicott et al., 1998; Goldman et al., 2009). Indeed, the version of the task used here was relatively difficult, as shown by the performance data, perhaps exaggerated because subjects were new to the task, and were given only brief instruction, and they completed it in the MEG scanner. Limiting our

study to young men with an above-average and restricted range of verbal IQ (NART scores ranged from 108-125) may also have contributed to our positive results, given gender (Harrison and Tunbridge, 2008; Jacobs and D'Esposito, 2011), age (Tunbridge et al., 2007; Diaz-Asper et al., 2008; Dumontheil et al., 2011), and possibly IQ related (Diaz-Asper et al., 2008) variation in COMT function. Other factors explaining differences between studies may include complications arising by other unmeasured and confounding genetic differences that may also distinguish Val and Met carriers. This includes both the functional epistasis (see Chapter 7, section 7.5) and possible differences in subpopulations of a particular genotype due to linkage disequilibrium (Meyer-Lindenberg et al., 2006; Tan et al., 2007); see Chapter 7, section 7.5 for more information of polymorphisms and how they may interact. Some previous meta-analyses may have failed to find associations if they included different variations of either WM tasks or indeed the N-back task itself. Not all cognitive tasks are sensitive to the Val^{158}Met polymorphism (Tunbridge et al., 2006). This means that meta-analyses including all studies in this field are unlikely to find statistically significant associations of COMT with WM and executive function (Barnett et al., 2008). Furthermore, in cognitive tasks requiring a certain amount of flexibility from the changing of rules during tasks, Val homozygous subjects have the advantage, thus Met homozygous subjects may suffer from impaired cognitive flexibility, suggesting a tension between stability and flexibility (Nolan et al., 2004). This suggests the task chosen here is heavily based on 'stability'.

3.4.2 COMT genotype x tolcapone interaction on accuracy scores

Two prior studies have investigated tolcapone and Val^{158}Met interactive effects on WM and executive functioning. As explained in Chapter 3, section 3.1.3, these studies gave more equivocal results than we report here (Apud et al., 2007; Giakoumaki et al., 2008). Overall, therefore, all studies agree that tolcapone and genotype have interactive effects on executive functioning, with tolcapone enhancing Val homozygous subjects showing either no improvement, or impairing, Met homozygous subjects. The demographic and methodological factors

mentioned above (section 3.4.1) are probably relevant in explaining why we identified more robust genotype x tolcapone interactions, specifically on N-back performance, than did the prior studies; moreover, in addition, our sample of homzoygotes (n=60) is considerably larger than the number included in the earlier studies (n=22-23).

The effects seen in this chapter were of notable size, with Val^{158}Met genotype and tolcapone together accounting for 19% of the variance in N-back performance. This is substantial, especially since this did not include the contribution of other genetic (Meyer-Lindenberg et al., 2006; Nackley et al., 2006; Roussos et al., 2009) and epigenetic (Ursini et al., 2011) sources of COMT variation, nor polymorphisms in interacting dopaminergic genes (Bertolino et al., 2006; Frank et al., 2007; Prata et al., 2009). Chapter 7, section 7.5 details the information on some of these other polymorphisms.

These data reported in this chapter comply with the inverted-U theory of cortical DA relating to cognitive performance. The relative accuracy scores for 2-back can be placed on, and understood in terms of, the inverted-U schematic shown in Figure 3.5. Essentially we see that tolcapone affects the two genotypes with opposite effects, Val homozygous subjects scoring more highly with tolcapone than their placebo counterparts, Met homozygous subjects scoring lower than their tolcapone counterparts. It is thought that the tolcapone inhibits the high activity enzyme in the Val genotype so that the enzyme is unable to break down as much prefrontal DA. The presumed increase in DA availability, and thence DA signals, translates into a greater capacity for WM. Met homozygous subjects, however, are thought to already have a near optimal level of PFC DA required for WM. This means the enhanced DA resulting from tolcapone causes supraoptimal levels of DA to occur in the PFC, which translates to worse WM performance in Met homozygous subjects. However, the same increase in DA in Val homozygous subjects, who start

from a lower baseline, take them nearer to the peak and thus improves performance (see Figure 3.5). Goldman-Rakic provides a mechanism for these three levels of DA (suboptimal, optimal and supraoptimal) in the PFC focussing on the effect of D_1 receptors (see Chapter 1, Figure 1.2). DA, acting at D_1 receptors can enhance glutamatergic inputs to both pyramidal cells and interneurons. At low levels of DA release, these inputs are not enhanced to either class. At moderate levels of DA release (i.e. what we may see in Met placebo or Val tolcapone subjects) it is the glutamatergic inputs to pyramidal cells which are primarily enhanced, leading to an increase in pyramidal cell delay activity and improved WM function. At high levels of DA release, the glutamatergic inputs are enhanced to both pyramidal cells and interneurons, leading to a reduction in pyramidal cell activity, by feed forward inhibition with resultant impairment of WM (Goldman-Rakic et al., 2000).

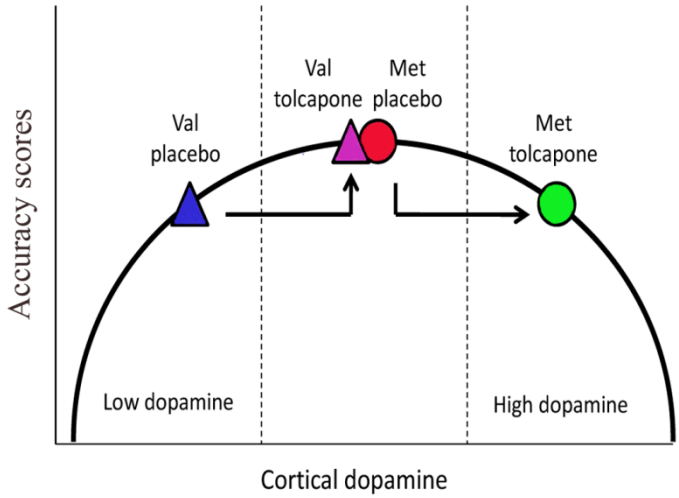


Figure 3.5 Inverted-U schematic. The relationship between cortical DA and accuracy scores during 2-back is shaped like an inverted-U. In the placebo condition, scores in this WM task are higher in Met homozygotes than Val homozygotes, but tolcapone reverses this, with the Met homozygote group scoring lower than the Val homozygotes.

These data can also be interpreted in terms of relating prefrontal DA to stability of representations (see Chapter 1, Figure 1.3). Met alleles (with relatively low COMT activity) allow the diffusion of DA further from the synaptic cleft due to the elevation of DA in the PFC. This further diffusion leads to activation of extrasynaptic D_1 receptors. It is predicted that D_1

stimulation should enhance the stability of PFC networks and thus the performance of cognitive tasks involving the maintenance of information, but possibly also leading to decreased cognitive flexibility as noted above (Bilder et al., 2004). The high COMT activity encoded by the Val allele would limit diffusion from the synapse, favouring activation of intrasynaptic D_2 receptors. D_2 activation transiently reduces local inhibitory interneuron activity, resulting in a relatively non-specific increase in pyramidal neuron excitability. This results in a reduction in the stability of PFC for the Val allele, manifesting in a decreased ability to maintain information in WM (Bilder et al., 2004; Winterer and Weinberger, 2004) but an enhanced ability to update the content of the WM with new information or switch cognitive sets (Cools and Robbins, 2004). If the N-back task instantiated here relies more on stability than flexibility, then this would explain why the Met placebo group scored higher than the Val placebo group. Similarly, the inhibition of COMT by tolcapone results in an increased level of extrasynaptic DA, thus Val homozygous subjects on tolcapone become more Met-placebo-like and improve their accuracy score.

3.4.3 Interpretation of reaction time data

Literature linking COMT to RT in N-back is inconclusive. Some data show a COMT genotype effect (Apud et al., 2007; Giakoumaki et al., 2008; Gong et al., 2011; Mueller et al., 2011), others find non-significant results regarding COMT-modulated RT (Goldberg et al., 2003). The trend effect in this data set shows COMT genotype affecting the data in a way that corroborates some of the previous results, that is, Val homozygous subjects are slower to respond than their Met counterparts. In addition, the data also show that after tolcapone administration, the Val but not Met homozygous subjects were faster to respond in the N-back task.

In any event, it is difficult to interpret the results in the RT data as some subjects reported a tactic of tapping out numbers in a rhythm, therefore the aim was not necessarily to press

answers as soon as possible during this task. This means that the RT data may not represent how quickly the participants can process and produce their answers (i.e. if they were holding back their choice so as to press in accordance with their self-set rhythm). However, given that the RTs increased with N-back load, this suggests that to some degree the RT data represents the time it might take for the brain to process the result. Regardless, accuracy is the primary outcome of interest for this task, and it is notable that the results were clear and robust in that respect.

3.4.4 Limitations and future studies

This study is limited to the use of tolcapone in a single dose, therefore not allowing exploration of whether similar results would persist, or be attenuated, or be exacerbated with chronic usage. The participant demographic used here was limited to healthy, young, above average IQ males. But this could be extended, amongst others, to females, the elderly or possibly patients with diseases that involve DA dysregulation. These issues are important in terms of the potential clinical relevance of the present results (see Chapter 7, section 7.2 for more detailed discussion).

Future studies may also wish to expand on the type of executive function task used. Using only N-back does not allow us to assess COMT's effect on 'stability' vs. 'flexibility' of representations. The test battery could include the Wisconsin Card Sorting Task, known to require 'flexibility' of cognition to a greater degree than the N-back. Alternatively, it is possible to separate and assess which components of WM that COMT influences, namely encoding, delay, or retrieval, by using a style of task that separates these subcomponents. Various studies have already begun to 'parse' these different WM subprocesses (Goldberg and Weinberger, 2004). For example, Bruder and colleagues (2005) examined the differential influence of COMT variability on four traditional WM tasks thought to be mediated by the dorsolateral PFC. The tasks were chosen

because of their different associations to specific WM subprocesses including simple information maintenance, temporal indexing of information, and 'online' manipulation and updating of WM contents. Met homozygous subjects only showed better performance on the task that demanded active manipulation, as well as maintenance, of information (using a letter number re-sequencing task). Another study failed to show performance difference between Val and Met homozygous subjects, but shows a greater fMRI activity among Val compared to Met homozygous subjects in the dlPFC during encoding but not retrieval processes (Tan et al., 2007).

3.4.5 Conclusion

These results provide a strikingly clear example of a genotype x tolcapone interaction during the N-back. Tolcapone differentially affects Val and Met homozygous subjects' ability to perform this WM task. This is largely due to the pharmacogenetically favourable set of circumstances (discussed in Chapter 7, section 7.2). These rare circumstances may explain why COMT provides the clearest demonstration to date of a qualitative pharmacogenetic effect whereby not just the magnitude but the direction of the behavioural effect of a drug is determined by a polymorphism. However, the fact that prior studies did not find such clear cut results attests to the many other factors which can obscure this relationship. Therapeutic and pharmacogenetic implications are discussed in Chapter 7, section 7.2.

Chapter 4

Exploring neural correlates of working memory performance using MEG

Chapter 4

4. Exploring neural correlates of working memory performance using MEG

4.1 Introduction

The preceding chapter demonstrated how COMT genotype in healthy volunteers influenced their performance on the N-back task, with poorer performance in Val homozygous subjects than Met homozygous subjects. An interaction between the COMT Val^{158}Met genotype and tolcapone saw tolcapone improve performance in the Val homozygote group, but impair it in the Met homozygote group (see Chapter 3). In this chapter the MEG approach is used to explore the neural signature of the N-back task and how COMT modulates this, (using principles outlined in Chapter 2 and this chapter, section 4.2) in subgroups matched for performance. The role of DA and COMT in WM, and the relationship to the inverted-U, has already been considered in Chapter 1, section 1.3.3. Below, the concept of WM is further explored providing details of how COMT affects the neural activity during the N-back task.

4.1.1 The PFC and working memory

The PFC is known to play an important role in higher cognitive functions (Miller and Cohen, 2001). Prefrontal lesions and inactivation disrupt cognitive tasks that depend on WM (Chafee and Goldman-Rakic, 2000; Buckley et al., 2009). The delayed-response paradigm has been used in animal experiments to study the cellular basis of WM. This has been researched most extensively in the primate dorsolateral PFC. Neurons in the PFC increase their activity during the cue, delay, and response phases of this task (Fuster and Alexander, 1971; Kubota and Niki, 1971; Rao et al., 1997). During the delay period, selectively enhanced firing rates occur in the absence of external cues, which has been interpreted as a neural basis for the active maintenance of memory items. The primate PFC is the only area where the firing rates of

neurons during the delay period between encoding and recall are closely correlated with behavioural performance. Hence, the PFC is thought to be a key area in WM processing.

It is important to note some theories suggest a link between WM and striatal levels of DA (Pribram and Tubbs, 1967; Weinberger, 1987). In this regard, it is entirely possible that altered levels of DA in the PFC will affect or modulate subcortical areas as has been demonstrated in animal studies (Pycock et al., 1980). Human imaging data corroborate the notion that cortico-striatal connections are important for WM. Direct measurement of DA transmission in humans, using PET, showed that striatal DA synthesis capacity can predict not just WM performance, but also PFC activity (specifically the left inferior prefrontal region) during WM performance (Landau et al., 2009). Clearly, the interpretation of WM data in terms of cortico-striatal circuitry is complicated. This considered and given the limited time (and analysis procedures) available, the role of the striatum in WM will not be a feature of this thesis. Instead the focus will be on the influence of COMT on cortical processing of WM during the performance of the N-back task.

4.1.2 The effect of COMT on neural substrates of N-back performance

The N-back task requires the activation of a wide network of brain regions, with dorsolateral PFC activation being the most consistently reported event in terms of the BOLD fMRI response (Fletcher and Henson, 2001). Importantly, Val^{158}Met genotype has been shown during the N-back task to modulate the dorsolateral PFC as well as a diverse cortical WM network (Callicott et al., 2000; Egan et al., 2001; Mattay et al., 2003) with a more recent suggestion emphasizing a role for the cingulate cortex (Stokes et al., 2011). As detailed in Chapter 1 section 1.3.3, studies report Met homozygous subjects to have lower activity in these areas than their Val counterparts, suggesting they are more efficient performers (Egan et al., 2001; Gallinat et al., 2003; Tsai et al., 2003; Blasi et al., 2005; Bertolino et al., 2006; Tan et al., 2007; Krach et al., 2010).

This result has been replicated a number of times and is significant by meta-analysis (Mier et al., 2009).

4.1.3 'Efficiency' hypotheses in imaging studies

The NEH is expounded in Chapter 1, section 1.5.1, with section 1.3.3 detailing fMRI studies of COMT and N-back that are relevant to this hypothesis. It has been suggested that Val^{158}Met effects on PFC efficiency may result from the role of DA in modulating the signal-to-noise ratio in the PFC (Winterer and Weinberger, 2004). Although this finding is robust, it is unclear what processes underlie efficiency. It is tempting to suggest that the relatively poorer PFC function associated with the Val allele, compared with the Met allele, may be due to suboptimal levels of PFC DA during WM, as theorized in the inverted-U-shaped curve hypothesis (Goldman-Rakic et al., 2000); see Chapter 1, section 1.2.

Mattay and colleagues (2003) demonstrated this relationship between DA and efficiency may be inverted-U shaped. The study administered amphetamine, which increases synaptic DA, to healthy volunteers. They reported genotype x tolcapone interactions for both WM performance and PFC efficiency, determined using fMRI. On the one hand, Val (but not Met) homozygote N-back scores and PFC efficiency improved at the 1- and 2-back levels following amphetamine. This is consistent with the idea that, compared to Met homozygotes, Val homozygous subjects have reduced DA levels due to high COMT activity, thus amphetamine increases DA signalling, moving the Val homozygous subject along and up towards the peak of the curve. This results in an improvement of memory and PFC performance in the Val homozygous subjects, analogous to the findings with tolcapone in Chapter 3. On the other hand, an increase in PFC activity was observed in Met homozygous subjects at the 3-back level, which was associated with a decrement in performance and increase in RT, consistent with a detrimental

effect of supra-optimal levels of PFC DA (due to low COMT activity in combination with augmented DA function induced by amphetamine) at high working memory loads. This can be represented by amphetamine pushing the Met homozygous subjects right along the inverted-U curve, up and over past the peak of the curve. See Chapter 1, section 1.5.2 for an overview on COMT and NEH.

Winterer found a possible way that EEG may be relevant to efficiency by analysing event-related potentials (ERPs) in samples of schizophrenia patients, their unaffected siblings, and healthy controls. Here, the measurement of electrophysiological response variability in a frontal P300 component during an auditory oddball task identified a 'noise' parameter that indexed the amount of activity not time-locked to the stimuli, i.e. spontaneous background activity. Electrophysiological prefrontal noise was positively associated with P300 amplitude, but inversely correlated with N-back performance across all subgroups (Winterer et al., 2004; Winterer and Weinberger, 2004; Winterer et al., 2006). This suggestion of a higher signal being 'less efficient' leading to a worse score, sits nicely with the similar relationship shown in fMRI studies of N-back. In the same paper, Val homozygous subjects (particularly males) showed significantly more PFC noise than Met carriers, although no difference was found between Met^{158}Met and Val^{158}Met subgroups.

4.1.4 Potential time components of interest for working memory and N-back

A key time point of interest during the N-back, and indeed all WM tasks, is the P300 (250-400ms post stimulus). P300 can be split into early and late time points, P3a and P3b respectively (Sutton et al., 1967). P3a has a maximum amplitude over frontal/central sites with peak latency between 250-280ms. It is generally associated with attention (Polich, 2007) and novelty (Hagen

et al., 2006). P3b has a peak between 250 and 500ms (though these boundaries vary within the literature) and amplitudes are thought to peak over parietal areas (Polich, 2007).

The amplitude of the P300 is known to increase as WM load increases (Donchin and Coles, 1988; Gevins and Cutillo, 1993; Segalowitz et al., 2001; Dolu et al., 2005), and is thought to reflect the updating component of WM which would be prompted in N-back when a new number is shown (Johnson and Donchin, 1985; Başar-Eroglu et al., 2001). Although the location of altered P300 is primarily in frontal regions, it also seems to extend beyond the PFC, e.g. a prominent posterior element has been shown at the Pz and Cz sites on the EEG map (Dolu et al., 2005), which correspond broadly to midline vertex and midline parietal areas of the local maps to which I will refer. Despite the general notion that the P300 may increase during increasing load of WM, some individual areas may exhibit a P300 reduction, assumed to be because more complex task requirements distract attention across more sub-processes than a simpler task (Houlihan et al., 1998).

4.1.4.1 COMT modulated P300

Some studies have found difference in auditory event-related P300 between COMT genotypes, the Met homozygous subjects demonstrating a higher amplitude than Val homozygous subjects over the centro-parietal area, constituting the first description of a genetic influence on the P300 wave (Golimbet et al., 2006). Since then, one study demonstrated COMT modulation of the P300 over the midline regions relating to 3-back (Yue et al., 2009) with Val homozygous subjects showing higher amplitude (and lower latency) than Val^{158}Met heterozygotes.

4.1.5 Summary

Evidence of a difference in efficiency between Val and Met homozygous subjects during the N-back is particularly prominent among the fMRI literature, especially in the dlPFC, but what this means at the neural level is yet to be addressed. EEG studies provide some evidence, albeit limited, of Val^{158}Met differences during N-back. These studies are sparse and somewhat preliminary. This chapter explores these potential Val^{158}Met differences in WM further with MEG using performance-matched individuals (to avoid confounds), focussing on the P300, the time component most relevant to WM according to previous literature.

4.1.6 Objectives

The main objective was to assess the hypothesis that the MEG signature associated with the N-back task will differ between genotype and drug groups, thus extending the limited EEG data pertaining to COMT and N-back.

4.2 Methods

The task used in this chapter is the same as that detailed in Chapter 3 section 3.3.1.

4.2.1 Principles of analysis approach

See Chapter 2 section 2.8.4 for the general approach to MEG analysis. Below includes N-back specific information.

4.2.1.1 Selection Criteria

Given the wealth of data accrued from the use of MEG in this study, various parameters have been selected to focus on key results:

1. The P300 focus

The P300 was the main time component of focus for reasons outlined above.

2. Contrasts

In line with the N-back fMRI literature (Egan et al., 2001; Mattay et al., 2003; Giakoumaki et al., 2008; Blanchard et al., 2011), all power maps and probabilities were prepared with contrasts that subtract the 0-back from any 1, 2 or 3-back condition. This assumes that the brain signal is additive and subtracting 0-back will subtract the 'non-WM load' components common across all back conditions. The 2-back will be the main focus, decided a priori based on both a wish to minimise the amount of comparisons, and previous literature demonstrating that genotype differences tend to present at this level of moderate difficulty. It is also relevant to note that the In House methods used could not, at this time, support a repeated measures analysis of the MEG data.

3. Performance matching

The N-back behavioural results (see Chapter 3), provided sufficient data to allow MEG analysis in either a performance-matched subgroup or all participants within each group. The former allows any differences in brain signal between groups to be attributed to differences in cognitive strategies or differing ways of processing that do not result in differing performance scores. Using non-performance matched subjects may confuse the interpretation of the data since the 'better scorers' may process information in ways which were unrelated to the particular drug/genotype groups in question. Given that my behavioural results show differences in performance scores (Chapter 3, section 3.4.3), the decision was made a priori to focus analysis on the performance-matched individuals. Table 4.1 shows the demographics table for the

performance-matched sample. There were no significant differences between groups for any of the values shown.

Table 4.1 Demographics of the Performance-matched study cohort

	Met placebo	Val placebo	Met tolcapone	Val tolcapone
Number	9	11	12	8
Ethnicity[a]	8C, 1I	8C, 2I, 1Ch	12C	5C, 2I, 1Ch
Age (y)	22.1 (2.9)	23.9 (4.9)	21.4 (2.8)	24.3 (3.2)
NART	117.5 (4.6)	118.0 (6.0)	115.6 (4.9)	117.8 (4.4)
Alcohol units/week	11.5 (8.8)	8.1 (5.1)	7.7 (5.5)	10.9 (11.1)
Cigarettes/day	0 (0)	0.5 (1.4)	0.5 (1.7)	0.39 (1.1)
BDI	2.3 (2.2)	4.2 (3.3)	3.8 (3.8)	3.9 (3.6)
STAI	26.4 (4.9)	33.6 (7.9)	30.9 (8.3)	34.5 (2.3)

Values are mean (s.d.).
NART: National Adult Reading Test. BDI: Beck depression inventory. STAI: Spielberger Trait Anxiety Inventory.
[a]C: Caucasian; I: Indian; Ch: Chinese; A: African Caribbean.

4.2.1.2 Course of analysis

The analysis protocol, and the sequence of results to be presented, were as follows:

1. Assessment of global plots for N-back load across the participant group as a whole to find key time components of interest, using global activity and probability plots (Chapter 2, section 2.8.5 describes the different plots and maps).

2. Use of global plots and probability maps (Chapter 2, section 2.8.4.3 describes how these statistics are calculated) to assess differences during the 2-back condition, among the four participant groups.
3. Selection of time components of interest for further analysis. To select the time points of interest, the global plots for 2-back were examined (using performance-matched data as selected by 2-back performance, contrasted with 0-back) separated by the four groups: Val placebo, Val tolcapone, Met placebo and Met tolcapone. The regional activation maps (local maps) derived from the two-way ANOVA were then examined to identify areas of significant activity difference. Further investigation of a time point took place if the component met the following criteria:

i. The levels of significance were $p< 0.01$ (as previously mentioned in section 2.8.5.2). For the global plots $p< 0.01$ was chosen over $p<0.05$ for a greater stringency to identify the most robust changes.

ii. The components found within the first 600ms are linked to WM as reported in previous literature i.e. P300 (Dolu et al., 2005).

iii. The time point is within the range shown to be modulated by back condition and therefore important in memory-load.

4. Characterisation of these time components in terms of local power maps and local probability maps. Local map information can be found in Chapter 2 section 2.8.5.3.

The scheme for MEG analysis is summarized in Figure 4.1

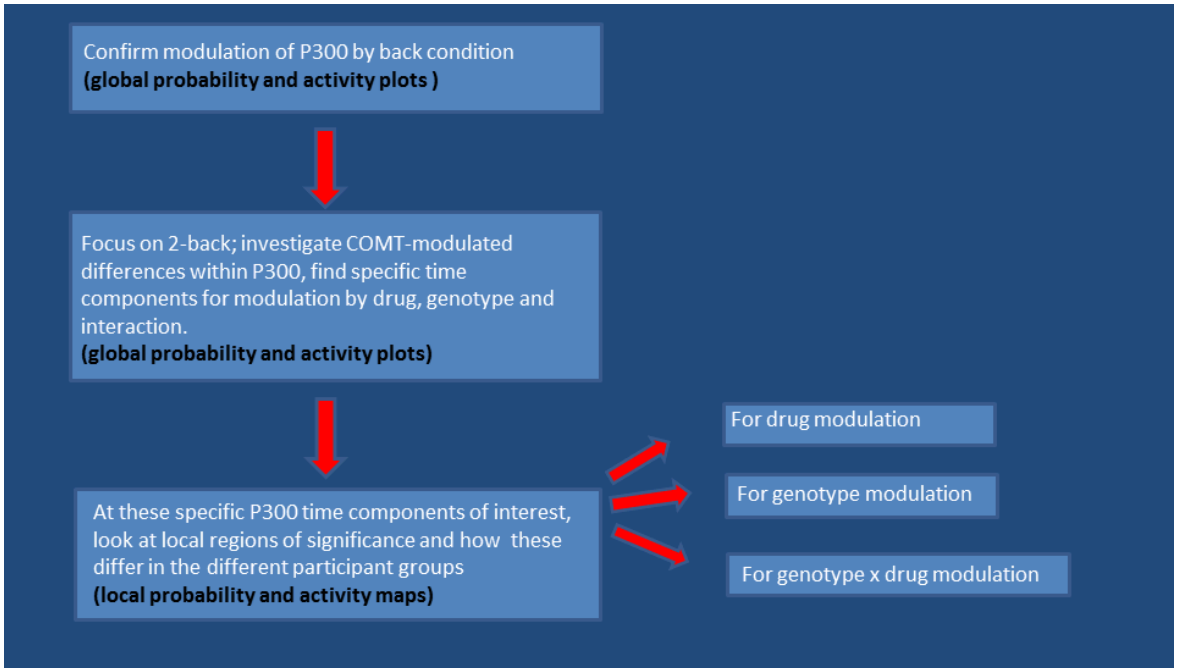


Figure 4.1 Strategy for MEG analysis of N-back processing. The process of analysis is summarised in the above flowchart. Time-components of significant difference among the N-back conditions were noted. The next step focused on the 2-back condition to investigate COMT modulated differences at P300 that were within the areas shown to be COMT modulated. Further investigation of COMT modulation uses local maps.

4.3 Results

4.3.1 Comparison of performance-matched and non-performance-matched data

Figure 4.2 shows a global probability plot (shown in a.) and global activity plots (shown in b. and c.). The global probability plot in a. has time on the x axis and probability on the y axis. Each of the curves represents the probability resulting from an ANOVA comparing the differences in evoked activity between the different back conditions. The black line corresponds to the non-performance-matched data (the corresponding activities to this are shown in b.) and the red line in a. corresponds to the performance-matched data (the corresponding activities of which are shown in c.). The y-axis represents the negative logarithm of probability, i.e. the value 2 represents a significance level of p=0.01, which is marked on the graph. Any line that goes above this marker has reached significance level. The global activity plots have time on the x axis and gradiometer root-mean-square signal (i.e. power) measured in fT/cm on the y axis. It shows lines, each representing the collective activity over the whole head, during a particular condition, in this case, a back condition, either 1-,2- or 3-back.

The global plots represent the activity elicited from each of the N-back conditions for both non-performance-matched (shown in Figure 4.2b.) and performance-matched (Figure 4.2c.) subjects. This is the only figure that will show non-performance-matched data. This figure does not separate the participants into the four participant groups (for data split by drug and/or genotype groups see 4.3.2), but instead looks at the participant group as a whole, separating by back condition. Each line represents a particular N-back condition, either 1-, 2- or 3-back (all contrasted with 0-back activity).

These activity plots demonstrate similar shaping in both performance-matched and non-performance matched versions. Thus the ability to compare between conditions is not diminished by using a performance-matched focus, yet it will reduce the likelihood that any differences seen between drug or genotype groups are just a function of performance score. This is particularly important in light of the behavioural results which show that the groups perform differently. The probability plot in Figure 4.2a. shows both performance-matched and non-performance matched versions have a similar P300 time component where WM load alters the signal, peaking in significance around 271ms. This is more significant for performance matched ($p=4.4x10^{-6}$) than non-performance-matched ($p=9.9x10^{-3}$), although both results are highly significant[7]. The activity maps (Figures 4.2b. and 4.2c.) at this time both show an increasing need for 'brain activity' as the N-back load increases, with 3-back yielding the most global activity, followed by 2-back and then 1-back. The peak on the performance-matched global plots around this time point occurs at 286ms and reached a height of 0.69fT/cm for 3-back, 0.63fT/cm, for 2-back and 0.59fT/cm for 1-back.

The one key difference between performance-matched and non-performance matched is the extra time component of interest in the non-performance-matched activity plot peaking at 441ms. This peak was not investigated further. The remainder of the analysis focuses entirely on the performance-matched subgroups shown in Table 4.1, and therefore is limited to the P300 since this is the only peak which meets the pre-specified criterion of $p<0.01$ (Figure 4.2a.).

N.B. Despite the focus on P300 for reasons already stated, the whole 600ms time period has been included in the global plots to show the extent of significant time components that this MEG analysis highlights. The P300 is highlighted with a blue strip.

[7] The high significance is a result of the effect being consistent across a number of channels.

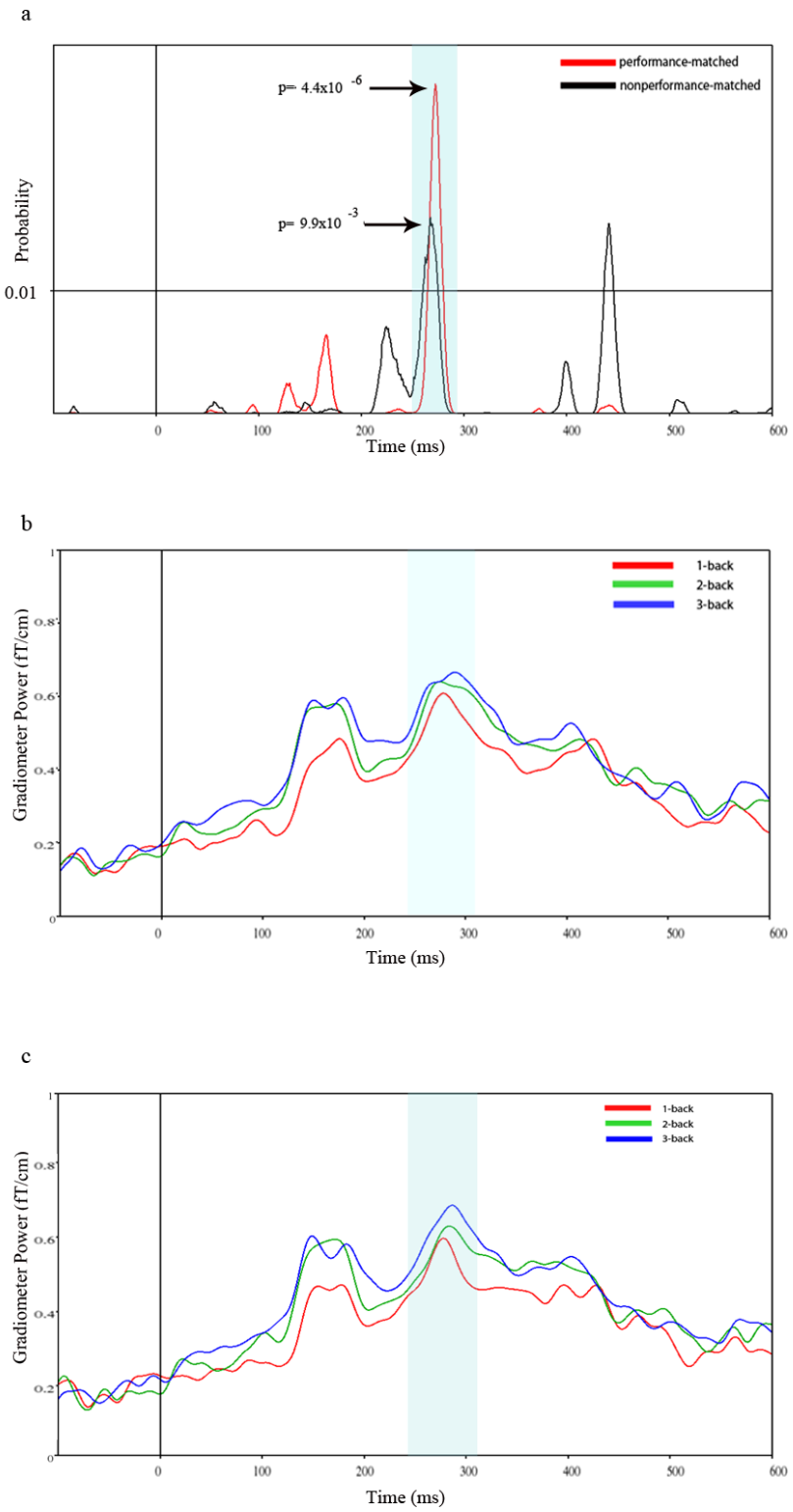


Figure 4.2 Global plots for performance-matched and non-performance-matched data. The probability plot shown in a. demonstrates the time points at which the back-conditions differ. The red line represents performance-matched data; the black line represents the non-performance-matched data. The graph is displayed so that the lines above the 0.01 value represent significant difference. Here, both performance-matched and non-performance-matched data demonstrate an early P300 component. This figure shows global power plots for whole brain activity elicited during the N-back task using non-performance-matched data (shown in b.) and performance-matched data (shown in c.). Each line represents a particular N-back condition, either 1-, 2- or 3-back.

4.3.2 Analysis of time components of interest in performance-matched data

Figure 4.3 shows the global plots for 2-back performance-matched data, illustrating the evoked response for subjects (see 4.3d.) in a particular group and how these differ between the different groups (see 4.3a., b. and c). These differences are split into a. 'drug differences', b. 'genotype differences' and c. 'genotype x tolcapone interaction' for clarity. The black line in Figure 4.3a. shows a placebo-tolcapone difference, with a peak significance of $p=3.7x10^{-20}$ observed at 275ms. Inspection of the blue line in Figure 4.3b identifies a main effect of genotype ($p=3.4x10^{-6}$) with a key peak at 280ms. The global activity plot in 4.3d suggests that this difference may be driven by the high peak in the Met placebo group at 278ms (1.1fT/cm). Figure 4.3c shows a 335ms peak of significance ($p=1.4x10^{-5}$) relating to a genotype x tolcapone interaction. Inspection of 4.3d. suggests that this interaction is due to the Val homozygote group showing increased activation with tolcapone when compared to placebo, but the Met homozygote group showing decreased activation in the tolcapone group as compared to the placebo group.

The temporal order of signals around this early P300 phase in the global plot is not significantly different between groups (data not shown) despite the values being slightly later in the Val homozygote groups (282ms and 293ms for placebo and tolcapone respectively) relative to the peaks in Met homozygote groups (278ms and 275ms for placebo and tolcapone respectively).

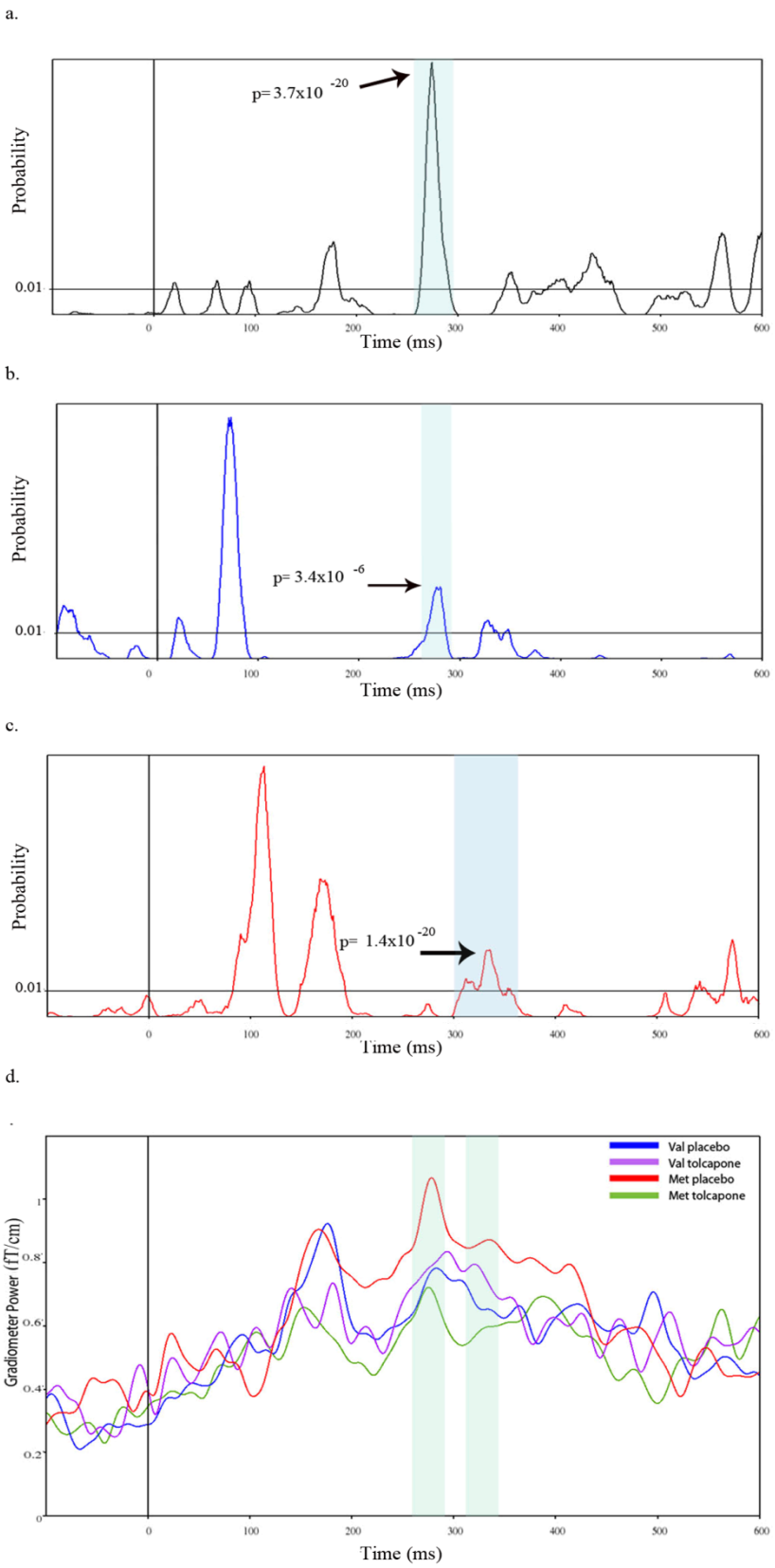


Figure 4.3 Participant group differences in 2-back. Images a. – c. show the probability plots: The lines on each plot represent time points of significant difference between a. the drug and placebo groups (black), b. the genotype groups (blue) and c. their interaction (red). The activity plots (shown in d.) are separated by Val placebo (blue), Val tolcapone (lilac), Met placebo (red) and Met tolcapone (green). The areas highlighted in blue strips corresponds to areas of significance for drug, genotype or their interaction.

Summary of 2-back global map findings

Three of the findings are highlighted in Figure 4.3:

1. Main effect of tolcapone around 275ms (Figure 4.3a.)
2. Main effect of genotype around 280ms (Figure 4.3b.)
3. Genotype x tolcapone interaction around 335ms (Figure 4.3c.)

Sections 4.8.3, 4.8.4 and 4.8.5 explore these P300 time components:

4.3.3 Effect of tolcapone on the 275ms time component

Figure 4.4 explores the basis for the significant effect of tolcapone found at 275ms, showing the local maps relating to 275ms post-stimulus onset. The probability map in 4.4i. depicts areas of significant difference between tolcapone and placebo groups, with red indicating areas where $p<0.01$. Here, and in similar local maps throughout this thesis, the arrows point to areas of significance in the probability maps and the ellipses highlight these same areas in the corresponding activity maps. Figures 4.4ii. and 4.4iii. show local activity maps for placebo and tolcapone administered subjects, respectively. In these activity maps, the red represents the higher activity levels. Inspection of probability maps (Figure 4.4i.) reveals a spread of significance over the vertex, through the right parietal to the right temporal lobe, labelled 1 ($p=0.0012$). This corresponds to a higher placebo activity far right and frontal (1.5fT/cm in the placebo group vs. 0.72fT/cm in the tolcapone group) whilst the midline vertex significance appears to come from a small area of higher activity in tolcapone groups over placebo groups (1.3fT/cm compared to 0.69fT/cm respectively, $p=0.013$).

Area 2 points to a significance explained by lower activity in the tolcapone group over left temporal areas ($p=0.0044$). This area has highest activity in the placebo activity, with a strength

of 1.2fT/cm, compared to 0.78fT/cm in tolcapone. Area three highlights a difference over the frontal regions, therefore likely to be relevant to the WM task. The activity difference causing this appears to come from higher activity in tolcapone in the posterior areas of this frontal region, but for the majority of this area, where the difference arises is not clear.

Summary of the differences between tolcapone and placebo groups at 275ms during the 2-back

There are clear differences in activity between placebo and tolcapone. The tolcapone effects extend beyond frontal areas. Regarding this effect, the direction of activity is not the same over all areas (i.e. in some areas, tolcapone has greater activity than placebo, in other areas the reverse).

4.3.4 The effect of genotype on MEG signal during 2-back at 280ms

Figure 4.5 shows the probability and activity maps of Val and Met homozygous subjects analysed at 280ms post-stimulus onset. This was shown to be significantly different between the genotypes in the previous global probability plot shown in Figure 4.3b. At areas 1 and 2, the Val homozygous subjects show greater left and right occipital cortex activity compared to Met homozygous subjects (1.2fT/cm vs. 0.76fT/cm respectively, p=0.0079). The right parietal region, labelled 3, is more active in Val homozygous subjects (0.75fT/cm) than Met homozygous subjects (0.67fT/cm), p=0.017. A further effect is seen in the anterior left temporal area, labelled 4, showing higher activity in the Val homozygote group (1.0fT/cm) than the Met homozygote (0.77fT/cm) group, p=0.032. Note the stretch of red on the right side over temporal regions in the Met activity local map, shown in aiii. This activity does not appear to be significantly different from the Val activity and therefore has been ignored.

Summary of the Val^{158}Met effects on MEG signal at 280ms during the 2-back

Val homozygous subjects exhibit higher activity than Met homozygous subjects over left temporal, occipital and right parietal areas (and frontal, see point 3).

4.3.5 Genotype x tolcapone interaction in the 335ms time component

Figure 4.3c demonstrated a genotype x tolcapone interaction at 335ms. Figure 4.6a shows probability (4.6ai.) and activity maps (4.6aii-v.) at 335ms for Val homozygous and Met homozygous subjects administered either tolcapone or placebo.

The two significant areas are labelled 1 and 2. The left fronto-temporal area, labelled 1 ($p=8.9x10^{-3}$), has the highest activity in Met placebo (1.4fT/cm) and Val tolcapone (1.5fT/cm) groups, followed by the Val placebo group (1.4fT/cm) and then the Met tolcapone group (1.1fT/cm). The second significant area of difference between the four groups occurs over the intersection of left temporal, occipital and parietal cortices, labelled 2 ($p=7.8x10^{-3}$). Inspection of the activity maps reveals that the highest signal in this area arises from the Met tolcapone group (shown in 4.6av.) with an activity peaking at 1.1fT/cm, followed by Val placebo (1.0fT/cm), with the lowest activity groups seen in Val tolcapone (0.68fT/cm) and Met placebo (0.71fT/cm). In general, the overall activity pattern of Met placebo and Val tolcapone are more similar than either Val placebo and Met tolcapone groups.

The first significant area to explore in post-hoc analyses was the left fronto-temporal difference, corresponding to the area labelled as 1 in Figure 4.6ai. Probability maps generated from the post-hoc analysis of signals at 335ms, are shown in Figure 4.6b. The greatest difference in activity was observed in Figure 4.6biii. between Val tolcapone (1.5fT/cm) and Met tolcapone

groups (1.1fT/cm) in the frontal cortex ($p=8.0x10^{-3}$) as shown by the corresponding activity maps in 4.6aiv and 4.6av. This same area shows significance in Figure 4.6ii. comparing Met placebo and Met tolcapone groups (1.4fT/cm vs. 1.1fT/cm respectively; $p=1.5x10^{-2}$). The second significant region in the post-hoc results relates to the left temporo-occipito-parietal area, labelled 2 in Figure 4.6ai. The biggest difference here is shown in 4.6bi, assessing Met placebo vs. Val placebo differences (p=0.008) with 4.6a showing that the Val placebo (seen at 4.6aii.) with an activity of 0.09fT/cm, is more active than the Met placebo (seen at 4.6aiii.) with an activity of 0.46fT/cm. Figure 4.6bii. shows the same region is significantly different between the Met placebo group (0.52fT/cm, see 4.6aiii.) and the Met tolcapone group (0.63fT/cm). Thus the Met tolcapone group is more active than the Met placebo group over this temporal region.

Summary of Post hoc data for genotype x tolcapone interaction at 335ms during 2-back

Overall, from this systematic assessment of the post-hoc data of the 335ms component we see that, over frontal regions, the Val tolcapone group exhibits higher activity than the Met tolcapone group and the Met placebo group exhibits higher activity than the Met tolcapone group. However, over the temporo-occipito-parietal border, the Val placebo group is more active than Met placebo group, and the Met tolcapone group is more active than the Met placebo group.

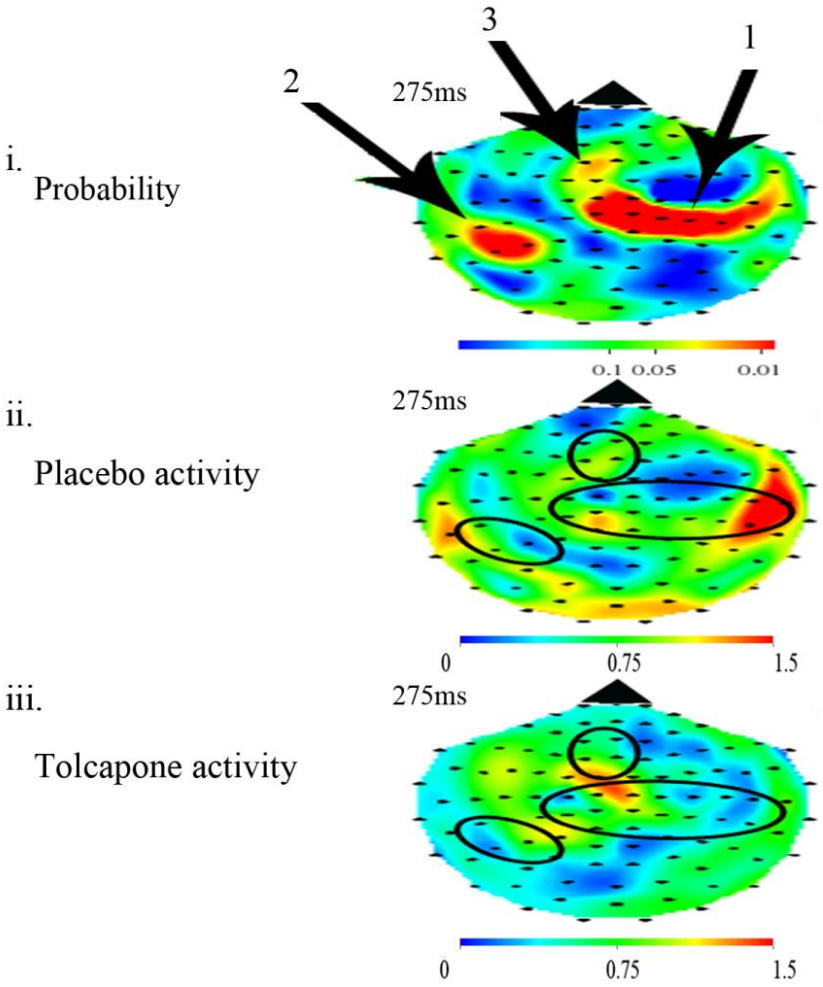


Figure 4.4 Local maps demonstrating differences in activity between placebo-treated and tolcapone-treated groups during performance of the 2-back task at 275ms. Figure 4.4i. represents the probability map, with red corresponding to highly significant areas. Activity plots in 4.4ii. and iii. show the areas active in the placebo and tolcapone groups respectively

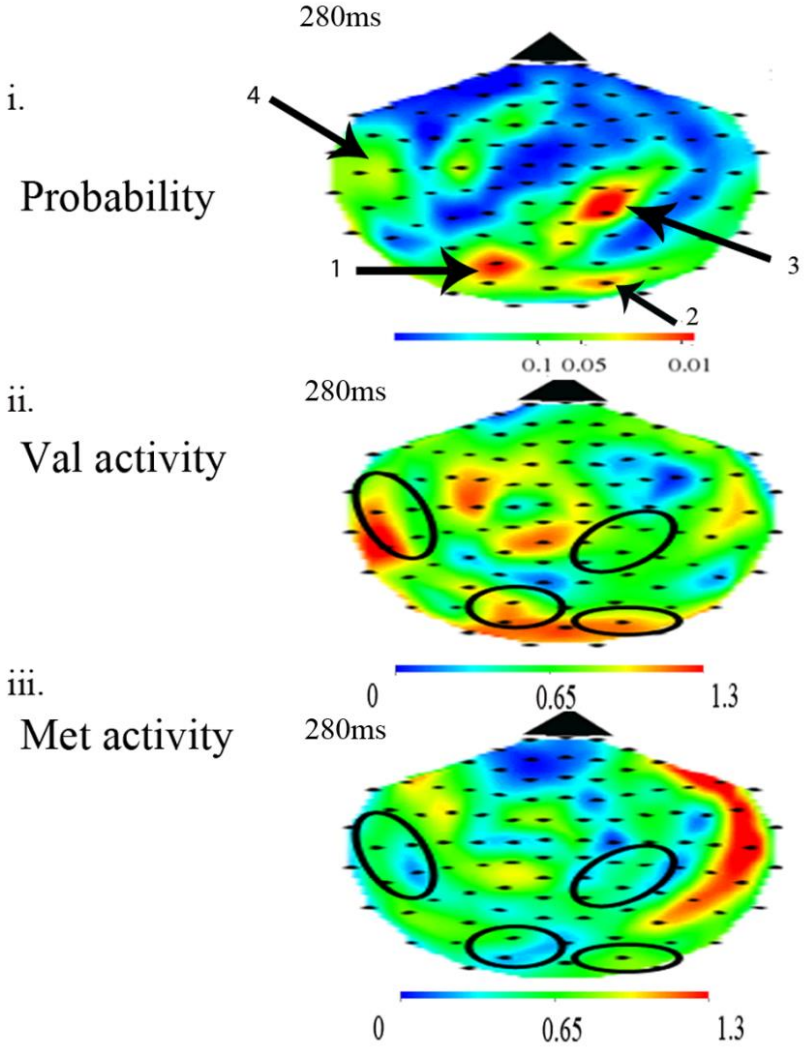


Figure 4.5 Local maps demonstrating genotype effects during 2-back at 280ms. Figure 4.4i. represents the probability map, with red reflecting highly significant areas. 4.5ii. and 4.5iii. show activity plots that allow us to see different areas are active in the Val (ii.) and Met (iii.) groups.

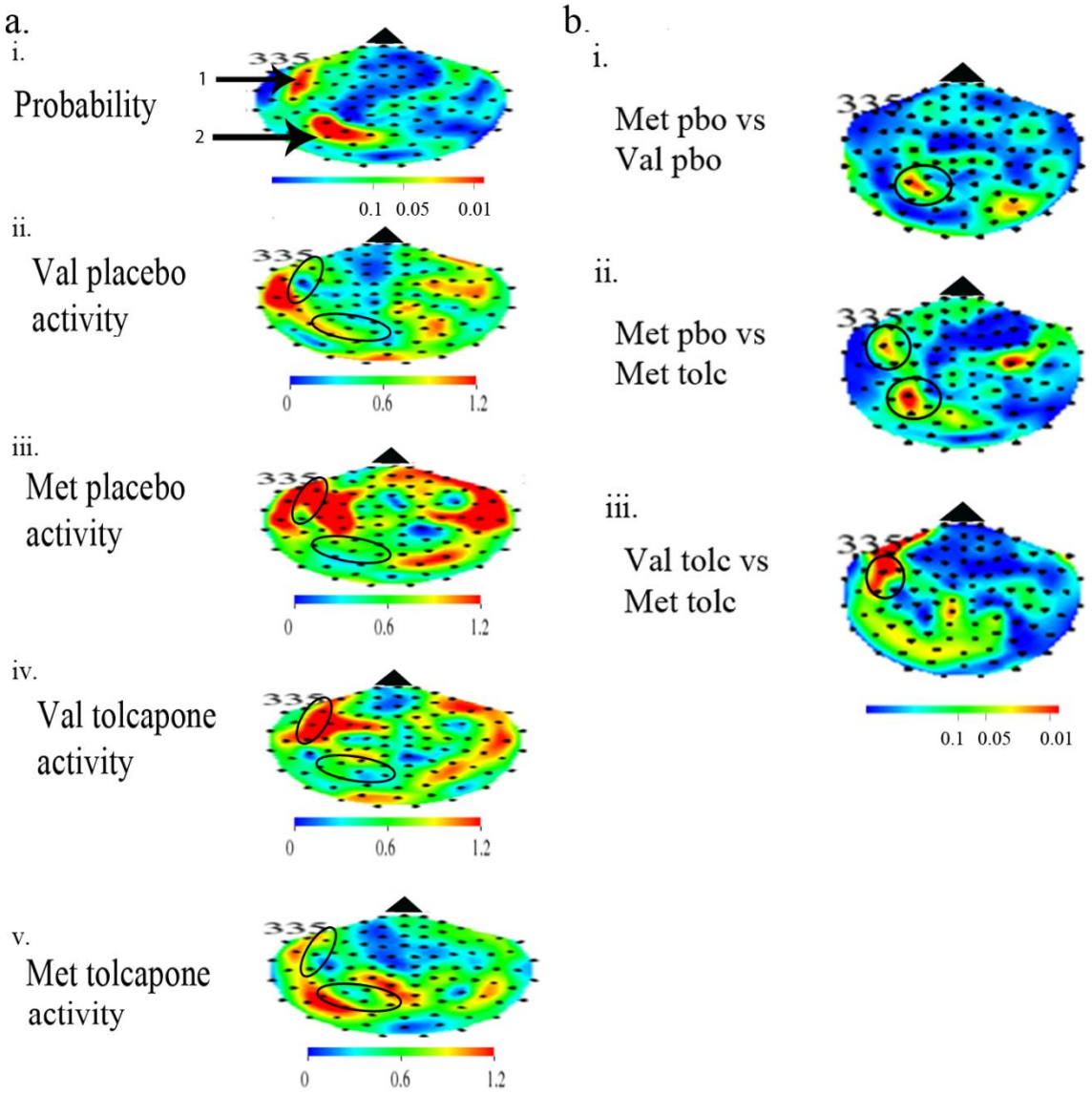


Figure 4.6 Interaction effects at 335ms during 2-back. Areas of significant difference between the four participant groups are shown in 4.6ai., labelled 1 and 2. The activity that corresponds with these areas of significance is shown in 4.6aii., iii., iv., and v. Figure 4.6b. highlights the significant comparisons from post-hoc analyses, with the circle highlighting areas of interest that come within those areas already seen in ai. The post-hoc comparisons are shown only in those cases where at least one area of significance matched the areas of significance shown in ai.

4.4 Discussion

This current investigation sought to explore how COMT modulation, via life-long (genotype) and short term (tolcapone-mediated) variation, affected the MEG signal during the N-back WM task. It focused on performance-matched groups, and on specific effects and time intervals as described earlier in section 4.2.1.1. The story is, as expected, complicated, but several interesting findings emerged from the data in this chapter:

1. The P300 shows modulation by COMT Val^{158}Met genotype, COMT inhibition, and interaction between genotype and tolcapone during 2-back (see Figure 4.3). These three effects occurred at different times within the P300.

2. Higher activity is seen over midline frontal regions in tolcapone subjects compared to placebo subjects at the 275ms component (see Figure 4.4).

3. The Val homozygous subjects (when both placebo and tolcapone groups are merged together) are generally more active than the Met homozygous subjects during the 280ms component (see Figure 4. 5).

4. Met placebo and Val tolcapone patterns are similar to each other in activity values and distribution, as are Met tolcapone and Val placebo groups. Post-hoc results suggest that the pattern of activity levels may be different depending on whether the areas are frontal or posterior. That is, frontal areas show the Met placebo group elicit a higher activity than Met tolcapone group, and the Val tolcapone group elicit higher activity than the Met tolcapone group. However, posterior activity shows the Met placebo group has lower activity than both the Val placebo and the Met tolcapone group (see Figure 4.6).

I will now discuss the important points regarding time components, areas of interest, genotype and drug effects, and possible relations to the 'efficiency' argument prominent in the fMRI literature. There is one important proviso in my discussion of the MEG results. As stated earlier, analyses were performed on performance-matched data in order to be able to titrate out the neural correlates of COMT-modulation without confusing those linked to specifically COMT-modulated performance with other factors that may contribute to performance. However, in order to compare genotypes, particularly in relation to 'efficiency', sometimes the participant groups are describe as 'better' or 'worse' performers, based on how they ranked in the behavioural data (see Chapter 3).

4.4.1 The P300 and its modulation by N-back load and COMT

An activity peak was observed coinciding with a component known to be related to WM, the P300, during the N-back task used in this thesis. This peak is modulated by N-back load with the higher difficulty conditions eliciting higher activity (Figure 4.2), suggesting greater resources are needed to compute a higher difficulty task.

This peak was also genotype modulated, with activity positively correlating with the 'better performing' groups. Over regions of significant difference at this time point, Met placebo activation was generally higher than that of Val placebo over temporo-frontal regions (see Figure 4.6a.). Over these temporo-frontal regions, Tolcapone affected the activation differentially depending on genotype, causing Met homozygous subjects to be less active and Val homozygous subjects to be more active (see section 4.4.3). This suggests that higher activation as measured by evoked responses in P300 amplitude is linked to better performance (even though the groups were matched for performance, as noted). This has been demonstrated in the EEG literature previously (Dolu et al., 2005) and suggests that better performers may have a greater

'recruitment capacity' or rather a better ability to activate synchronous firing of neurons. It is possible that this higher activation therefore, represents optimal levels of DA in the network, which translates to higher magnetic signal, presumably driven by a high-signal-to-noise ratio. This is relevant to (and apparently conflicting with) the argument that Val homozygous subjects are less efficient (see section 4.4.5). However, posterior regions show the opposite genotype x tolcapone relationship (more akin to NEH); with the Met placebo group exhibiting lower activity than Val placebo and Met tolcapone groups (Figure 4.6). COMT modulation of regions outside the PFC is discussed further in the next section.

4.4.2 COMT-related differences in N-back MEG signal for P300 are shown over both frontal and temporo-parieto-occipital areas

WM is thought to be a function that takes place primarily in frontal regions (Fletcher and Henson, 2001), and to be modulated therein by COMT (Callicott et al., 1998; Egan et al., 2001; Mattay et al., 2003). Areas relating to P300 are thought to be frontal in earlier P300 (P3a) and parietal in the later P300 (P3b) (Spencer et al., 1999), and as previously explained, it has been well-documented that COMT modulates frontal brain regions (see section 4.1.2). Therefore we would have expected COMT differences in WM to include, but not be exclusive to, frontal areas, specifically during early P3a. At 275ms post-stimulus onset (which can be classed as P3a) an area of significance has been highlighted over a frontal region (see Figure 4.4). Here, higher placebo activity (compared to the tolcapone group) was seen far right extending to frontal areas. The P3b also showed some frontal activity modulated by COMT i.e. Met placebo and Val tolcapone groups were more active in frontal areas than the other two groups at 335ms (Figure 4.6). It is perhaps surprising that genotype comparisons did not yield any differences over frontal regions, though possible differences may well be more apparent outside of evoked analysis (see Chapter 7, section 7.3).

Activity over the left temporo-parieto-occipital region was particularly apparent in the P3b (335ms in the present data) with the Val placebo group exhibiting higher activity than the Met placebo group, and the Met tolcapone group exhibiting higher activity than their placebo counterparts (see Figure 4.6). Early components also showed COMT modulation of areas outside the PFC. In the 280ms component (Figure 4.5), these areas followed a pattern where Val homozygous subjects had higher activity than Met homozygous subjects.

To summarise, although perhaps more heavily weighted towards the COMT-modulation over posterior regions, the regions of significance highlighted in the present data are consistent with current research in this field where areas of COMT modulation are found outside the PFC including the posterior parietal cortex (Stokes et al., 2011) and the medial temporal lobe (de Frias et al., 2009; Qin et al., 2012), and an appreciation that other regions (e.g. parietal cortex; striatum) contribute to WM. Specific to the modulation by COMT Val^{158}Met genotype, episodic memory tests[8] have shown Val homozygous subjects increased memory-related activation not only in the right PFC but also in portions of the medial occipital cortex that extended into the primary visual cortex (Schott et al., 2006). This further evidences COMT's effect in posterior regions in relation to memory.

At first sight, the effect of COMT over posterior/visual regions might seem unexpected given the anterior-posterior DA gradient. It is well known that DA is differentially distributed throughout the cortex, decreasing along the anterior-posterior gradient, with the highest concentration prefrontally and the lowest concentration in the occipital cortex (Brown et al., 1979; Lidow et al., 1991). Although the regional distribution of COMT activity in the cortex is not clear, this suggests that COMT's effects are likely to be lesser in the posterior, compared to the

[8] This task first presents words ('encoding') and then requires 'remembering' and 'forgetting'.

anterior, cortex. Thus, the present results showing COMT modulated activity at the back of the brain (for example, Figure 4.5) suggest such effects might be indirect, perhaps through cortico-cortical connections from the frontal cortex. Since such connections can be anatomically convergent, this may also explain how significant posterior effects on MEG signal could be seen, even in the absence of significant changes frontally. It is also consistent with evidence for DA modulated 'top down' control of visual attention, with prefrontal regulation of the other areas of the brain (Feredoes et al., 2011; Zanto et al., 2011; Gazzaley and Nobre, 2012). DA has also been shown to modulate the strength of cortico-cortical connections in Parkinson's disease patients (Stoffers et al., 2008).

The possibility of parietal cortex involvement during WM is particularly intriguing, granting multiple interpretations of the data. Firstly, it allows us to look not only at anterior-driven fronto-parietal connections during WM, but parietal-driven parieto-frontal connections, the latter of which may better explain the patterns of activity suggested in Figure 4.6. Previous fMRI studies of WM demonstrate it is the 'rule updating' aspect of a paradigm that preferentially activates the PFC, whereas the parietal cortex is responsible for the stimulus encoding aspect (Montojo and Courley 2008). However, the coupling between parietal and frontal cortex is reportedly crucial for both processes (Ma et al. 2011; Sauseng et al. 2005; Gazzaley et al. 2004). Therefore, these data could be interpreted as a parietal-driven encoding of incoming information, with the worse performers requiring a greater activity in the parietal regions to reach a similar level of N-back accuracy scoring (see Figure 4.6).

Alternatively, it is also possible that, despite controlling for attention differences between groups through the subtraction of brain activity during the 0-back blocks, some of the activity captured, particular in the more difficult conditions, may represent a greater (or lesser) ability to

attend and focus on the task in question. If the greater activity in the worse performers, as seen over these posterior areas (see Figure 4.6), is explained predominantly by parietal activity, then it is possible that greater activity represents a less efficient attentional processing, as it is well documented that attentional processing is linked to parietal areas (Pessoa et al. 2002; Posner and Dehaene 1994). Source localisation is required to document exactly where and how much of the brain activity comes from these parietal areas, which will dictate the importance of this possible attention effect.

4.4.3 A putative inverted-U relationship between MEG signal 'activity' and frontal DA

Results pertaining to frontal areas at 335ms suggest an inverted-U relationship between DA and MEG signal. The summary of these results appear in point 4 at the start of this discussion chapter. They suggest a relationship as drawn out in the schematic below, with Met placebo and Val tolcapone groups having the highest frontal activity, and Val placebo and Met tolcapone groups the lowest.

The groups with higher activity over frontal regions are those associated with better performance (in the full sample) i.e. Val homozygous subjects given tolcapone and Met homozygous subjects given placebo. These are the same groups associated with 'optimum DA levels' pertaining to Goldman-Rakic's inverted-U shaped curve (see Chapter 3, section 3.4.2). This suggests that more activity over the frontal region reflects optimal DA levels, relating to the highest possible synchronous firing of pyramidal neurons to engage better in the task, again, aligning with the idea of 'higher recruitment capacity' represented by a higher peak amplitude.

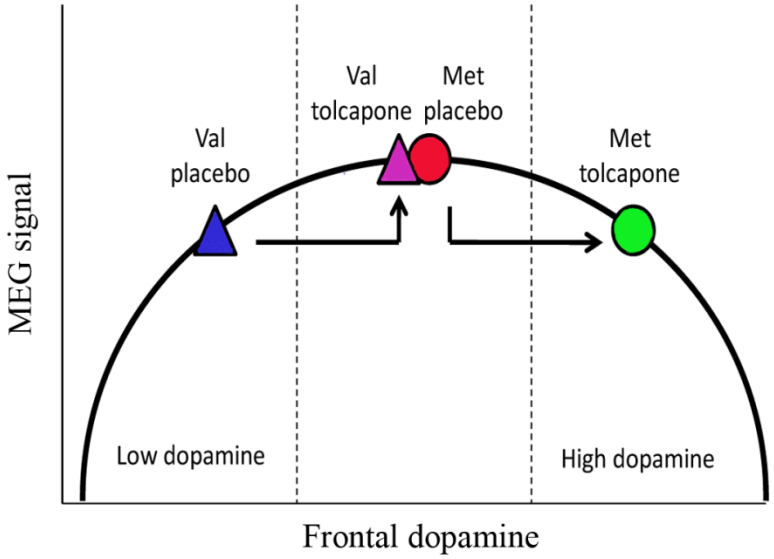


Figure 4.7 Inverted-U schematic for frontal regions at 335ms. The relationship between frontal DA and MEG signal during 2-back may be shaped like an inverted-U. In the placebo condition, MEG activity in this WM task is higher in Met homozygous subjects than Val homozygous subjects, but tolcapone reverses this, with the Met homozygous subjects having a lower MEG signal than the Val.

4.4.4 Efficiency

The following discusses to what extent the data from this chapter support the hypothesis that Val homozygous subjects are 'less efficient' than Met homozygous subjects during the N-back task.

Firstly, it is important to reiterate that MEG measures a very different parameter of 'activity' to that detected by the BOLD response (see Chapter 1, section 1.5 for more information on what these two modalities measure). Notably, MEG signal is a direct measure of neural activity whereas BOLD is indirect; also, the methods have very different temporal resolutions, therefore it is unlikely that we would see a one-to-one relationship. It is also possible, with the millisecond resolution of MEG, that the neural efficiency correlates might relate to either a specific time component, or the average of the whole 1800ms time span between stimuli.

Secondly, the Val^{158}Met effects seen in my results are not limited to the frontal regions (see sections 4.4.1 and 4.4.2) that might comply with the dorsolateral PFC and anterior cingulate areas reported in the fMRI literature (Egan et al., 2001). This exemplifies how other imaging modalities provide extra information outside that detected from the BOLD signal. For example, my results show robust changes in different parts of the brain including left temporal occipital and right parietal areas (see Figure 4.5).

Thirdly, I report the relationship between Val and Met homozygous subjects to be largely in the direction intuitive to that which pertains to efficiency in areas outside frontal regions i.e. the Val homozygote group show greater activity in all areas of significant difference at 280ms (see Figure 4.5) and at 335ms over the parieto-temporo-occipital border, the Val placebo group has a greater activity than the Met placebo group, and the Met tolcapone group shows a higher activity than the Met placebo group (Figure 4.6). However, over frontal regions the interaction effect seems to be the opposite of this, i.e. higher performing groups show higher MEG signal indicative of 'recruitment capacity'. Evidence for this includes Figure 4.6, where the Met placebo group exhibits greater activity than the Met tolcapone group over the tempero-frontal region. Although these contrasting relationships within the same paradigm are not highlighted in most fMRI studies (e.g. Egan et al., 2001), section 1.5.1 has already outlined a fMRI study that found these contrasting relationships in differing areas during another cognitive paradigm (Green et al., 2012).

To recapitulate, although a weak and imperfect comparison, it is possible that the different activity levels of the Val and Met homozygote groups seen in the MEG data during the 2-back largely pertain to efficiency, at least in some areas, with the greater MEG activity in the Val homozygote group (Figure 4.5 and 4.6) indicative of inefficient processing, though this not the case for a left frontal region shown in Figure 4.6. Future analyses may wish to focus on other

time components including longer latencies, or cumulative effects, as it is possible they will approximate better to the notion of efficiency shown in fMRI. It is also possible that assessing efficiency using MEG is not best analysed using evoked data, perhaps frequency maps would better show differential activation. To see if these MEG-'efficiency' correlates are confined just to WM, or also include other cognitive domains, tasks outside the realm of WM that can be graded in terms of 'better' or 'worse' performance, especially those that tap into regions outside the PFC, could also be investigated for COMT modulation in the MEG scanner.

4.4.5 Limitations and future studies

The N-back task used in this investigation does not separate different aspects of WM, (viz. encoding, maintenance and retrieval), and so it is possible that COMT affects only one or some of these aspects. Further WM tasks would be required in order to isolate individual components such as a delayed-match task to investigate maintenance in isolation.

The analyses here were limited to performance-matched data. The non-performance-matched global plot for the different N-back conditions (see Figure 4.2) demonstrated an extra time component of interest peaking at 441ms in the non-performance-matched data only. This time component could be of interest in a future study, possibly investigating if this peak is COMT-modulated and whether the height of the peak correlates with the accuracy scores in the behavioural data.

The evoked averages used in analysis include all trials, correct or incorrect. It is assumed that any differences in processing an impeding incorrect response will be outweighed by those processes preceding a correct response due to the averaging process, particularly as incorrect

responses are fewer in number. Isolation and analysis of correct trials was considered, but decided against due to the particular paradigm used in this thesis. Subjects deal with an overlap of new numbers before previous responses are given, and there is no clear set time to answer a pre-set question. Therefore it would be likely that any attempt to isolate correct responses would unknowingly isolate out brain signal that include processes preceding an 'incorrect response as subjects are processing both the correct and incorrect response at the same time.

Despite contrasting with 0-back, it is possible that a COMT effect on attention could confound the putative effect COMT has on WM. A way to tease out the specific WM and possible attentional contributions to the MEG activity would be to create a task that focuses on a particular N-back load, say 2-back, but varies in amount of on-screen distractors, providing the subject with a diminishing capacity to attend. Analysis of the P300 amplitudes in the varying conditions would suggest whether COMT modulation of attention affected this peak. Initial piloting would be required to ensure subjects are not distracted to a level that alters their WM performance.

4.4.6 Conclusion

This chapter demonstrates that neuronal activity, as indexed by MEG signal, shows clear modulation by COMT activity of the P300 during the N-back task. The detailed effects differ in different regions and at different times within the P300, and are related to genotype, to COMT inhibition, and their interaction. These effects are not limited to the PFC.

Chapter 5

The role of COMT in the MEG correlates of emotional face processing

Chapter 5

5. The role of COMT in the MEG correlates of emotional face processing

5. 1 Introduction

5.1.1 What is emotion?

Emotion is the assignment of valence (positive and negative meaning) to objects, states and situations (Bevilacqua and Goldman, 2011). Emotion is critical to most aspects of human behaviour, and maladaptation can lead to mood and anxiety disorders (McEwan, 2000). Happiness, sadness, anger, fear, disgust and surprise are considered the six basic emotions (Ekman and Friesen, 1971). As initially proposed by Darwin in 1872, these emotions are recognised in the same way across cultures (Andrew, 1963; Ekman, 1969). A set of photographs entitled 'Pictures of Facial Affect' (Ekman and Friesen, 1976) have since been used in a variety of paradigms to investigate emotional processing.

5.1.2 A role for DA in emotional processing

Research focussing on the VTA and efferent targets in the nucleus accumbens (mesolimbic pathway), and PFC (mesocortical pathway) reveal an important role for DA in the regulation of emotion (Laviolette, 2007). It has been shown that electrical stimulation of the VTA increases DA release and produces fear reactions in cats (Levita et al., 2002) and conditioned fear responses are disrupted when dopaminergic projections to the nucleus accumbens are inactivated (Haralambous and Westbrook, 1999). Providing a possible mechanism for this, in rats, D_1 receptor activation attenuates the inhibitory action of the medial PFC on the basolateral amygdala, whilst enhancing other inputs by increasing postsynaptic excitability of basolateral projection neurons, thereby enhancing amygdala activity (Grace and Rosenkranz, 2002). Consistent with these findings, the same study also showed that the plasticity of odour-evoked fear conditioning responses that come prior to foot shocks is blocked by antagonism of DA

receptors and by stimulation of PFC (Grace and Rosenkranz, 2002). For a review on DA and fear conditioning see Pezze and Feldon (2004). Despite the focus to date on negative emotions such as fear in animal studies, emotional regulation by DA may extend to positive emotions. For example, microinjection of dopaminergic agonists in the rat nucleus accumbens elicits the 50khz ultrasonic vocalization, which is a measure of pleasure in these animals (Burgdorf et al., 2007).

Human studies, using fMRI to compare subjects given either amphetamine or placebo, have shown that DA potentiates the response of the amygdala, and a number of other areas, to emotional stimuli such as angry and fearful facial expressions (Hariri et al., 2002; Tessitore et al., 2002). In another study, healthy volunteers who were administered sulpiride (a D_2 receptor antagonist) showed decreased responses in limbic areas (amygdala, hippocampus and anterior cingulate) to unpleasant pictures but increased activation in cortical frontal, temporal and parietal areas (Takahashi et al., 2005), though a similar study failed to find an effect (Franken, et al., 2008). PET studies have since reported an increase in DA release in the left amygdala, left medial temporal lobe and left inferior frontal gyrus during presentation of emotional stimuli, particularly negative, e.g. words with negative affect such as 'fire' and 'blood' were compared to neutral words such as 'park' or 'life' (Badgaiyan et al., 2009; Badgaiyan, 2010). Regarding positive expression, several studies have examined the effects of D_2 antagonists such as haloperidol. One reported a 'blunting' in emotional response by those individuals who took part (Mizrahi et al., 2007), though others show a self-reported 'enhancement' in emotional response (Saeedi et al., 2006; Willner et al., 2005). Thus, evidence suggests DA modulation of emotional processing, though it is not clear whether this is limited to negative emotions.

Consistent with those studies suggesting a role for DA in emotional processing, there are disturbances in the ability to process a range of positive and negative emotions in psychiatric disorders affecting the DA system. For example, impaired emotion is documented among patients

with schizophrenia in relation to the classifying of emotional faces (Whittaker et al., 2001) and emotional facial responses to happy and sad films (Mattes et al., 1995). It has also been documented that both positive and negative (but mainly negative) emotional recognition is worsened in Parkinson's patients; for reviews see Gray and Tickle-Degnen (2010), and Peron and Dondaine (2011).

5.1.3 COMT and emotional processing

Studies described in Chapter 1 section 1.3.3 and Chapter 4 section 4.1.2 demonstrate that the low activity COMT Met allele is associated with better prefrontal function and efficiency than the Val allele during cognitive processing. However, conversely, the Met allele also appears to be associated with negative emotional states and affective disorders. For example, the Met allele has been linked (albeit inconsistently in some cases) with increased levels of anxiety in women (Enoch et al., 2003), obsessive-compulsive disorder in men (Karayiorgou et al., 1997; Pooley et al., 2007), panic disorder (Woo et al., 2004), type 1 alcoholism (Tiihonen et al., 1999), aggressiveness and anger-related traits, (Rujescu et al., 2003; Volavka et al., 2004); bipolar affective disorder (Mynett-Johnson et al., 1998), and major depression (Ohara et al., 1998). The Met allele also leads to reduced pain thresholds and increased emotional response to pain (Zubieta et al., 2003; Diatchenko et al., 2005), apparently via diminished regional opiod responses (Zubieta et al., 2003). While these results are, in places, sparse and inconsistent, they arouse the possibility of a functional trade-off between genetic variation that concurrently results in 'better' cognition, but 'worse' emotional function.

In addition to these possible behavioural effects, functional imaging studies show Val^{158}Met differences in the neural correlates of emotional processing. For example, Met homozygous individuals present increased reactivity and greater functional connectivity of brain circuitry implicated in generating and regulating emotional responses to faces (Drabant et al.,

2006). This includes greater activation in the right hippocampal formation, and the right ventrolateral prefrontal cortex. Subjects with the Met homozygous genotype also showed greater functional connectivity between the amygdala and orbitofrontal cortex (OFC)/ventrolateral PFC (vlPFC), the hippocampus and OFC/vlPFC, and vlPFC and parahippocampal gyrus relative to Val homozygous individuals. The regions with the largest main effect of COMT (the PFC and hippocampal formation) also show the most abundant expression of COMT, both in terms of messenger RNA (Matsumoto et al., 2003; Chen et al., 2004) and enzyme activity (Masuda et al., 2003), thus supporting the interpretation that these imaging findings represent changes in COMT-modulated DA neurotransmission. However, COMT is minimally expressed in the amygdala (Hong et al., 1998) where termination of DA effects are largely mediated by the DAT (Revay et al., 1996), yet fMRI studies show COMT Val^{158}Met genotype influences emotion-related amygdala BOLD response e.g. during fearful face processing (Domschke et al., 2012). These amygdala findings serve as a precedent for anatomically indirect effects of COMT genotype on neural activity, given the lack of COMT expression in the amygdala (Hong et al., 1998). See also the discussion on local vs. distant causes of differential MEG signals (Chapter 4, section 4.4.2).

5.1.4 Face processing

Faces are often used in tasks to assess emotion. Visual cues for recognition of an individual and the emotion they display have advantages over auditory cues, which depend on the individual vocalising, and olfactory cues, which are spatially limited.

The perception of a face is decoded by multiple bilateral brain regions. These include; the occipitotemporal cortex, capturing general features; projecting to the fusiform gyrus for analysis of the constant aspects of the face that do not vary, allowing recognition of unique faces; and superior temporal sulcus for assessing the changing aspects of the face e.g. mouth movements (Haxby and Gobbini, 2002). Activity of the fusiform gyrus is also thought to identify emotional

valence (Drabant et al., 2006; Kawasaki et al., 2011). The superior temporal sulcus connects to the intraparietal sulcus, which monitors the head movement and direction of eyes; and the auditory cortex, which processes phonetic content from the mouth. The fusiform gyrus is connected to the lateral anterior temporal gyrus and is thought to identify a person and any concomitant biographical information. All these structures are connected to limbic areas, in particular the amygdala and the insula (Del-Ben and Graeff, 2010). The activation of the amygdala has been extensively studied for fearful faces in neuroimaging studies (Breiter et al., 1996). However, amygdala activation is also observed with the expression of anger (Hariri et al., 2000), disgust (Sprengelmeyer et al., 1998), sadness (Surguladze et al., 2003), happiness (Breiter et al., 1996; Surguladze et al., 2003) and negative surprise (Kim et al., 2003). The insula cortex has afferent and efferent connections with the medial and orbitofrontal cortices, anterior cingulate, and amygdala (Augustine, 1996). The insula activation is frequently associated with disgust (Phillips et al., 2004), and there is evidence of a broader emotional processing role (Phan et al., 2002), particularly regarding making judgments about emotions based on facial expression (Gorno-Tempini et al., 2001).

5.1.4.1 Temporal components in face processing

Evoked response potentials (ERPs) are often measured in response to different emotional facial expressions in order to probe early emotional processing. A well characterized response to facial stimuli, known as the N170[9] (occurring 170ms post-stimulus onset), is thought to correspond to the structural processing of a face (Bentin et al., 1996; Cauquil et al., 2000; Itier and Taylor, 2004; Eimer and Holmes, 2007) and has been localized to the fusiform face area in MEG studies (Deffke et al., 2007) and over occcipitotemporal electrode sites in EEG studies (Jacques et al., 2007), thought to be back projections from the amygdala (Dolan et al., 1996; Blair et al., 1999; Liu et al., 1999; Mayberg et al., 1999). This N170 is seen as the hallmark of the

[9] 'N' refers to EEG components with a negative peak at a given time component.

viewing of a face. Some studies suggest this component elicits activity differences in response to different emotions, usually with response peaks shorter for positive than negative affects (Campanella et al., 2002; Batty and Taylor, 2003; Krombholz et al., 2007). However, other studies find the N170 to be unaffected by facial emotional expression (Eimer et al., 2003; Holmes et al., 2003; Ashley et al., 2004; Balconi and Lucchiari, 2005).

Other time components of suggested importance in face recognition include the N120, which shows a marked difference in activity following the presentation of a fearful, compared with a neutral, face (Eimer and Holmes, 2002; Pizzagalli et al., 2002). Linkenkaer-Hansen and colleagues showed clearly that this component is separate from the N170 as the N120 is found at posterior occipital areas, and the N170 slightly anterior and lateral to this location (Linkenkaer-Hansen et al., 1998).

Two time components are thought to be key in processing of the face after the initial N170 structural encoding. A more detailed perceptual analysis on the affective significance of the face takes place at 250ms (Streit et al., 2000; Eimer et al., 2003) and a more cognitive evaluation which used 'prototype' knowledge about emotion occurs at the N400 (Adolphs, 2002). Both these components have been seen as negative deflections over posterior temporal regions in EEG studies and appear to be modulated by repetition (Begleiter et al., 1995; Eimer, 2000; Herzmann et al., 2004; Itier and Taylor, 2004) and pre-existing facial familiarity (Schweinberger et al., 1995; Schweinberger et al., 2002; Schweinberger et al., 2002; Jemel et al., 2010). Interestingly, at the N400, negativities for grimaces (i.e. non-prototypical emotional faces) were significantly stronger over posterior sites than when compared with neutral and prototypical emotional faces (Paulmann and Pell, 2009). Paulmann interprets that this may mean different circuitry underlies the processing of typical and non-typical affective faces.

A much earlier, contentious, time component has been associated with face processing before 100ms post face presentation. Some of these studies suggest a time component as early as 50ms, but fail to show that this response is face-specific (George et al., 1997; Seeck et al., 1997; Debruille et al., 1998). Braeutigam et al. (2001) found components in the right occipitotemporal cortex around 42ms, the right anterior temporal at 48ms, and the bilateral posterior areas at 54ms. These latency responses were also found upon presentation of a coffee mug, but much weaker and at a different topographic distribution (Braeutigam et al., 2001). These putative early components could indicate that fast face processing originates from a monosynaptic pathway from eye to cortex (Johnson, 2005). More recently it has been demonstrated in EEG (using faces and cars in both scrambled and non-scrambled versions), that a pre-100ms time component, approximately 95ms, is associated with low-level visual cues to a face, whereas the more typical N170 is associated with the higher-level representation of a face (Rossion and Caharel, 2011). It has also been suggested that this early visual processing is limited to 'known' faces, with a recent MEG study showing brain responses at 30-60ms in the bilateral ventral occipital temporal regions and the right anterior medial temporal region, depending on previously associated emotional context (Morel et al., 2012). The authors suggest that the memory trace of a face integrates positive emotional cues present in the context of a prior encounter and that this emotional memory can influence the very first stages of face processing.

5.1.5 Objectives

The objectives for this chapter are best described as a series of questions. The first section of analysis does not split the groups by drug and genotype. The following questions were asked, and are addressed in section 5.4.

1. Do we observe the N170 face-specific response?
2. If so, is the N170 sensitive to facial emotion?

3. Which time components during the task are modulated by emotion? i.e. finding time components of the MEG signal that are significantly different between neutral faces and emotional faces.
4. Is there one emotion (either fear or happy) that has a greater effect on MEG signal? As noted, the literature suggests that unpleasant or fearful stimuli may be more pertinent, and particularly so for these negative emotions in relation to COMT modulation.

The second section of analysis splits the participants into the Val^{158}Met genotype and tolcapone vs. placebo groups. The following question is asked (and addressed in 5.3.2):

5. Are the emotion-modulated components of MEG signal (see questions 3 and 4) also affected by genotype or tolcapone or their interaction?

5.2 Methods

To explore how MEG signals differ during emotional processing, 58 subjects split into four participant groups (Val placebo, Met placebo, Val tolcapone, Met tolcapone) viewed a series of emotional face images (task described in 5.2.1). Methods of analysis specific to this faces task are detailed here. Methods common to all chapters can be found in Chapter 2, in particular see section 2.8.4 for information on preprocessing, analysis and map production. The task and strategy for analysis are described below.

5.2.1 The faces task

As explained above, fMRI studies show differences in BOLD signal which are modulated by COMT Val^{158}Met genotype during passive viewing of negative emotions (Smolka et al., 2005; Drabant et al., 2006). Due to prefrontal inhibition, limbic responses are reduced when explicit

labelling of an emotional expression is required (Critchley et al., 2000; Hariri et al., 2002). Thus a passive task has been used in this thesis and therefore, no behavioural data appear in this chapter.

Subjects were asked to look centrally at the screen as images of faces (40 neutral, 40 happy and 40 fearful expressions) and motorbikes (60 images) appeared before them in random order. Each image was presented twice, giving a total of 360 trials. The image remained on the screen for 1000ms. It is thought that face recognition is a holistic processing that looks at relative spatial arrangement of the eyes, nose, mouth etc. and not just the presence of such features (Farah et al., 1998; Maurer et al., 2002). Motorbikes are used as a control to the face as they provide similar spatial frequencies (Swithenby et al., 1998). A crosshair is presented for 1000ms in between each image shown. On 40 of the 360 trials, this crosshair changes from red to blue halfway through. The subject is instructed to press a button with their index finger on these blue crosshair trials in order for us to ascertain that they were paying attention to this otherwise passive face processing task. The whole task lasts approximately 15 minutes.

The set of face stimuli were developed by N. Tottenham and supported by the MacArthur Foundation Research Network on Early Experience and Brain Development (Tottenham et al., 2002). These faces were matched across expressions for gender and ethnicity. Motorbike images were taken from Kylliainen et al. (2006). Dr Susannah Murphy adapted these images to the faces task instantiated here for use in the MEG scanner at OHBA.

5.2.2 Characterising time points of interest

Chapter 2, section 2.8.4 describes how the time points of interest were selected. As with the N-back data, all results shown here are limited to information taken from gradiometer data,

thus focussing on closer (i.e. cortical) sources. The analysis focuses on the first 600ms period after a face appears on the screen. There is a 1200 millisecond interval between presentations, but the face and emotion literature does not generally discuss beyond 500ms (Bergersen et al., 2011).

To select time points of interest, global probability plots for the three face conditions were first examined, pooling the data from all subjects. Time components that differed between emotional and neutral faces were noted. Further activity and probability global plots then explored the four groups, Val placebo, Val tolcapone, Met placebo and Met tolcapone. The regional activation maps (local maps) were derived from two-way ANOVAs. The maps were examined to identify areas of significant activity difference and how they differed between groups. An overview of analysis strategy can be seen below in Figure 5.2.

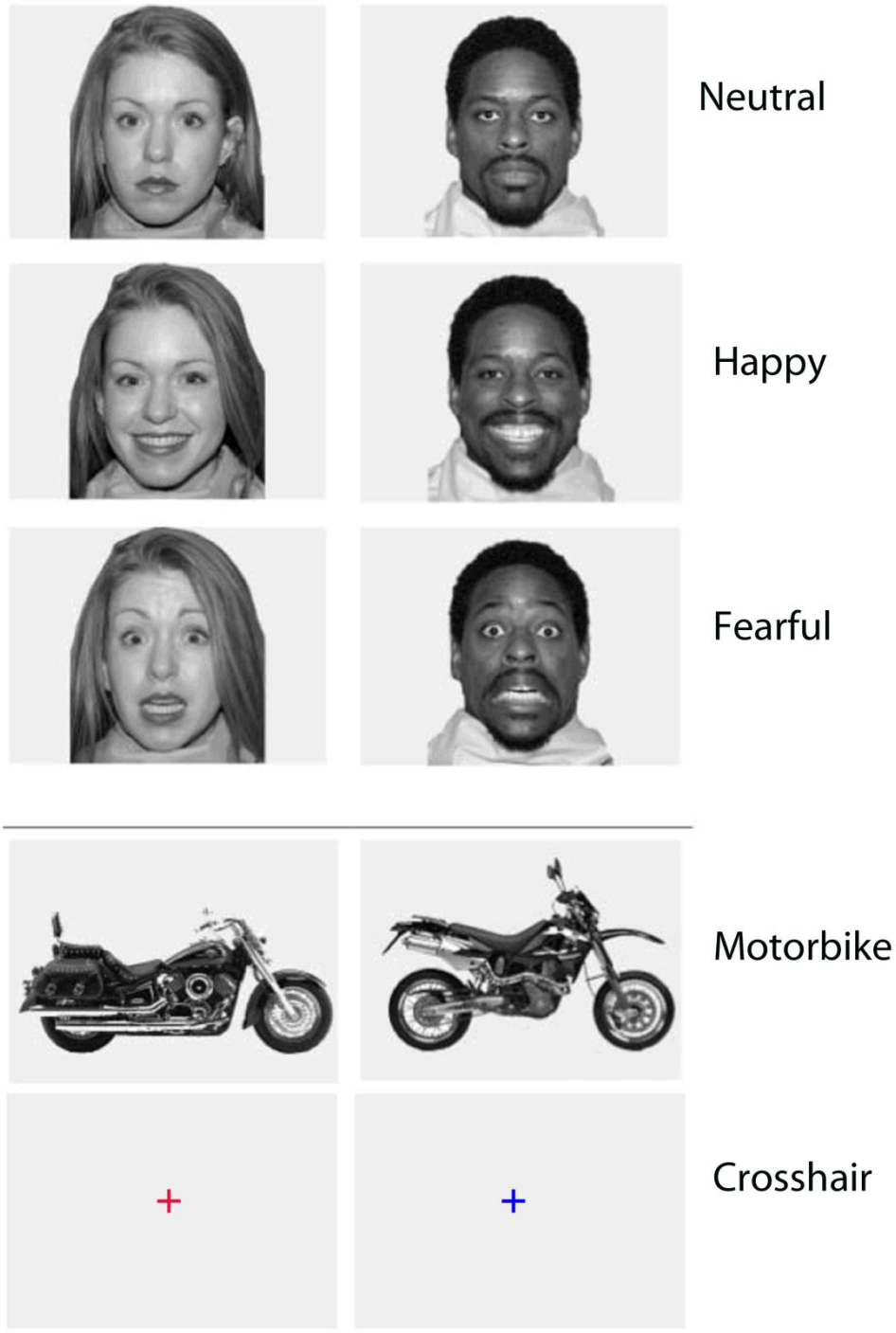


Figure 5.1 Images of the emotional processing task. Images of faces (neutral, happy or fearful) and motorbikes are displayed in turn on a grey background. Crosshairs are interspersed between these images. Participants are instructed to press a button when crosshairs are blue in order to prove they are attending to the stimuli.

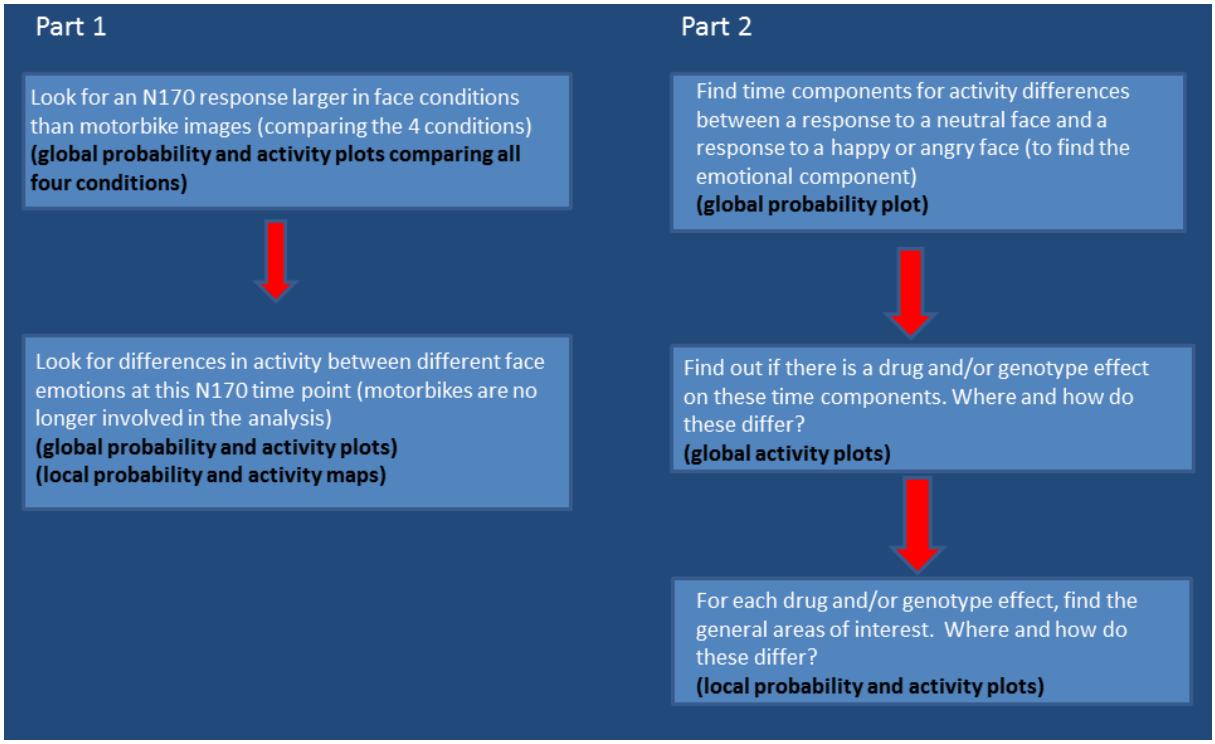


Figure 5.2 Strategy for MEG analysis of face processing. Part 1 represents analysis that takes place with pooled data. Part 2 splits the data into four participants to see how COMT affects MEG signal during face processing.

5.2.3 Displaying the results

Following fMRI based approaches (Smolka et al., 2005; Drabant et al., 2006), contrasted images have been prepared. This involves subtracting the response to neutral faces from the emotion of interest (i.e. happy or fear). Non-contrasted images were also prepared to compare motorbike with faces, neutral with happy, or neutral with fear conditions. Unless stated otherwise, all local power maps and probabilities shown are with contrasts that subtract the neutral face condition from fearful condition. This assumes that the brain signal is additive and subtracting the activity elicited upon presentation of a neutral face will subtract the 'non-emotional face' elements of the activity common across all face conditions, leaving us with a clearer idea of just the emotional component.

The MEG analysis will not show negative deflections due to the squaring that takes place upon signal calculation. However, although the 'N' refers to the negative potential picked up regarding EEG patterns of activity, the 'N' terminology will be used during this chapter.

5.2.4 Demographics

Table 5.1 shows the demographic information specific for participants used in analysis of the faces task. This differs from the full participant group due to poor signal-to-noise ratio and other artefacts causing some of the subjects' data to be omitted. There were no significant differences between groups for any of the variables represented in Table 5.1.

Table 5.1: Demographics of subjects

	Met placebo	**Val placebo**	**Met tolcapone**	**Val tolcapone**
Number	16	13	12	17
Ethnicity[a]	15C, 1I	10C, 2I, 1Ch	12C	14C, 2I, 1Ch
Age (y)	22.7 (3.4)	24.5 (5.4)	25.3 (10.2)	24.3 (3.6)
NART	118 (4.2)	117 (5.2)	115 (4.9)	117 (5.3)
Alcohol units/week	101.8(10.0)	7.5 (4.6)	9.0 (4.8)	7.8 (8.6)
Cigarettes/day	0.8 (3)	0.4 (1.4)	0.5 (1.7)	0.1 (0.04)
BDI	4.5 (6.9)	3.23 (3.3)	3.8 (3.8)	3.3 (2.7)
STAI	29.9 (8)	32.6 (8.1)	31.8 (7.5)	31.5 (4.5)

Values are mean (s.d.).
NART: National Adult Reading Test. BDI: Beck depression inventory. STAI: Spielberger Trait Anxiety Inventory.
[a]C: Caucasian; I: Indian; Ch: Chinese

5.3 Results

The first set of results (shown in 5.3.1) show MEG signal during emotional face processing found in the data set as a whole. The second set of results (shown in 5.3.2) shows how COMT and tolcapone affect MEG activity.

5.3.1 Emotional processing over all participant groups

5.3.1.1 Attending to cross hairs

The rate of correct button presses to blue crosshairs was above 94% and did not differ between groups. This would suggest that in 6% of the trials, participants were not attentive to the task (not looking at the screen), and so may not have even responded to images appearing before and after the cross hair. As this is difficult to determine, and given the high percentage of button presses overall, all trials were included in the analyses.

5.3.1.2 Global plots show clear N170 response

Figure 5.3a shows a global activity plot for the average response to a given condition, namely 'Neutral', 'Happy', 'Fearful' and the control, 'Motorbike'. This graph shows a clear peak 170ms after a subject was presented with a face (2.5fT/cm happy; 2.5fT/cm fearful; 2.61fT/cm neutral). A significantly different, much lower activity response is elicited on presentation of motorbike vs. faces, $p=7.1x10^{-7}$ (statistical data not shown).

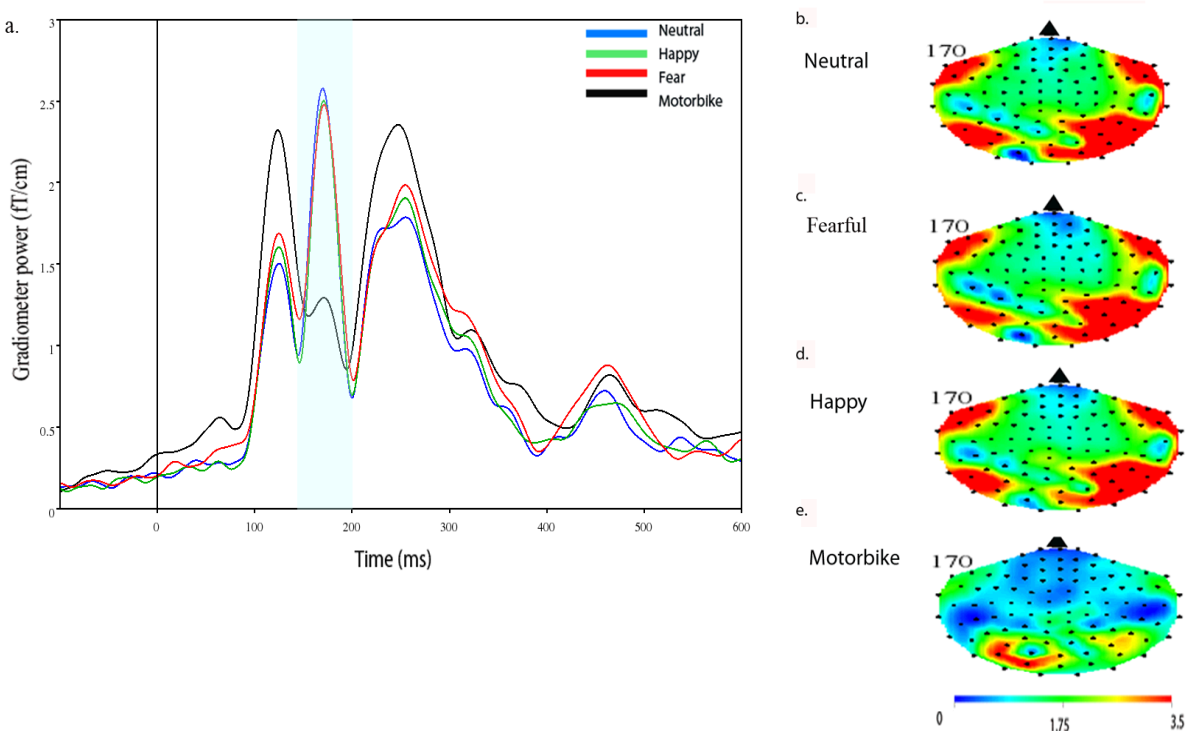


Figure 5.3 The N170 face response. The blue shading in a. highlights the N170 time component, which is significantly different ($p<0.001$) between neutral face and motorbike responses. Higher activity is present in neutral (blue), happy (green) and fearful (red) face responses, compared to the motorbike response. Figures b., c., d., and e. show the higher activity elicited upon face presentation is present over temperofrontal and temperoparietal regions.

Local maps corresponding to the previous global map (Figure 5.3a.) are shown in Figure 5.3b., c., d. and e. These show similar distributions for all faces (Figure 5.3b.-d.), which are distinct from the motorbike image (Figure 5.3e.). All faces produce a bilateral response over frontal and anterior temporal, as well as bilateral occipitotemporal areas, which could potentially correspond to the fusiform cortex. Hints of a right hemisphere dominance is suggested for occipitoparietal areas. Further source localisation and statistical measures would be needed to investigate these suggestions.

5.3.1.3 Time components corresponding to emotional face processing

Here, the aim was to determine whether the N170 was sensitive to facial emotion, and which time components were modulated by emotion (see section 5.1.5). The probability map in Figure 5.4a illustrates a one-way ANOVA showing the time component of significant difference in the processing of the fearful face versus a neutral face. The processing of fearful is significantly different to that of neutral around the N4-like component, specifically peaking at 388ms (ranging from 373ms- 414ms), $p=4.8x10^{-12}$. Figure 5.4b shows the local probability plot at this peak, with the significance shown in 5.4a. appearing over two main regions, a left frontal-parietal area labelled 1 ($p=2.2x10^{-4}$) and a right frontal area labelled 2 ($p=1.8x10^{-4}$). The activity differences relating to this are not clear in the pooled data.

There were no significant differences for the happy vs. neutral contrast (data not shown), thus demonstrating that MEG responses to the processing of a happy face were similar to neutral faces throughout the processing of the first 600ms. All subsequent analyses thus focus on the processing of fearful faces.

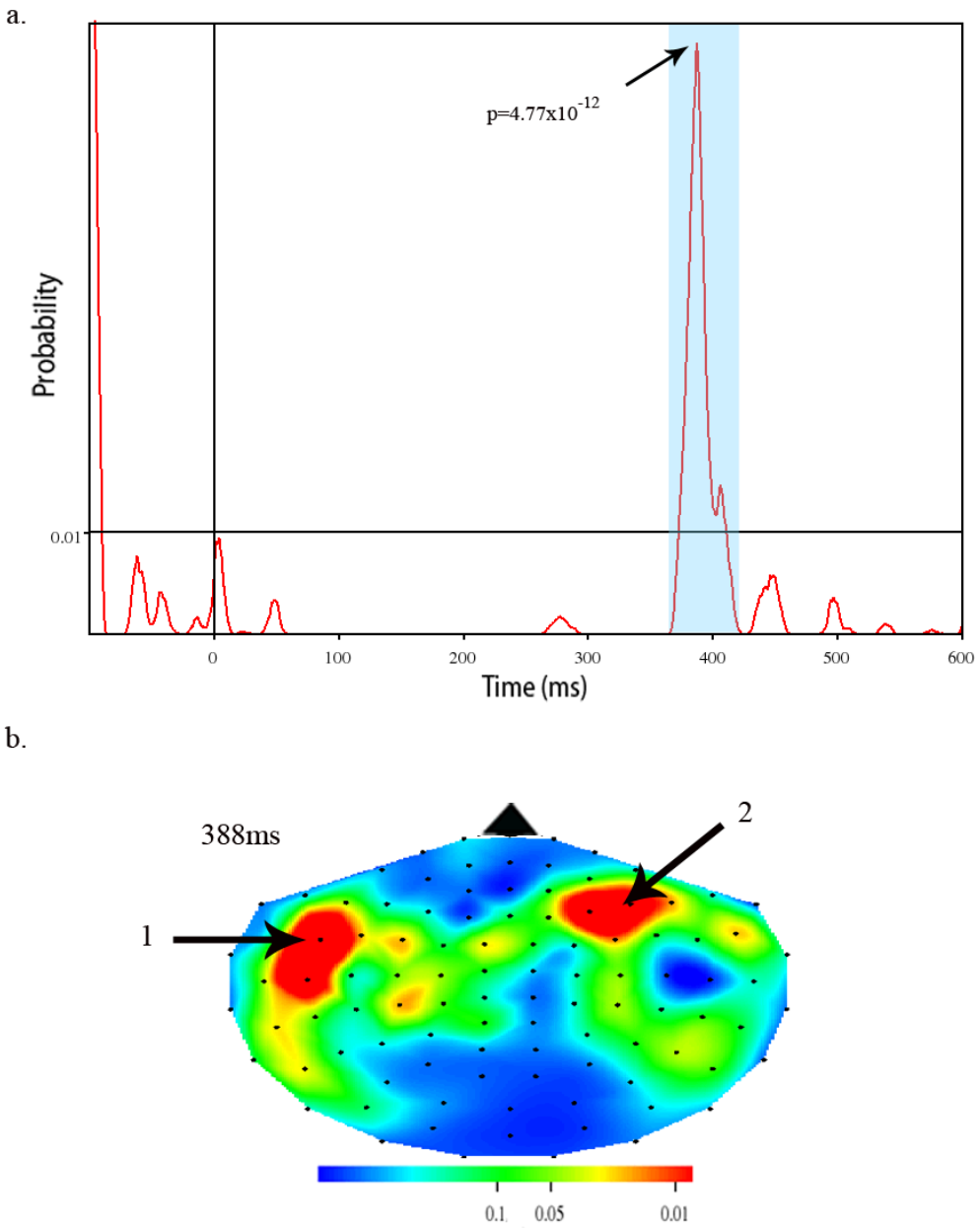


Figure 5.4 An N4-like component in fearful processing. The time component of significant difference in activity between fearful and neutral faces is shown in 5.4a. Here we see only one significant component, the N4-like component. The local probability plot in 5.4b demonstrated that the differences arise mainly from two highly significant regions; right frontal (labelled 2) and left fronto-temporal (labelled 1) regions.

5.3.2 COMT modulation of fearful faces

The aim of this part of the study is to determine whether COMT modulates the N4-like component for fearful face processing. This seeks to answer MEG Objective 5 including (a.) how genotype modulates and (b.) how tolcapone modulates the emotional component (see section 5.1.5).

5.3.2.1 Fearful activity maps show different peak activities for different groups

The global probability maps shown in Figure 5.5 represent the two-way ANOVA performed on fearful data split by the four participant groups. Individual significance levels for a. main effect of drug (black), b. main effect of genotype (blue) and c. the genotype x tolcapone interaction (red) are presented. The focus will be on the N4-like peak defined in Figure 5.4 (373-414ms), since this is the interval found to be significant for fearful face processing. Figure 5.5a. shows a significant difference between drug and placebo groups peaking at 390ms (ranging from 385ms to 394ms), p=0.0017. In Figure 5.5b activity is significantly different between Val and Met homozygous subjects with a peak at 393ms (the significant component ranging from 381-400ms), $p=9.0x10^{-6}$. The interaction peak is shown in 5.5c at 373ms (from 364ms to 385ms), $p=2.3x10^{-14}$, representing the time point at which tolcapone differentially affects the two genotype groups. Figure 5.5d depicts global brain activity for each individual drug and/or genotype group. The Met tolcapone group shows the highest activity at 384ms (0.60fT/cm), shortly followed by the Val placebo group at 380ms with a peak of 0.49fT/cm. The lowest activity level in this highlighted time frame is the Met placebo group (at 0.38fT/cm). The Val tolcapone group has an activity ranging from 3.9fT/cm to 4.8fT/cm within the highlighted time component, making it the second lowest activity. However, this group peaks outside the time component of significance at 0.54fT/cm. In addition to having the two lowest activities, the Val tolcapone group (426ms) and the Met placebo group (394ms) appear to exhibit their N4-like peak later than the other peaks

(Val placebo at 388ms and Met tolcapone at 390ms), though this difference is not significant (data not shown).

5.3.2.2 Characterising the difference in MEG signal at 390ms between placebo and tolcapone groups

Figure 5.6 shows the differences between placebo and tolcapone groups at 390ms. Label 1 shows an effect (p=0.008) of tolcapone at the midline frontal area, appearing more active in the tolcapone condition with 0.62fT/cm at the highest area of activity compared to the peak of placebo activity in this area at 0.40fT/cm.

This pattern of the tolcapone group exhibiting higher activity is also shown in left frontal regions labelled 2 (p=0.0042), the greatest activity difference in this area shows tolcapone peaking at 6.2fT/cm compared to 0.45fT/cm for placebo. The midline parietal area, labelled 3, appears more active in placebo (0.57fT/cm), compared to tolcapone (0.48fT/cm), p=0.0069.

Summary of placebo vs. tolcapone at 390ms

The tolcapone group was more active over the two frontal areas of significance, and the placebo group more active over the parietal area of significance.

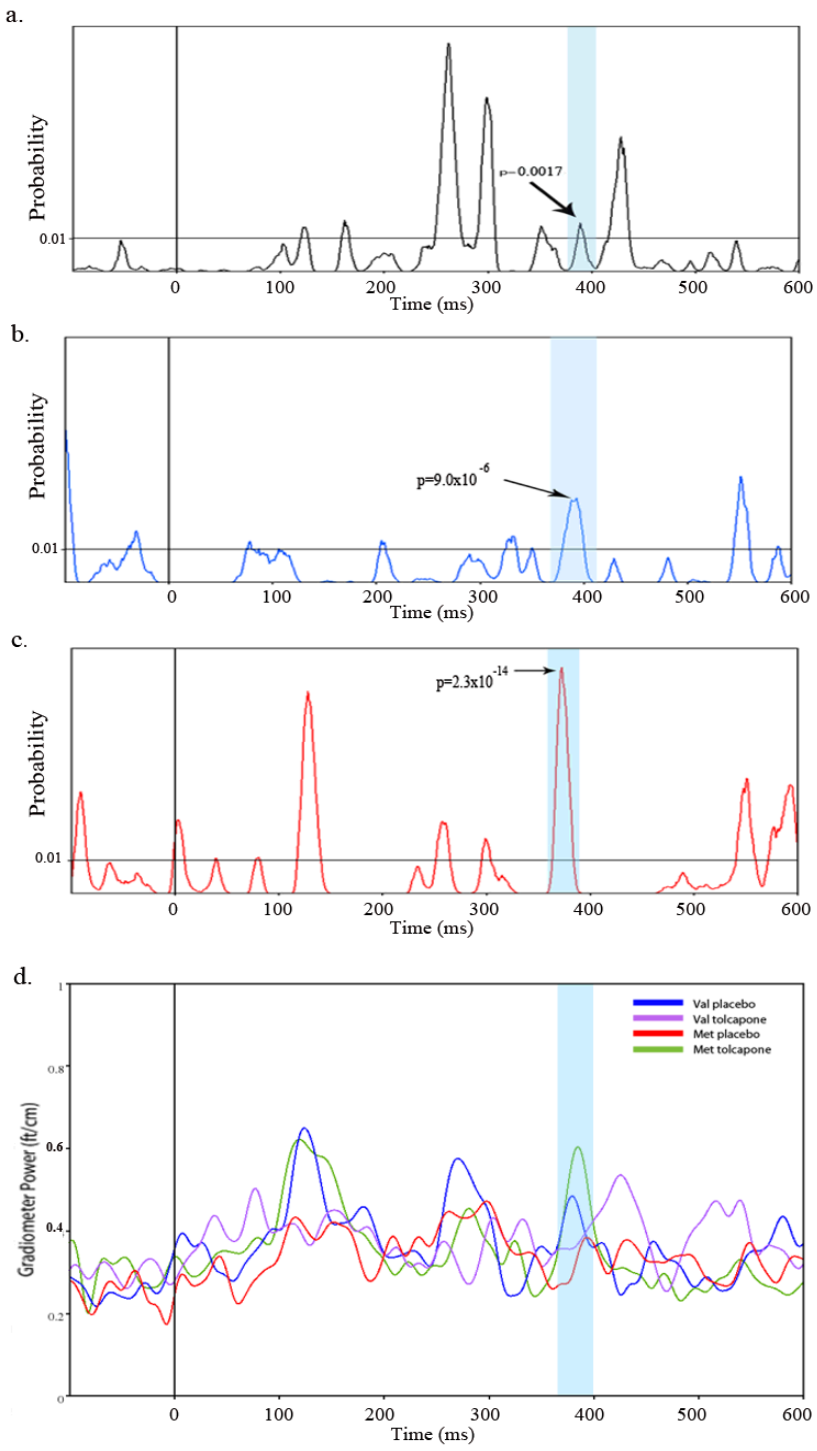


Figure 5.5 Global plots showing COMT modulation of fearful processing. The blue shaded regions highlight the N4-like region of interest; significant components outside of this region are omitted from further analysis as they did not show the initial difference between fearful and neutral faces. The probability plots are shown for a. the effect of tolcapone, b. the effect of genotype and c. the interaction effect. The corresponding activities of the four participant groups are shown in d. Met tolcapone has the highest activity (0.60fT/cm) followed by Val placebo (0.49fT/cm). Met placebo has the lowest peak (0.38fT/cm). The Val tolcapone activity in this significant region ranges from 3.9fT/cm to 4.8fT/cm.

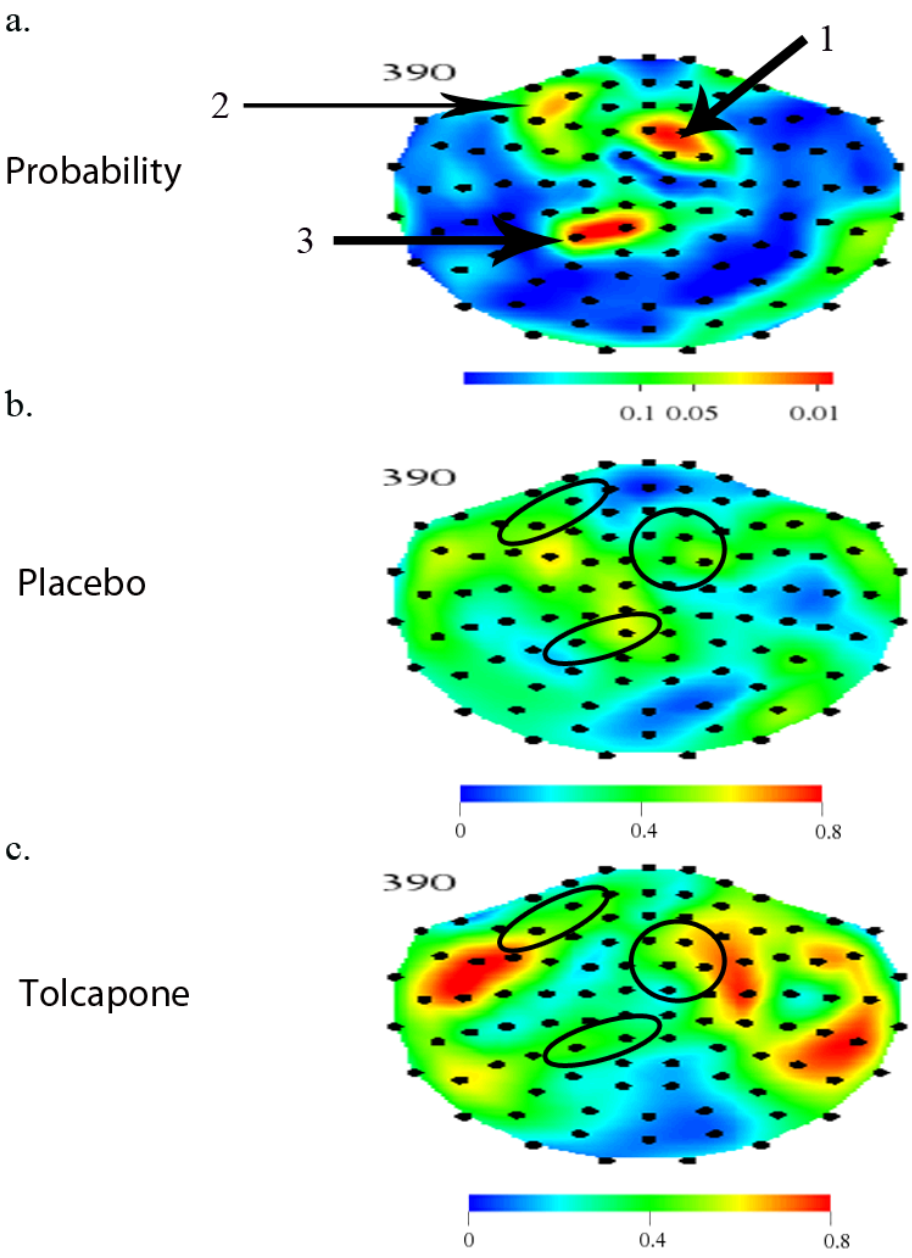


Figure 5.6 Tolcapone effect on fearful faces at 390ms. The local probability map shown in 5.6a. represents the difference in processing a fearful face between the placebo and tolcapone groups. Three key areas of significance are shown; 1, midline frontal moving towards the vertex; 2, left frontal; 3, left parietal. The activity maps in 5.6b. and c. correspond to this probability plot for placebo and tolcapone groups respectively. Areas 1 and 2 are more active in the tolcapone group, area 3 is more active in the placebo group.

5.3.2.3 Characterising Val^{158}Met genotype differences in fearful processing at 390ms

Figure 5.7 depicts the genotype main effect peak at 390ms that was noted in Figure 5.5b. A highly significant right temporal area, labelled '1' ($p=4.6x10^{-4}$) appears to arise from the greater activation in Met homozygous subjects; 0.61fT/cm compared to 0.43fT/cm in Val homozygous subjects. Label 2, marks an occipitotemporal area (p=0.039). This region demonstrates higher activation in Met homozygous subjects, reaching an activity of 0.69fT/cm compared to 0.60fT/cm in Val homozygous subjects. Second, the frontal region labelled 3 (p=0.034) can be explained by higher activity in Val homozygous subjects, with a peak activation of 0.56fT/cm, compared to 0.28fT/cm for Met homozygous subjects.

Summary of Val^{158}Met genotype differences at 390ms

Over frontal areas, higher activity is seen in Val homozygous subjects than Met homozygous subjects. Over occipital and temporal areas, higher activity is seen in Met homozygous subjects than Val homozygous subjects.

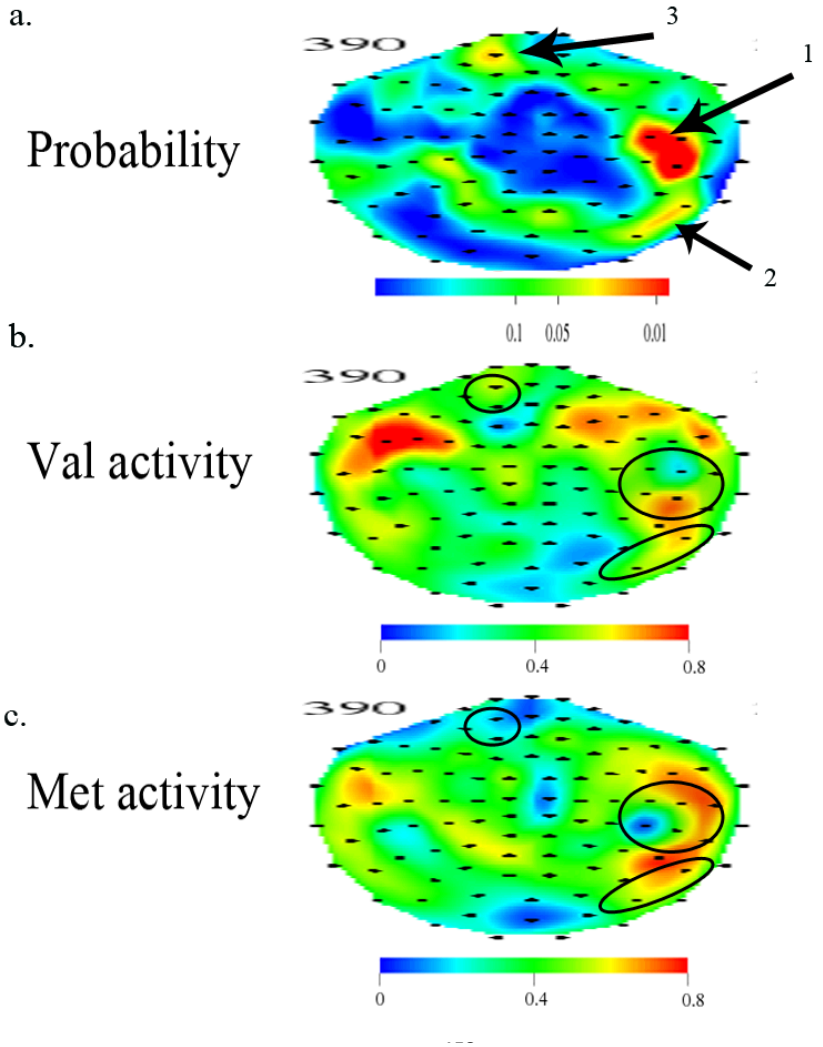


Figure 5.7 The effect of COMT genotype on fearful face processing at 390ms. Significant areas of difference in activity are shown in a. and occur over areas including the right temporal area, labelled 1. A comparison of b. Val homozygous activity and c. Met homozygous activity suggests differences arise from higher activation in Met homozygous subjects. Label 2 marks an occipitotemporal area of significance, differing due to higher activation in Met compared to Val homozygous subjects. Label 3 points to an area of frontal significance explained by higher activation in Val compared to Met homozygous subjects.

5.3.2.4 Characterising the genotype x tolcapone interaction at 370ms

Figure 5.8a shows five main locations of interaction between tolcapone and genotype at 370ms. The post-hoc data for comparisons shown to contain significant regions are shown in Figure 5.8b. The areas of significance that reached threshold in Figures 5.8ai. and bi. and ii., were the left frontal ($p=1.8x10^{-4}$), and the right frontal stretching to temporal areas ($p=3.3x10^{-3}$), labelled '1' and '2', respectively. Other areas in Figure 5.8a (labelled 3-5) include an anterior temporal area, labelled '3' ($p=1.1x10^{-2}$), a right parietal area labelled '4'($p=2.1x10^{-3}$), and a left tempo-occipital ($p=6.3x10^{-3}$), labelled '5'. These did not show significant differences between the groups analysed during post-hoc testing. However, over the five 'significant' areas, Val homozygous subjects on placebo show the highest activity, closely followed by the Met homozygous subjects on tolcapone, with Met placebo and Val tolcapone groups showing very little activity in these areas.

The post-hoc results shown in 5.8b. are the comparisons of groups that demonstrated significant effects within the five areas already highlighted in Figure 5.8a(i). The activity difference between Met placebo and Met tolcapone groups to fearful faces (bi.) highlights two regions; left frontal ($p=0.0078$), labelled 1, and right frontal ($p=0.0096$) labelled 2. In these two key areas, the relevant activity plots shown in Figures 5.8aiii. and 5.8v. indicate that the Met tolcapone group are generally more active than the Met placebo group, in both areas. The left frontal area has an activity level of 0.41fT/cm for the Met placebo group compared with the higher level of 0.57fT/cm for the Met tolcapone group. The right frontal area has an activity level of 0.32fT/cm for the Met placebo group compared to a higher level of 0.60fT/cm for the Met tolcapone group.

Figure 5.8bii. shows that the activity elicited upon fearful face presentation differs between Val tolcapone and Val placebo groups, in the same two areas (left frontal labelled 1, p=0.0045; and right frontal labelled 2, p=0.020). The Val homozygous subjects show the opposite pattern in activity to the Met homozygous subjects (Figures 5.8aii. and iv.) i.e. Val homozygous subjects on tolcapone exhibit less activity than Val homozygous subjects on placebo. The left frontal area has a relatively low activity level of 0.29fT/cm for the Val tolcapone group but a higher activity of 0.73fT/cm for the placebo group. The right frontal area also has a low activity for Val tolcapone than placebo group (0.41fT/cm vs. 0.73fT/cm respectively).

Summary of genotype x tolcapone interaction at 370ms

In summary, tolcapone has opposite effects in the two genotype groups: the Met tolcapone group is more active than the Met placebo group, and the Val placebo group is more active than the Val tolcapone group.

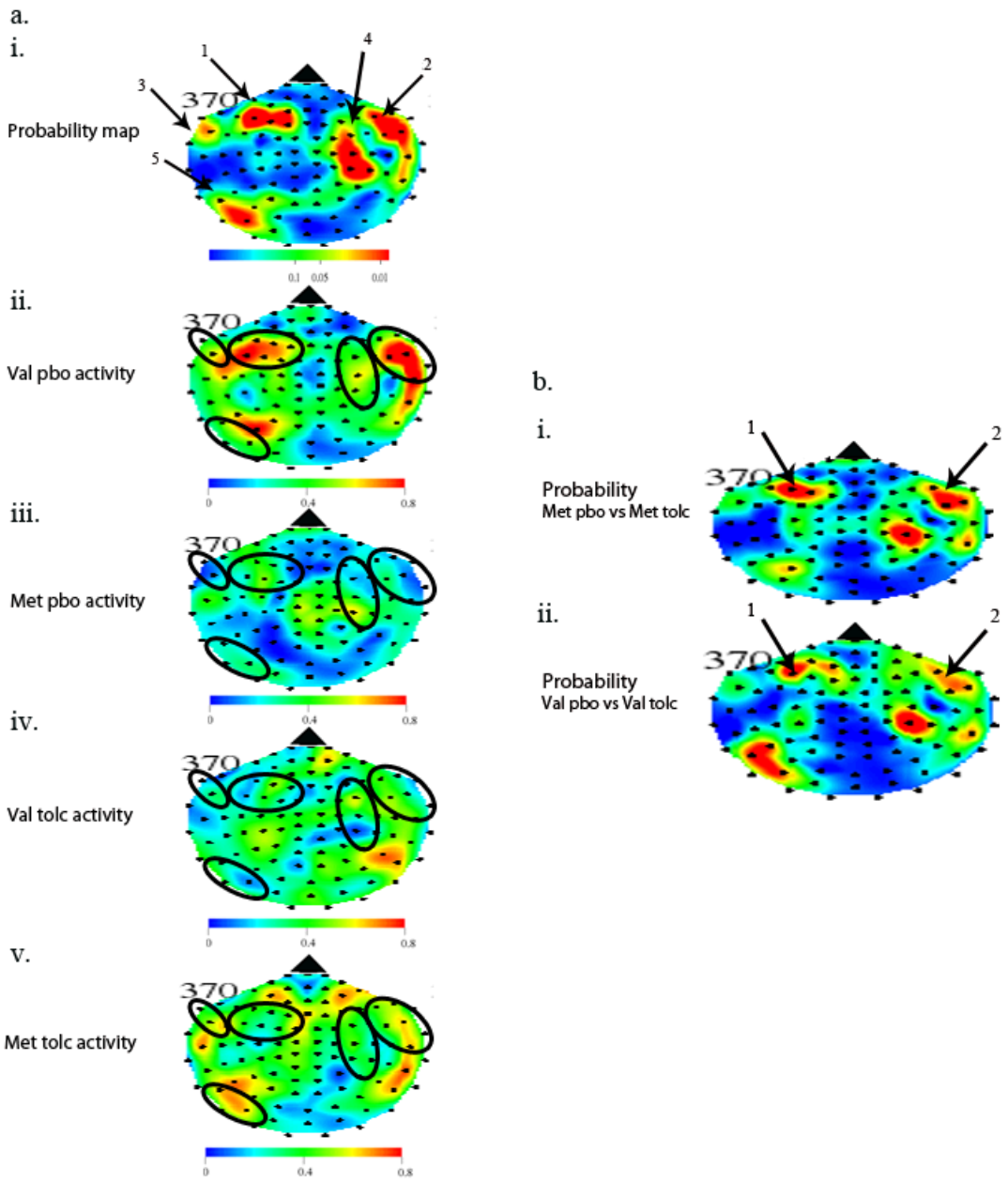


Figure 5.8 Local maps exploring genotype x tolcapone interaction effects of processing fearful faces at 370ms. Significant interaction effects are seen over five regions in ai. The activity maps for each group are shown in 5.8aii-v. Post-hoc tests that relate to those regions labelled 1 and 2 are shown in b. The significant areas in bi. can be explained by the higher activity of the Met tolcapone group (av.) compared to the Met placebo group (shown in aiii.). The significant areas in bii. can be explained by the Val tolcapone group exhibiting less activity than the Val placebo group.

5.4 Discussion

The results obtained in this chapter support a face-specific response at the N170 (Objective 1; Figure 5.4), with no emotional modulation of this time component (Objective 2; Figure 5.5). The N4-like component is exposed as an emotional processing component, specifically for the fear response (Objectives 3 and 4; Figure 5.6). COMT is shown to modulate this fearful processing at the N4-like component (Objective 5; Figure 5.7). Tolcapone groups are more active over frontal areas, whereas, placebo groups are more active over the parietal region of significance (Objective 5; Figure 5.8). Genotype differences were also found, with the activity levels of Met homozygous subjects generally higher than that of the Val homozygous subjects over the temporal areas, though the activity over frontal regions of Val homozygous subjects was generally higher than their Met counterparts (Objective 5a; Figure 5.9). An interaction between genotype and tolcapone demonstrates that tolcapone affects Val and Met homozygous subjects in opposite directions (Figure 5.10) over lateral frontal regions. Thus the Met homozygote group on tolcapone showed greater than the Met homozygous group on placebo, but the Val homozygous group on placebo was more active than the Val homozygous group on tolcapone. The following discussion will consider each part of this investigation in turn.

5.4.1 Face-specific response

The N170 local activation at occipitotemporal sites agrees with an extensive face processing literature (Bentin et al., 1996; Clark et al., 1996; Kanwisher et al., 1997; Haxby et al., 2000; Carmel and Bentin, 2002). The plots in Figure 5.5 show bilateral activity but with possible right hemisphere dominance, arguably in an area that could be broadly interpreted as the fusiform face area. These data may also support the theories that suggest the right hemisphere is dominant in the perception of facial expression, regardless of valence (Lane et al., 1995; Jessimer and Markham, 1997; Lane and Nadel, 2000) in patients (Mills, 1912), healthy controls (Borod et

al., 1998) and animals such as monkeys (Hauser, 1993). However, both the putative asymmetry and the localization of the signal to the fusiform region would require further analysis in order to be substantiated.

5.4.2 No modulation of the N170 or 250ms components by facial emotional expression

As briefly explained in section 5.1.4.1, although it was initially thought that the N170 was unaffected by emotional expression, there is a body of EEG literature suggesting the contrary (Blau et al., 2007; Wronka and Walentowska, 2011). The N170 emotional modulation literature is perhaps stronger for negative emotion e.g. angry over happy (Krombholz et al., 2007), especially over the extrastriate cortex (Fruehholz et al., 2011). However, the data in this chapter shows there to be no significant differences between neutral, happy and fearful expressions at the N170 component. As the task is passive, it is possible the lack of emotional modulation in N170 is due to the participants processing the faces implicitly. There is unresolved debate as to whether implicit and explicit processing of faces differ in results. Some studies show emotional modulation at these earlier time components during processing of a face only during explicit tasks i.e. those where the main goal of the task is to recognise the emotion as opposed to, for example, the gender (Babiloni et al., 2010; Wronka and Walentowska, 2011). However, a recent study finds both N170 and 250ms time components to vary according to emotion in both explicit and implicit tasks (Fruehholz et al., 2011).

5.4.3 The N4-like component for emotional face processing

The current study observes an N4-like component of the MEG signal as the only time component to have significantly different activity for response to a fearful face vs. a neutral face. The N4-like component has previously been demonstrated to be involved in emotional

processing, showing greater activity for negative words than positive and neutral ones (De Pascalis et al., 2009) and, for subjects in a pessimistic mood, a bias to expect negative outcomes was seen as a 300-400ms component over posterior scalp regions (Chung et al., 1996). As noted below, these are not the same regions as our MEG data would suggest. The N4-component has also been established for face processing, particularly during semantic processing involved in recognising a previously seen face, or a non-prototypical emotion such as a grimace (see section 5.1.4.1), the latter of which seems more plausible for the task instantiated here. Interestingly, it has been shown in a word based task (but not in a faces task in the same study) looking at the N170, 250ms and N400 responses, that the N400 is the only component to maintain the emotional modulation in the 'implicit' task, when the 'explicit tasks' showed all three components to be emotion-modulated (Paulmann and Pell, 2009).

At the N4-like component, the local maps (seen in Figures 5.7 and 5.8) show an expanse of temporo-parietal, occipito-temporal and frontal areas to be active during the 370 and 390 peaks. This not only demonstrates areas likely to include the fusiform, but also shows activity differences to extend beyond those expected.

These data suggest that this N4-like emotional component is involved in processing fearful, but not happy, emotions. The emphasis on negative emotions and the N4-like component has been stressed previously (Chung et al., 1996; De Pascalis et al., 2009). However, given that studies show happy emotion does modulate responses in EEG and fMRI (Breiter et al., 1996; Canli et al., 2002; Pessoa et al., 2002; Yang et al., 2002), it is interesting that the MEG data fail to show significant difference between happy and neutral responses. There is more than one possible explanation for the fearful-specific emotional modulation observed here:

1. Both happy faces and fearful faces were not equally arousing. This would mean the N4-like component could represent not the valence, but the arousal. If fearful faces are more arousing then this may elicit a component with greater activity, as opposed to just its valence alone. Given that stronger activity correlates with more emotionally competent stimuli i.e. intensity of stimulus (Rudrauf and Damasio, unpublished data reported in Bergersen et al. (2011)), it is plausible that the degree of fear exhibited in the images was of higher intensity than the level of happiness expressed. If we showed moving images, where the emotions were clearer, or more complex pictures with scenarios, we might see delineation between happy and neutral faces. However, throwing this theory into doubt, previous work has demonstrated that subjects have judged a set of fearful and happy faces to be equally arousing, but still produced a much greater response to fearful (Drabant et al., 2006).

2. The interpretation of the failure to find differences between fearful and happy face processing may be an inaccurate representation i.e incorrect as, for example, it is only based on global power. Thus, if the analysis had been limited to a specific region such as the occipital cortex, results may have been different, and modulation of happy expression may have been apparent. It has been demonstrated that fearful modulation generally produces higher activities than happy modulation (Breiter et al., 1996; Whalen et al., 1998), and so we were more likely to still see this difference in the global plots, whereas smaller differences in the happy modulation may have been obscured.

5.4.4 The effect of tolcapone on processing fearful faces at the N4-like component

The tolcapone group showed higher MEG activity over frontal regions compared to the placebo group, but lower outside of frontal areas. This could be interpreted as a selective enhancement of frontal cortex neural activity due to the increased DA tone produced by tolcapone. To a certain extent, it is expected that there would be a tolcapone effect on MEG

signal as it is known that DA drugs affect other imaging modalities in emotional processing tasks. For example, dextroamphetamine increases BOLD response in the amygdala during the processing of angry and fearful facial expressions (Hariri et al., 2002). The putative MEG basis for the tolcapone and placebo differences (via the effect on DA and in turn, glutamate) is outlined in Chapter 1, section 1.5.2.

5.4.5 Effect of Val^{158}Met genotype and genotype x tolcapone interactions on fearful faces at the N4-like component

We do not see peak latency differences between any of the participant groups at the N4-like time components. Latency changes have been seen during faces tasks in previous literature e.g. upside down faces are processed 40ms slower (Eimer and Holmes, 2002); though not, as far as I am aware, during the processing of the same kind of face by different participant groups.

The current data suggest that Met homozygous subjects show higher activity compared to Val homozygous subjects during processing of fearful faces over posterior regions. Over frontal regions the data suggest Val homozygous subjects show greater activation compared to Met homozygous subjects. To my knowledge this is also the first study showing a genotype x tolcapone interaction effect on emotional processing, or more generally, that genotype can influence a drug's effect on neural activity during emotional processing. I interpret the interaction as reflecting an increase in Met homozygote activity on tolcapone, and a decrease in Val homozygote activity on tolcapone, over cortical bilateral frontal regions at the N4-like component (Figure 5.8).

Genotype modulated areas during the N4-like component include a frontal region (see Figure 5.7, labelled 3). These analyses do not, and were not, expected to detect other deeper

structures commonly associated with emotional processing (such as the amygdala or hippocampus), in fMRI. However, the cortical areas shown in Figure 5.7 have been suggested to play key roles in emotional processing. The PFC appears to be the predominant region over which COMT modulation occurs in the COMT-emotional processing literature (along with the hippocampus), particularly the vlPFC (Smolka et al., 2005; Drabant et al., 2006) in response to unpleasant vs. neutral affective pictures (Smolka et al., 2005) and affective faces (Drabant et al., 2006). Outside of the COMT literature, the PFC has been shown to be involved in emotional processing (Gur et al., 1994; Grodd, 1995; Dolan et al., 1996; Kosslyn et al., 1996; Reiman et al., 1997; Sprengelmeyer et al., 1998; Blair et al., 1999; Kimbrell et al., 1999; Damasio et al., 2000; Northoff et al., 2000; Esslen et al., 2004), with Fuster (2001) detailing that within the PFC, the orbital and medial parts were found to be predominantly involved in emotional processing and the lateral part in cognitive functions. Research indicates that the vlPFC participates in the processing of negative emotions. Activation of the vlPFC has been reported during induction of sadness (Levesque et al., 2003) and recollection of sad autobiographical memories (Pelletier et al., 2003; Markowitsch et al., 2003). Increased activation in the vlPFC was also found in patients suffering from major depression (Drevets, 2000; Brody et al., 2001). However, the analyses in this thesis do not allow us to conclude if it is ventral, dorsal, lateral or central parts of the PFC in which differences are seen, and source localisation would be needed to further investigate the extent to which the data in this chapter comply with previous studies.

A larger region of genotype modulation shown in Figure 5.7, over the temporal cortex, is also consistent with prior literature linking the temporal cortex to emotional processing. Esslen found extended activation in both temporal lobes during perception of happiness, sadness and fear, but not during disgust and anger (Esslen et al., 2004). Many functional imaging papers report activation in temporal areas during emotional processing (Breiter et al., 1996; Kosslyn et

al., 1996; Reiman et al., 1997; Sprengelmeyer et al., 1998; Blair et al., 1999; Mayberg et al., 1999; Pizzagalli et al., 2000). The temporal activity seen here also spreads towards occipital regions. Pizzagali and colleagues (2000) found activation in bilateral occipitotemporal regions, including lingual and fusiform gyri and extending to inferior temporal gyri in response to emotional faces (Pizzagalli et al., 2000). These results were suggested to indicate facial configuration extraction processes. Activation specifically of both left and right fusiform gyrus has been reported by Breiter et al. (1996) during response to happy and fearful faces. This occipital activity is usually interpreted as back projections from the amygdala reaching the fusiform face area (Morris et al., 1998; Vuilleumier et al., 2002).

5.4.6 Limitations and future studies

Analyses from this chapter focus heavily on 'fearful' face processing. COMT may not modulate other negative emotions in the same way as it modulates fearful faces in this task. It is possible, for example, that COMT only modulates fear. However, there are studies to suggest COMT modulation of activity elicited upon viewing angry faces (Defrancesco et al., 2011; Lonsdorf et al., 2011). Further studies are required using a range of negative facial and non-face-based emotions to see if similar responses to that found in this chapter can be replicated. It is, of course, entirely possible that different emotions are processed at different time components, thus time components outside the N4-like component may be more important for the other emotions not already studied here. Even within a time component, different emotions have previously been shown to occur at slightly different latencies (Batty and Taylor, 2003).

Together, the results show a main effect of drug, a main effect of genotype and a genotype x tolcapone interaction on fearful face processing. The robust results may, in part, reflect the homogenous and all-male sample. The latter is particularly important in emotional

processing given evidence of sexual dimorphism in emotional face processing. Thus, the inclusion of females may have yielded a very different activity profile. Some research shows a greater responsiveness of women to facial expressions (Dimberg and Lundquist, 1990; Orozco and Ehlers, 1998; Campanella et al., 2004), whilst men are faster than women to recognize faces showing fear (Campanella et al., 2004) and anger (Williams and Mattingley, 2006). Moreover, a sexually dimorphic effect of COMT is particularly prominent for emotional phenotypes (e.g. OCD, panic) as noted earlier. Thus, limiting the study to men may have contributed to the robustness of these current data which should not be extrapolated to women without considerable caution.

This passive viewing task does not contain a behavioural measure of how well emotions are being processed. This information would also allow us to make some comments on how MEG signals compare to the putative fMRI 'efficiency' signals. It is possible that COMT alleles do not produce an effect at the behavioural scoring level, as shown in the lack of effect during the Penn Emotion Recognition Test and the Penn Emotion Acuity Test (Defrancesco et al., 2011), and the faces task (Drabant et al., 2006). Future studies could involve participants pressing buttons that correspond to a happy, sad, or neutral face. This alone may not provide a difficulty level high enough to evince a difference between the two genotypes, therefore it would be necessary to pilot the study in order to find tasks where emotions are more difficult to recognise e.g. by masking the face slightly. Collection of data on implicit vs. explicit processing is also a possibility. For this, half of the time subjects could respond with whether the face is male or female (implicit processing of emotion) and the rest of the time subjects respond to say whether the emotion is neutral, happy or sad (explicit processing of emotion). This would also allow us to assess whether the emotional modulation of the N4-like component occurs exclusively in either explicit or implicit tasks (or both).

It is worth noting that DA involvement in emotional processing, whilst heavily studied, is not the predominant neurochemical involved. This chapter focuses on COMT, which metabolises DA (and possibly noradrenaline). However, a body of literature also exists for the serotonin (5-HT) signalling and emotional processing. For example, studies show differences in BOLD response to emotional faces related to a 5-HT transporter promoter (5-HTT) polymorphism which is thought to affect 5-HT reuptake (Cools et al., 2005; Anderson et al., 2007; Fusar-Poli et al., 2007; van der Veen et al., 2007). It would therefore, be of interest in future studies to stratify subjects by this genotype, as well as COMT Val^{158}Met, as part of a broader characterisation of the genetic contributions to face processing. However, initial studies of this kind have not identified interactions between COMT and 5-HTT genotypes (Defrancesco et al., 2011; Lonsdorf et al., 2011), although these authors measured different aspects of emotional processing from that tested here, and their sample sizes were small.

Finally, detection of activation in the amygdala is of particular value in the study of emotional processing. Capturing amygdala activation is difficult in MEG because it is a deep brain structure. As MEG analysis progresses, it will incorporate algorithms that may well allow assessment of deeper structures, such as the Beamformer techniques currently in development (see Chapter 7, section 7.3).

5.4.7 Conclusion

This chapter demonstrates that COMT, and by inference DA, modulation of face processing does not occur at the N170, but rather at later N4-like components, specifically for fear processing. This COMT modulation of facial fear processing happens both at the 'trait' level i.e. genotype, and 'state' level i.e. with tolcapone. Tolcapone affects the two genotype groups

differently. All of these modulations are regionally located. This speaks to the complexity of the underlying circuits involved, and thus, the need for further studies.

Chapter 6

The role of COMT in reward processing and decision making

Chapter 6

6. The role of COMT in reward processing and decision making

The role of COMT in WM and emotional processing is covered in Chapters 3-5. This chapter explores its impact on behaviour and neural correlates of decision making and reward processing.

6.1 Introduction

Although COMT has been shown to modulate WM and executive function, its significance for other cognitive domains remains relatively unexplored. I chose to include a reward-processing task in the test battery as DA is a critical mediator of reward processing which includes risk-taking, decision-making and gambling (Schultz et al., 1997; Berridge and Robinson, 1998; Gan et al., 2010). The particular paradigm used in this chapter has already been shown to be affected by the COMT Val^{158}Met polymorphism (Marco-Pallarés et al., 2009).

I will first briefly outline links between DA and reward. I will then note the literature surrounding COMT's impact on reward processing and related phenotypes, focussing on the reward delivery, since it is most relevant to the data presented here.

6.1.1 Dopamine and reward

Reward processing involves multiple brain regions including the ventral striatum (VSTR), midbrain, orbitofrontal cortex, (OFC), anterior cingulate cortex (ACC), PFC, ventral palladium and thalamus (Haber and Knutson, 2010). DA is a key neurotransmitter in this processing, particularly with respect to the mesolimbic DA pathway from the VTA to the VSTR. This is supported by

experiments that show DA antagonists to selectively reduce the motivation of animals to pursue rewards, without affecting their preferences for such reward when they can be obtained without effort (Berridge and Robinson, 1998). It has also been shown that the phasic activity of many midbrain DA cells, and DA transmission in the nucleus accumbens region of the VSTR, is modulated by parameters of the value of an upcoming reward, including its size, probability and the delay to its occurrence (Gan et al., 2010).

Although it has been ascertained that DA plays a prominent role in reward processing, its precise function is yet to be fully elucidated. This problem is fuelled by the close link between reward and many other domains, including motivation, pleasure, reinforcement, and most pertinent for the task in this thesis, risk-taking. DA may only be necessary for some of these processes.

Current understanding of DA in reward expounds that rather than conveying the direct incentive or hedonic properties of reward to an animal, midbrain dopaminergic activity is used to guide learning by conveying the discrepancy between past and current reward expectations, known as 'reward prediction errors' (Montague et al., 1996). This guides reinforcement learning, as neatly demonstrated in the Pavlovian conditioning paradigms that see animals learn that a particular cue is associated with reward. Initially the phasic increase in DA activity and transmission is observed only in response to reward delivery; however, following training, these signals come to be elicited by the cue and not just the reward (Schultz et al., 1997; Clark et al., 2010). In humans, either boosting or blocking DA transmission can also accelerate or retard reward learning (Pessiglione et al., 2006). For reviews on DA and reward see the following references (Schultz, 1997; Berridge and Robinson, 1998; Wise, 2004; Schultz, 2010).

6.1.2 COMT and reward

Given the evidence that DA is involved in reward processing, the question arises as to whether it is also modulated by COMT. For a full review on findings regarding COMT and reward see Tunbridge et al. (2012). Due to the many facets of reward, terminology of subcategories is complicated. Here I will use the phrase reward 'performance' to encompass any reward-related behavioural result, the phrase 'risky decision making' to refer to the behavioural quality of selecting a large bet over a small bet, and the phrase 'reward processing' to refer to the neural processes that occur upon the reveal of the outcome of a bet to the participant.

6.1.2.1 Evidence that COMT affects reward 'performance'

Evidence for a COMT effect on reward come indirectly in the form of COMT association studies for addiction or risk-taking behaviour and more directly, COMT effects on decision-making (i.e. gambling) tasks.

Drugs of abuse increase mesolimbic DA transmission therefore mimicking the physiological effect of natural reward (Di Chiara and Imperato, 1988). This is thought to be the mechanism by which some people become addicted (Hyman et al., 2006). Many associations have been made between COMT activity and various aspects of addiction. These papers suggest COMT plays a minor role in addiction. The Val allele may be associated with an increased likelihood of smoking, and possibly with methamphetamine abuse in the Han Chinese population (Li et al., 2004). After smoking, Val homozygous subjects show a larger decrease in the VSTR D_2 receptor binding (a measure thought to be related to an increase in DA release), compared with Met homozygous subjects. Val homozygotes are also associated with greater alleviation after smoking following a craving (Brody et al., 2006).

Turning specifically to the role of COMT in risky decision making, three tasks have been used to explore the effect of COMT. For a gambling task, participants were given a choice of either low (5) or high (25) which represented the amount in dollars that they would be gambling with; they then received feedback to tell them whether they had won or lost (Gehring and Willoughby, 2002). This is the task I will be using and is further detailed below (see section 6.2). Multiple studies failed to find a significant difference in genetic subgroups with regards to selection of '5' or '25', either overall or after influence from prior results i.e. a previous loss or gain, suggesting Val^{158}Met does not influence selection between large and small bets, even in light of previous losses and wins (Yacubian et al., 2007; Marco-Pallares et al., 2009; Camara et al., 2010).

However, a trend effect has been found in the Iowa Gambling task, which gives a choice of playing with immediate large rewards in two long-term losing decks or immediate small rewards in two long-term winning decks. Met homozygous subjects chose cards from the disadvantageous decks more than Val-allele carriers (van den Bos et al., 2009). This has been interpreted as high attention to wins compared to losses among the Met homozygous subjects, thus adding more subjective value to the immediate gains over the general losses in the deck. A further study in adolescent females demonstrated a relationship between risk taking propensity and Val^{158}Met using the Balloon Anxiety Risk Task-Youth Version (Amstadter et al., 2012). The task accrues higher monetary gains the more a participant chooses to blow up a balloon, but a loss of money if the balloon bursts. Here, in contrast to the Gehring and Willoughby paper, Met allele carriers had higher risk-taking propensity scores compared to Val homozygous subjects. In summary, there is currently no clear pattern between Val^{158}Met and decision making.

6.1.2.2 Evidence that COMT affects brain activity during reward processing

As with the WM literature, other studies have focussed on the fMRI or EEG correlates of COMT Val^{158}Met during reward-related tasks rather than upon behavioural differences. Most relevant here, Marco-Pallares and colleagues (Marco-Pallarés et al., 2009) used EEG to investigate the neural response to reward outcome in the Gehring and Willoughby gambling task (outlined in the above paragraph and detailed in section 6.2) and whether COMT Val^{158}Met influenced this. There were several key findings:

1. A medial frontal negativity (MFN) occurred from 250-300msec post presentation of win or loss[10].
2. This peak (maximal at approximately 265ms) was modulated by valence: it was greater for losses, compared to wins.
3. Val homozygous subjects showed a larger difference in MFN activity for losses vs. wins compared to Met homozygous subjects.
4. Val homozygous subjects showed a greater difference in MFN activity for maximum (25) when compared to minimum (5) losses, compared to Met homozygous subjects.
5. Oscillatory activity demonstrated greater activation in the beta range for wins than losses.
6. This beta activation was modulated by genotype, with Val homozygous subjects showing a greater difference for wins vs. losses than Met homozygous subjects.

[10] This time component is related to error-related negativity (ERN) documented for performance errors (Gehring et al., 1993). In 1997, postulating it to be generated by the same mechanism, Miltner et al. (1997) described a component in the human ERP associated with negative-related feedback. A task was designed by Gehring and Willoughby (2002) to assess the difference between loss and error or alternatively win and gain, they found that the 'loss' component characterised this signal better than 'error'.

Thus, Val homozygous subjects showed greater differences in both MFN magnitude and beta activation for losses vs. wins, compared with Met homozygous subjects.

An fMRI study used the same task, except 20% of trials were 'boost' trials in which either '5' or '25' was replaced by '125' (Camara et al., 2010). They found the VSTR was activated for valence (in this case a contrast using wins minus losses) for both the standard and boost trials. A Val^{158}Met genotype effect on this valence-related activation of the VSTR was shown during boost trials (but not standard trials). This effect was again due to a significantly greater difference between VSTR activation in wins trials minus loss trials in Val homozygous subjects, compared with Met homozygous subjects, agreeing with the aforementioned EEG findings. This greater difference seen in Val homozygous subjects was due to a greater reduction in BOLD response to losses, compared with Met homozygous subjects; BOLD responses to wins were similar in Val and Met homozygous subjects.

Taken together, these studies indicate that COMT modulates the relative activation to wins vs. losses, with the Val allele associated with greater differential activation between win and loss conditions. It is possible that the effect reported by Camara et al. was limited to 'boost' trials due to the difference in temporal resolution between EEG and MEG, and fMRI: whilst differences over milliseconds can be detected using EEG and MEG, fMRI requires changes to occur over several seconds for it to be detected. Thus, it is conceivable the standard trials produce small and short acting changes in neural activation insufficient to alter BOLD response but detectable using EEG and MEG.

Forbes and colleagues (Forbes et al., 2009) investigated the effect of several dopaminergic genes on VSTR activation during outcome. In this task participants had to guess the outcome but did not win or lose a specific amount on a trial-by-trial basis. Participants did, however, receive positive or negative feedback after each trial, and were told that their performance would determine the amount of money they would receive afterwards. Although this task robustly activated the VSTR, reward-related VSTR activation was unaffected by COMT genotype (these results were corroborated in a follow up task by Nikolova and colleagues (2011). However, reward-related VSTR activation was modulated by a multilocus dopaminergic genetic profile, consisting of functional polymorphisms in the Val^{158}Met and DAT, as well as DA receptor genes, DRD2 and DRD4. It appears the effects of COMT are less prominent when the relevant reward is not directly linked to on-going performance, since in this task participants were unable to monitor how they performed on each individual trial with a post-task reward. The effect of COMT on brain activation during reward outcome is likely to be moderated by polymorphisms in other dopaminergic genes, as has also been reported for reward anticipation (Yacubian et al., 2007; Dreher et al., 2009). Consistent with this, interactive effects of COMT and DAT on cortical activation during reward outcome have been reported (Dreher et al., 2009).

6.1.2.3 Summary of COMT reward outcome data

Performance results are not significantly different between COMT genotypes, however, some neural differences have been demonstrated. Firstly, the Val allele is associated with greater discrimination between wins and losses, compared with the Met allele, at least at the level of brain activation, and this is mediated at least in part by the VSTR (Camara et al., 2010). Secondly, the relative insensitivity to wins vs. losses associated with the Met allele might be due to differences in processing losses between Val and Met carriers. The negative findings reported by others (Forbes et al., 2009; Nikolova et al., 2011) emphasise that the relative importance of COMT

during reward outcome may depend on the task employed. In addition to the aforementioned findings, COMT also appears to modulate OFC activation during the delivery of uncertain rewards, with the Met allele associated with greater activation, compared with the Val allele.

6.1.3 Objectives

The objectives of this study are presented as a series of questions below and the strategy of analysis summarised in the flowchart shown in Figure 6.2[11].

a. Behavioural Objectives

Given prior studies, do we see an increasing percentage of conservative choices (over the whole group) with time? Do we see any differences in the proportion of high risk '25' or low risk '5' choices in the different participant groups? A previous study found no differences so we expected no difference, at least in the placebo groups (Marco-Pallares et al., 2009).

b. MEG Objectives

Do we replicate, as far as possible given our MEG data and evoked analyses, the responses from other studies mentioned in section 6.1.2.2 in terms of genotype effects on timing, location, valence and magnitude? Moreover, how are these parameters altered by tolcapone administration in the different genotype groups? To reiterate, earlier studies focussed on a medial frontal negativity (MFN) occurring from 250-300msec, modulated by valence (showing greater in magnitude for losses, compared to wins). For this component, Val homozygous subjects showed larger difference in MFN magnitude for losses vs. wins, compared with Met

[11] As with the other tasks used in this thesis, many other questions could be posed and analysed, but here we selected based on those evoked analyses that were most similar to the previous COMT study that used this task (Marco-Pallares et al., 2009).
.

homozygous subjects. Furthermore, Val homozygous subjects showed a greater difference in MFN amplitude for maximum (25) vs minimum (5) losses, compared with Met homozygous subjects.

6.2 Methods

The subjects used in this study came from the whole sample described in Chapter 2, Table 2.1.

6.2.1 The Gambling Task

The task used in this thesis involves monetary decision making based on a choice between two numerals '5' and '25' (Gehring and Willoughby, 2002) and has previously been used to demonstrate COMT-modulated effects on reward processing (Marco-Pallares et al., 2010). In this task subjects are given £10 to play with, and in each cycle are asked to choose between a box that allows them to gamble with 5 pence or a box that allows them to gamble with 25 pence. The boxes change colour 1000ms after the choice is made (green=win, red=loss) to show whether they have won or lost, they are also able to see the colour of the box they did not choose. The outcome is random and independent of the participant's choice. This coloured display remains on the screen for 1800ms. Every so often the screen shows the participant how much money they currently have banked and the participant presses a button to signal the start of the next round. The task lasts approximately 30 minutes depending on how long it takes the subject to decide on which box they wish to choose.

This task has been used extensively for neuroeconomics and decision-making studies, and, as mentioned in 6.1.2.2, a recent EEG study has shown that responses to wins and losses

during the task are sensitive to the COMT genotype of participants, as described above (Marco-Pallares et al., 2009).

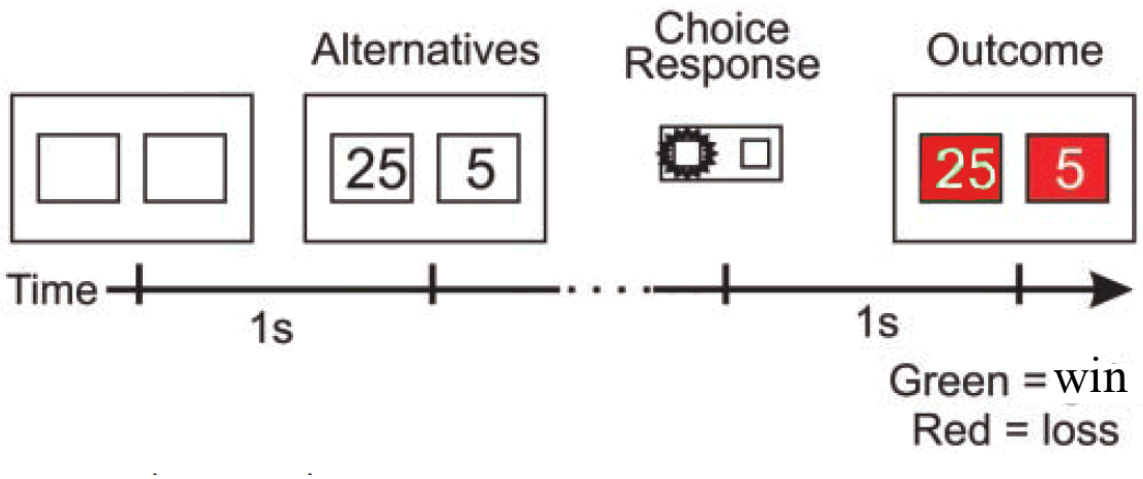


Figure 6.1 Gambling task. Participants are presented with two boxes. They select one to gamble with, representing either '5' or '25' pence. Colours then appear to show whether a win or loss was incurred (green representing win, red representing loss). The participant can also see the outcome of the alternate choice (taken from Gehring and Willoughby, 2002).

Outcomes of this task can be as follows:

1. 'Loss-5-(loss-25)': (both numeral boxes in red)- This represents losing 5 pence when the alternative choice would have lost the participant 25 pence. This can be described as a loss-correct situation. A loss was made, but the participant made the best possible choice.

2. 'Loss-25-(loss-5)': (both numeral boxes in red)- This represents losing 25 pence when the alternative choice would have lost the participant 5 pence. This can be described as a loss-error situation. A loss was made and the participant would have been better off choosing the alternative choice.

3. ‘Win-5-(win-25)’: (Both numeral boxes in green)- This represents winning 5 pence when the alternative choice would have won the participant 25 pence. This can be described as a win-error situation. A win was made but the participant would have been better off choosing the alternative choice.

4. ‘Win-25-(win-5)’: (Both numeral boxes in green)- This represents winning 25 pence when the alternative choice would have won the participant 5 pence. This can be described as a win-correct situation. A win was made and the participant made the best possible choice.

5. ‘Loss-5-(win-25)’: This represents losing 5 pence when the alternative choice would have won the participant 25 pence. This can be described as a loss-error situation. A loss was made and the participant would have been better off choosing the alternative choice.

6. ‘Loss-25-(win-5)’: This represents losing 25 pence when the alternative choice would have won the participant 5 pence. This can be described as a loss-error situation. A loss was made and the participant would have been better off choosing the alternative choice.

7. ‘Win-5-(loss-25)’: This represents winning 5 pence when the alternative choice would have lost the participant 25 pence. This can be described as a win-correct choice. A win was made and the participant made the best possible choice.

8. ‘Win-25-(loss-5)’: This represents winning 25 pence when the alternative choice would have lost the participant 5 pence. This can be described as a win-correct choice. A win was made and the participant made the best possible choice.

I limit the outcome conditions studied in this thesis to those numbered 1-4, as per Marco-Pallares and colleagues (2009), thus eliminating those that differ in both magnitude and valence simultaneously, allowing the assessment of the effect of COMT on either of these without the other present as a confounding factor (and to enable direct comparison with other findings).

6.2.2 Analysis of behavioural data

Number of '5' choices made and '25' choices made were totalled for each person and turned into percentages of total responses. Two and three-way ANOVAs i.e. genotype x tolcapone and genotype x tolcapone x valence (win vs. loss) were used to investigate main effects and interactions. LSD post-hoc tests were used to investigate significant interactions.

Reaction times between presentation of stimulus and button press were also taken and averaged for both '5' and 25', again by group.

6.2.3 MEG analysis

See Chapter 2, section 2.8.4 for information on preprocessing, analysis and map production. Analyses were specifically designed to address the following questions (summarised in Figure 6.2):

1. **Do the different outcome conditions (i.e. wins vs. losses) influence MEG activity?** To assess whether participants process wins and losses differently, I produced global plots of all individuals averaged together, separated by the reward outcomes, along with a corresponding probability map reflecting ANOVA analysis to assess if differences between these conditions were significant. This provided a quick overview of amplitudes of peaks of interest. The analysis focused on peaks within the 250-300ms time period highlighted in the

literature. Local maps for the peaks of interest were then produced to see general regions over which these differences occur.

2. **Does COMT genotype modulate MEG response to valence?** Global maps were produced separately for each of the four participant groups, representing a previously used parameter, namely average win-minus-loss (Marco-Pallares et al., 2009). To calculate this contrast, the activity of outcomes from the two win conditions were averaged to create a 'win average' (i.e. the average of win-25-win and win-5-win). The corresponding loss average was then subtracted from this. A corresponding probability map was made to show where these groups differed. The time component of interest was limited to 250-300ms. Local maps of this component were constructed to give further insight into the differences between genotype groups.

3. **Does COMT genotype modulate the MEG signal response to processing magnitude (i.e. '5' vs. '25')?** A similar procedure for investigating the effect of valence was conducted for investigating 'magnitude' (5 vs. 25) of losses. The comparison of loss-25-loss and loss-5-loss was used so that comparisons could be made to previous literature (Marco-Pallares et al., 2009).

4. **How does tolcapone influence MEG activity during reward processing?** The impact of tolcapone and the interaction of the Val^{158}Met polymorphism and tolcapone inhibition on the parameters described above were explored, as in Chapters 4 and 5. There were no explicit predictions regarding main or interactive effects of tolcapone since prior findings did not exist, nor is it clear whether an inverted-U applies to this task.

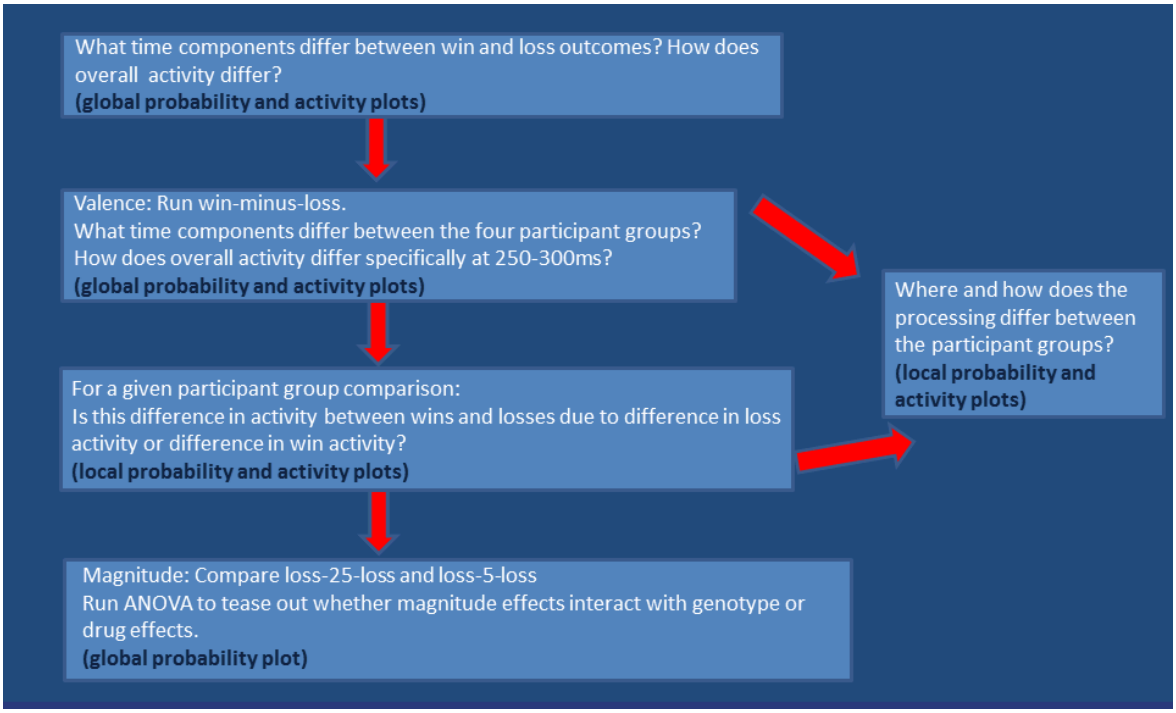


Figure 6.2 Strategy for MEG analysis of reward processing. The flow chart shows that the analysis first looked at global plots demonstrating whole-head activity differences between processing a win and a loss. A contrast was then created to assess differences between the four participant groups. Finally COMT modulation of magnitude was assessed.

6.3 Behavioural results

6.3.1 Participants' 'riskiness' (percentage 'high bets') decreases over time.

After splitting the duration of the task into four quadrants, the percentage of time participants selected '25' was calculated for each quadrant (as performed in previous literature). Percentages differed between quadrants [main effect of quadrant: $F_{(2.3,145.3)}$=4.6, p=0.008] and Figure 6.3 demonstrates that the percentage of selection of '25' is lower in quadrant four than quadrant one (p=0.05), dropping from 54.4% (s.d. 19.0) to 48.8% (s.d. 24.0), suggesting that participants become more risk averse with time. This change in riskiness is not altered by drug or genotype groups, nor their interaction (data not shown).

6.3.2 Bets placed were unaffected by prior outcomes

The percentage of risky bets made were no different depending on the prior outcomes, e.g. whether the individual had experienced one win, two wins, one loss or two losses prior to the bet did not alter their likelihood of choosing '25' or '5'. Over all subjects, 52.0 % of bets placed were '25' (s.d. 18.5). Results were not altered by prior outcome since the genotype x tolcapone interaction remained significant in bets placed after one or two losses or one or two wins (Fs>7.5; ps<0.01).

6.3.3 Val^{158}Met genotype x tolcapone interaction influences size of bet chosen

There was no main effect of genotype or tolcapone on measures of size of bet chosen e.g. the percentage of '25' chosen ('high' bets). However, strikingly, there was a genotype x tolcapone interaction [$F_{(1,61)}$=7.91, p=0.007]. In the subjects given placebo, Met homozygous subjects made fewer 'high' bets than Val homozygous subjects (p=0.05); this difference was reversed in subjects given tolcapone ($p=2.5x10^{-2}$; Figure 6.4), with tolcapone-treated Val homozygous subjects making fewer 'high bets' i.e. being more risk averse than tolcapone-treated

Met homozygous subjects ($p=8.0x10^{-3}$). Drug and genotype together explained 8.5% of the variance. A post hoc power calculation of these data revealed that the sample sizes used provided an observed power of 0.79. The main effects and interactions shown in Figure 6.4 were also seen for trials following two wins or two losses or other combinations of prior outcomes (data not shown).

6.3.4 No COMT effect on reaction time

We found no main effects of drug or genotype, nor interactions, on gambling task RT (Table 6.1; all $F<1.5$, $p>0.3$). Hence, the amount of time taken to choose between a high and low-risk bet was not COMT-modulated.

Table 6.1 Gambling task reaction times

	Met Pbo	Val Pbo	Met Tolc	Val Tolc
RT (ms)	730 (333)	715 (331)	884 (557)	667 (187)

Values are mean (s.d.) RT: reaction time. ms: milliseconds; RT data were not available for 10 subjects.

6.3.5 No correlations between gambling performance and other COMT-modulated behavioural measures

As Chapter 3 showed findings of genotype x tolcapone interactions on N-back performance (section 3.3.3), and Chapter 2 showed genotype x tolcapone interactions on the happiness VAS rating (section 2.6.3), I investigated whether these variables correlated with the gambling data found in this chapter. However, there were no significant correlations as measured by Pearson's correlation coefficient (all $r<0.23$; $p>0.05$). The inclusion of happiness

VAS score as a covariate did not affect the N-back or gambling task behavioural results (data not shown).

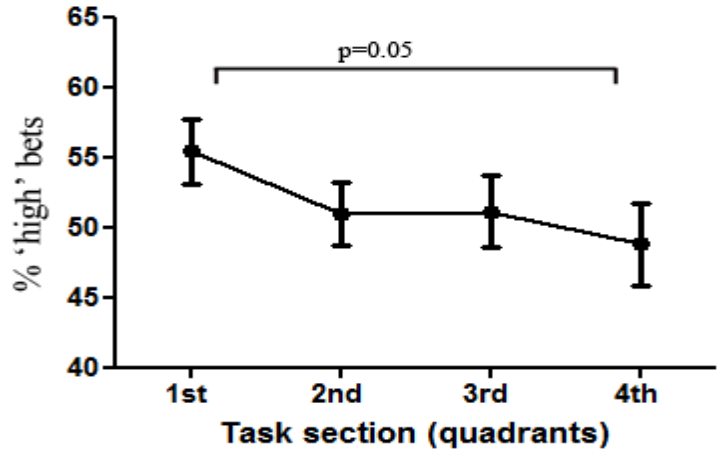


Figure 6.3 Propensity for making 'risky' bets reduces over time. 'Riskiness' is measured by the percentage of high bets placed (i.e choosing '25' over '5' pence). The above graph shows that the percentage of high bets, and thus 'riskiness' decreases with time, with a significant reduction between the 1st and 4th quarter of the task.

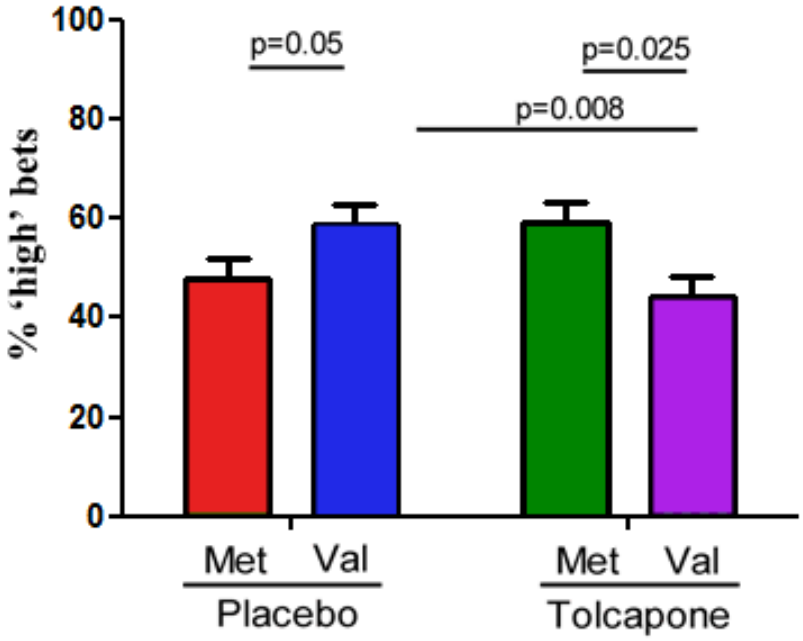


Figure 6.4 Gambling task behavioural results. This figure shows a genotype x tolcapone interaction, with Val homozygous subjects choosing small bets less than Met homozygous subjects in the placebo condition, but more than Met homozygous subjects in the tolcapone condition.

6.4 MEG results for investigating the neural correlates of reward processing

The first set of results (6.4.1 and 6.4.2) look at the data in the whole cohort, without separating them between drug and genotype groups. Section 6.4.3 onwards introduces the drug and genotype comparisons. Note that, unlike the N-back results (Chapter 4), MEG analysis was not limited to groups matched for 'performance'- all subjects were included. Performance matching was unnecessary because the 'performance' results here do not present a confounding factor to the COMT influence on processing wins and losses, as these aforementioned results do not directly pertain to the neural aspect of outcome processing that is being analysed. This was suggested in section 6.3.2 where previous outcomes have no bearing on the next numeral selection.

6.4.1 Global maps show neural response to losses is larger than to wins

Figure 6.5 represents the impact of wins vs. losses on global MEG signal. The probability maps shown in Figure 6.5a demonstrate a significant difference between processing wins and losses. The 250-300ms time period associated with processing losses and wins by Marco-Pallares and colleagues (2009) is found in this data between 257-305ms, with a maximal value of $p=1.4x10^{-25}$ occurring 274ms post stimulus. Figure 6.5b shows the neural activity corresponding to this, depicting power of signal elicited when processing the outcome. The blue shaded area highlights the time of interest, which peaks at 0.95fT/cm for wins, 265ms post stimulus onset (where stimulus is presentation of coloured boxes), and a higher value of 1.2fT/cm for losses, 262ms post stimulus onset.

Figure 6.6 is similar to 6.5 but breaks the data down further into the two types of wins and losses i.e. win-25-win, loss-25-loss, win-5-win and loss-5-loss. The significant difference between these four conditions is, again, seen at the 250-300ms time span, peaking at $p=3.3x10^{-59}$,

274ms. Figure 6.6b demonstrates that activity was highest for the loss-25-loss condition, peaking at 1.2fT/cm, 262ms post stimulus, followed by loss-5-loss (1.2fT/cm 262ms), then win-25-win (1.0fT/cm, 249ms). The lowest activity was seen for win-5-win (0.90fT/cm, 249ms). Note there are hints of a temporal difference between losses and wins, with the two loss conditions peaking in activity later than the two win conditions. However, repeated measure ANOVAs looking at the average latency of the peak for each participant shows this does not reach significance (data not shown).

N.B. The other peaks shown in Figures 6.5 and 6.6 are omitted from further investigation within this thesis as they do not fall within the time period of interest as defined by previous literature.

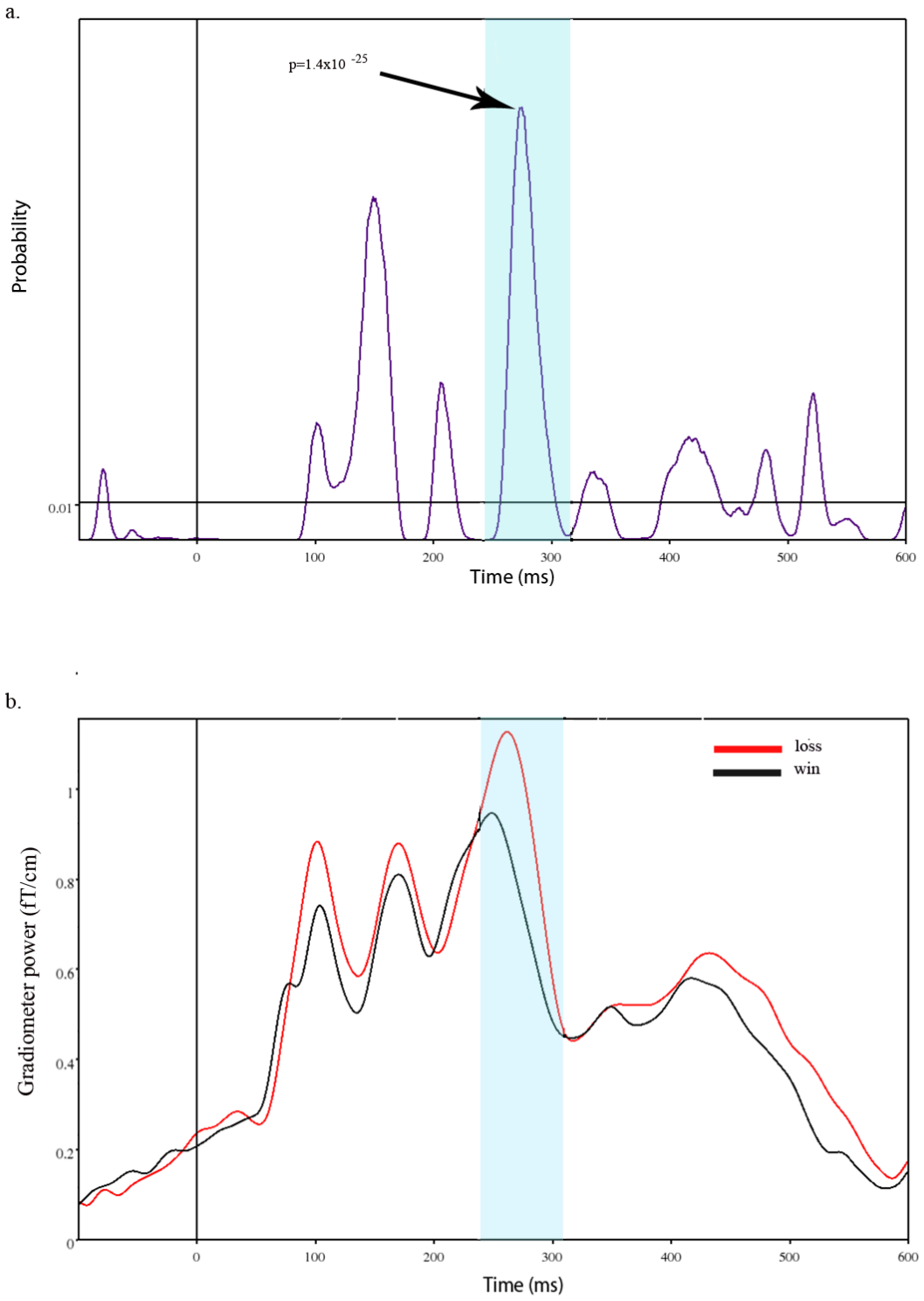


Figure 6.5 Probability plot and activity plot showing differences between losses and wins. a. The time components denoting a difference between processing wins and losses protrude above the line x=0.01. The blue shaded column highlights the peak (257-305ms) that fell within the 250-300ms period of interest. b. Activity over the whole brain following the presentation of losses (shown in red) and wins (shown in black). The blue shaded area corresponds to the significant peak highlighted in a.

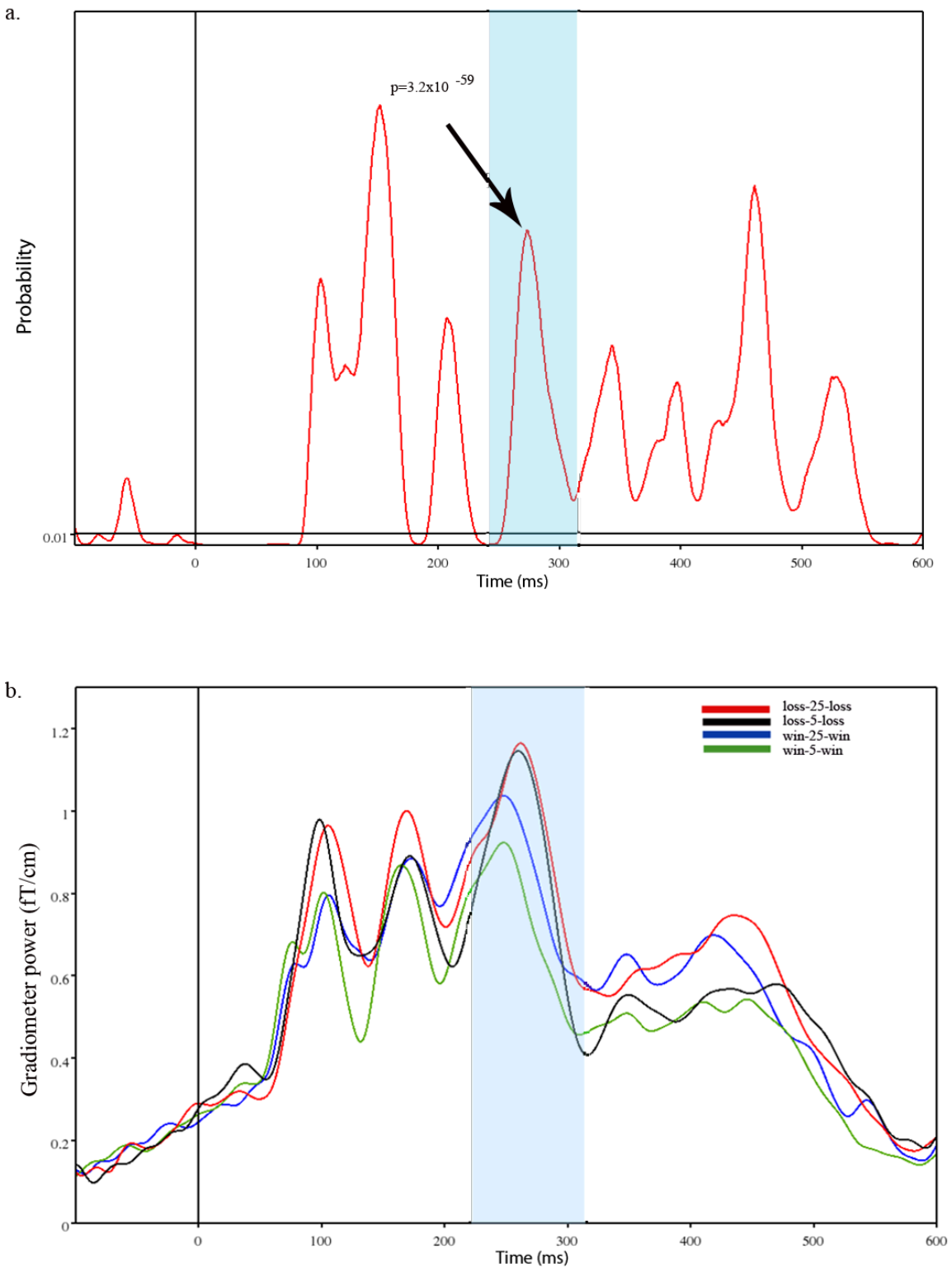


Figure 6.6 Global activity elicited from the four outcome conditions. a. The shaded blue area highlights a peak where processing differs between the four outcome conditions within the 250-300ms time component of interest. b. At the time of interest (shaded blue), both loss conditions elicit a higher peak than the two win conditions (win-5-win shown in green; win-25-win in blue).

6.4.2 Local plots for the four different loss and win conditions

Figure 6.7 shows the different local maps for the four task outcomes at the time of peak significance, 275ms[12]. Figure 6.7a shows the areas of significant difference between them and the maps below this show the activity for these aforementioned outcomes. Label 1 points to a midline frontal region noted (p=0.02), as this potentially corresponds to the MFN reported in the original report of the task in EEG (Gehring and Willoughby, 2002). Activities in this region are higher for the two loss conditions, (1.5fT/cm for loss-5-loss; 1.3fT/cm for loss-25-loss) than for the two win conditions (0.6fT/cm for win-5-win; 1.2fT/cm for loss-5-loss).

Widespread bilateral temporo-occipital areas are also shown to differ significantly between conditions. The occipital areas, labelled 2 ($p=6.0x10^{-5}$) show loss-5-loss and loss-25-loss have the highest activities (1.2fT/cm and 1.4fT/cm respectively) compared to 0.69fT/cm for win-25-win and for win-5-win. The left temporal area, labelled 3, ($p=1.5x10^{-4}$), reveals a complicated picture as, although the highest activity is seen in the loss-5-loss condition (2.2fT/cm), loss-25-loss exhibits the lowest activity (1.4fT/cm- although the distribution of activity is greater than the two win conditions) with win-5-win (1.6fT/cm) and win-25-win (1.7fT/cm) having the intermediate values.

Summary of sections 6.4.1 and 6.4.2

These results so far speak to the question 'Do different reward outcomes elicit different activity in MEG?', demonstrating that participants process the win and loss conditions differently, with higher activity responses to losses, particularly over bilateral temporal, occipital and medial frontal areas.

[12] Peak significance demonstrated by Figure 6.6 was 274ms, but the local maps were produced at every five second interval early on in analysis, therefore the closest time frame to this was chosen, i.e. 275ms.

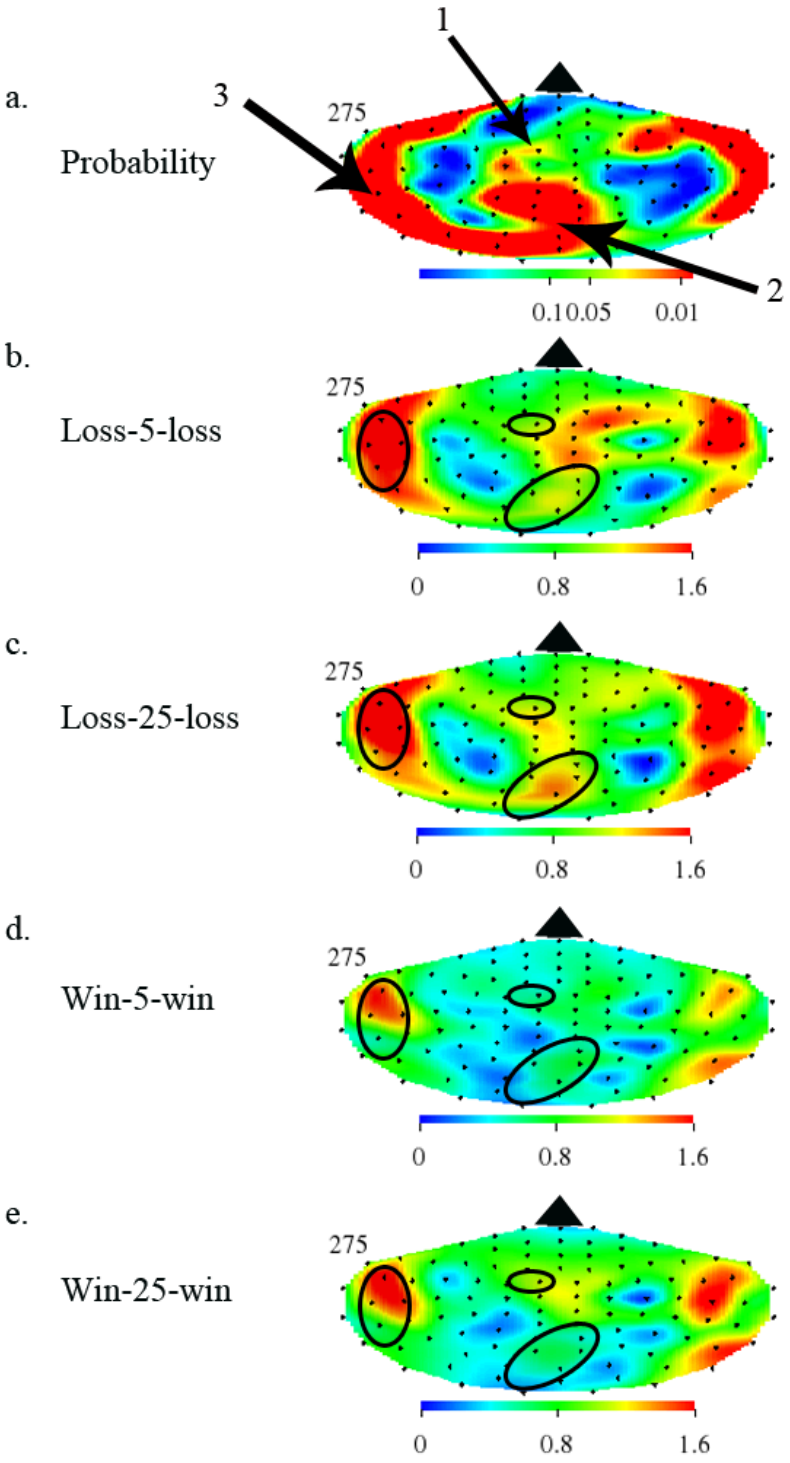


Figure 6.7 Local maps for the four outcome conditions. The probability map in 6.7a. has a broad sweep of regions with significant difference. Three key areas are highlighted; medial-frontal, temporal and occipital. Despite bilateral significance, only the left temporal region has been highlighted for brevity's sake, selected as it shows the highest contrast between the conditions. Areas of activation for each condition of interest are shown in 6.7b., c., d., and e. They correspond to the greater activation in loss conditions over win conditions.

6.4.3 COMT modulation of wins and losses

The next section looks at MEG objectives listed in part b. of section 6.13, investigating COMT-modulation of the MEG signal in relation to valence. This part of the investigation looks at a 'win-minus-loss' contrast (of the two activity curves shown in Figure 6.5b.) and focuses on how it differs between participant groups during the 250-300ms time period. Specifically, it is the activity generated upon processing a win, minus the activity generated upon processing a loss[13].

6.4.3.1 Global probability plots show genotype x tolcapone interaction

The probability map in Figure 6.8a depict the time points at which there are main or interactive effects of genotype x tolcapone. The global activity plot for the win-minus-loss parameter is shown in Figure 6.8b, depicting the four different genotype and tolcapone groups separately. The peaks in global power represent the peak 'difference in activity' between the average of the win conditions and the average of the loss conditions.

Two peaks of significance are highlighted with a blue strip in Figure 6.8a. The first demonstrates a tolcapone vs. placebo difference peaking at 274ms, $p=5.8x10^{-18}$, with a curve extending from 230-303ms. This activity peak corresponds to a higher tolcapone activity peak (243ms, 2.3fT/cm) compared to the placebo group (227ms, 1.7fT/cm). The second peak of interest represents a genotype x tolcapone interaction at 288ms ($p=5.4 \times 10^{-4}$). It is interesting that the Val placebo group seem to elicit higher activity (2.3fT/cm) than the Met placebo group (1.4fT/cm) akin to the data from the Marco-Pallares study. This is investigated further using local maps, in section 6.4.3.3. Although it appears that there may be between-group differences in the latencies of the peaks, this was not statistically significant (data not shown).

[13] Note that GRMS values are always positive due to squaring. Thus, even if the minuend has less power (GRMS) than the subtrahend, the resultant power curves for the contrast will be positive for all times. Win-minus-loss was chosen over loss-minus-win to follow the methods of Marco-Pallares et al. (2009)

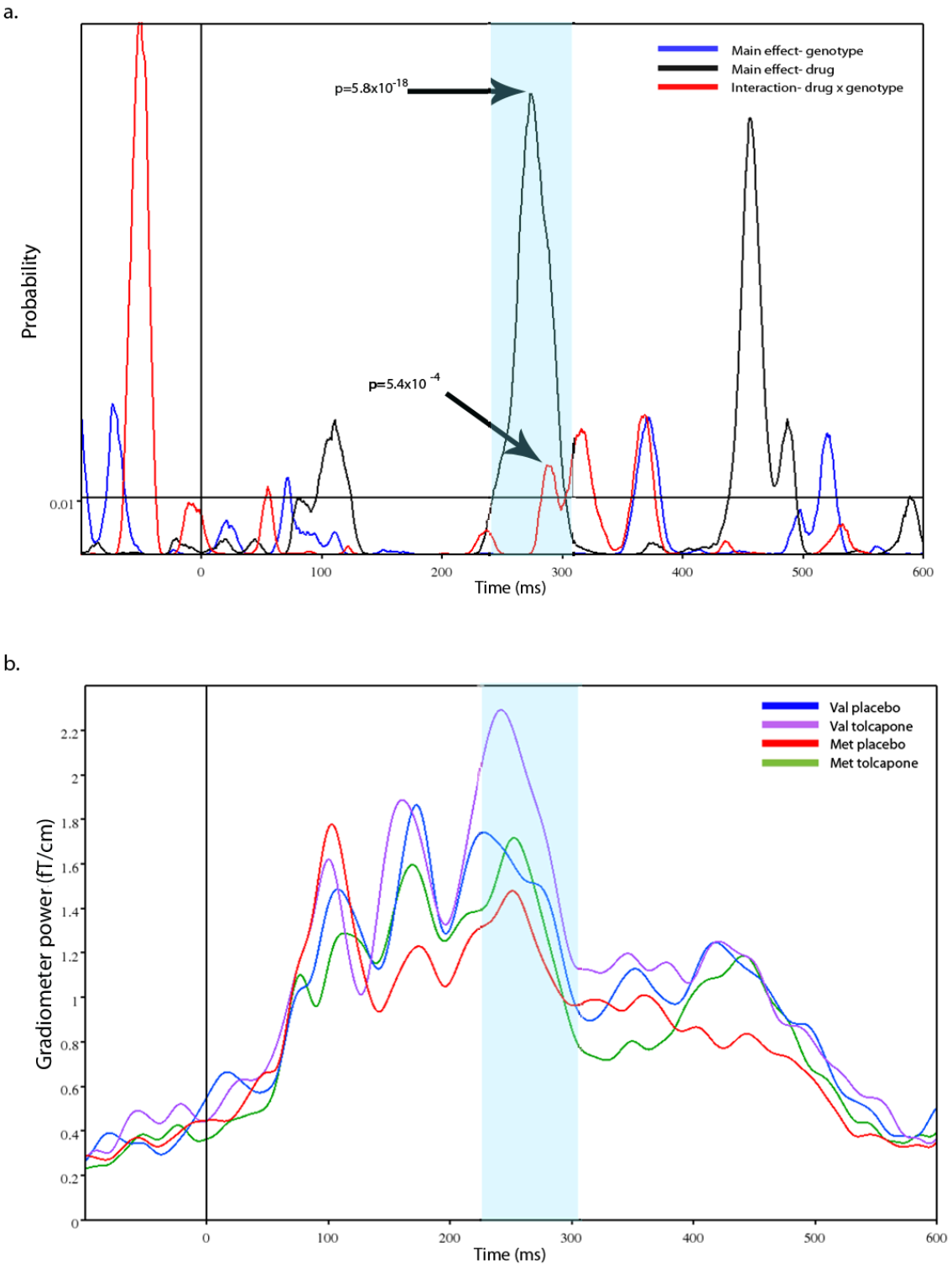


Figure 6.8 Win-minus-loss contrast separated by the four participant groups. a. Time points of significant difference between tolcapone and placebo groups (represented in black), Val homozygotes vs. Met homozygotes (blue), and the interaction between these genotype and drug groups (red) are shown. The light blue shading highlights the time component selected for further investigation and shows a noticeable absence of genotype main effect. b. The time point of focus (highlighted in blue shading) shows the tolcapone (lilac and green) groups have a higher activity than their placebo counterparts (red and blue). The Val placebo (blue) looks to be higher than the Met placebo group (red).

6.4.3.2 Local plots of drug effects on wins and losses at 275ms

Figure 6.9 illustrates the main effect of drug at the 275ms time component for wins-minus-losses. Three key regions are shown on the probability map. The first two large regions, labelled 1 and 2, demonstrate higher activity in tolcapone vs. placebo. Label 1 points to a large area left frontal, with the placebo group having a lower difference of 0.58fT/cm and the tolcapone group having an activity of 1.8fT/cm (p=0.0014). The left tempero-parieto-occipital border, labelled 2, has an activity of 0.95fT/cm in placebo but a higher value of 1.4fT/cm in the tolcapone condition (p=0.0086).

The right occipital area, labelled three (p=0.019), shows the placebo group to have a higher activity difference than tolcapone (2.85fT/cm vs. 2.19fT/cm; p=0.019).

Summary of tolcapone effects on the win-minus-loss contrast

Areas of significant difference between placebo and tolcapone are extensive. Tolcapone activity is higher than placebo over the majority of these significant areas.

6.4.3.3 Characterisation of genotype x tolcapone interaction at 285ms

The probability plot in Figure 6.10 shows the local map corresponding to the interaction between genotype and tolcapone at 285ms (see Figure 6.8). There are two key areas of significance; the left temporal section edging onto frontal areas, labelled 1 ($p=3.7x10^{-3}$), and the left parietal area, labelled 2 (p=0.01). However, only area 1 produced significant results in post-hoc analyses, though area 2 does appear to arise from the greater activity shown in Met placebo than the other three participant groups. Significant differences in area 1 were found between the Val placebo and Met placebo groups (p=0.032); between the Val tolcapone and Met tolcapone groups ($p=4.1x10^{-3}$); and between the Val placebo and Met tolcapone groups (p=0.021) as shown in Figure 6.10b. These differences in activity are shown in aii-v. The higher activity in the Val

placebo group, shown in aii. (1.9fT/cm), compared to Met placebo (1.3fT/cm), shown in aiv., is responsible for the significant difference demonstrated in bi., whereas the higher activity levels in the Met tolcapone group, shown in av. (1.26fT/cm), compared to the Val tolcapone group, (0.9fT/cm) shown in aiii., is responsible for the significant difference shown in bii. The lower activity of the Met tolcapone group (1.26fT/cm) over the Val placebo group (1.9fT/cm) is responsible for the significant difference shown in biii.

Summary of section 6.4.3

Answering MEG objectives 2, 3 and 4 (see section 6.2.3); I showed that COMT activity, determined both by the Val^{158}Met genotype and with tolcapone, modulates the neural response to valence. People given tolcapone show higher activity for the win-minus-loss contrast in both the left frontal and left temporo-parieto-occipital border, compared to those given placebo (Figure 6.9). A genotype x tolcapone interaction (Figure 6.10) was seen in the temporo-frontal border showing a higher Val placebo activity compared to Met placebo, but a reversal of these relative activities was seen in the tolcapone condition.

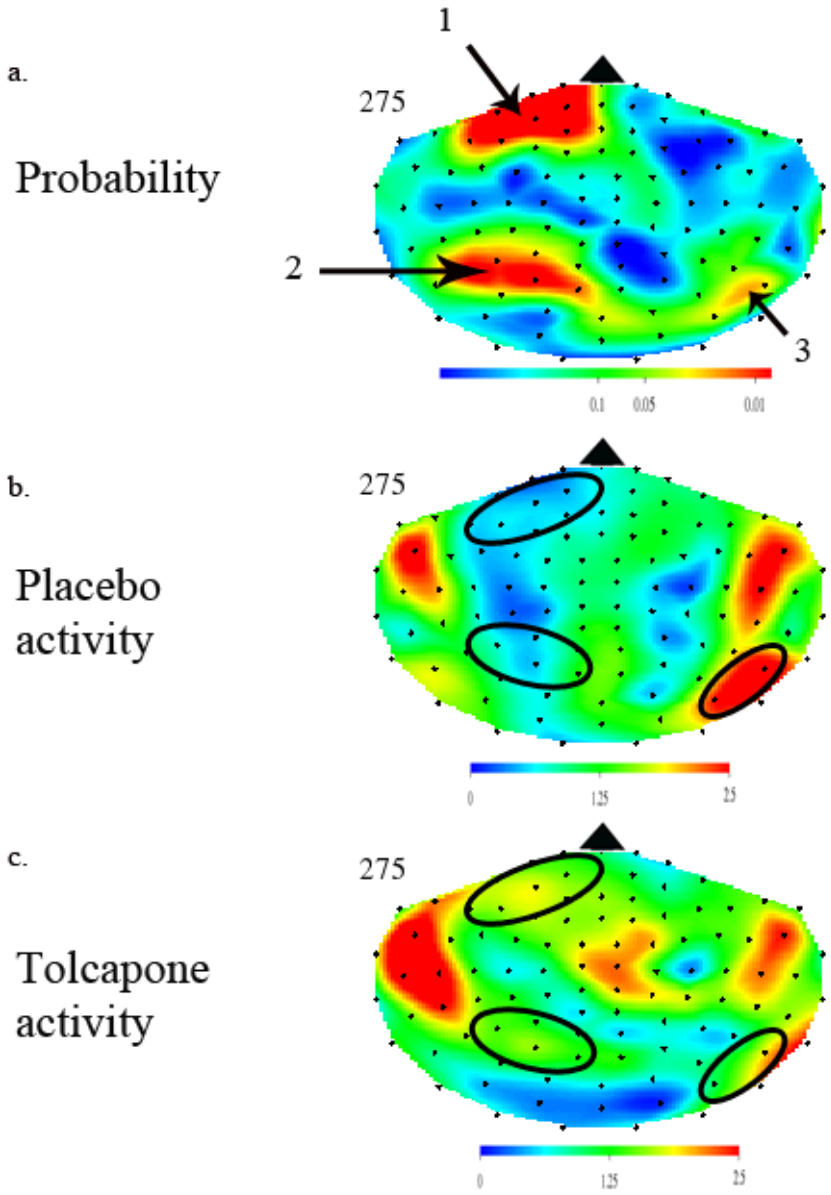


Figure 6.9 Tolcapone effect on valence (win-minus-loss contrast). Significant areas of difference between the tolcapone and placebo groups are represented in a., the activities of which are shown in b. and c. respectively. Areas labelled 1 and 2 demonstrate higher activity in tolcapone than placebo. Area 3 shows higher activity in placebo than tolcapone.

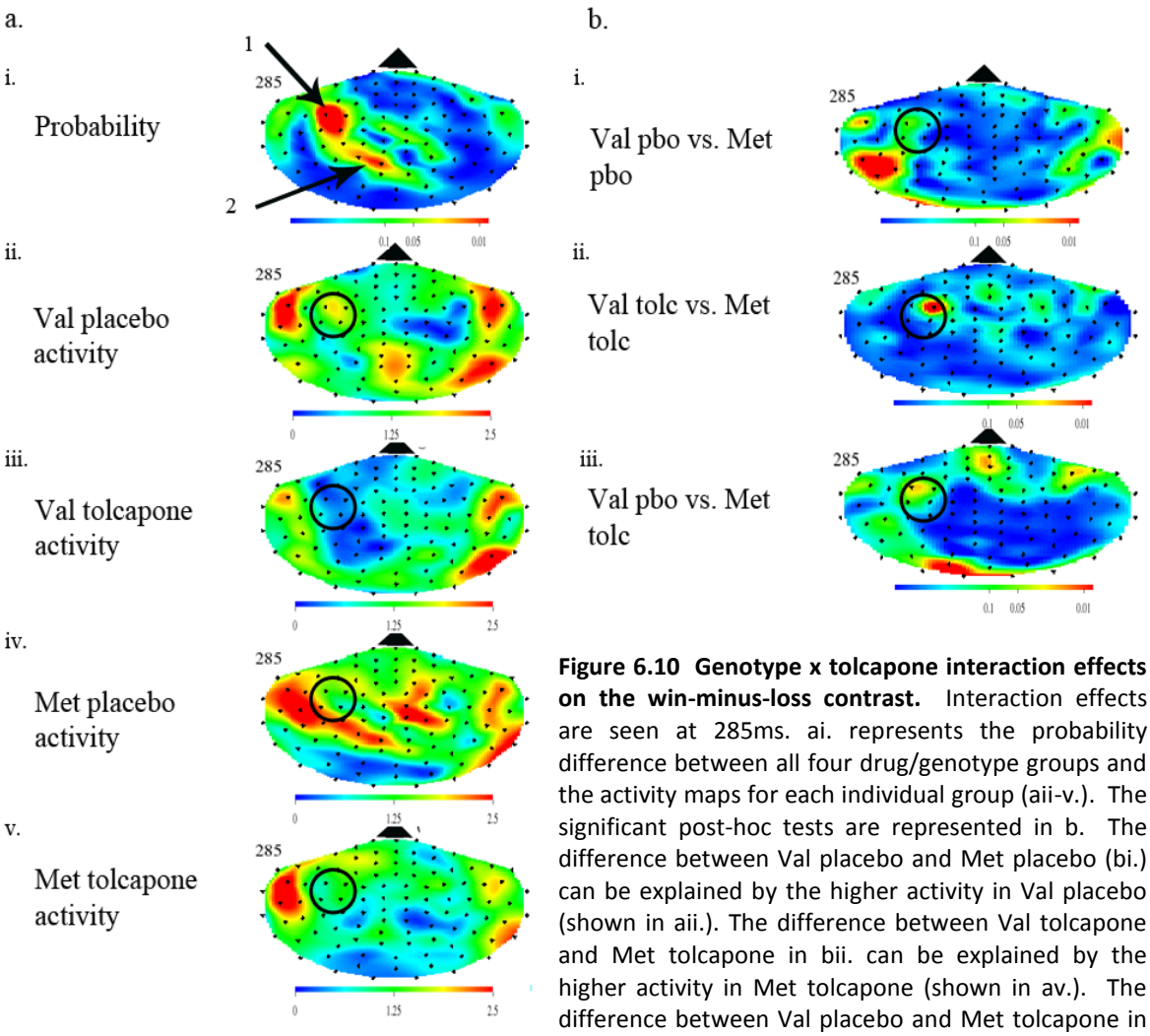


Figure 6.10 Genotype x tolcapone interaction effects on the win-minus-loss contrast. Interaction effects are seen at 285ms. ai. represents the probability difference between all four drug/genotype groups and the activity maps for each individual group (aii-v.). The significant post-hoc tests are represented in b. The difference between Val placebo and Met placebo (bi.) can be explained by the higher activity in Val placebo (shown in aii.). The difference between Val tolcapone and Met tolcapone in bii. can be explained by the higher activity in Met tolcapone (shown in av.). The difference between Val placebo and Met tolcapone in bii. can be explained by the higher activity in Val placebo (shown in aii).

6.4.4 No COMT effects on MEG signal in response to magnitude of bet

This section aims to answer the MEG objective (see section 6.1.3) asking 'Does COMT modulate the neural response to magnitude?' i.e. whether COMT genotype or tolcapone alter the difference between processing a high value or a low value. I only look at this for the loss condition, again emulating Marco-Pallares (2009) as far as possible.

Figure 6.11 compares the two loss conditions, 'loss-25' and 'loss 5' to enable a comparison of the magnitude-related results shown by Marco-Pallares. Figure 6.11a shows the probability plot demonstrating a lack of statistically significant difference between low and high loss magnitude on activity at the time component of interest and no effect of drug or genotype, nor their interaction, on the neural response to magnitude. It is noted that there are some effects of magnitude at later time intervals (379ms, $p=8.6x10^{-9}$; 466ms, $p=1.8x10^{-9}$) but these are not modulated by tolcapone or genotype, nor do they correspond to the 250-300ms of focus in this chapter.

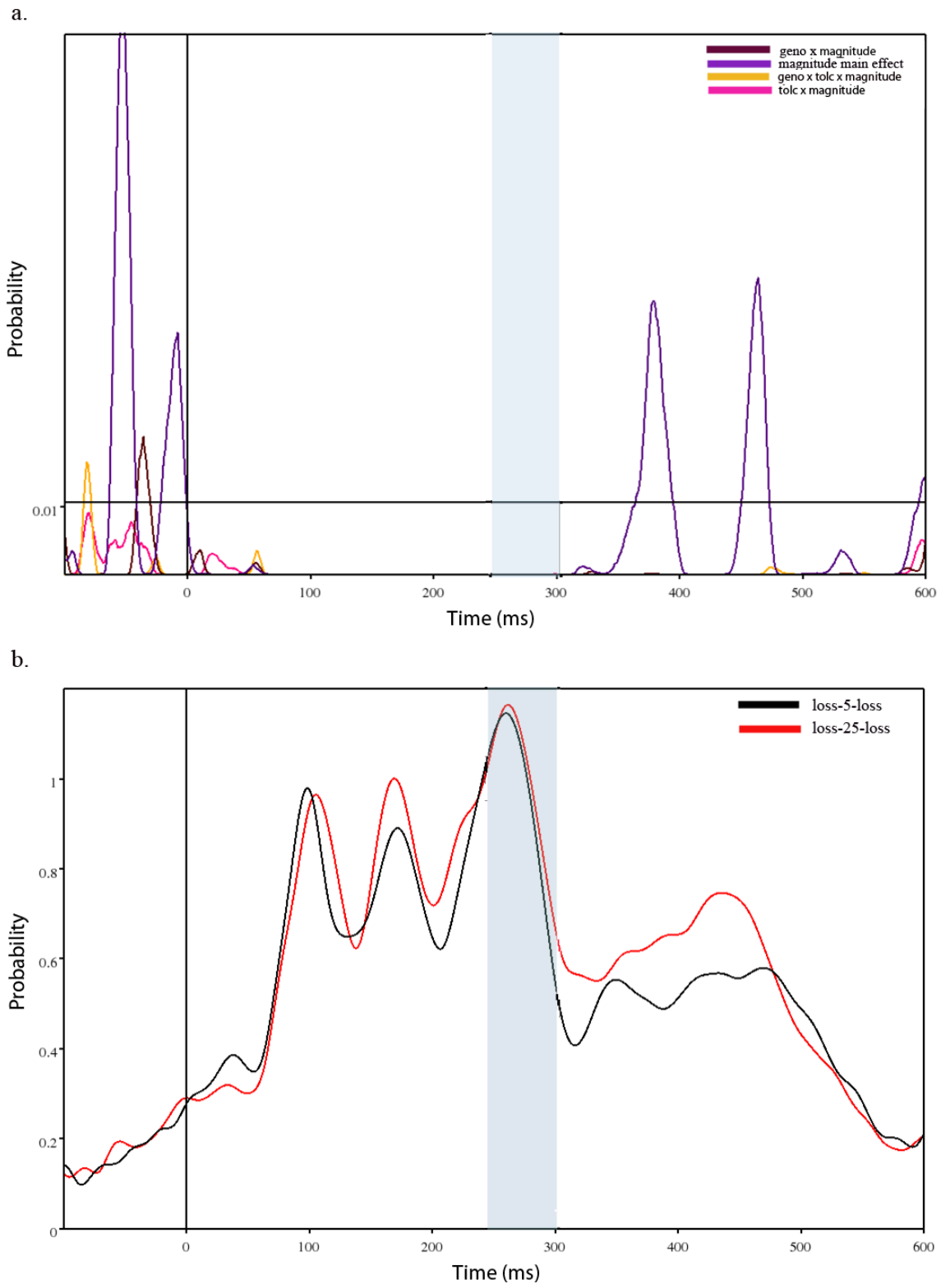


Figure 6.11 No COMT effects on magnitude. This figure explores the possibility of COMT effects on magnitude. The probability map in a. compares loss-5-loss and loss-25-loss, showing no differences between the two conditions at our time component of interest (shown in blue shading). The probability plot shown in a. also shows no COMT-modulated differences in this time period. The activity plots for the two conditions are represented in b. Note that a. depicts differences in processing '25' and '5' that occur between 350-470ms post-stimulus onset. These look to arise from a higher activity response to big losses, though they are not COMT-modulated.

6.5 Discussion

In both the behavioural and MEG sections of this discussion, I first discuss any results relating to the participant group as a whole and then discuss those results relating to COMT-modulated differences.

6.5.1 Behavioural Discussion

To summarise, participants became more risk averse over time and the size of bet placed was not affected by prior win or loss outcome. These findings did not differ between drug and genotype groups. However, a COMT Val^{158}Met genotype x tolcapone interaction was seen for size of bet placed, with the Val tolcapone group significantly more risk averse than the Val placebo group, but the Met tolcapone group significantly less risk averse than the Met placebo group. The Val placebo group also demonstrated a significantly higher risk aversion than the Met placebo group.

6.5.1.1 Risk aversion tendency increases across time

Overall participants showed a slight inclination to select the riskier option. This slight inclination ('25' selected 51.7% of the time) corroborates other studies that show participants choose '25' slightly more than '5' (Marco-Pallarés et al., 2009; Camara et al., 2010). The overall preference for riskiness is marginal and may reflect the fact that both values are relatively low, with '5' pence being a negligible amount to win or lose, therefore perhaps not seen as worth playing with.

Our findings of increasing risk aversion over time across all groups during this task agree with prior findings (Gehring and Willoughby, 2002). As time goes on and money accumulates, the participants take a more protective approach to their winnings, switching to small bets to

conserve gains rather than attempting to win more. This strategy may speak to the greater aversion that people feel towards a loss of a particular value than a win of the same value or to put succinctly 'losses loom larger than gains' (Tversky and Kahneman, 1992). Alternatively, shown in Figure 6.3, low and high bets become equally probable fairly soon into the experiment suggesting that subjects might have figured out that the outcomes are random.

6.5.1.2 COMT effect on risk behaviour

There are two main COMT-related behavioural findings. First, on placebo, Met homozygous subjects are more risk averse than Val homozygous subjects. Second, genotype interacts with COMT inhibition by tolcapone to affect this index: compared with those given placebo, tolcapone made Met homozygous subjects less risk averse and Val homozygous subjects more so. These interactions were robust and the drug effect sufficient to reverse baseline genotype differences. These findings highlight that COMT has a role in phenotypes beyond executive function and WM (Bilder et al., 2004; Tunbridge et al., 2006). Involvement of COMT in dopaminergic contributions to decision making and reward has previously been demonstrated in relatively complex paradigms as mentioned earlier in this chapter (Yacubian et al., 2007; Krugel et al., 2009; Frank and Fossella, 2011), but the gambling task data presented here show even the simple trait of risky decision making is affected by COMT. High COMT activity, such as that in the Val placebo group, is associated with making a higher proportion of large gambles (risky choices) compared with low COMT activity in the Met placebo group. The finding may relate to the demonstration in rodents that enhanced DA signalling via D_2 receptors attenuates risky choices (Simon et al., 2010). Rats chose between small 'safe' rewards and large 'risky' rewards. Systemic activation of D_2-like receptors attenuated risky choices, whereas drugs acting on D_1-like receptors had no effect. This suggests that it is the level of D_2-like receptor signalling that is particularly important for risk aversion.

The genotype x tolcapone interaction on the size of bet placed is consistent with an inverted-U model of DA function underlying this behaviour (see Figure 6.13). It can be interpreted that, after tolcapone, Val homozygous subjects move up the curve both in terms of cortical DA and risk aversion. Met homozygous subjects in the placebo condition start with higher cortical DA levels (and further along the curve) than their Val counterparts, thus they move down the curve following an increase in DA after tolcapone administration, and exhibit a concomitant decrease in risk aversion. This is the first positive evidence that the Inverted-U applies to COMT phenotypes outside of WM.

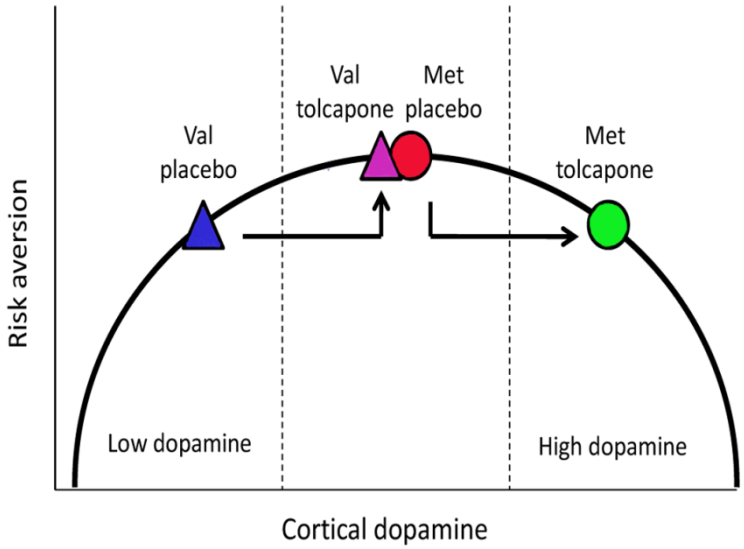


Figure 6.12 Inverted-U relationship between DA and risk aversion. The curve is a schematic suggesting that the levels of 'risk aversion', as measured by percentage of 'small bets' chosen, is related to cortical DA levels in an inverted-U shaped manner. The groups with the putative lowest (Val placebo) and highest (Met tolcapone) DA levels have the higher riskiness choosing higher bets of '25' over '5' more often than the Val tolcapone and Met placebo groups, which putatively have the middling levels of cortical DA.

Taken together, these studies suggest that COMT genotype can affect behaviour in an economically relevant context, but the precise nature and consistency of this effect remains unclear. In particular, the data here did, but others including Marco-Pallares et al. did not, observe an effect of COMT genotype on making risky decisions, despite nearly identical

experimental settings in both studies (Camara et al., 2010; Marco-Pallares et al., 2010; Farrell et al., 2012). The main points and comparisons are summarised in Table 6.2 in section 6.5.2.2, including a further very recent study using the same task (Heitland et al., 2012) which, similar to the Marco-Pallares study, found no significant COMT-modulated differences in sizes of bets placed.

Causes of differences between Marco-Pallares et al. (2009) and this study

The discrepancy between the Marco-Pallares study and that used in this thesis may be due to several factors. Firstly, the Marco-Pallares study contained (though did not present in analysis, therefore it was not included in the paradigm for this thesis) a richer set of choice outcomes, originally proposed by Gehring and Willoughby, including scenarios that involve a choice between two boxes displaying identical magnitudes e.g. '5' and '5'. Arguably, this manipulation may alter perceived incentives such that the COMT effect comes to the fore. In other words, although the propensity for making risky choices is somehow 'hard-wired' in the genes, a specific context is required in order to trigger the behaviour. More generally, the difference might reflect the higher level anxiety as the participants in this study completed the task in the MEG scanner, which may have been more stressful than an EEG task. The greater stress may have led to higher levels of DA (Reinhard et al., 1982) and thereby affected the subjects' position on the inverted-U curve. Thus, a more stressful setting might tease out behavioural differences. As mentioned previously with regard to that task, the effect of limiting the study to young men with an above-average and restricted range of verbal IQ (NART scores ranged from 108-125) may also have contributed, given gender (Harrison and Tunbridge, 2008; Jacobs and D'Esposito, 2011) and age (Tunbridge et al., 2007; Diaz-Asper et al., 2008; Dumontheil et al., 2011) variation in COMT function. Further factors explaining differences between studies have been mentioned previously in the Chapter 3, section 3.4.1 and include other unmeasured and confounding genetic differences. This includes difference in effects of the Val^{158}Met

genotype due to haplotype background and epistasis with other genes (Meyer-Lindenberg et al., 2006; Tan et al., 2007).

6.5.2 MEG results discussion

MEG results firstly showed a difference in global activity in the processing of wins and losses. Losses yield higher activity than wins during the 250-300 time period of interest. A putative MFN area (medial frontal) is shown at the 265ms but we also demonstrated effects outside of this, including occipitotemporal areas.

Secondly, previously demonstrated COMT genotype effects on reward processing in EEG were replicated, in that the Val placebo group showed greater activity for the win-minus-loss contrast. Subjects given tolcapone showed a bigger difference between wins and losses than subjects given placebo in the majority of areas of significant difference. In addition, a genotype x tolcapone interaction in frontal areas produced higher activity in Val placebo than Met placebo, but lower in Val tolcapone than Met tolcapone. This suggests tolcapone has opposing effects on the Val and Met homozygous groups. Assessment of the effect of COMT on magnitude of bets produced negative results, in contrast to the earlier reports (Marco-Pallares et al., 2009).

6.5.2.1 Losses loom larger than gains, including in the putative MFN

The data suggest that the impact of a loss outcome on neural activity in cortical areas is greater than the impact of wins, thus answering MEG objective 1 'Do different reward outcomes elicit different activity in MEG?'. This result applies to the global analysis (Figure 6.5 and 6.6) and over most significant areas on the local maps (Figure 6.7), including an area potentially corresponding to the putative MFN identified in the original EEG-based report of the task (Gehring and Willoughby, 2002). The areas of significance shown in these analyses suggest that

areas both within and outside those areas associated with the MFN may be important in processing reward. A recent MEG source localisation performed on this task confirms that the MFN region (posterior cingulate cortex) can be detected with MEG signals around 250-315ms (Doñamayor et al., 2011).

Initially it was thought that these medial-frontal regions implicated in the MFN were focused on error-related signals rather than loss-related signals, but the task used here allowed the separation of a loss from an error, by creating a loss-correct (losing '5' when the alternative was to lose '25') and a win-error (winning '5' when the alternative was to win '25') condition. The present data showed that this MFN activity was more accurately predicted by a loss or win, rather than error or correct. The only significant area not to comply with this 'losses higher than wins' pattern of activity is the anterior temporal area (in Figure 6.7). Here, the two 'correct choice' conditions have higher activity than the 'error-choice' conditions, suggesting one area for specifically processing errors (as distinct from losses).

6.5.2.2 The effect of COMT genotype on processing outcomes

Assessing Valence

The MEG data did not show a specific COMT main effect on the win-minus-loss contrast, however, a genotype effect (which may well include the MFN region) may be masked by the interaction effects. A recent COMT-EEG study (Heitland et al., 2012) used the same task instantiated in this thesis and thus allows comparison between Marco-Pallares (2009), Heitland (2012) and the data in this thesis relating to the Val^{158}Met effect on neural processing upon win and loss outcomes. Table 6.2 summarises behavioural as well as neural results and discusses the differences.

Assessing magnitude

The lack of COMT modulation on magnitude is a stark contrast to the Marco-Pallares data (see Table 6.2). Heitland et al. (2012) also fail to find a genotype modulation of magnitude within the 250-300ms time period, instead attributing the effect of magnitude to the later P300 time component, and hence outside the focus of this study. However, Figure 6.11 suggests no COMT modulation during the entire 0-600ms period assessed. Differences between papers can be attributed to those listed in 6.5.1. Specifically regarding the comparison between this study and that of Heitland et al. (2012), our study had much higher number of participants, all of whom were male, compared to the six female participants in Heitland (see also Table 6.2).

6.5.2.3 COMT genotype x tolcapone interaction and tolcapone main effect modulate MEG signals associated with reward processing

Interaction effect of valence

A genotype x tolcapone interaction and main effect of tolcapone on the neural correlates of processing wins vs. losses, as in the faces task (Chapter 5), demonstrate the importance of COMT beyond WM paradigms, thus answering the MEG objectives 'Does COMT genotype modulate response to valence?' and 'How does tolcapone affect the MEG activity?' (see section 6.1.3). Figure 6.9 suggests tolcapone increases activity in two of the three areas shown to be significant. Figure 6.10 suggests that, at least over the area labelled 1 (the left temporo-frontal area), the Val placebo group shows a greater differential response to wins vs. losses (i.e. the win-minus-loss contrast) compared to the Met placebo group. However, this genotype difference is reversed in those given tolcapone. Hence the relative activities for the four different drug and genotype groups suggest that tolcapone may affect the Val^{158}Met genotypes differently, in opposing directions, consistent with the behavioural results presented above. The possible basis of this has been discussed previously in Chapter 1, section 1.5.2.

Table 6.2 COMT Val^{158}Met genotype difference in behaviour and evoked responses to valence and magnitude

	Behavioural	ERP/ERF to valence	ERP/ERF to magnitude
Marco-Pallares et al. (2009)[1]	No overall COMT effect on size of bets chosen or reaction time	COMT significantly modulated MFN values. Win-minus-loss activity was higher in Val than Met homozygotes at 250-300ms.	Higher amplitude for loss-25 than loss-5. Val homozygotes showed greater difference between the two conditions than Met homozygotes. This was within the 250-300ms time period, focused around the MFN.
Heitland et al. (2012)[2]	No overall COMT effect on size of bet. Met homozygotes showed progressive increase in risk taking with longer task duration towards the end of the task.	No COMT genotype x valence interaction within 250-300ms, but activity appeared larger in Val homozygotes than Mets in frontal areas for both outcomes.	Significant effect at a late P300 of COMT on frontal amplitudes between '25' and '5', following both loss and win comparisons. Amplitude for Met homozygotes was increased compared to Val homozygotes.
This thesis[3]	Size of bet placed not affected by prior win or loss outcome. In placebo subjects, Met homozygotes made a higher number of small bets than Val homozygotes. Therefore Val homozygotes are prone to riskier behaviour. No COMT effect on reaction time.	No genotype main effect on win-minus-loss contrast but the Val placebo group have higher activity than the Met placebo group for the win-minus-loss contrast at 285ms over the head as a whole. When assessed separately in wins and losses, Vals show greater activity over the putative MFN region and also outside i.e. occipital and parietal areas (data not shown for last clause). COMT genotype looks to affect both outcomes the same.	No COMT modulation of magnitude at any stage (though analysis restricted to losses, as in Marco-Pallares). No magnitude difference at all between 250-300ms, but there is a magnitude difference at a late P300 (though not COMT modulated).
Comparison	The data in this thesis is the only set to find COMT genotype modulation in size of bet placed. Differences could be due to the number of participants and demographics, or the use of MEG rather than EEG.	Both Heitland and this thesis suggest COMT genotype affects both win and loss valences. All three data sets suggest Val homozygotes show higher activity than Met homozygotes. All three data sets suggest the MFN as a key region here, though the data from this thesis is perhaps the least convincing. The data in this thesis is the only one to suggest areas outside the MFN to be important in COMT modulation of valence within the 250-300ms time component.	COMT modulation of response to magnitude is seen in frontal regions in both the Marco-Pallares data and the Heitland data, but not the data shown in this thesis. However, the Marco-Pallares data shows this response is within the 250-300ms component and shows a greater difference in the Val homozygotes compared to the Met homozygotes, whereas Heitland suggests this effect is limited to a late P300 and is higher in Met homozygotes.

[1] Mixed gender EEG study. N=40
[2] All female EEG study. N=6
[3] All male MEG study. N=67

No interaction effect of magnitude

The data in this chapter failed to demonstrate an effect of COMT interaction on processing a loss of '5' vs. a loss of '25' at the 250-200ms time period, thus addressing the MEG objective 'Does COMT modulate magnitude?' (section 6.1.5). This does not agree with a previous study that found a higher amplitude of signal for maximum losses compared to minimum losses, with the Val homozygous subjects presenting a greater difference (Marco-Pallares et al., 2009). The recent Heitland study differs from both of these studies, suggesting an effect of COMT on magnitude but at a late time component, with the greater difference between minimum and maximum magnitude occurring in Met homozygous subjects compared to Val homozygous subjects (see Table 6.2). The differences may appear for several reasons as already stated in 6.5.1.

6.5.2.4 The effects of COMT over the putative MFN region

Effects of tolcapone and a genotype x tolcapone interaction are shown for both anterior (label 1, Figure 6.9 and label 1, Figure 6.10) and posterior regions (label 2, Figure 6.9 and label 2, Figure 6.10) regions. Although we do not observe evidence of COMT's modulation of the MFN during the win-minus-loss contrast in either genotype, tolcapone or their interaction, there is demonstration of frontal modulation by genotype shown in Figure 6.10 between Val and Met placebo groups. Despite a lack of significant findings regarding COMT genotype effects during the win-minus-loss contrast (therefore demonstrating absence of effect over a putative MFN region), further analysis has shown Val placebo and Met placebo do show differences in processing outcomes when wins and losses are assessed separately. Significant effects can be seen over a putative MFN region (data not shown as these analyses were not within the pre-specified criteria i.e. performed by Marco-Pallares). This suggests that the MFN is involved in processing outcome irrespective of whether it is a win or a loss[14]. This suggestion is corroborated by similar results in

[14] Though source analysis is necessary to confirm this area as the MFN, and this putative MFN area is one of several regions of significance between the genotype groups.

another study (Heiltand et al., 2012), see Table 6.2. It is also possible that activity arising from the MFN may not be well detected by gradiometers thus limiting the ability to pick up any COMT modulated differences at the MFN from the present analyses.

6.5.2.5 The effects of COMT are widespread

Section 6.5.2.3 gives examples of COMT effects on regions outside of the frontal cortical areas. Thus, COMT's effects seem to be widespread, beyond those frontal regions thought to be directly modulated by COMT. COMT's indirect effects on MEG signal over regions outside the PFC are discussed in Chapter 4, section 4.4.2.

6.5.2.6 Possible therapeutic effect of tolcapone

The MEG objectives outlined in section 6.1.5 required an exploration of the effect of tolcapone on the participants. Drugs abused recreationally can increase mesolimbic DA transmission (Dichiara and Imperato, 1988) thus producing the physiological effect of natural rewards. This mimicking is acknowledged to underpin addictive disorders (Hyman et al., 2006). Given its importance for regulating DA function and its role in modulating brain activation during reward processing, COMT is an attractive candidate gene for addictive disorders due to its importance for regulating DA function and the growing body of literature suggesting its importance in modulating brain activity during reward processing. Given that the propensity toward risky behaviour relates to addiction, data from both the behavioural and neural analysis suggests tolcapone might be 'anti-addictive' in Val homozygotes but 'pro-addictive' in Met homozygotes. Broader therapeutic implications are discussed in the Chapter 7, section 7.2.

6.5.2.7 Limitations and future studies

Further clarification of the similarities and differences between our results and previous findings require source localisation algorithms and time frequency analysis. This would allow us firstly to test how well the ACC (MFN) source is represented as the main area of COMT-modulated activity (Yacubian et al., 2007; Marco-Pallares et al., 2009) along with searching for deeper sources (see below). Secondly, it would allow us to add to ambiguous literature currently including a MEG study that finds theta power to be associated to monetary gains (Doñamayor et al., 2011) and EEG studies finding theta power mainly associated with monetary losses (Cohen et al., 2007; Marco-Pallares et al., 2008). It would also be possible to establish whether COMT modulates such effects.

The analyses in this study were limited to reward outcome. However, this task also yields data on processes associated with reward anticipation, i.e. signals detected during the time between choosing '5' and '25' and awaiting the outcome. fMRI studies show VSTR activation during reward anticipation, as measured by BOLD response, is modulated by the Val^{158}Met allele during the task that most resembles the one used in this thesis (Yacubian et al., 2007) with higher activation in Met compared to Val homozygous subjects. Similar results were found in two other studies, one using anticipation of uncertain rewards on a slot machine gambling task (Dreher et al., 2009) and the other, a monetary incentive delay task where participants could win or lose money of different magnitudes depending on subsequent motor task performance (Schmack et al., 2008). This is potentially interesting as in this case, it is Met homozygous subjects that elicit higher activity (Yacubian et al., 2007; Camara et al., 2010), which is different from the reward outcome studies (such as that mentioned in this thesis) that document Val homozygous subjects to exhibit higher activity than their Met counterparts. It would be interesting to see if this discrepancy still remained in a multimodal imaging study using the same participants and task in both fMRI and MEG.

The role of the striatum in reward processing is well known from fMRI studies and animal studies (see section 6.1.1) but is mostly overlooked in EEG and MEG studies due to source localisation issues. It would be particularly helpful for this task if MEG allowed us to detect striatal signal. As my results show that COMT affects reward processing, it is possible that some of this COMT involvement takes place in the VSTR (a region heavily involved in reward processing), especially given previous studies suggest COMT might have a striatal effect (Yacubian et al., 2007; Camara et al., 2010; Boot et al., 2011; Nikolova et al., 2011). The manipulation of COMT itself has not been shown to directly affect DA levels in the striatum. However, it is possible that COMT does have a direct, but small, effect that is either below the limit of detection of these studies or only present under certain conditions (e.g. such as increased dopaminergic transmission). Alternatively, the role of COMT in the striatum could act via an indirect effect. For example, COMT may modulate striatal regions downstream of the cortex, since there is an inverse relationship between cortical and striatal DA function (Pycock et al., 1980; Kellendonk et al., 2006). This is evidenced in the Val genotype association with increased midbrain DA synthesis, demonstrated both in post-mortem tissue (Akil et al., 2003) and in vivo (Meyer-Lindenberg et al., 2005). Akil indicated the effect of COMT is limited to neurons in the ventral tier of the substantia nigra. These project to the dorsal striatum, rather than the VSTR including the nucleus accumbens (Haber and Knutson, 2010). This suggests indirect effects of COMT might be more prominent in this region. Although COMT does not affect basal DA transmission in the striatum, a COMT effect might become significant under conditions of 'elevated transmission'. For the dorsal striatum, evidence for this theory is mixed. COMT inhibition potentiates the increase in DA levels induced by DAT blockade (Kaakkola and Wurtman, 1992; Budygin et al., 1999) but not by amphetamine (Tuomainen et al., 1996). For the VSTR regions (including the nucleus accumbens) evidence is sparse and unexplored.

6.5.3 Summary

In summary, the behavioural genotype x tolcapone interaction on reward processing was striking yet unexpected and speaks to the need for further studies of the extent to which COMT modulates wins and losses. The MEG data suggest that the MFN is an area important in reward processing but illustrates extensive areas outside this region may also be important. COMT was shown to modulate the MEG signal at a time component associated with reward processing. The robust MEG findings replicate (to a certain extent) and extend previous EEG findings relating to valence and COMT modulation, but fail to replicate a reported COMT genotype effect on signal differences relating to reward magnitude. Novel findings include the role of tolcapone in altering MEG activity in Val and Met homozygous subjects in opposite directions.

Chapter 7

Discussion

Chapter 7

7. Discussion

7.1 Summary

The aim of this thesis was to explore how the COMT Val^{158}Met polymorphism and the pharmacological manipulation of COMT with tolcapone affects humans during tasks thought to be modulated by DA and specifically by COMT. These tasks were performed in the MEG scanner and investigated WM, emotional processing of faces, and the processing of wins and losses in a gambling task.

Chapter 1 introduced background information on DA, and looked at theories of DA to explain this involvement. It then detailed COMT modulation of DA (leaving evidence of COMT's involvement in each of the tasks to their relevant chapters). Finally it gave a brief conceptual and technical introduction to MEG and its analysis. **Chapter 2** took a step-by-step approach to explaining how participants were selected and scanned, and the principles of, and approach to, data analysis (leaving task-specific details to their relevant chapters). **Chapter 3** was the behavioural study of the N-back task and described a striking example of a genotype x drug interaction on WM performance: tolcapone improved N-back accuracy in Val homozygous subjects but impaired it in Met homozygous subjects. **Chapter 4** assessed MEG signal during the N-back task and showed significant effects of genotype and tolcapone on the P300 component during the 2-back, including an interaction in which tolcapone had the opposite effects on MEG signal in the two genotypes. **Chapter 5** first looked at the N170 response to faces, finding no emotional or COMT modulation at this time point. A COMT effect was found on an N4-like component during fearful face processing. Tolcapone, genotype and interaction effects were shown. The relationship between COMT and MEG signal changed depending on the region and time of focus. **Chapter 6** studied a simple gambling task, and demonstrated a clear genotype x

tolcapone interaction for size of bets placed: on placebo, Met subjects were more risk averse than the Val subjects, but this difference was reversed in subjects given tolcapone. There were also separate and interactive genotype and tolcapone effects on MEG signal during the task. Key MEG findings firstly confirm a difference in neural processing of wins and losses at 265ms, in areas including the putative MFN area identified in prior studies, but also in occipitotemporal regions. Some of the previously demonstrated COMT genotype effects on reward processing in EEG were replicated in MEG; the Val placebo group showed a bigger difference in activity between wins and losses than their Met counterparts, although this chapter reports a lack of COMT effect on processing the magnitude of the outcome. Additionally, novel effects of tolcapone and genotype x tolcapone interactions were seen on several aspects of MEG signal during the task. A summary of findings and suggested interpretations appear in Table 7.1.

I will now discuss two broader issues arising from the results of this thesis, not covered in previous chapters: the potential therapeutic implications of the data, and consideration of the role of MEG in future pharmacological and pharmacogenetic studies of this kind.

7.2 Therapeutic implications of the Val^{158}Met genotype x tolcapone interaction

The conceptual and experimental explanations for the striking pharmacogenetic interaction between tolcapone and genotype upon WM and risk aversion were discussed in Chapters 3 and 6 respectively; here I discuss briefly the potential therapeutic implications of these findings.

To date, tolcapone (and other COMT inhibitors) have only been used clinically to augment L-DOPA in the treatment of Parkinson's disease, although they are also being trialled for several

Table 7.1 Summary of findings and their interpretation

	Main Findings	Suggested Interpretation
Working Memory	**Behavioural**- On pbo, Met homozygotes outperformed Val homozygotes. A geno x tolc interaction affected WM performance; the Val group improved on tolc, but the Met group worsened. **MEG**- P300 was modulated by geno, tolc and the interaction of these two. Higher midline frontal activity appeared in the tolc group compared to the pbo group. Generally, Val homozygotes showed higher activity than Met homozygotes. Over frontal regions, the Met pbo group had a higher activity than the Val tolc group, and the Val tolc group had a higher activity than the Met tolc group. Over posterior regions, the Met pbo group had lower activity than the Val pbo and Met tolc groups.	An inverted-U relationship appears between frontal DA levels and WM performance. This relationship may also hold for DA levels and neural activity over frontal regions. It is possible that neural activity is related to efficiency over the posterior regions and 'recruitment capacity' over the frontal regions. Alternatively the parietal involvement could be linked to attentional aspects of processing or encoding of incoming information.
Emotional Faces Processing	COMT modulation of fearful face processing was demonstrated at N400 (but not N170 or 250ms, also previously associated with face recognition). Over frontal regions, the tolc group exhibited higher activity than the pbo group, and Val homozygotes exhibited higher activity than Met homozygotes. An interaction effect over frontal areas occurred; Met homozygotes increased in activity on tolcapone, but Val homozygotes decreased in activity. In parietal regions the pbo group was more active than the tolc group. In temporal regions the Met homozygote group was more active than the Val homozygote group.	The faces shown here may be processed as more complex non-prototypical faces. Over frontal regions, tolc has opposing effects on MEG signal in response to fearful face processing, depending on the genotype in question.
Reward Processing	**Behavioural**- A geno x tolc interaction occurred for size of bet placed. The Met (compared to Val) pbo group was more risk averse, but the Met tolc group was less risk averse than the Met pbo group. **MEG**- Losses exhibited greater activity than wins around 250-300ms, including over the putative MFN region, but also occipitotemporal areas. Val (compared to Met) homozygotes showed higher activity for the win-minus-loss contrast. The tolc group showed higher activity than the pbo group. Over frontal areas a geno x tolc interaction occurred; the Val placebo group has greater activity than the Met pbo group, but the Val tolc group showed lower activity than the Met tolc group. COMT did not modulate magnitude of losses.	An inverted-U relationship between DA and risk behaviour is suggested. During the processing of outcomes (in the 250-300ms time period), COMT modulates neural activity of both losses and wins, but not the magnitude of losses.
Commonalities	A geno x tolc interaction occurred in every task for neural, and where relevant behavioural, measures. These neural effects appeared over frontal regions. However, the effect of COMT in all three tasks extended beyond the frontal regions.	COMT modulates tasks beyond WM and in regions beyond the PFC. DA affects WM and reward processing at specific time components in an inverted-U manner.

N.B. pbo= placebo tolc=tolcapone geno=genotype

other neuropsychiatric indications including pathological gambling and schizophrenia (for a list of the eight trials see www.clinicaltrials.gov/ct2/results?term=tolcapone, last accessed August 2012). My findings that tolcapone has clear effects on WM (Chapter 3) and risk aversion (Chapter 6), suggest that the drug might also have a broader potential therapeutic value, specifically in Val homozygous subjects. For example, tolcapone may aid Val homozygous subjects as a WM 'cognitive enhancer' or, more speculatively, to treat excessive risk taking. Clearly, all subjects should be genotyped for Val^{158}Met prior to any study or use of the drug for cognitive or behavioural indications; currently only two of the eight trials include genotype assessment. Trials which do not include genotype run the risk of a 'false negative' result, whereby opposing results in Val and Met homozygous subjects (i.e. beneficial in one, harmful in the other) cancel each other out. However, there are a number of important issues, and limitations to the current data, which need to be taken into account before any such therapeutic possibilities could be implemented in practice.

First, my data only pertain to young men. There are significant sex differences in COMT activity and function (Harrison and Tunbridge, 2008), and so it would be necessary to extend the work to women. Similarly, it is possible that the behavioural and cognitive effects of tolcapone might differ in individuals with a lower (or higher) IQ than the men studied here.

Second, it would be valuable to study other polymorphisms in addition to just Val^{158}Met, since together they may explain more of the variance in performance and thus their measurement might improve the pharmacogenetic predictive power. Several additional loci within the COMT gene modify the effect of the Val^{158}Met SNP including the rd2097603 p2 promoter region SNP and the rs165599 SNP in the 3' region (Chen et al., 2004; Meyer-Lindenberg et al., 2006). A particularly interesting haplotype involving the Val^{158}Met allele (that better accounts for pain sensitivity) stems from variations in mRNA secondary structure and subsequent

differences in COMT protein translation, leading to approximately 10% of Val homozygotes having more Met-like activity (Nackley et al., 2009). Similarly, polymorphisms affecting DA signalling beyond COMT may also help explain more of the variance in the data. Polymorphisms in both the DAT and D_4 receptor have been shown to produce epistatic interactions with COMT in cognitive processing tasks (Bertolino et al., 2008; Heinzel et al., 2012). Increases in D_4 receptor functioning displayed an inverted-U relationship between go/no-go task performance and increased COMT-dependent DA levels; whereas subjects with decreased D_4-sensitivity showed an inverted-U relationship (Heinzel et al., 2012). A further example can be found in the effect on striatal activation of the DAT 9-repeat allele, which goes in the exact opposite direction of the DAT 10-repeat allele based on COMT genotype during reward anticipation (Yacubian et al., 2007; Bertolino et al., 2008; Dreher et al., 2009).

In summary, genotyping of polymorphisms other than just the $Val^{158}Met$ allele has the potential to better explain and predict response to tolcapone, because it can explain better where an individual is likely to lie on the inverted-U. However, the benefits of this information would need to be traded off against the much large number of subjects who would need to be screened for a study where recruitment is based on a particular combination of genotypes. The results would also be less generalisable. In this regard it is worth mentioning that, given the use of homozygotes only, I do not know how $Val^{158}Met$ heterozygotes would respond to tolcapone. On theoretical grounds (given the codominance of the alleles) I would expect any effects of tolcapone on WM or risk taking to be limited, since they will be intermediate between the opposing results of the two homozygote groups.

Reflecting the two factors discussed above, there may be situations in which tolcapone alters performance of Val and Met homozygotes in the same way for both genotypes, depending on where the groups lie initially on the inverted-U curve. For example, if all subjects started with

cortical DA levels that lay on the extreme left of the curve then the effect of tolcapone would be to move subjects towards the optimal curve peak, but not tip over. Depleted cortical DA levels could arise in patients with Parkinson's (Ehringer and Hornykiewicz, 1960; Olanow and Tatton, 1999), patients with schizophrenia (Davis et al., 1991), or older people, who have been shown to have lower D_1 (Suhara et al., 1991) and D_2 receptor availability (Volkow et al., 1996) and lower DAT availability (Volkow et al., 1996). The schematic in Figure 7.1b. illustrates how, in this situation, both genotypes move towards, but not beyond, optimal levels on the curve, and so improved performance would occur regardless of genotype. This can be compared to Figure 7.1c. exemplifying what might occur in individuals with high levels of DA, e.g. during stress (Roth et al., 1988; Abercrombie et al., 1989). All individuals start much higher on the curve, such that the effect of tolcapone is to worsen both genotypes as they move to supraoptimal levels of DA.

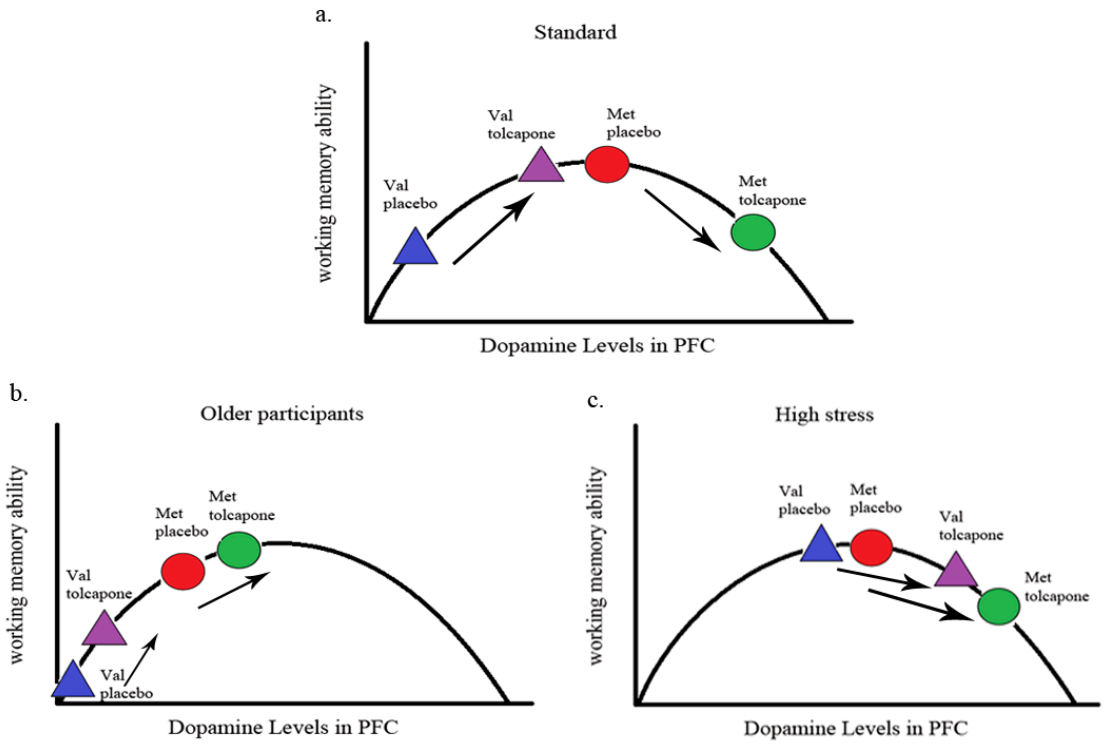


Figure 7.1 Schematics of possible COMT genotype x tolcapone interactions. The images represent different versions of how tolcapone could affect genotype depending on where Val homozygotes and Met homozygotes initially sit on the curve. a. The general pattern of DA and WM ability as shown in this thesis is represented. b. Possible alterations of where individuals would sit if older. c. Possible alterations of where individuals would sit if under high stress.

A third consideration relevant to the therapeutic implications of the current data is that they relate to the acute effects of a single dose of tolcapone. It is possible that the effects seen here might attenuate due to adaptive changes after chronic use, or they might be enhanced. Thus, studies using repeated administration (e.g. for four weeks) need to be conducted. However, this would raise concerns about hepatotoxicity of tolcapone. Although the risk is now thought to be negligible (Leegwater-Kim and Waters, 2006), for a review see Olanow et al. (2007), there are restrictions on prescribing, with a 'black box' warning in the British National Formulary and a requirement for regular blood tests. This would significantly impact on the feasibility of such a study. In this regard, the development of novel and non-hepatotoxic COMT inhibitors is relevant (see below).

Fourth, the existing data are limited to a single test of WM, and a single measure of gambling behaviour. It is essential to broaden the range of tasks into different aspects of cognition and risk taking if the clinical potential – and the possible harms – of tolcapone are to be understood. For example it is already clear that COMT affects some other forms of memory (Dickinson and Elvevag, 2009), and emotional (Smolka et al., 2005; Domschke et al., 2012) and reward (Tunbridge et al., 2012) processing, but the impact of tolcapone on these domains has not been assessed. My finding of a possible effect of tolcapone on happiness ratings indicates that future studies should also assess mood.

Fifth, the present results relate to tolcapone. Other COMT inhibitors are being developed, in part because of the liver toxicity of tolcapone, and the lack of central penetration of the other licensed COMT inhibitor, entacapone (Barrow, 2012). It will be valuable to study these emerging drugs in a similar fashion, since if they differ in some respects from tolcapone (e.g. in terms of potency, or selectivity for MB vs. S-COMT) the behavioural effects might be different. A recent review suggests tolcapone is still the most effective COMT inhibitor (Marsala et al., 2012).

Finally, given the promise of tolcapone in this study and studies prior, it is surpising tolcapone does not feature more heavily in clinical trials. This may be for two reasons, firstly the perception that tolcapone leads to hepatotoxicity when used clinically. In fact, over 40 000 patient years has presented only three deaths from tolcapone-related hepatotoxicity (Olanow et al. 2007). Perhaps the clinical popularity of tolcapone has suffered from the arguably unfair reputation formed prior to advice that patients should receive regular liver enzyme tests, and hence explains why many potential documented beneficial effects of this drug, such as the improvement in accuracy during WM tasks, have been slow to reach clinical stage. Secondly, the highly significant effect of tolcapone may only be useful clinically if the effect size of the drug results in a noteable improvement in patient condition. However, Chapter 3 demonstrates effect size is great enough to make a large difference to patient accuracy scores (58% to 70%). Comparisons for each individual are required, rather than the averages, to examine if this effect is consistent across all Val subjects.

Despite these many uncertainties, my behavioural results do suggest that further trials of COMT inhibitors, in genetically informed designs, are warranted for a range of DA-related neuropsychiatric indications.

7.3 Implications for the use of MEG in studies of COMT and pharmacogenetics

To my knowledge, this is the first MEG study relating to COMT, and the first pharmacogenetic MEG study, also one of the first to use MEG to study drug effects (Mendez et al., 2012; Wilson et al., 2012). This section first sums up the MEG work across Chapters 4, 5 and 6 of this thesis, then focuses on what conclusions can be drawn from this study about the role and value of MEG for further studies of this kind.

The COMT modulated MEG activity elicited over frontal areas is interpreted as activity generated from the PFC, relating to either attentional aspects of the task not subtracted via the contrasting used in analysis, or, in the case of the N-back task, recruitment capacity associated with better maintenance of WM representations. COMT-modulated MEG activity outside of the PFC is assumed to originate predominantly from the parietal cortices in WM, relating to the efficiency of signal during specific encoding aspects of the WM task, and also attentional aspects, required specifically for those higher order levels of the paradigm. For the emotional processing task, the activity elicited specifically over the posterior areas relate to the occipital regions associated with the visual system and face identification; the temporal cortex relevant for emotional processing; and again, there is a possibility that the parietal areas pertain to attention during the task. Interestingly these putative parietal regions do not appear as a COMT-modulated area during reward processing, with a fronto-temporal area showing higher activity in the groups thought to have higher prefrontal DA. Recognising similarities and differences between brain processing over all three tasks presents further directions for future work to resolve unanswered questions not otherwise covered in the respective discussion chapters. Central to these unanswered questions is the confirmation of the parietal cortex involvement and its relation to attention and/or information encoding. This will require source analysis and further tasks able to isolate out attentional and encoding aspects (suggestions for which have been previously discussed in the relevant chapters).

More generally, this study is encouraging for future pharmacogenetic MEG work. Most importantly, the data show that it is feasible to use MEG to reveal genetic and pharmacological influences (and their interaction) on neural activity, and thus in principle MEG may be a valuable imaging modality in this field. The particular value of MEG is its temporal resolution, helping to dissect the sequence of events (over tens of milliseconds) by which a drug or genotype influences

neural activity in response to a stimulus or task. In this regard it can complement fMRI, as discussed previously. For example, my data show clearly that the Val^{158}Met genotype impacts on neural activity during the N-back in a temporal and spatial pattern which is clearly more complex than, and extends well beyond, the Val^{158}Met difference in PFC activity identified from the BOLD fMRI studies.

Notably, the MEG responses I observed were task specific, and affected time points – and to some extent locations– which were consistent with expectations and prior data. These considerations together give more confidence that the observed effects are biologically meaningful. For example, significant differences in COMT during the N-back directly map onto time frames associated with WM i.e. the P300, or with viewing a face i.e. N400. There was some evidence for task-specific activity over the putative MFN region in the gambling task.

Thus, MEG has utility and value in neuropsychopharmacological and pharmacogenetic studies. Its potential extends well beyond the limited analyses conducted here, to take fuller advantage of the unique attributes of MEG and the novel analytical methodologies which are emerging. Time constraints for this thesis and logistical difficulties associated with being one of the first MEG studies in a new unit with development of analysis protocols in their infancy, contributed to certain restrictions on analyses (beyond that described in Chapter 2, section 2.8.4), this includes the focus on evoked data i.e. averaged over trials and participants. Even within evoked data, assessment was limited to peak height for preselected time components. It is also possible that differences in onset and duration of a curve may occur between groups. This requires testing the significance of any differences in times of similar-looking peaks and measuring areas underneath peaks of significance.

Extension beyond evoked analyses to time-frequency calculations will allow assessment of non-phase-locked data, allowing more opportunity to explore differences between the genotype and drug groups. Non-phase-locked data of interest would be missed in the current analysis, for example, if two individuals engage in a cognitive process in similar ways, but waveforms are not in synchrony, i.e. the millisecond where person A is experiencing a peak in, say, gamma, person B is experiencing a trough, this would cancel out upon averaging. Non-time-locked data of interest may be missed if two people process in the same way, but perhaps person A starts this processing several milliseconds earlier or later. One paper demonstrated the use of time-frequency analysis with relation to COMT, showing alpha frequencies may differ between Val^{158}Met genotypes (Bodenmann et al., 2009). Assessing gamma oscillations may also be particularly relevant given they are known to be key during WM tasks (Barr et al., 2010; Missonnier et al., 2010; Barr et al., 2011).

The analysis used in this thesis is thought to best pick up MEG signals from cortical sources, as explained in Chapter 2, section 2.8.1. However, this is limited in terms of exactly where in the cortex, and investigation into source-space is required to have greater confidence as to where the regions of interest lie. A range of approaches are under development; popular source localisation packages include Beamforming[15] and Minimum Norm estimates[16].

To identify deeper sources, both source localisation and assessment of magnetometer coils would be required. Demonstrating COMT differences in deeper sources may help to answer whether the striatum plays a role (as may be suggested from the growing literature demonstrating COMT's effects outside cortical regions e.g. possible limbic areas). This deeper source analysis would be particularly useful in the faces task where structures such as the

[15] Available from https://www.ynic.york.ac.uk/events/new-meg-software-available-within-ynic/ last accessed August 2012
[16] Available from http://www.martinos.org/mne/ last accessed August 2012

amygdala and hippocampus are known to exhibit COMT modulation (Drabant et al., 2006). This further investigation into sources could also include the exploration of hemispheric differences in locations of interest. When limited to the focality of gradiometers, a rough distinction of left and right is possible but it is difficult to be precise without source space, though it would be possible to define what channels are left and right, extract a measure, then compare the two hemispheres as two separate groups.

To summarise, the strength of MEG lies in its temporal resolution. Its relative weaknesses include spatial localisation, its ability to look at deep sources, and the time-consuming nature of analyses. It is best seen as a complement to other imaging techniques, not a replacement. This may be particularly true as fMRI studies move towards connectivity analysis, hence temporal limitations in fMRI can be remedied using MEG. Indeed, MEG may be most useful as part of a crossmodal imaging approach[17] to brain imaging e.g. MEG with fMRI. This will also allow investigation of the relationships between MEG findings and other measures of 'neural activity' such as the BOLD response (for a review see Singh, 2012).

7.4 Conclusion

The work in this thesis extends the evidence that genetic and pharmacological variation in COMT activity impact on behaviour and neural activity. The effects of COMT are not limited to WM tasks or prefrontal regions, but extend to emotional and reward processing, and to posterior brain regions. The study shows the value, and the complexity, of using MEG for studies of this kind. There is much more to achieve both in terms of further analysis of the current data, and extensions of findings in future studies.

[17] The subjects from the present study also underwent a resting state MRI scan, although analysis of the data was beyond the scope of this thesis.

Appendices

Appendices

Appendix 1 Questionnaire sheets

The following are examples of the questionnaire sheets given to participants.

MEG study: Health screening questions

If you answer 'yes' to any of the questions, please give brief details. The information you provide will be kept confidential. If you do not take part in the study, it will be destroyed.

1. Have you ever suffered from jaundice, hepatitis, or any other disease of the liver?

2. Have you ever been referred to a psychiatrist or a neurologist?

3. Have you ever been diagnosed with, or treated for, depression?

4. Are you on any medication now, or have you been in the past six months?

5. a) How many units of alcohol do you drink in an *average* week? (1 unit = ½ pt beer, or glass of wine, or single measure of spirits)

b) In the past, have you regularly drunk much more than this?

c) Have you ever had alcohol-related health problems?

6. Do you smoke? If so:
How many per day on average?
When was your last cigarette?

Thank you for your time.

NART (2nd edition)

CHORD
ACHE
DEPOT
AISLE
BOUQUET
PSALM
CAPON
DENY
NAUSEA
DEBT
COURTEOUS
RAREFY
DRACHM
AEON
PLACEBO
ABSTEMIOUS
DÉTENTE
IDYLL
PUERPERAL
AVER
HIATUS
SUBTLE
PROCREATE
GIST
GOUGE
SUPERFLUOUS
SIMILE
BANAL
QUADRUPED
CELLIST
FACADE
ZEALOT
GAUCHE
TOPIARY
LEVIATHAN
BEATIFY
PRELATE
SIDEREAL
EQUIVOCAL
NAIVE
CATACOMB
GAOLED
THYME
HEIR
RADIX
ASSIGNATE
DEMESNE
SYNCOPE
LABILE
CAMPANILE

Verbal IQ = 129 – (No. of Incorrect * 0.919)

SUBJECT # DATE

BECK INVENTORY

On this questionnaire are groups of statements. Please read each group of statements carefully, then pick out the one statement in each group which best describes the way you have been feeling in *the pa week including today*.
Circle the number beside the statement you have picked. If several statements apply equally well, circl each one. Be sure to read all the statements in each group before making your choice.

1.
0 I do not feel sad
1 I feel sad
2 I am sad all the time and can't snap out of it
3 I am so sad or unhappy that I can't stand it

2.
0 I am not particularly discouraged about the future
1 I feel discouraged about the future
2 I feel I have nothing to look forward to
3 I feel that the future is hopeless and that things cannot improve

3.
0 I do not feel like a failure
1 I feel I have failed more than the average person
2 As I look back on my life, all I can see is a lot of failures
3 I feel I am a complete failure as a person

4.
0 I get as much satisfaction out of things as I used to
1 I don't enjoy things the way I used to
2 I don't get real satisfaction out of anything anymore
3 I am dissatisfied or bored with everything

5.
0 I don't feel particularly guilty
1 I feel guilty a good part of the time
2 I feel quite guilty most of the time
3 I feel guilty all of the time

6.
0 I don't feel I am being punished
1 I feel I may be punished
2 I expect to be punished
3 I feel I am being punished

7.
0 I don't feel disappointed in myself
1 I am disappointed in myself
2 I am disgusted with myself
3 I hate myself

SUBJ# DATE

8.	0	I don't feel I am any worse than anybody else
	1	I am critical of myself for my weaknesses or mistakes
	2	I blame myself all the time for my faults
	3	I blame myself for everything bad that happens
9.	0	I don't have any thoughts of killing myself
	1	I have thoughts of killing myself, but I would not carry them out
	2	I would like to kill myself
	3	I would kill myself if I had the chance
10.	0	I don't cry any more than usual
	1	I cry more now than I used to
	2	I cry all the time now
	3	I used to be able to cry, but now I can't cry even though I want to
11.	0.	I am no more irritated now than I ever am
	1	I get annoyed or irritated more easily than I used to
	2	I feel irritated all the time now
	3	I don't get irritated at all by the things that used to irritate me
12.	0	I have not lost interest in other people
	1	I am less interested in other people than I used to be
	2	I have lost most of my interest in other people
	3	I have lost all of my interest in other people
13.	0	I make decisions about as well as I ever could
	1	I put off making decisions more than I used to
	2	I have greater difficulty in making decisions than before
	3	I can't make decisions at all anymore
14.	0	I don't feel I look any worse than I used to
	1	I am worried that I am looking old and unattractive
	2	I feel that there are permanent changes in my appearance that make me look unattractive
	3	I believe that I look ugly
15.	0	I can work about as well as before
	1	It takes an extra effort to get started at doing something
	2	I have to push myself very hard to do anything
	3	I can't do any work at all

SUBJ# DATE

16. 0 I can sleep as well as usual
1 I don't sleep as well as I used to
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep
3 I wake up several hours earlier than I used to and cannot get back to sleep

17. 0 I don't get more tired than usual
1 I get tired more easily than I used to
2 I get tired from doing almost anything
3 I am tired too tired to do anything

18. 0 My appetite is no worse than usual
1 My appetite is not as good as it used to be
2 My appetite is much worse now
3 I have no appetite at all anymore

19. 0 I haven't lost much weight, if any, lately
1 I have lost more than 5 pounds
2 I have lost more than 10 pounds
3 I have lost more than 15 pounds

20. 0 I am no more worried about my health than usual
1 I am worried about physical problems such as aches and pains; or upset stomach; or constipation
2 I am very worried about physical problems and it is hard to think about much els
3 I am so worried about my physical problems that I cannot think about anything else

21. 0 I have not noticed any recent changes in my interest in sex
1 I am less interested in sex than I used to be
2 I am much less interested in sex now
3 I have lost interest in sex completely

Subj# Date

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

Subj# Date

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 mu
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

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**

Subj# Date

Befindlichkeits Scale

Below you will find pairs of words. Please decide, without thinking too much, which of the two words corresponds most closely to your present state or the way you feel now. Put a cross in the box to the right of the word. Only if you are completely unable to make a decision, mark the box in the 'neither-nor' column. Do not skip any lines.

I am feeling more:

				Neither-Nor
Alert		Listless		
Uninterested in others		Interested in others		
Cheerful		Downcast & blue		
Successful		Unsuccessful		
Irritated		Calm		
Lacking decisiveness		Decisive		
Joyful		Tearful		
Good-humoured		Ill-humoured		
Without an appetite		With a good appetite		
Sociable		Withdrawn		
Unworthy		Worthwhile		
Relaxed		Tense		
Happy		Unhappy		
Shy		Bold		
Sinful & wicked		Pure		
Secure		Threatened		
Abandoned		Cared for		
Even-tempered		Short-tempered		
Confident		Insecure		
Miserable		Jolly		
Flexible		Rigid		
Tired		Rested		

Subj# Date

I am feeling more:

				Neither-Nor
Hesitant		Sure		
Composed		Restless		
Without energy		Energetic		
Useless		Useful		
Sluggish		Animated		
Superior		Inferior		
Outgoing		Inhibited		
In a good mood		Gloomy		
Lacking in drive		Always on the go		
Sickly		Healthy		
Determined		Aimless		
Serious		Happy-go-lucky		
Lacking in ideas		Full of ideas		
Easily hurt		Not easily hurt		
Pessimistic		Optimistic		
Carefree		Brooding		
Worn out		Peppy		
Able to love		Unable to love		
Guilty		Guiltless		
Exhausted		Refreshed		
Fed-up with life		Enjoying life		
Good		Bad		
Merry		Sad		
Loved		Unloved		
Lazy		Active		
Reserved		Responsive		
Lively		Lifeless		
Perky		Lacking in energy		
Attentive		Absent-minded		

Subj# Date

I am feeling more:

				Neither-Nor
Desperate		Hopeful		
Contended		Discontented		
Timid		Adventurous		
Strong		Weak		
Well-balanced		Unbalanced		

Appendix 2 Visual analogue scale scores

Visual analogue scale scores		T1	T2	T3
Alertness	Met Placebo	6.7 (1.6)	7.0 0(2.3)	6.3 (2.3)
	Val Placebo	6.0 (2.1)	5.9 (2.1)	5.8 (2.6)
	Met Tolcapone	5.9 (2.2)	5.8 (2.0)	5.1 (2.4)
	Val Tolcapone	6.0 (1.6)	6.2 (2.0)	5.5 (2.0)
Drowsiness	Met Placebo	2.2 (2.2)	2.6 (2.6)	3.0 (2.9)
	Val Placebo	3.8 (3.0)	3.4 (2.3)	2.9 (2.7)
	Met Tolcapone	3.7 (2.6)	3.1 (2.5)	4.0 (2.3)
	Val Tolcapone	3.4 (2.0)	2.8 (1.9)	3.5 (2.0)
Anxiety	Met Placebo	1.1 (1.0)	0.9 (1.1)	0.5 (0.6)
	Val Placebo	2.0 (1.9)	1.5 (1.6)	1.4 (1.8)
	Met Tolcapone	2.0 (1.8)	1.7 (1.3)	1.5 (1.7)
	Val Tolcapone	1.2 (0.8)	1.2 (1.4)	1. 0(1.1)
Happiness	Met Placebo	6.9 (1.5)	7.5 (1.3)	7.1 (1.7)
	Val Placebo	6.1 (1.7)	6.4 (1.6)	5.9 (1.9)
	Met Tolcapone	7.3 (1.6)	7.2 (1.6)	7.1 (2.3)
	Val Tolcapone	5.9 (2.1)	6.4 (1.5)	6.9 (1.4)
Nausea	Met Placebo	0.6 (1.8)	0.8 (1.8)	0.5 (1.2)
	Val Placebo	0.9 (1.6)	0.8 (1.8)	0.8 (2.0)
	Met Tolcapone	0.9 (1.2)	0.7 (1.2)	1.1 (1.5)
	Val Tolcapone	0.9 (1.8)	0.6 (0.8)	0.8 (1.3)
Sadness	Met Placebo	0.7 (0.8)	0.7 (0.8)	0.3 (0.4)
	Val Placebo	1.1 (1.2)	1.1 (1.8)	1.0 (1.8)
	Met Tolcapone	1.0 (1.3)	0.9 (1.0)	1.2 (1.4)
	Val Tolcapone	1.6 (1.7)	1.6 (1.9)	1.7 (2.0)

Mean values and s.d. to 1.d.p.

Appendix 3 Befeindlichkeit scores

Befeindlichkeit scores		T1	T2	T3
Personality	Met Placebo	4.7 (5.2)	5.5 (6.4)	4.1 (5.3)
	Val Placebo	6.5 (3.9)	6.5 (5.7)	6.7 (5.2)
	Met Tolcapone	5.9 (6.7)	6.3 (6.2)	4.3 (5.5)
	Val Tolcapone	7.3 (5.3)	6.5 (4.1)	6.8 (5.4)
Mood	Met Placebo	14.4 (17.9)	14.6 (17.3)	12.9 (16.3)
	Val Placebo	17.5 (13.1)	16.0 (14.3)	16.2 (13.9)
	Met Tolcapone	18.1 (20.6)	18.8 (21.7)	14.4 (17.5)
	Val Tolcapone	19.9 (17.2)	16.7 (14.2)	16.2 (15.1)
Overall	Met Placebo	19.3 (21.3)	20.1 (24.6)	17.3 (21.0)
	Val Placebo	23.9 (15.6)	22.5 (18.6)	22.8 (17.7)
	Met Tolcapone	24.0 (25.4)	25.1 (25.2)	18.7 (20.4)
	Val Tolcapone	27.2 (20.6)	23.1 (16.4)	23.6 (19.2)

Mean values and s.d. to 1d.p.

Appendix 4 Pearson correlations between N-back performance and happiness VAS scores.

	Happiness VAS Score		
	T1	**T2**	**T3**
N-back Accuracy			
0-back	-0.03	0.03	-0.15
1-back	0.08	0.03	-0.13
2-back	0.14	0.12	0.04
3-back	0.12	0.09	0.04

Values are Pearson R correlation coefficients.
All correlations non-significant.

References

Abele-Brehm, A. and W. Brehm (1986). "Zur Konzeptualisierung und Messung von Befindlichkeit. Die Entwicklung der "Befindlichkeitsskalen" Diagnostica **32**(3): 209-228.

Abercrombie, E. D., Keefe, K.A. DiFrischia, D. S. and M. J. Zigmond (1989). "Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal-cortex." Journal of Neurochemistry **52**(5):1655-1658.

Abi-Dargham, Mawlawi, A.O., Lombardo, I. Gil, R. Martinez, D. Huang, Y. Hwang, D. R., Keilp, J. Kochan, L. Van Heertum, R. Gorman, J. M., and M. Laruelle (2002). "Prefrontal dopamine D1 receptors and working memory in schizophrenia." Journal of Neuroscience **22**(9):3708-3719.

Adolphs, R. (2002). "Neural systems for recognizing emotion." Current Opinion in Neurobiology **12**(2):169-177.

Akil, M., Kolachana, B. S., Rothmond, D. A., Hyde, T. M., Weinberger, D. R., and J. E. Kleinman. (2003). "Catechol-O-methyltransferase genotype and dopamine regulation in the human brain." Journal of Neuroscience **23**(6):2008-2013.

Amstadter, A. B., MacPherson, L., Wang, F., Banducci, A. N. Reynolds, E. K., Potenza, M. N., Gelernter, J., and C. W. Lejuez (2012). "The relationship between risk-taking propensity and the COMT Val158Met polymorphism among early adolescents as a function of sex." Journal of Psychiatric Research **46**(7):940-945.

Anderson, I. M., Del-Ben, C. M., Mckie, S., Richardson, P., Williams, S. R., Elliott, R., and J. F. Deakin (2007). "Citalopram modulation of neuronal responses to aversive face emotions: a functional MRI study." NeuroReport **18**(13):1351-1355.

Andrew, R. J. (1963). "Evolution of facial expression." Science **142**(7):1034.

Apud, J. A., Mattay, V., Chen, J., Kolachana, B. S., Callicott, J. H., Rasetti, R., Alce, G., Iudicello, J. E., Akbar, N., Egan, M. F., Goldberg, T. E., and D. R. Weinberger (2006). "Tolcapone improves cognition and cortical information processing in normal human subjects." Neuropsychopharmacology **32**(5):1011-1020.

Ashley, V., Vuilleumier, P., and D. Swick (2004). "Time course and specificity of event-related potentials to emotional expressions." NeuroReport **15**(1): 211-216.

Atkinson, R. C. and R. M. Shiffrin (1968). "Human memory: A proposed system and its control processes". In Spence, K. W., and J.T. Spence The psychology of learning and motivation (Volume 2)." New York: Academic Press. **pp. 89–195.**

Augustine, J. R. (1996). "Circuitry and functional aspects of the insular lobe in primates including humans." Brain Research Reviews **22**(3):229-244.

Babiloni, C.,Vecchio, F., Buffo, F., Buttiglione, M. Cibelli, G., and P. M. Rossini (2010). "Cortical responses to consciousness of schematic emotional facial expressions: A high-resolution EEG study." Human Brain Mapping **31**(10):1556-1569.

Baddeley, A. (2000). "The episodic buffer: a new component of working memory?" Trends in Cognitive Sciences **4**(11):417-423.

Baddeley, A. (2010). "Working memory." Current Biology **20**(4):136-140.

Baddeley, A. D., Hitch, G. (1974). "Working Memory." in G.H. Bower (Ed.) The Psychology of Learning and Motivation San Diego: Academic Press **pp47-87**.

Badgaiyan, R. D. (2010). "Dopamine is released in the striatum during human emotional processing." NeuroReport **21**(18):1172-1176

Badgaiyan, R. D., Fischman, A. J., and N. M. Alpert (2009). "Dopamine release during human emotional processing." Neuroimage **47**(4):2041-2045.

Balconi, M. and C. Lucchiari (2005). "Event-related potentials related to normal and morphed emotional faces." Journal of Psychology **139**(2):176-192.

Barnett, J. H., Heron, J., Ring, S. M., Goding, J., Goldman, D., Xu, K., and P. B. Jones (2007). "Gender-specific effects of the catechol-O-methyltransferase Val108/158Met polymorphism on cognitive function in children." American Journal of Psychiatry **164**(1):142-149.

Barnett, J. H., Jones, P. B., Robbins, T. W., and U. Muller (2007). "Effects of the catechol-O-methyltransferase Val(158)Met polymorphism on executive function: a meta-analysis of the Wisconsin Card Sort Test in schizophrenia and healthy controls." Molecular Psychiatry. **12**(5):502-509

Barnett, J. H., Scoriels, L., and M. R Munafo (2008). "Meta-analysis of the cognitive effects of the catechol-O-methyltransferase gene Val158/108Met polymorphism." Biological Psychiatry. **64**(2):137-144.

Barr, M. S., Farzan, F., Arenovich, T. Chen, R., Fitzgerald, P. B., and Z. J. Daskalakis (2011). "The effect of repetitive transcranial magnetic stimulation on gamma oscillatory activity in schizophrenia." PloS One **6**(7): e22627.

Barr, M. S., Farzan, F., Tran, L. C., Chen, R., Fitzgerald, P. B., and Z. J. Daskalakis (2010). "Evidence for excessive frontal evoked gamma oscillatory activity in schizophrenia during working memory." Schizophrenia Research **121**(1–3):146-152.

Barrow, J.C. (2012) "Inhibitors of catechol-O-methyltransferase. CNS and Neurological Disorders-Drug Targets **11**(3):324-332

Başar-Eroglu, C., Demiralp, T., Schurmann, M., and E. Basar (2001). "Topological distribution of oddball 'P300' responses." International Journal of Psychophysiology **39**(2–3):213-220.

Batty, M. and M. J. Taylor (2003). "Early processing of the six basic facial emotional expressions." Cognitive Brain Research **17**(3):613-620.

Beato, R., Levy, R., Pillon, B., Vidal, C., du Montcel, S. T., Deweer, B., Bonnet, A. M., Houeto, J. L., Dubois, B., and F. Cardoso (2008). "Working memory in Parkinson's disease patients: clinical features and response to levodopa." Arquivos de Neuro-Psiquiatria **66**(2)**:**147-151.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., and J. Erbaugh (1961). "An inventory for measuring depression." Archives of General Psychiatry **4**(6):561-571.

Begleiter, H., Porjesz, B., and W. Wang (1995). "Event-related brain potentials differentiate priming and recognition to familiar and unfamiliar faces." Electroencephalography and Clinical Neurophysiology **94**(1):41-49.

Bentin, S., Allison, T., Puce, A., Perez, E, and G. McCarthy (1996). "Electrophysiological studies of face perception in humans." Journal of Cognitive Neuroscience **8**(6): 551-565.

Bergersen, L. H., Sander, M., and J. Storm-Mathisen (2011). "What the nose knows, what the eyes see, how we feel, how we learn, how we understand motor acts, why "YY" is essential for ion transport, how epigenetics meet neurobiology in Rett syndrome: seven topics at the 2010 Kavli Prize Symposium on Neuroscience." Neuroscience **190**:1-11.

Berridge, K. C. and T. E. Robinson (1998). "What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?" Brain Research Reviews **28**(3):309-369.

Bertolino, A., Blasi, G., Latorre, V., Rubino, V., Rampino, A., Sinibaldi, L., Caforio, G., Petruzzella, V., Pizzuti, A., Scarabino, T., Nardini, M., Weinberger, D. R., and B. Dallapiccola (2006). "Additive effects of genetic variation in dopamine regulating genes on working memory cortical activity in human brain." Journal of Neuroscience **26**(15):3918-3922.

Bertolino, A., Caforio, G., Blasi, G., De Candia, M., Latorre, V., Petruzzella, V., Altamura, M., Nappi, G., Papa, S., Callicott, J. H., Mattay, V. S., Bellomo, A., Scarabino, T., Weinberger, D. R., and M. Nardini (2004). "Interaction of COMT (Val(108/158)Met) genotype and olanzapine treatment on prefrontal cortical function in patients with schizophrenia." American Journal of Psychiatry **161**(10):1798-1805.

Bertolino, A., Di Giorgio, A., Blasi, G., Sambataro, F., Caforio, G., Sinibaldi, L., Latorre, V., Rampino, A., Taurisano, F., Fazio, L., Romano, R., Douzgou, S., Popolizio, T., Kolachana, B., Nardini, M., Weinberger, D. R., and B. Dallapiccola (2008). "Epistasis between dopamine regulating genes identifies a nonlinear response of the human hippocampus during memory tasks." Biological Psychiatry **64**(3):226-234.

Bertolino, A., Rubino, V., Sambataro, F., Blasi, G., Laorre, V., Fazio, L., Caforio, G., Petruzzella, V., Kolachana, B., Hariri, A., Meyer-Lindenberg, A., Nardini, M., Weinberer, D. R., and T. Scarabino (2006). "Prefrontal-hippocampal coupling during memory processing is modulated by COMT Val158Met genotype." Biological Psychiatry **60**(11):1250-1258.

Bevilacqua, L. and D. Goldman (2011). "Genetics of emotion." Trends in Cognitive Sciences **15**(9):401-408.

Bilder, R. M., Volavka, J., Lachman, H. M., and A. A. Grace (2004). "The catechol-O - methyltransferase polymorphism: Relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes." Neuropsychopharmacology **29**(11): 1943-1961.

Bishop, S. J. (2008). "Neural mechanisms underlying selective attention to threat." Annals of the New York Academy of Sciences **1129**:141-152.

Blair, R. J. R., Morris, J. S., Frith, C. D., Perrett, D. I., and R. J. Dolan (1999). "Dissociable neural responses to facial expressions of sadness and anger." Brain **122**(5)**:**883-893.

Blanchard, M. M., Chamberlain, S. R., Roiser, J., Robbins, T. W., and U. Muller (2011). "Effects of two dopamine-modulating genes (DAT1 9/10 and COMT Val/Met) on n-back working memory performance in healthy volunteers." Psychological Medicine **41**(3):611-618.

Blasi, G., Mattay, V. S., Bertolino, A., Elvevag, B., Callicott, J. H., Das, S., Kolachana, B. S., Egan, M. F., Goldberg, T. E., and D. R. Weinberger (2005). "Effect of catechol-O-methyltransferase Val158Met genotype on attentional control." Journal of Neuroscience **25**(20): 5038-5045.

Blau, V., Maurer, U., Tottenham, N., and B. D. McCandliss (2007). "The face-specific N170 component is modulated by emotional facial expression." Behavioral and Brain Functions **3**(1):7

Bodenmann, S., Rusterholz, T., Durr, R., Stoll, C., Bachman, V., Geissler, E., Jaggi-Schwarz, K., and H. F. Landolt (2009). "The functional Val158Met polymorphism of COMT predicts interindividual differences in brain α oscillations in young men." The Journal of Neuroscience **29**(35):10855-10862.

Boot, E., Booij, J., Zinkstok, J. R., Baas, F., Swillen, A., Owen, M. J., Murphy, D. G., Murphy, K. C., Linszen, D. H., and T. A. Van Amelsvoort (2011). "COMT Val158Met genotype and striatal D2/3 receptor binding in adults with 22q11 deletion syndrome." Synapse **65**(9):967-970.

Borod, J. C., Cicero, B. A., Obler, L. K., Welkowitz, J., Erhan, H. M., Santschi, C., Grunwald, I. S., Agosli, R. M, and J. R. Whalen (1998). "Right hemisphere emotional perception: Evidence across multiple channels." Neuropsychology **12**(3):446-458.

Braeutigam, S., Bailey, A. J., and S. J. Swithenby (2001). "Task-dependent early latency (30-60 ms) visual processing of human faces and other objects." NeuroReport **12**(7):1531-1536.

Braver, T. S., Cohen, J. D., Nystrom, L. E., Jonides, J., Smith, E. E., and D. C. Noll (1997). "A parametric study of prefrontal cortex involvement in human working memory." Neuroimage **5**(1):49-62.

Breiter, H. C., Etcoff, N. L., Whalen, P. J., Kennedy, W. A., Rauch, S. L., Buckner, R. L., Strauss, M. M., Hyman, S. E., and B. R. Rosen (1996). "Response and abituation of the human amygdala during visual processing of facial expression." Neuron **17**(5):875-887.

Brinker, G., Bock, C., Busch, E., Krep, H., Hossmann, K. A., and M. Hoehn-Berlage (1999). "Simultaneous recording of evoked potentials and T*(2)-weighted MR images during somatosensory stimulation of rat." Magnetic Resonance in Medicine **41**(3):469-473.

Brody, A. L., Mandelkern, M. A., Olmstead, R. E., Scheibal, D., Hahn, E., Shiraga, S., Zamora-Paja, E., Farahi, J., Saxena, S., London, E. D., and J. T. McCracken (2006). "Gene variants of brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens." Archives of General Psychiatry **63**(7):808-816.

Brody, A. L., Saxena, S., Mandelkern, M. A., Fairbanks, W. L., Ho, M. L., and L. R. Baxter (2001). "Brain metabolic changes associated with symptom factor improvement in major depressive disorder." Biological Psychiatry **50**(3):171-178.

Brown, R. M., Crane, A. M., and P. S. Goldman (1979). "Regional distribution of monoamines in the cerebral cortex and subcortical structures of the rhesus monkey: Concentrations and in vivo synthesis rates." Brain Research **168**(1):133-150.

Brozoski, T. J., Brown, R. M., Rosvold, H. E., and P. S. Goldman (1979). "Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey." Science **205**(4409): 929-932.

Bruder, G. E., Keilp, J. G., Xu, H., Shikhman, M., Schori, E., Gorman, J. M. and T. C. Gilliam (2005). "Catechol-O-methyltransferase (COMT) genotypes and working memory: associations with differing cognitive perations." Biological Psychiatry **58**(11):901-907.

Buckley, M. J., Mansouri, F. A., Hoda, H., Mahbubi, M., Browning, P. G., Kwok, S. C., Phillips, A., and K. Tanaka (2009). "Dissociable components of rule-guided behavior depend on distinct medial and prefrontal regions." Science **325**(5936): 52-58

Budygin, E. A., Gainetdinov, R. R., Kilpatrick, M. R., Rayevsky, K. S., Mannisto, P. T., and R. M. Wightman (1999). "Effect of tolcapone, a catechol-O-methyltransferase inhibitor, on striatal dopaminergic transmission during blockade of dopamine uptake." European Journal of Pharmacology **370**(2):125-131.

Burgdorf, J., (2006). "The neurobiology of positive emotions." Neurosci Biobehav Rev **30** (2):173-87.

Burgdorf, J., Wood, P. L., Kroes, R. A., Moskal, J. R., and J. Panksepp (2007). "Neurobiology of 50-kHz ultrasonic vocalizations in rats: Electrode mapping, lesion, and pharmacology studies." Behavioural Brain Research **182**(2):274-283.

Buzsáki, G., Anastassiou, C. A., and C. Koch (2012). "The origin of extracellular fields and currents — EEG, ECoG, LFP and spikes." Nature Reviews Neuroscience **13**(6):407-420.

Callicott, J. H., Bertolino, A., Mattay, V. S., Langheim, F. J., Duyn, J., Coppola, R., Goldbert, T. E., and D. R. Weinberger (2000). "Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited." Cerebral Cortex **10**(11): 1078-1092.

Callicott, J. H., Ramsey, N. F., Tallent, K. Bertolino, A., Knable, M. B., Coppola, R., Goldbert, T., Van Gelderen, F., Mattay, V. S., Frank, J. A., Moonen, C. T., and D. R. Weinberger (1998). "Functional magnetic resonance imaging brain mapping in psychiatry: methodological issues illustrated in a study of working memory in schizophrenia." Neuropsychopharmacology **18**(3):10.

Camara, E., Kramer, U. M., Cunillera, T. Marco-Pallares, J. Cucurell, D., Nager, W., Mestres-Misse, A., Bauer, P., Schule, R., Schols, L. Tempelmann, C., Rodriguez-Fornells, A., and T. F. Munte (2010). "The effects of COMT (Val108/158Met) and DRD4 (SNP-521) dopamine genotypes on brain activations related to valence and magnitude of rewards." Cerebral Cortex **20**(8):1985-1996.

Campanella, S., Quinet, P., Bruyer, R., Crommelinck, M., and J. M Guerit (2002). "Categorical perception of happiness and fear facial expressions: an ERP study." Journal of Cognitive Neuroscience **14**(2):210-227.

Campanella, S., Rossignol, M., Mejias, S. Joassin, F., Maurage, F., Debalisse, D., Bruyer, R., Crommelinck, M., and J. M Guerit (2004). "Human gender differences in an emotional visual oddball task: an event-related potentials study." Neuroscience Letters **367**(1):14-18.

Canli, T., Sivers, H., Whittfield, S. L., Gotlib, I. H., and J. D. Gabrieli (2002). "Amygdala response to happy faces as a function of extraversion." Science **296**(5576):2191

Carlsson, A., Lindqvist, M., Magnusson, T., and B. Waldeck (1958). "On the presence of 3-hydroxytyramine in brain." Science **127**(3296):471.

Carmel, D. and S. Bentin (2002). "Domain specificity versus expertise: factors influencing distinct processing of faces." Cognition **83**(1):1-29.

Cauquil, A. S., Edmonds, G. E., and M. J. Taylor (2000). "Is the face-sensitive N170 the only ERP not affected by selective attention?" NeuroReport **11**(10):2167-2171.

Ceravolo, R., Piccini, P., Bailey, D. L., Jorga, K. M., Bryson, H., and D. J. Brooks (2002). "18F-dopa PET evidence that tolcapone acts as a central COMT inhibitor in Parkinson's disease." Synapse **43**(3):201-207.

Chafee, M. V. and P. S. Goldman-Rakic (2000). "Inactivation of parietal and prefrontal cortex reveals interdependence of neural activity during memory-guided saccades." Journal of Neurophysiology **83**(3):1550-1566.

Charney, D. S. (2004). "Discovering the neural basis of human social anxiety: a diagnostic and therapeutic imperative." American Journal Psychiatry **161**(1): 2.

Chen, J. S., Lipska, B. K., Halim, N., Ma, Q. D., Matsumoto, M. Melhem, S., Kolachana, B. S., Hyde, T. M., Herman, M. M., Apud, J. Egan, M. F., Kleinman, J. E., and D. R. Weinberger (2004). "Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): Effects on mRNA, protein, and enzyme activity in postmortem human brain." American Journal of Human Genetics **75**(5): 807-821.

Chen, J. S., Song, J., Yuan, F., Tian, Q., Ji, Y., Ren-Patterson, R., Liu, G., Sei, Y., and D. R. Weinberger (2011). "Orientation and cellular distribution of membrane-bound catechol-O-methyltransferase in cortical neurons implications for drug developments." Journal of Biological Chemistry **286**(40):34752-34760.

Chung, G., Tucker, D. M., West, P., Potts, G. F., Liotti, M., Luu, P., and A. L. Hartry (1996). "Emotional expectancy: brain electrical activity associated with an emotional bias in interpreting life events." Psychophysiology **33**(3):218-233.

Clark, J. J., Sandberg, S. G., Wanat, M.J., Gan, J.O., Horne, E.A. Hart, A.S. Akers, C.A., Parker J.G., Willuhn, I., Martinez, V., Evans, S.B., Stella, N., and P.E Phillips (2010). "Chronic microsensors for longitudinal, subsecond dopamine detection in behaving animals." Nature Methods **7**(2):126-129.

Clark, V. P., Keil, K., Maisog, J.M., Courtney, S., Ungerleider, L.G., and J.V. Haxby (1996). "Functional magnetic resonance imaging of human visual cortex during face matching: a comparison with positron emission tomography." Neuroimage **4**(1):1-15.

Cohen, M. X., Elger, C.E., and C. Ranganath (2007). "Reward expectation modulates feedback-related negativity and EEG spectra." Neuroimage **35**(2):968-978.

Collins, P., Wilkinson, L.S., Everitt, B.J., Robbins, T.W., and A.C. Roberts (2000). "The effect of dopamine depletion from the caudate nucleus of the common marmoset (Callithrix jacchus) on tests of prefrontal cognitive function." Behavioral Neuroscience **114**(1): 3-17.

Congdon, E., Constable, R.T., Lesch, K.P., and T. Canli (2009). "Influence of SLC6A3 and COMT variation on neural activation during response inhibition." Biological Psychology **81**(3):144-152.

Cools, R., Calder, A.J., Lawrence, A.D., Clark, L., Bullmore, E., and T.W. Robbins (2005). "Individual differences in threat sensitivity predict serotonergic modulation of amygdala response to fearful faces." Psychopharmacology **180**(4): 670-679.

Cools, R. and M. D'Esposito (2011). "Inverted-U–shaped dopamine actions on human Working Memory and Cognitive Control." Biological Psychiatry **69**(12): e113-125.

Cools, R., Gibbs, S.E., Miyakawa, A., Jaqust, W., and M. D'Esposito (2008). "Working memory capacity predicts dopamine synthesis capacity in the human striatum." Journal of Neuroscience **28**(5):1208-1212.

Cools, R. and T. W. Robbins (2004). "Chemistry of the adaptive mind." Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences. **362**(1825):17.

Critchley, H. D., Daly, E.M., Bullmore, E.T., Williams, S.C., Van Amelsvoort, T., Robertson, D.M., Rowe, A., Philips, M., McAlonan, G., Howlin, P., and D.G. Murphy (2000). "The functional neuroanatomy of social behaviour: Changes in cerebral blood flow when people with autistic disorder process facial expressions." Brain **123**(11):2203-2212.

Crofts, H. S., Dalley, J.W., Collins, P., Van Denderen, J.C., Everitt, B.J., Robbins, T.W., and A.C. Roberts (2001). "Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set." Cerebral Cortex **11**(11):1015-1026.

Da Prada, G., Zurcher, R., Kettler, R., and A. Colzi (1991). "New therapeutic strategies in Parkinson's disease: Inhibition of MAO-B by Ro 19-6327 and of COMT by Ro 40-7592." Advanced Behavioral Biology **39**:723

da Silva, F. H. (2010). "Electrophysiological basis of MEG signals." In Hansen, P., Kringelback, M.O., and R. Salmelin (Eds.) "MEG: an introduction to methods" Oxford University Press **pp. 1-23**

Damasio, A. R., Grabowski, T.J., Bechara, A., Damasio, H., Ponto, L.L., Parvizi, J., and R.D Hichwa, (2000). "Subcortical and cortical brain activity during the feeling of self-generated emotions." Nature Neuroscience **3**(10):1049-1056.

Davis, K. L., Kahn, R.S., Ko, G., and M. Davidson (1991). "Dopamine in schizophrenia – a review and reconceptualization." American Journal of Psychiatry **148**(11): 1474-1486.

de Frias, C. M., Marklund, P., Eriksson, E., Larsson, A., Oman, L., Annerbrink, K., Backman, L., Nilsson, L. G., and L. Nyberg (2010). "Influence of COMT gene polymorphism on fMRI-assessed sustained and transient activity during a working memory task." Journal of Cognitive Neuroscience **22**(7):1614-1622.

De Pascalis, V., Arwari, B., D'Antuono, L., and L. Cacace (2009). "Impulsivity and semantic/emotional processing: An examination of the N400 wave." Clinical Neurophysiology **120**(1):85-92.

Debruille, J. B., Guillem,F., and B. Renault (1998). "ERPs and chronometry of face recognition: following-up Seeck et al. and George et al." NeuroReport **9**(15): 3349-3353.

Defrancesco, M., Niederstätter, H., Parson, W., Oberacher, H., Hinterhuber, H., Canazel, M., Bidner, J., Deisenhammer, E. A., Kemler, G., Weiss, E. M., and J. Marksteiner (2011). "Influence of serotonin transporter genotype and catechol-O-methyltransferase Val158Met polymorphism on recognition of emotional faces." Journal of International Neuropsychological Society. **17**(6):1014-20.

Del-Ben, C. M. and F. G. Graeff (2010). "Modulation of emotional faces processing and its implication for depression and anxiety." in Del-Ben, C.M. Neuroimaging : Chapter **3**

Di Chiara, G. and A. Imperato (1988). "Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats." Proceedings of the National Academy of Sciences **85**(14):5274-5278.

Dias, R., Robbins, T. W., and A. C. Roberts (1996). "Dissociation in prefrontal cortex of affective and attentional shifts." Nature **380**(6569):69-72.

Dias, R., Robbins, T.W., and A. C. Roberts (1996). "Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset." Behavioral Neuroscience **110**(5):872-886.

Diatchenko, L., Slade, G.D., Nakley, A. G., Bhalang, K., Sigurdsson, A. , Belfer, I., Goldman, D., Xu, K., Shabalina, S. A., Shagin, D., Max, M. B., Makarov, S. S., and W. Maixner (2005). "Genetic basis for individual variations in pain perception and the development of a chronic pain condition." Human Molecular Genetics **14**(1):135-143.

Diaz-Asper, C. M., Goldberg, T.E., Kolachana, B. S., Straub, R. E., Egan, M. F., and D. R. Weinberer (2008). "Genetic variation in catechol-O-methyltransferase: effects on working memory in schizophrenic patients, their siblings, and healthy controls." Biological Psychiatry **63**(1):72-79.

Dichiara, G. and A. Imperato (1988). "Drugs abused by humans preferentially increase synaptic dpamine concentrations in the mesolimbic system of freely moving rats ." Proceedings of the National Academy of Sciences of the United States of America **85**(14):5274-5278.

Dickinson, D. and B. Elvevag (2009). "Genes, cognition and brain through a COMT lens." Neuroscience **164**(1):72-87.

Dimberg, U. and L. O. Lundquist (1990). "Gender differences in facial reactions to facial expressions." Biological Psychology **30**(2):151-159.

Dingemanse, J., Jorga, K.M., Schmitt, M., Geischke, R., Fotteler, B., Zurcher, G., Da Prada, M., and P. van Brummelen (1995). "Integrated pharmacokinetics and pharmacodynamics of the novel catechol-O-methyltransferase inhibitor tolcapone during first administration to humans[ast]." Clinincal Pharmacology and Therapeutics **57**(5): 508-517.

Dolan, R. J., Fletcher, P., Morris, J., Kapur, N., Deakin, J.F and C.D. Frith (1996). "Neural activation during covert processing of positive emotional facial expressions." Neuroimage **4**(3):194-200.

Dolu, N., and C. Başar-Eroğlu (2005). "An assessment of working memory using P300 wave in healthy subjects." International Congress Series **1278**(0):7-10.

Domschke, K., Baune, B.T., avlik, L., Stuhrmann, A., Suslow, T., Kugel, H., Zwanzger, P., Grotegerd, D., Sehlmeyer, C., Arolt, V., and U. Dannlowski (2012). "Catechol-O-methyltransferase gene variation: Impact on amygdala response to aversive stimuli." Neuroimage **60**(4):2222-2229.

Doñamayor, N., Marco-Pallarés, J., Heldmann, M., Schoenfeld, M.A., and T.F. Munte (2011). "Temporal dynamics of reward processing revealed by magnetoencephalography." Human Brain Mapping **32**(12):2228-2240.

Donchin, E. and M. G. H. Coles (1988). "Is the P300 component a manifestation of context updating?" Behavioral and Brain Sciences **11**(03):357-374.

Drabant, E. M., Hariri, A.R., Meyer-Lindenberg, A., Munoz, K.E., Mttay V.S., Kolachana, B.S., Egan, M.F and D.R. Weinberger (2006). "Catechol O-methyltransferase Val(158)Met genotype and neural mechanisms related to affective arousal and regulation." Archives of General Psychiatry **63**(12):1396-1406.

Dreher, J. C., Kohn, P., Kolachana, B., Weinberger, D.R., and K.F., Berman (2009). "Variation in dopamine genes influences responsivity of the human reward system." Proceedings of the National Academy of Sciences of the United States of America **106**(2): 617-622.

Drevets, W. C. (2000). "Neuroimaging studies of mood disorders." Biological Psychiatry **48**(8):813-829.

Dumontheil, I., Roggeman, C., Ziermans, T., Peyrard-Janvid, M., Matsson, H., Kere, J., and T. Klinberg (2011). "Influence of the COMT genotype on working memory and brain activity changes during development." Biological Psychiatry **70**(3): 222-229.

Durstewitz, D. and J. K. Seamans (2008). "The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-O-methyltransferase genotypes and schizophrenia." Biological Psychiatry **64**(9):739-749.

Egan, M. F., Goldberg, T.E., Kolachn, B.S., Callicott, J.., Mazznti, C.M., Straub R.E., Goldman D. and D.R. Weinberger (2001). "Effect of COMT Val 108/158 Met genotype on frontal lobe function and risk for schizophrenia." Proceedings of the National Academy of Sciences of the United States of America **98**(12):6917-6922.

Ehringer, H. and O. Hornykiewicz (1960). "Verteilung von noradrenalin und dopamin (3-hydroxytyramin) im gehirn des menschen und ihr verhalten bei erkrankungen des extrapyramid alen systems." Klinische Wochenschrift **38**(24):1236-1239.

Eimer, M. (2000). "Event-related brain potentials distinguish processing stages involved in face perception and recognition." Clinical Neurophysiology **111**(4):694-705.

Eimer, M. and A. Holmes (2002). "An ERP study on the time course of emotional face processing." NeuroReport **13**(4):427-431.

Eimer, M. and A. Holmes (2007). "Event-related brain potential correlates of emotional face processing." Neuropsychologia **45**(1):15-31.

Eimer, M., Holmes, A., and F.P. McGlone (2003). "The role of spatial attention in the processing of facial expression: An ERP study of rapid brain responses to six basic emotions." Cognitive Affective & Behavioral Neuroscience **3**(2):97-110.

Ekman, P. and W. V. Friesen (1971). "Constants across cultures in the face and emotion." Journal of Personality and Social Psychology **17**(2):124-129.

Ekman, P., Sorenson, E.R. and W.V., Friesen (1969). "Pan-cultural elements in facial displays of emotion." Science **164**(88):86.

Enoch, M. A., Xu, K., Ferro, E., Harris, C.R. and D. Goldman (2003). "Genetic origins of anxiety in women: a role for a functional catechol-O-methyltransferase polymorphism." Psychiatric Genetics **13**(1):33-41.

Esslen, M., Pascual-Marqui, R.D., Hell, D., Kochi, K., and D. Lehmann (2004). "Brain areas and time course of emotional processing." Neuroimage **21**(4):1189-1203.

Farah, M. J., Wilson, K.D., Drain, M., and J.N. Tanaka (1998). "What is "special" about face perception?" Psychological Review **105**(3):482-498.

Farde, L., Halldin, C., Stone-Elander, S., and G. Sedvall (1987). "Pet analysis of human dopamine receptor subtypes using C-11 SCH 23390 and C-11 Raclopride." Psychopharmacology **92**(3):278-284.

Farrell, S. M., Tunbridge, E.M., Brautigam, S. and P.J. Harrison (2012). "COMT Val(158)Met genotype determines the direction of cognitive effects produced by catechol-O-methyltransferase inhibition." Biological Psychiatry **71**(6):538-544.

Fassbender, C., Schweitzer, J.B., Cortes, C.R, Tagamets, M.A., Reeves, G.M., and R. Gullapalli (2011). "Working memory in attention deficit/hyperactivity disorder is characterized by a lack of specialization of brain function." PLoS One **6**(11):e27240.

Feredoes, E., Heinen, K., Weiskopf, N., Ruff, C., and J. Driver (2011). "Causal evidence for frontal involvement in memory target maintenance by posterior brain areas during distracter interference of visual working memory." Proceedings of the National Academy of Sciences of the United States of America **108**(42):17510-17515.

Fletcher, P. C. and R. N. A. Henson (2001). "Frontal lobes and human memory." Brain **124**(5):849-881.

Forbes, E. E., Brown, S.M., Kimak, M., Ferrell, R.E., Manuck, S.B., and A.R. Hariri (2009). "Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity." Molecular Psychiatry **14**(1):60-70.

Fox, P. T. and M.E. Raichle (1986). "Focal physiological uncoupling of cerebral blood-flow and oxidative-metabolism during somatosensory stimulation in human-subjects." Proceedings of the National Academy of Sciences of the United States of America **83**(4):1140-1144.

Fox, P. T., Raichle, M.E., Mintun, M.A., and C. Dence (1988). "Nonoxidative glucose consumption during focal physiologic neural activity." Science **241**(4864):462-464.

Frank, M. J. and J. A. Fossella (2011). "Neurogenetics and pharmacology of learning, motivation, and cognition." Neuropsychopharmacology **36**(1): 33-152.

Frank, M. J., Moustafa, A.A., Haughey, H.M., Curran, T., and K.E. Hutchinson (2007). "Genetic triple dissociation reveals multiple roles for dopamine in reinforcement learning." Proceedings of the National Academy of Sciences of the United States of America **104**(41):16311-16316.

Franken, I. H., Nijs, I., and L. Pepplinkhuizen (2008) “Effects of dopaminergic mdulation on electrophysiological brain response to affective stimuli.” Psychopharmacology **195** (4):137-46

Fruehholz, S., Jellinghaus, A., and M., Herrmann (2011). "Time course of implicit processing and explicit processing of emotional faces and emotional words." Biological Psychology **87**(2):265-274.

Fusar-Poli, P., Allen, P., Lee, F., Surguladze, S., Tunstall, N., Fu, C.H., Brammer, M.J., Cleare, A.J., and P.K. McGuire (2007). "Modulation of neural response to happy and sad faces by acute tryptophan depletion." Psychopharmacology **193**(1):31-44.

Fuster, J. M. (2001). "Physiology of executive functions." Brain and Cognition **47**(1-2): 13-14.

Fuster, J. M. and J. E. Alexander (1971). "Neuron activity related to short term memory." Science **173** (3997):2.

Gallinat, J., Bajbouj, M. Sander, T., Schlattmann, P., Xu, K., Ferro, E. F., Goldman, D., and G.Winterer (2003). "Association of the G1947A COMT (Val(108/158)Met) gene polymorphism with prefrontal P300 during information processing." Biological Psychiatry **54**(1):40-48.

Gan, J. O., Walton, M. E., and P. E. Phillips (2010). "Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine." Nature Neuroscience **13**(1):25-27.

Gaspar, P., Ruiz, S., Zamorano, F., Altayo, M., Perez, C., Bosman, C. A., and F. Aboitiz (2011). "P300 amplitude is insensitive to working memory load in schizophrenia." BMC Psychiatry **11**(1):29.

Gasparini, M., Fabrizio, E., Bonifati, V., Meco, G (1997). "Cognitive improvement during tolcapone treatment in Parkinson's disease." Journal of Neural Transmission **104**(8):887-894.

Gazzaley, A. and A. C. Nobre (2012). "Top-down modulation: bridging selective attention and working memory." Trends in Cognitive Sciences **16**(2): 29-135.

Gehring, W. J. and A. R. Willoughby (2002). "The medial frontal cortex and the rapid processing of monetary gains and losses." Science **295**(5563):2279-2282.

George, N., Jemel, B., Fiori, N., and B. Renault (1997). "Face and shape repetition effects in humans: A spatio-temporal ERP study." NeuroReport **8**(6): 1417-1423.

Gevins, A. and B. Cutillo (1993). "Spatiotemporal dynamics of component processes in human working memory." Electroencephalography Clinical Neurophysiology. **87**(3): 15.

Giakoumaki, S. G., Roussos, P., and P. Bitsios (2008). "Improvement of prepulse inhibition and executive function by the COMT inhibitor tolcapone depends on COMT Val(158)Met polymorphism." Neuropsychopharmacology **33**(13):3058-3068.

Gibbs, S. E. B. and M. D'Esposito (2005). "Individual capacity differences predict working memory performance and prefrontal activity following dopamine receptor stimulation." Cognitive, Affective and Behavioral Neuroscience **5**(2): 212-221.

Girault, J.-A. and P. Greengard (2004). "The neurobiology of dopamine signaling." Archives of Neurology **61**(5):641-644.

Gogos, J. A., Morgan, M., Luine, V., Santha, M., Ogawa, S., Pfaff, D., and M. Karayiorgou (1998). "Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior." Proceedings of the National Academy of Sciences of the United States of America **95**(17): 9991-9996.

Goldberg, T. E., M. F. Egan, et al. (2003). "Executive subprocesses in working memory - Relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia." Archives of General Psychiatry **60**(9): 889-896.

Goldberg, T. E. and D. R. Weinberger (2004). "Genes and the parsing of cognitive processes." Trends in Cognitive Sciences **8**(7):325-335.

Goldman-Rakic, P. S. (1995). "Cellular basis of working memory." Neuron **14**(3):477-485.

Goldman-Rakic, P. S., Muly, E. C. 3rd., and G. V. Williams (2000). "D(1) receptors in prefrontal cells and circuits." Brain Research Reviews **31**(2-3):295-301.

Goldman, D., D. R. Weinberger, Malhotra, A. D. and T. E. Goldberg (2009). "The role of COMT Val158Met in cognition." Biological Psychiatry **65**(1):e1-2

Golimbet, V., Gritsenko, I., Alfimova, M., Lebedeva, I., Lezheiko, T., Abramova, L., Kaleda, V., and R. Ebstein (2006). "Association study of COMT gene Val158Met polymorphism with auditory P300 and performance on neurocognitive tests in patients with schizophrenia and their relatives." World Journal of Biological Psychiatry **7**(4):238-245.

Gong, P., Li, J., Chi, W., Wang, J., Yao, T., Zhang, K., Gao, X., and F. Zhang (2011). "Variants in COMT and DBH influence on response inhibition ability in Chinese Han females." Cellular and Molecular Neurobiology **31**(8):1163-1169.

Gorno-Tempini, M. L., Pradelli, S., Serafini, M., Pagnoni, G., Baraldi, P., Porro, C., Nicoletti, R., Umita, C., and P. Nichelli (2001). "Explicit and incidental facial expression processing: an fMRI study." Neuroimage **14**(2):465-473.

Grabner, R. H., Stern, E., and A. C. Neubauer (2003). "When intelligence loses its impact: neural efficiency during reasoning in a familiar area." International Journal of Psychophysiology **49**(2):89-98.

Grace, A. A. (1995). "The tonic/phasic model of dopamine system regulation: its relevance for understanding how stimulant abuse can alter basal ganglia function." Drug and Alcohol Dependence **37**(2):111-129.

Grace, A. A. and J. A. Rosenkranz (2002). "Regulation of conditioned responses of basolateral amygdala neurons." Physiology and Behavior **77**(4–5):489-493.

Gray, H. M., and L. Tickle-Degnen (2012). "A meta-analysis of performance on emotion recognition tasks in Parkinson's disease." Neuropsychology **24**(2):176-191

Green, A. E., Kraemer, D. J. M., Deyoung, C. G., Fossella, J. A., and J. R. Gray (2012). "A gene–brain–cognition pathway: prefrontal activity mediates the effect of COMT on cognitive control and IQ." Cerebral Cortex. [epub ahead of print]

Greengard, P. (2001). "The Neurobiology of Slow Synaptic Transmission." Science **294**(5544):1024-1030.

Grodd W, S. F., Klose U, and T. Nägele (1995). "[Functional magnetic resonance tomography of psychological functions exemplified by experimentally induced emotions]." Der Radiologue. **35**(4):283-9

Grossman, M. H., Emanuel, B. S., and M. L. Budarf (1992). "Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11.1-->q11.2." Genomics **12**(4):822-825.

Gulledge, A. T. and D. B. Jaffe (1998). "Dopamine decreases the excitability of layer v pyramidal cells in the rat prefrontal cortex." Journal of Neuroscience **18**(21):9139-9151.

Gur, R. C., Skolnick, B. E., and R. E. Gur (1994). "Effects of emotional discrimination tasks on cerebral blood-flow – regional activation and its relation to performance." Brain and Cognition **25**(2):271-286.

Haber, S. N. and B. Knutson (2010). "The reward circuit: linking primate anatomy and human imaging." Neuropsychopharmacology **35**(1):4-26.

Hagen, G. F., Gatherwright, J. R., Lopez, B. A., and J. Polich (2006). "P3a from visual stimuli: task difficulty effects." International Journal of Psychophysiology **59**(1): 8-14.

Haier, R. J., Siegel, B. V., Nuechterlein, K. H., Hazlett, Erin., Wu, J. C., Paek, J., Browning, H. L., and M. S. Buchsbaum (1988). "Cortical glucose metabolic-rate correlates of abstract reasoning and attention studied with positron emission tomography." Intelligence **12**(2): 199-217.

Haier, R. J., White, N. S., and M. T. Akire (2003). "Individual differences in general intelligence correlate with brain function during nonreasoning tasks." Intelligence **31**(5):429-441.

Hall, H., Sedvall, G., Magnusson, O., Kopp, J., Halldin, C., and L. Farde (1994). "Distribution of D-1-dopamine and D-2-dopamine receptors and dopamine and its metabolites in the human brain." Neuropsychopharmacology **11**(4):245-256.

Hämäläinen, M., Hari, R., Risto, J., Illmoniemi, Knuutila, J., and O. V. Lounasmaa (1993). "Magnetoencephalography;theory, instrumentation, and applications to noninvasive studies of the working human brain." Reviews of Modern Physics **65**(2):413.

Haralambous, T. and R. F. Westbrook (1999). "An infusion of bupivacaine into the nucleus accumbens disrupts the acquisition but not the expression of contextual fear conditioning." Behavioral Neuroscience **113**(5):925-940.

Hardware manual Elekta Neuromag (2005) NM 20125A-G

Hari, R. and O. V. Lounasmaa (1989). "Recording and interpretation of cerebral magnetic fields." Science **244**(4903):432-436.

Hariri, A. R., Bookheimer, S. Y., and J. C. Mazziotta (2000). "Modulating emotional responses: effects of a neocortical network on the limbic system." NeuroReport **11**(1): 43-48.

Hariri, A. R., Mattay, V. S., Tessitore, A., Fera, F., Smith, W. G., and D. R. Weinberger (2002). "Dextroamphetamine modulates the response of the human amygdala." Neuropsychopharmacology **27**(6):1036-1040.

Hariri, A. R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M. F., and D. R. Weinberger (2002). "Serotonin transporter genetic variation and the response of the human amygdala." Science **297**(5580):400-403.

Harrison, P. J. and E. M. Tunbridge (2008). "Catechol-O-methyltransferase (COMT): A gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders." Neuropsychopharmacology **33**(13):3037-3045.

Hauser, M. D. (1993). "Right-hemisphere dominance for the production of facial expression in monkeys." Science **261**(5120):475-477.

Haxby, J. V. and M. I. Gobbini (2002). "Human neural systems for face perception and social communication." International Journal of Psychophysiology **45**(1-2):56-57.

Haxby, J. V., Hoffman, E. A., and M. I. Gobbini (2000). "The distributed human neural system for face perception." Trends in Cognitive Sciences **4**(6):223-233.

Heinz, A. and M. N. Smolka (2006). "The effects of catechol O-methyltransferase genotype on brain activation elicited by affective stimuli and cognitive tasks." Reviews in the Neurosciences **17**(3):359-367.

Heinzel, S., Dresler, T., Baehne, C. G., Heine, M., Boreatti-Hummer, A., Jacob, C. P., Renner, T. J., Reif, A., Lesch, K. P., Fallgatter, A. J., and A. C. Ehlis (2012). "COMT × DRD4 epistasis impacts prefrontal cortex function underlying response control." Cerebral Cortex [Epub ahead of print]

Heitland, I., Oosting, R., Baas, J. M., Massar, S. A., Kenemans, J. L., and K. B. Bocker (2012). "Genetic polymorphisms of the dopamine and serotonin systems modulate the neurophysiological response to feedback and risk taking in healthy humans." Cognitive, Affective, & Behavioral Neuroscience: [Epub ahead of print]

Herzmann, G., Schweinberger, S. R., Sommer, W., and L. Jentzsch (2004). "What's special about personally familiar faces? A multimodal approach." Psychophysiology **41**(5):688-701.

Holmes, A., Vuilleumier, P and M. Eimer (2003). "The processing of emotional facial expression is gated by spatial attention: evidence from event-related brain potentials." Brain research. Cognitive brain research **16**(2):174-184.

Hong, J., Shu-Leong, H., Tao, X., and Y. Lap-Ping (1998). "Distribution of catechol-O-methyltransferase expression in human central nervous system." NeuroReport **9**(12):2861-2864.

Houlihan, M., Stelmack, R., and K. Campbell (1998). "Intelligence and the effects of perceptual processing demands, task difficulty and processing speed on P300, reaction time and movement time." Intelligence **26**(1):16.

http://www.neurobs.com/last accessed August 2012

http://www.clinicaltrials.gov/ct25/results?terms=tolcapone last accessed August 2012

http://www.ynic.york.ac.uk/events/new-meg-software-available-within-ynic/ last accessed August 2012

http://www.martinos.org/mne/ last accessed August 2012

Hurd, Y. L., Suzuki, M., and G. C. Sedvall (2001). "D1 and D2 dopamine receptor mRNA expression in whole hemisphere sections of the human brain." Journal of Chemical Neuroanatomy **22**(1-2):127-137.

Hyman, S. E., Malenka, R. C., and E. J. Nestler (2006). Neural mechanisms of addiction: The role of reward-related learning and memory. Annual Review of Neuroscience. **29:**565-598.

Itier, R. J. and M. J. Taylor (2004). "Effects of repetition and configural changes on the development of face recognition processes." Developmental Science **7**(4): 469-487.

Jacobs, E. and M. D'Esposito (2011). "Estrogen shapes dopamine-dependent cognitive processes: implications for women's health." Journal of Neuroscience **31**(14):5286-5293.

Jacques, C., d'Arripe, O., and B. Rossion (2007). "The time course of the inversion effect during individual face discrimination." Journal of Vision **7**(8):3

Jemel, B., Schuller, A. M., and V. Goffaux (2010). "Characterizing the spatio-temporal dynamics of the neural events occurring prior to and up to overt recognition of famous faces." Journal of Cognitive Neuroscience **22**(10):2289-2305.

Jessimer, M. and R. Markham (1997). "Alexithymia: A right hemisphere dysfunction specific to recognition of certain facial expressions?" Brain and Cognition **34**(2): 246-258.

Johnson, M. H. (2005). "Subcortical face processing." Nature Reviews Neuroscience **6**(10):766-774.

Johnson, R. and E. Donchin (1985). "Second thoughts: multiple P300s elicited by a single stimulus." Psychophysiology **22**(2):12.

Joober, R., Gauthier, J., Lal, S., Bloom, D., Lalonde, F., Rouleau, G., Benkelfat, C., and A. Labelle (2002). "Catechol-O-methyltransferase Val-108/158-Met gene variants associated with performance on the Wisconsin Card Sorting Test." Archives of General Psychiatry **59**(7):662-663.

Kaakkola, S. and R. J. Wurtman (1992). "Effects of comt inhibitors on striatal dopamine metabolism – a microdialysis study." Brain Research **587**(2):241-249.

Kanwisher, N., McDermott, J., and M. M. Chun (1997). "The fusiform face area: a module in human extrastriate cortex specialized for face perception." The Journal of Neuroscience **17**(11):4302-4311.

Karayiorgou, M., Altemus, M., Galke, B. L., Goldman, D., Murphy, D. L., Ott, J., and J. A. Gogos (1997). "Genotype determining low catechol-O-methyltransferase activity as a risk factor for obsessive-compulsive disorder." Proceedings of the National Academy of Sciences **94**(9):4572-4575.

Karoum, F., Chrapusta, S. J., and M. F. Egan (1994). "3-Methoxytyramine is the major metabolite of released dopamine in the rat frontal cortex: reassessment of the effects of antipsychotics on the dynamics of dopamine release and metabolism in the frontal cortex, nucleus accumbens, and striatum by a simple two pool model." Journal of Neurochemistry **63**(3):972-979.

Kastner, A., Anglade, P., Bounaix, C., Damier, F., Javoy-Agid, F., Bromet, N., Agid, Y., and E. C. Hirsch (1994). "Immunohistochemical study of catechol-O-methyltransferase in the human mesostriatal system." Neuroscience **62**(2):449-457.

Kawasaki, H., Tsuchiya, N., (2012). "Processing of facial emotion in the human fusiform gyrus." Journal of Cognitive Neuroscience **24**(6):1358-70

Kehagia, A. A.,Barker, R. A., and T. W. Robbins (2010). "Neuropsychological and clinical heterogeneity of cognitive impairment and dementia in patients with Parkinson's disease." Lancet Neurology **9**(12):1200-1213.

Kellendonk, C., Simpson, E. H., Polan, H. J., Malleret, G., Vronskaya, S., Winiger, V., Moore, H., and K. R. Kandel (2006). "Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning." Neuron **49**(4):603-615.

Kereszturi, E., Tarnok, Z., Bognar, E., Lakatos, K., Farkas, L., Gadoros, J., Sasvari-Szekely, M., and Z. Nemoda (2008). "Catechol-O-methyltransferase Val158Met polymorphism is associated with methylphenidate response in ADHD children." American Journal of Medical Genetics Part B: Neuropsychiatric Genetics **147B**(8): 1431-1435.

Kim, H., Somerville, L. H., Johnstone, T., Alexander, A. L., and P. J. Whalen (2003). "Inverse amygdala and medial prefrontal cortex responses to surprised faces." NeuroReport **14**(18): 2317-2322.

Kimberg, D. Y., Aguirre, G. K., Lease, J., and M. D'Esposito (2001). "Cortical effects of bromocriptine, a D-2 dopamine receptor agonist, in human subjects, revealed by fMRI." Human Brain Mapping **12**(4):246-257.

Kimberg, D. Y. and M. D'Esposito (2003). "Cognitive effects of the dopamine receptor agonist pergolide." Neuropsychologia **41**(8):1020-1027.

Kimberg, D. Y., D'Esposito, M., and M. J. Farah (1997). "Effects of bromocriptine on human subjects depend on working memory capacity." NeuroReport **8**(16):3581-3585.

Kimbrell, T. A., George, M. S., Parekh, P. I., Ketter, T. A., Podell, D. M., Danielson, A. L., Repella, J. D., Benson, B. E., Willis, M. W., Herscovitch, P., and R. M. Post (1999). "Regional brain activity during transient self-induced anxiety and anger in healthy adults." Biological Psychiatry **46**(4):454-465.

Kimoto, S., Muraki, K., (2012). "Selective overexpression of Comt in prefrontal cortex rescues schizophrenia-like phenotypes in a mouse model of 22q11 deletion syndrome." Translational Psychiatry **2**:e146.

Klimesch, W., Doppelmayr, M., and S. Hanslmayr (2006). "Upper alpha ERD and absolute power: their meaning for memory performance." Event-Related Dynamics of Brain Oscillations **159**:151-165.

Klimesch, W., Doppelmayr, M., Pachinger, T., and H. Russegger (1997). "Event-related desynchronization in the alpha band and the processing of semantic information." Brain research. Cognitive Brain Research **6**(2):83-94.

Klimesch, W., Doppelmayr, M., Schwaiger, J., Auinger, P., and T. Winkler (1999). "'Paradoxical' alpha synchronization in a memory task." Brain Research. Cognitive Brain Research **7**(4):493-501.

Klimesch, W., Schimke, H., (Doppelmayr, M., Ripper, B., Schwaiger, J., and G. Pfurtsheller 1996). "Event-related desynchronization (ERD) and the Dm effect: Does alpha desynchronization during encoding predict later recall performance?" International Journal of Psychophysiology **24**(1-2):47-60.

Knauff, M., Fangmeier, T., Ruff, C. C., and P. N. Johnson-Laird (2003). "Reasoning, models, and images: Behavioral measures and cortical activity." Journal of Cognitive Neuroscience **15**(4):559-573.

Koch, C. and A. Zador (1993). "The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization." Journal of Neuroscience **13**(2):413-422.

Koerts, J., Leenders, K. L., and W. H. Brouwer (2009). "Cognitive dysfunction in non-demented Parkinson's disease patients: controlled and automatic behavior." Cortex **45**(8):922-929.

Kosslyn, S. M., Shin, L. M., Thompson, W. L., McNally, R. J., Rauch, S. L., Pitman, R. K., and N. M. Alpert (1996). "Neural effects of visualizing and perceiving aversive stimuli: A PET investigation." NeuroeRport **7**(10):1569-1576.

Krach, S., Jansen, A., Krug, A., Markov, V., Thimm, M., Sheldrick, A. J., Eggermann, T., Zerres, K., Stocker, T., Shah, N. J., and T. Kircher (2010). "COMT genotype and its role on hippocampal-prefrontal regions in declarative memory." Neuroimage **15**:53(3):978-84

Krimer, L. S., Jakab, R. L., and P. S. Goldman-Rakic (1997). "Quantitative three-dimensional analysis of the catecholaminergic innervation of identified neurons in the macaque prefrontal cortex." Journal of Neuroscience **17**(19):7450-7461.

Krombholz, A., Schaefer, F., and W. Boucesein (2007). "Modification of N170 by different emotional expression of schematic faces." Biological Psychology **76**(3): 156-162.

Krugel, L. K., Biele, G., Mohr, P. N., Li, S. C., and H. R. Heekeren (2009). "Genetic variation in dopaminergic neuromodulation influences the ability to rapidly and flexibly adapt decisions." Proceedings of the National Academy of Sciences of the United States of America **106**(42):17951-17956.

Kubota, K. and H. Niki (1971). "Prefrontal cortical unit activity and delayed alternation performance in monkeys." Journal of Neurophysiology **34**(3):337-347.

Kwong, K. K., Belliveau, J. W., Chesler, D. A., Goldberg, I. E., Weisskoff, R. M., Poncelet, B. P., Kennedy, D. N., Hoppel, B. E., Cohen, M. S., and R Turner (1992). "Dynamic mr imaging of brain activity during primary sensory stimulation."Proceedings of the National Academy of Sciences USA **15**:89(12):5675-9 .

Kylliäinen, A., Braeutigam, S., Hietanen, J. K., Swithenby, S. J., and A. J. Bailey (2006). "Face and gaze processing in normally developing children: a magnetoencephalographic study." European Journal of Neuroscience **23**(3):801-810

Lachman, H. M., Papolos, D. F., Saito, T., Yu, Y. M., Szumlanski, C. L., and R. M. Weinshilboum (1996). "Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders." Pharmacogenetics **6**(3):243-250

Lachowicz, J.E., and D.R. Sibley (1997) "Molecular characteristics of mammalian dopamine receptors. Pharmacology & toxicology **81**(3):105-113

Lamm, C., Fischmeister, F. P., and Bauer, H. (2005). "Individual differences in brain activity during visuo-spatial processing assessed by slow cortical potentials and LORETA." Brain Research. Cognitive Brain Research **25**(3):900-912.

Landau, S. M., Lal, O'Neil, J. P., Baker, S., and W J. Jaqust (2009). "Striatal dopamine and working memory." Cerebral Cortex **19**(2):445-454.

Lane, R. D., Kivley, L. S., Du Bois, M. A., Shamasundara, F., and G. E. Schwartz (1995). "Levels of emotional awareness and the degree of right hemispheric dominance in the perception of facial emotion." Neuropsychologia **33**(5):525-538.

Lane, R. D. and L. Nadel (2000). "Cognitive neuroscience of emotion." New York: Oxford University Press

Laviolette, S. R., (2007). "Dopamine modulation of emotional processing in cortical and subcortical neural ciruits: evidence for a final common pathway in schizophrenia?" Schizophrenia Bulletin **33**(4):971-981.

Lee, J. H., Durand, R., Gradinaru, V., Zhang, F., Goshen, I., Kim, D. S., Fenno, L. E., Ramakrishnan, C., and K. Deisseroth (2010). "Global and local fMRI signals driven by neurons defined optogenetically by type and wiring." Nature **465**(7299): 788-792.

Leegwater-Kim, J. and C. Waters (2006). "Toicapone in the management of Parkinson's disease." Expert Opinion on Pharmacotherapy **7**(16):2263-2270.

Levesque, J., Eugene, F., Joanette, Y., Paquette, V., Mensour, B., Beaudoin, G., Leroux, J. M., Bourgouin, P., and M. Beauregard (2003). "Neural circuitry underlying voluntary suppression of sadness." Biological Psychiatry **53**(6):502-510.

Levita, L., Dalley, J. W., and T. W. Robbins (2002). "Nucleus accumbens dopamine and learned fear revisited: a review and some new findings." Behavioural Brain Research **137**(1–2):115-127.

Li, J., Yu, C., Li, Y., Liu, B., Liu, Y., Shu, N., Song, M., Zhou, Y., Zhu, W., Li, K. and T. Jiang (2009). "COMT Val158Met modulates association between brain white matter architecture and IQ." American Journal of Medical Genetics Part B-Neuropsychiatric Genetics **150B**(3):375-380.

Li, T., Chen, C. K., Hu, X., Ball, D., Lin, S. K., Chen, W., Sham, P. C., Loh el-W., Murray, R. M., and D. A. Collier (2004). "Association analysis of the DRD4 and COMT genes in methamphetamine abuse." American Journal of Medical Genetics Part B-Neuropsychiatric Genetics **129B**(1):120-124.

Lidow, M. S., Goldman-Rakic, P. S., Gallager, D. W., Rakic, P (1991). "Distribution of dopaminergic receptors in the primate cerebral cortex: Quantitative autoradiographic analysis using [3H]raclopride, [3H]spiperone and [3H]SCH23390." Neuroscience **40**(3):657-671.

Lindvall, O. and A. Bjorklund (1984). "Dopamine containing systems in the CNS." Handbookof Chemical Neuroanatomy: Classical Transmitterin The Rat **2**:55-122.

Linkenkaer-Hansen, K., Palva, J. M., Sams, M., Hietanen, J. K., Aronen, H. J., and R J Ilmoniemi (1998). "Face-selective processing in human extrastriate cortex around 120 ms after stimulus onset revealed by magneto- and electroencephalography." Neuroscience Letters **253**(3):147-150.

Liu, C. H., Collin, C. A., Burton, A. M., and A. Chaudhuri (1999). "Lighting direction affects recognition of untextured faces in photographic positive and negative." Vision Research **39**(24):4003-4009.

Logothetis, N. K. and B. A. Wandell (2004). "Interpreting the BOLD signal." Annual Review of Physiology **66**:735-769.

Lonsdorf, T. B., Golkar, A., Lindstom, K. M., Fransson, P., Schalling, M., Ohman, A., and M. Ingvar (2011). "5-HTTLPR and COMTval158met genotype gate amygdala reactivity and habituation." Biological Psychology **87**(1):106-112.

Lotta, T., Vidgren, J., Ulmanen, I., Melen, K., Julkunen, I., and J. Taskinen (1995). "Kinetics of human soluble and membrane-bound catechol-O-methyltransferase – a revised mechanism and description of the thermolabile variant of the enzyme." Biochemistry **34**(13):4202-4210.

Luciana M, Depue RA, P Arbisi, and A. Leon (1992). " Facilitation of working memory in humans by a D2 dopamine receptor agonist." Journal of Cognitive Neuroscience **4**(1):58-68.

Lundstrom, K., Salminen, M., Jalanko, A., Savolainen, R., and I. Ulmanen (1991). "Cloning and characterization of human placental catchol-O-methyltransferase CDNA." DNA and Cell Biology **10**(3):181-189.

Ma, L., Steinberg, J.L., Hasan, K.M., Narayana, P.A., Kramer, L.A., and F.G. Moeller (2011) "Working memory load modulation of parieto-frontal connections: evidence from dynamic causal modelling". Human Brain Mapping **33**(8):1859-67

Malhotra, A. K., Kestler, L. J., Mazzanti, C., Bates, J. A., Goldberg, T., and D. Goldman (2002). "A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition." American Journal of Psychiatry **159**(4):652-654.

Manoach, D. S., Schlaug, D. Siewert, B., Darby, D. G., Bly, B. M., Benfield, A., Edelman, R. R., and S. Warach (1997). "Prefrontal cortex fMRI signal changes are correlated with working memory load." NeuroReport **8**(2):545-549.

Marco-Pallares, J., Cucurell, D., Cunillera, T., Garcia, R., Andres-Pueyo, A., Munte, T. F., and A. Rodriquez-Fornells (2008). "Human oscillatory activity associated to reward processing in a gambling task." Neuropsychologia **46**(1):241-248.

Marco-Pallares, J., Cucurell, D., Cunillera, T., Kramer, U. M., Camara, E., Nager, W., Bauer, P., Schule, R., Schols, L., Munte, T. F., and A. Rodriquez-Fornells (2009). "Genetic variability in the dopamine system (dopamine receptor D4, Catechol-O-methyltransferase) modulates neurophysiological responses to gains and losses." Biological Psychiatry **66**(2):154-161.

Marco-Pallares, J., Nager, W., Kramer, U. M., Cunillera, T., Camara, E., Cucurell, D., Schule, R., Schols, L., Rodriquez-Fornells, and T. F. Munte (2010). "Neurophysiological markers of novelty processing are modulated by COMT and

DRD4 genotypes." Neuroimage **53**(3):962-969.

Markowitsch, H. J., Vandekerckhove, M. M., Lanfermann, H., and M. O. Russ (2003). "Engagement of lateral and medial prefrontal areas in the ecphory of sad and happy autobiographical memories." Cortex **39**(4-5):643-65

Marsala, S. Z., Gioulis, M., Ceravolo, R., and M. Tinazzi (2012). "A systematic review of catechol-O-methyltransferase inhibitors: efficacy and safety in clinical practice." Clinical Neuropharmacology **35**(4):185-190.

Masuda, M., Tsunoda, M., and K. Imai (2003). "High-performance liquid chromatography-fluorescent assay of catechol-O-methyltransferase activity in rat brain." Analytical and Bioanalytical Chemistry **376**(7):1069-1073.

Mathiesen, C., Caesar, K., Akgoren, N., and M. Lauritzen (1998). "Modification of activity-dependent increases of cerebral blood flow by excitatory synaptic activity and spikes in rat cerebellar cortex." Journal of Physiology-London **512**(2):555-566.

Matsumoto, M., Weickert, C. S., Akil, M., Lipska, B. K., Hyde, T. M., Herman, M. M. Kleinman, J. E., and D. R. Weinberger (2003). "Catechol-O-methyltransferase mRNA expression in human and rat brain: Evidence for a role in cortical neuronal function." Neuroscience **116**(1):127-137.

Matsumoto, M., Weickert, C. S., Beltaifa, S., Kolachana, B., Chen, J., Hyde, T. M., Herman, M. M., Weinberer, D. R., and J. E. Kleinman (2003). "Catechol-O-methyltransferase (COMT) mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia." Neuropsychopharmacology **28**(8):1521-1530.

Mattay, V. S., Callicott, J. H., Bertolino, A., Heaton, I., Frank, J. A., Coppola, R., Berman, K. f., Goldberg, T. E., and D. R. Weinberger (2000). "Effects of dextroamphetamine on cognitive performance and cortical activation." Neuroimage **12**(3):268-275.

Mattay, V. S., Goldberg, T. E., Fera, F., Hariri, A. R., Tessitore, A., Egan, M. F., Kolachana, B., Callicott, J. H., and D. R. Weinberger (2003). "Catechol-O-methyltransferase Val158-Met genotype and individual variation in the brain response to amphetamine." Proceedings of the National Academy of Sciences of the United States of America **100**(10):6186-6191.

Mattay, V. S., Tessitore, A., Callicott, J. H., Bertolino, A., Goldberg, T. E., Chase, T. N., Hyde, T. M., and D. R. Weinberger (2002). "Dopaminergic modulation of cortical function in patients with Parkinson's disease." Annals of Neurology **51**(2):156-164.

Mattes, R.M., Schenider, F., Heimann, H., and N. Birbaumer (1995) “Reduced emotional response of shizophrenic patients in remission during social interaction” Schizophrenia research **17**(3):249-55

Maurer, D., Grand, R. L., and C. J. Mondloch (2002). "The many faces of configural processing." Trends in Cognitive Sciences **6**(6):255-260.

Mayberg, H. S., Liotti, M., Brannan, S. K., McGinnis, S., Mahurin, R. K., Jerabek, P. A., Silva, J. A., Tekell, J. L., Martin, C. C., Lancaster, J. L., and P. T. Fox (1999). "Reciprocal limbic-cortical function and negative mood: Converging PET findings in depression and normal sadness." American Journal of Psychiatry **156**(5):675-682.

McEwan, E., S. (2000). "Allostasis and allostatic load: implications for neuropsychopharmacology." Neuropsychopharmacology **22**(2):108-124

Mendez, M. A., Zuluaga, P., Hornero, R., Gomez, C., Escudero, J., Rodriquez-Palancas, A., Ortiz, T., and A Fernandez (2012). "Complexity analysis of spontaneous brain activity: effects of depression and antidepressant treatment." Journal of Psychopharmacology **26**(5):636-643.

Meyer-Lindenberg, A., Kohn, P. D., Kolachana, B., Kippenhan, S., McInerney-Leo, A., Nussbaum, R., Weinberger, D. R., and K. F. Berman (2005). "Midbrain dopamine and prefrontal function in humans: interaction and modulation by COMT genotype." Nature Neuroscience **8**(5):594-596.

Meyer-Lindenberg, A., Nichols, T., Callicott, J. H., Ding, J., Kolachana, B., Buckholtz, J., Mattay, V. S., Egan, M., and D. R. Weinberger (2006). "Impact of complex genetic variation in COMT on human brain function." Molecular Psychiatry **11** (9):867-877.

Mier, D., Kirsch, P., and A. Meyer-Lindenberg (2009). "Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis." Molecular Psychiatry. **15**(9):918-27

Millar, A., Smith, D., Choueiry, J., Fisher, D., Albert, P., and V. Knott (2011). "The moderating role of the dopamine transporter 1 gene on P50 sensory gating and its modulation by nicotine." Neuroscience **180**:148-156.

Miller, E. K. and J. D. Cohen (2001). "An integrative theory of prefrontal cortex function." Annual Review of Neuroscience **24**(1):167-202.

Mills, C. K. (1912). "The cortical representation of emotion, with a discussion of some points in the general nervous system mechanism of expression in its relation to organic nervous disease and insanity." Proceedings of American Medico-Psychological Association **19**(3):297.

Missonnier, P., Herrmann, F. R., Michon, A., Fazio-Costa, L., Gold, G., and F. Giannakopoulos (2010). "Early disturbances of gamma band dynamics in mild cognitive impairment." Journal of Neural Transmission **117**(4):489-498.

Mizrahi, R., Rusjan, P., gid, O., Graff, A., Mamo , D.M., Zipursky, R.S., and S. Kapur (2007) "Adverse subjective experience with antipsychotics and its relationship to striatal and extrastriatal D2 receptors: A PET study in schizophrenia." American

Journal of Pyschiatry **164**(4):630-637

Montague, P. R., Dayan, P., and T. J. Sejnowski (1996). "A framework for mesencephalic dopamine systems based on predictive hebbian learning." Journal of Neuroscience **16**(5):1936-1947.

Montojo, C.A., and S.M. Courtney (2008). "Differential neural activation for updating rule versus stimulus information in working memory" **59** (1):173-182

Morel, S., Beaucousin, V., Perrin, M., and N. George (2012). "Very early modulation of brain responses to neutral faces by a single prior association with an emotional context: Evidence from MEG." Neuroimage **61**(4):1461-1470.

Morris, J. S., Friston, K. J., Buchel, C., Frith, C. D., Young, A. W., Calder, A. J., and R. J. Dolan (1998). "A neuromodulatory role for the human amygdala in processing emotional facial expressions." Brain **121**(1):47-57

Mueller, E. M., Makeig, S., Stemmler, G., Henning, J., and J. Wacker (2011) "Dopamine effects on human error processing depend on catechol-O-methyltransferase Val 158Met genotype." Journal of Neuroscience **31**(44):15818-24

Muller, W. and J. A. Connor (1991). "Dendritic spines as individual neuronal compartments for synaptic Ca2+ responses." Nature **354**(6348):73-76.

Muly, E. C., Szigeti, K., and P.S. Goldman-Rakic (1998). "D1 receptor in interneurons of macaque prefrontal cortex: distribution and subcellular localization." Journal of Neuroscience **18**(24):10553-65

Mynett-Johnson, L. A., Murphy, V. E., Claffey, E., Shields, D. C., and P McKeon (1998). "Preliminary evidence of an association between bipolar disorder in females and the catechol-O-methyltransferase gene." Psychiatric Genetics **8**(4):221-5

Myohanen, T. T., Schendzielorz, N., and P. T. Mannisto (2010). "Distribution of catechol-o-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice." Journal of Neurochemistry **113**(6): 1632-1643.

Nackley, A. G., Shabalina, S. A., Lambert, J. E., Conrad M. S., Gibson, D. G., Spiridonov, A. N. Satterfield, S. K., and L. Diatchenko (2009). "Low enzymatic activity haplotypes of the human catechol-o-methyltransferase gene: enrichment for marker SNPs." PloS One **4**(4):e5237

Nackley, A. G., Shabalina, S. A., Tchivileva, I. E., Satterfield, K., Korchynskyi, O., Makarov, S. S. Maixner, W., and L. Diatchenko (2006). "Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure." Science **314**(5807):1930-1933.

Nelson (1982). "The National Adult Reading Test (NART): test manual: NFER-Nelson." NFER-Nelson.

Neubauer, A. C. and A. Fink (2009). "Intelligence and neural efficiency." Neuroscience and Biobehavioral Reviews **33**(7):1004-1023.

Nikolova, Y. S., Ferrell, R. E., Manuck, S. B., and A. R. Hariri (2011). "Multilocus genetic profile for dopamine signaling predicts ventral striatum reactivity." Neuropsychopharmacology **36**(9):1940-1947.

Nolan, K. A., Bilder, R. M., Lachman, H. M., and J. Volavka (2004). "Catechol O-methyltransferase Val158Met polymorphism in schizophrenia: differential effects of Val and Met alleles on cognitive stability and flexibility." American Journal Psychiatry **161**(2):359-361.

Northoff, G., Richter, A., Gessner, M., Schlagenhauf, F., Fell, J., Baumgart, F., Kaulisch, T., Kotter, R., Stephan, K. E., Leschinger, A., Hagner, T., Bargel, B., Witzel, T., Hinrichs, H., Bogerts, B., Scheich, H., and H. J. Heinze (2000). "Functional dissociation between medial and lateral prefrontal cortical spatiotemporal activation in negative and positive emotions: a combined fMRI/MEG study." Cerebral Cortex **10**(1):93-107.

Ohara, K., Nagai, M., Suzuki, Y., and K. Ohara (1998). "Low activity allele of catechol-o-methyltransferase gene and Japanese unipolar depression." NeuroReport **9**(7): 1305-1308.

Olanow, C. W. and W. G. Tatton (1999). "Etiology and pathogenesis of Parkinson's disease." Annual Review of Neuroscience **22**:123-144.

Olanow, C.W (2002). "The role of dopamine agonists in the treatment of early Parkinson's disease." Neurology **February 26**:58(4 suppl (1)):33-41.

Olanow, C. W. and P. B. Watkins (2007). "Tolcapone: an efficacy and safety review (2007)." Clinical Neuropharmacology **30**(5):287-294.

Olton, D. S. and B. C. Papas (1979). "Spatial memory and hippocampal function." Neuropsychologia **17**(6):669-682.

Orozco, S. and C. L. Ehlers (1998). "Gender differences in electrophysiological responses to facial stimuli." Biological Psychiatry **44**(4):281-289.

Owen, A. M. (2004). "Cognitive dysfunction in Parkinson's disease: the role of frontostriatal circuitry." Neuroscientist **10**(6):525-537.

Palladino, P. and M. Ferrari (2011). "Interference control in working memory: comparing groups of children with atypical development." Child Neuropsychology: [Epub ahead of print].

Palmatier, M. A., Kang, A. M. and K. K. Kidd (1999). "Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles." Biological Psychiatry **46**(4):557-567.

Papaleo, F., Crawley, J. N., Song, J., Lipska, B. K., Pickel, J., Weinberger, D. R., and J. Chen (2008). "Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice." Journal of Neuroscience **28**(35):8709-8723.

Parra, J., S. Kalitzin, S. N., and F. H. da Silva (2004). "Magnetoencephalography: an investigational tool or a routine clinical technique?" Epilepsy & Behavior **5**(3): 277-285.

Paulmann, S. and M. D. Pell (2009). "Facial expression decoding as a function of emotional meaning status: ERP evidence." NeuroReport **20**(18):1603-1608

Pelletier, M., Bouthillier, A ., Levesque, J., Carrier, S., Breault, C., Paquette, V., Mensour, B., Leroux, J. M., Beaudoin, G., Bourgouin, F and M. Beauregard (2003). "Separate neural circuits for primary emotions? Brain activity during self-induced sadness and happiness in professional actors." NeuroReport **14**(8):1111-1116.

Perlstein, W. M., Carter, C. S., Noll, D. C., and J. D. Cohen (2001). "Relation of Prefrontal cortex dysfunction to working memory and symptoms in schizophrenia." American Journal of Psychiatry **158**(7): 1105-13

Peron, J. and T. Dondaine (2011). "Emotional processing in Parkinson's disease: a systematic review". Movement Disorders **27**(2):186-189.

Pessiglione, M., Seymour, B., Flandin, G., Dolan, R. J., and C. D. Frith (2006). "Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans." Nature **442**(7106):1042-1045.

Pessoa, L., Kastner, S., and L. G., Ungerleider (2002). "Attentional control of the processing of neutral and emotional stimuli." Cognitive Brain Research **15**(1):31-45.

Pezze, M.A. and J. Feldon (2004). "Mesolimbic dopaminergic pathways in fear conditioning." Progress in Neurobiology **74**(5):301-20

Phan, K. L., Wager, T., Taylor, S. F., and I. Liberzon (2002). "Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI." Neuroimage **16**(2):331-348.

Phillips, M. L., Williams, L. M., Heining, M., Herba, C. M., Russell, T., Andrew, C., Bullmore, E. T., Brammer, M. J., Williams, S. C., Morgan, M., Young, A. W., and J. A. Gray (2004). "Differential neural responses to overt and covert presentations of facial expressions of fear and disgust." Neuroimage **21**(4):1484-1496.

Pizzagalli, D., Lehmann, D., Koenig, T., Regard, M., and R. D. Pascual-Marqui (2000). "Face-elicited ERPs and affective attitude: brain electric microstate and tomography analyses." Clinical Neurophysiology **111**(3):521-531.

Pizzagalli, D. A., Lehmann, D., Hendrick,A. M., Regard, M., Pascual-Marqui, R. D., and R. J. Davidson (2002). "Affective judgments of faces modulate early activity (not similar to 160 ms) within the fusiform gyri." Neuroimage **16**(3):663-677.

Polich, J. (2007). "Updating P300: An integrative theory of P3a and P3b." Clinical Neurophysiology **118**(10):2128-2148.

Pooley, E. C., Fineberg, N., and P. J. Harrison (2007). "The met(158) allele of catechol-O-methyltransferase (COMT) is associated with obsessive-compulsive disorder in men: case-control study and meta-analysis." Molecular Psychiatry **12**(6):556-561.

Posner, M.I., and S. Dehaene (1994). "Attentional networks" Trends in Neuroscience **17**(2):75-9

Prat, C. S., Keller, T. A., and M. A. Just (2007). "Individual differences in sentence comprehension: A functional magnetic resonance imaging investiagation of syntactic and lexical processing demands." Journal of Cognitive Neuroscience **19**(12):1950-1963.

Prata, D. P., Mechelli, A., Fu, C. H., Picchioni, M., Toulopoulou, T., Bramon, E., Walshe, M., Murray, R. M., Collier, D. A. and F. Mcguire (2009). "Epistasis between the DAT 3 ' UTR VNTR and the COMT Val158Met SNP on cortical function in healthy subjects and patients with schizophrenia." Proceedings of the National Academy of Sciences of the United States of America **106**(32):13600-13605.

Pribram, K. H. and W. E. Tubbs (1967). "Short-term memory, parsing, and the primate frontal cortex." Science **156**(3783):1765-1767.

Pycock, C. J., Kerwin, R. W., and C. J. Carter (1980). "Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats." Nature **286**(5768): 74-76.

Qin, S., Cousijn, H., Rijpkema, M., Luo, J., Franke, B., Hermans, E. J., and G. Fernandez (2012). "The effect of moderate acute psychological stress on working memory-related neural activity is modulated by a genetic variation in catecholaminergic function in humans." Frontiers In Integrative Neuroscience **6**:16.

Ragland, J. D., Turetsky, B. I., Gur, R. C., Gunning-Dixon, F., Turner, T., Schroeder, L., Chan, R., and R. E. Gur (2002). "Working memory for complex figures: An fMRI comparison of letter and fractal n-back tasks." Neuropsychology **16**(3):370-379.

Ramsey, N. F., Jansma, J. M., Jager, G., Van, Raalten, T., and R. S. Kahn (2004). "Neurophysiological factors in human information processing capacity." Brain **127**:517-525.

Rao, S. C., Rainer, G., and E. K. Miller (1997). "Integration of what and where in the primate prefrontal cortex." Science **276**(5313):821-4

Raxworthy, M. J., Gulliver, P. A., and P. J. Hughes (1982). "The cellular location of catechol-O-methyltransferase in rat-liver." Naunyn-Schmiedebergs Archives of Pharmacology **320**(2):182-188.

Rees, G., Friston, K., and C. Koch (2000). "A direct quantitative relationship between the functional properties of human and macaque V5." Nature Neuroscience **3**(7):716-723.

Reichle, E. D., Carpenter, P. A., and M. A. Just (2000). "The neural bases of strategy and skill in sentence-picture verification." Cognitive Psychology **40**(4):261-295.

Reiman, E. M., Lane, R. D., Ahern, G. L., Schwartz, G. E., Davidson, R. J., Friston, K. J., Yun, L. S. and K. Chen (1997). "Neuroanatomical correlates of externally and internally generated human emotion." American Journal of Psychiatry **154**(7): 918-925.

Reinhard, J. F., Bannon, M. J., and R. H. Roth (1982). "Acceleration by stress of dopamine synthesis and metabolism in prefrontal cortex: antagonism by diazepam." Naunyn-Schmiedebergs Archives of Pharmacology **318**(4):374-377.

Revay, R., Vaughan, R., Grant, S., and M. J. Kuhar (1996). "Dopamine transporter immunohistochemistry in median eminence, amygdala, and other areas of the rat brain." Synapse **22**(2):93-9

Robbins, T. W. (2000). "Chemical neuromodulation of frontal-executive functions in humans and other animals." Experimental Brain Research **133**(1):130-138.

Roberts, A. C., De Salvia, M. A., Wilkinnson, L. S., Collins, F., Muir, J. L., Everitt, B. J., and T. W. Robbins (1994). "6-Hydroxydopamine lesions of the prefrontal cortex in monkeys enhance performance on an analog of the Wisconsin Card Sort Test: possible interactions with subcortical dopamine." Journal of Neuroscience **14**(5 Pt 1):2531-2544.

Rosa, A., Peralta, V., Cuesta, K. J., Zarzuela, A., Serrano, F., Martinez-Larrea, A., and L. Fananas (2004). "New evidence of association between COMT gene and prefrontal neurocognitive function in healthy individuals from sibling pairs discordant for psychosis." American Journal of Psychiatry **161**(6):1110-1112.

Rossetti, Z. L. and S. Carboni (2005). "Noradrenaline and dopamine elevations in the rat prefrontal cortex in spatial working memory." Journal of Neuroscience **25**(9): 2322-2329.

Rossion, B. and S. Caharel (2011). "ERP evidence for the speed of face categorization in the human brain: Disentangling the contribution of low-level visual cues from face perception." Vision Research **51**(12):1297-1311.

Roth, J. A. (1992). "Membrane-bound catechol-O-methyltransferase – a reevaluation of its role in the O-Methylation of the catecholamine neurotransmitters." Reviews of Physiology Biochemistry and Pharmacology **120**:1-29.

Roth, R. H., Tam, S. Y., Ida, Y., Yang, J. X., and A. Y. Deutch (1988). "Stress and the mesocorticolimbic dopamine systems." Annals of the New York Academy of Sciences **537**:138-147.

Roussos, P., Giakoumaki, S. G., and P. Bitsios (2009). "Tolcapone effects on gating, working memory, and mood interact with the synonymous catechol-O-methyltransferase rs4818C/G polymorphism." Biological Psychiatry **66**(11):997-1004.

Rujescu, D., Giegling, I., Gietl, A., Hartmann, A. M., and H. J. Moller (2003). "A functional single nucleotide polymorphism (V158M) in the COMT gene is associated with aggressive personality traits." Biological Psychiatry **54**(1):34-39.

Rypma, B., Berger, J. S., and M. D'Esposito (2002). "The influence of working-memory demand and subject performance on prefrontal cortical activity." Journal of Cognitive Neuroscience **14**(5):721-731.

Sauseng, P., Klimesch, W., Schabus, M. and M. Doppelmayr (2005). "Fronto-parietal EEG coherence in theta and upper alpha reflect cetral executive fuctions of working memory". Iternational Journal of Psychophysiology **57** (2):97-103

Sawaguchi T, G.-R. P. (1991). "D1 dopamine receptors in prefrontal cortex: involvement in working memory." Science **251**:947-950.

Sawaguchi, T. and P. S. Goldman-Rakic (1991). "D1 dopamine receptors in prefrontal cortex: involvement in working memory." Science: **25**(4996):947-50

Schmack, K., F. Schlagenhauf, et al. (2008). "Catechol-O-methyltransferase Val(158)Met genotype influences neural processing of reward anticipation." Neuroimage **42**(4): 1631-1638.

Schott, B. H., Seidenbecher, C.I., Fenker, D.B., Lauer, C.J., Bunzeck, N., Bernstein, H.G., Tischmeyer, W., Gundelfinger, E.D., Heinze, H.J., and E. Duzel (2006). "The dopaminergic midbrain participates in human episodic memory formation: Evidence from genetic imaging." Journal of Neuroscience **26**(5):1407-1417.

Schultz, W. (1997). "Dopamine neurons and their role in reward mechanisms." Current Opinion in Neurobiology **7**(2):191-197.

Schultz, W. (2010). "Dopamine signals for reward value and risk: basic and recent data." Behavioral and Brain Functions **6**(24):1-9

Schultz, W., Dayan, P., and P.R. Montgue (1997). "A neural substrate of prediction and reward." Science **275**(5306):1593-1599.

Schweinberger, S. R., Pfütze, E.M., and W. Sommer (1995). "Repetition priming and associative priming of face recognition: evidence from event-related potentials." Journal of Experimental Psychology: Learning, Memory, and Cognition **21**(3):722-736.

Schweinberger, S. R., Pickering, E. C., Burton, A. M., and J. M. Kaufmann (2002). "Human brain potential correlates of repetition priming in face and name recognition." Neuropsychologia **40**(12):2057-2073.

Schweinberger, S. R., Pickering, E. C., Jentzsch, I., Burton, A., M., Kaufmann, and J. M. Kaufmann (2002). "Event-related brain potential evidence for a response of inferior temporal cortex to familiar face repetitions." Cognitive Brain Research **14**(3):398-409.

Seamans, J. K.,. Durstewitz, D., Christie, B. R., Stevens, C. F., and T. J. Sejnowski (2001). "Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons." Proceedings of the National Academy of Sciences of the United States of America **98**(1):301-306.

Seamans, J. K. and C. R. Yang (2004). "The principal features and mechanisms of dopamine modulation in the prefrontal cortex." Progress in Neurobiology **74**(1): 1-58.

Seeck, M., . Michel, C. M., Mainwaring, N., Cosgrove, R., Blume, H., Ives, J., Landis, T., and D. L. Schomer (1997). "Evidence for rapid face recognition from human scalp and intracranial electrodes." NeuroReport **8**(12):2749-2754.

Segalowitz, S. J., Wintink, A. J., and L. J. Cudmore (2001). "P3 topographical change with task familiarization and task complexity." Cognitive Brain Research **12**(3): 451-457.

Simon, N. W., Montgomery, K. S., Beas, B. S., Mitchell, M. R., LaSare, C. L., Mendez, I. A., Banuelos, C., Vokes, C. M., Taylor, A. B., Haberman, R. P., Bizon, J. L., and B. Selow (2010). "Dopamine modulation of risky decision-making." Journal of Neuroscience **31**(48):17460-17470

Singh, K.D. (2006) "Magnetoencephalography" In Senior, C., Russell, T., Gazzaniga, M.S., Methods in Mind. The MIT Press. **pp.291-326**

Singh, K. D. (2012). "Which "neural activity" do you mean? MRI, MEG, oscillations and neurotransmitters." Neuroimage **62**(2):1121-1130.

Slifstein, M., Kolachana, B., Simpson, E.H., and A. Abi-Dargham, (2008). "COMT genotype predicts cortical-limbic D1 receptor availability measured with [11C]NNC112 and PET." Molecular Psychiatry **13**:821-827.

Smolka, M. N., Schumann, G., Wrase, J., Grusser S. M., Flor, H., Mann, K., Braus, D. F., Goldman, D., Buchel, C., and A. Heinz (2005). "Catechol-O-methyltransferase Val 158 Met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex." Journal of Neuroscience **25**(4):836-842.

Spencer, K. M., Dien, J., and E. Donchin (1999). "A componential analysis of the ERP elicited by novel events using a dense electrode array." Psychophysiology **36**(3): 409-414.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., and G. A. Jacobs (1983). "Manual for the State-Trait Anxiety Inventory (Form Y)." Consulting Psychologists Press, Inc.: Palo Alto.

Sprengelmeyer R, Rausch M, Eysel, U. T., and H. Przuntek (1998). "Neural structures associated with recognition of facial expressions of basic emotions." Proceedings. Biological Sciences **265**(1409):1927-31

Stoffers, D., Bosboom, J. L., Wolters, Ech., Stam, C. J., and H. W. Berendse (2008). "Dopaminergic modulation of cortico-cortical functional connectivity in Parkinson's disease: an MEG study." Experimental Neurology **213**(1):191-195.

Stokes, P. R. A., Rhodes, R. A., Grasby, P. M., and M. A. Mehta (2011). "The effects of the COMT Val(108/158) Met polymorphism on BOLD activation during working memory, planning, and response inhibition: a role for the posterior cingulate cortex?" Neuropsychopharmacology **36**(4):763-771.

Streit, M., Wolwer, W., Brinkmeyer, J., Ihl, R., and W. Gaebel (2000). "Electrophysiological correlates of emotional and structural face processing in humans." Neuroscience Letters **278**(1-2):13-16.

Suhara, T., Fukuda, H., Inoue, O., Itoh, T., Suzuki, K., Yamasaki, T. and Y. Tateno (1991). "Age-related-changes in human D1-Dopamine receptors measured by positron emission tomography." Psychopharmacology **103**(1):41-45.

Surguladze, S. A., Brammer, M. J., Young, A. W., Andrew, C., Travis, M. J., Williams, S. C., and M. L. Phillips (2003). "A preferential increase in the extrastriate response to signals of danger." Neuroimage **19**(4):1317-1328.

Sutton, S., Tueting, P., Zubin, J., nd E. R. John (1967). "Information delivery and the sensory evoked potential" Science **155**(3768):1436-1439

Swithenby, S. J., Bailey, A. J. Braeutigam, S., Josephs, O. E., Jousmaki, V., and C. D. Tesche (1998). "Neural processing of human faces: a magnetoencephalographic study." Experimental Brain Research **118**(4):501-510.

Takahashi, H., M. Yamada, N., Koeda, M., Takano, A., Asai, K., Suhara, T., and Y. Okubo (2005) “Effects of dopaminergic and serotonergic manipulation on emotional processing: a pharmacological fMRI study” Neuroimage **27**(4):991-1001

Takahashi, H., Yamada, M., and T. Suhara (2012). "Functional significance of central D1 receptors in cognition: beyond working memory." Journal of Cerebral Blood Flow and Metabolism **32**(7):1248-1258.

Tan, H.-Y., Chen, Q., Goldberg, T. E., Mattay, V. S., Meyer-Lindenberg,A., Weinberger, D. R., and J. H. Callicott (2007). "Catechol-O-methyltransferase Val158Met modulation of prefrontal–parietal–striatal brain systems during arithmetic and temporal transformations in working memory." The Journal of Neuroscience **27**(49):13393-13401.

Tan, H. Y., Chen, Q., Sust, S., Buckholtz, J. W., Meyers, J. D., Egan, M. F., Mattay, V. S., Meyer-Lindenberg, A, Weinberger, D. R., and J. H. Callicott (2007). "Epistasis between catechol-O-methyltransferase and type II metabotropic glutamate receptor 3 genes on working memory brain function." Proceedings of the National Academy of Sciences of the United States of America **104**(30):12536-12541.

Tanaka, M., Yoshida, M., Emoto, H., and H. Ishii(2000). "Noradrenaline systems in the hypothalamus, amygdala and locus coeruleus are involved in the provocation of anxiety: basic studies." European Journal of Pharmacology **405**(1–3):397-406.

Tessitore, A., Hariri, A. R., Fera, F., Smith, W. G., Chase, T. N., Hyde, T. M., Weinberger, D. R., and V. S. Mattay (2002). "Dopamine modulates the response of the human amygdala: a study in Parkinson's disease." The Journal of Neuroscience **22**(20):9099-9103.

Tessitore, A., Hariri, A.R., Fera, F., Smith, W.G., Das, S., Weinberger, D.R., and V.S. Mattay (2005) "Functional changes in the activity of brain regions underlying emotion processing in the elderly" Psychiatry Research **139**(1):9-18

Tiihonen, J., Hallikainen, T., Lachman, H., Saito, T., Volavka, J., Kauhanen, J., Salonen, J. T., Ryynanen, O. P., Koulu, M., Karvonen, M. K., Pohjalainen, T., Syvalahti, E., and J. Hietala (1999). "Association between the functional variant of the catechol-O-methyltransferase (COMT) gene and type 1 alcoholism." MolecularPsychiatry. **4**(3):286-289

Tottenham, N., Borscheid, A., Ellertsen, K., Marcus, D. J., and C. A. Nelson (2002). "Categorization of facial expressions in children and adults: Establishing a larger stimulus set. [Abstract] " Journal of Cognitive Neuroscience: **14** 74.

Trantham-Davidson, H., Neely, L. C., Lavin, A., Seamans, J. K (2004). "Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex." The Journal of Neuroscience **24**(47):10652-10659.

Tsai, S. J., Yu, Y. W., Chen, T.J., Liou, Y.J., Chen, M.C., and C.J. Hong (2003). "Association study of a functional catechol-O-methyltransferase-gene polymorphism and cognitive function in healthy females." Neuroscience letters **338**(2):123-126.

Tseng, K. Y. and P. O'Donnell (2004). "Dopamine–glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms." The Journal of Neuroscience **24**(22):5131-5139.

Tunbridge, E., P. Burnet, W. J., Sodhi, M. S., and P. J. Harrison (2004). "Catechol-o-methyltransferase (COMT) and proline dehydrogenase (PRODH) mRNAs in the dorsolateral prefrontal cortex in schizophrenia, bipolar disorder, and major depression." Synapse **51**(2):112-118.

Tunbridge, E. M., Bannerman, D. M., Sodhi, M. S., and P. J. Harrison (2004). "Catechol-O-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex." Journal of Neuroscience **24**(23):5331-5335.

Tunbridge, E. M., Harrison, P. J., and D. R. Weinberger (2006). "Catechol-o-methyltransferase, cognition, and psychosis: Val 158 Met and beyond."Biological Psychiatry **60**(2):141-151.

Tunbridge, E. M., Huber, A. Farrell, S. M., Stumpenhorst, K., Harrison, P. J., and M. E. Walton (2012). "The role of catechol-O-methyltransferase in reward processing and addiction." CNS & Neurological Disorders-Drug Targets **11**(3):306-323.

Tunbridge, E. M., Weickert, C. S., Kleinman, J. E. Herman, M. M., Chen, J., Kolachana, B. S., Harrison, P. J., and D. R. Weinberger (2007). "catechol-O-methyltransferase enzyme activity and protein expression in human prefrontal cortex across the postnatal lifespan." Cerebral Cortex **17**(5):1206-1212.

Tuomainen, P., Tornwall, M., and P. T. Mannisto (1996). "Minor effect of tolcapone, a catechol-O-methyltransferase inhibitor, on extracellular dopamine levels modified by amphetamine or pargyline: A microdialysis study in anaesthetized rats." Pharmacology & Toxicology **78**(6):392-396.

Tversky, A. and D. Kahneman (1992). "Advances in prospect-theory – cumulative representation of uncertainty." Journal of Risk and Uncertainty **5**(4):297-323.

Ulmanen, I., Peranen, J., Tenhunen, J., Tilgmann, C., Karhunen, T., Panula, F., Bernasconi, L., Aubry, J. F., and K. Lundstrom (1997). "Expression and intracellular localization of catechol-O-methyltransferase in transfected mammalian cells." European Journal of Biochemistry **243**(1-2):452-459.

Ursini, G., Bollati, V., Fazio, L. Porcelli, A., Iacovelli, L., Catalani, A., Sinibaldi, L., Gelao, B., Romano, R., Rampino, A., Taurisano, P., Mancini, M., Di Giorio, A., Popolizio, T., Baccarelli, A., De Blasi, A., Blasi, G., and A. Bertolino (2011). "Stress-related methylation of the catechol-O-methyltransferase Val158 allele predicts human prefrontal cognition and activity." The Journal of Neuroscience **31**(18):6692-6698.

van den Bos, R., Homberg, J., Gijsbers, E., den Heijer, E., and E. Cuppen (2009). "The effect of COMT Val(158) Met genotype on decision-making and preliminary findings on its interaction with the 5-HTTLPR in healthy females." Neuropharmacology **56**(2):493-498.

van der Schaaf, M. E., Warmerdam, E., Crone, E. A., and R. Cools (2011). "Distinct linear and non-linear trajectories of reward and punishment reversal learning during development: Relevance for dopamine's role in adolescent decision making." Developmental Cognitive Neuroscience **1**(4):578-590.

van der Veen, F. M., Evers, E. A. T., Deutz, N. E., and J. A. Schmitt (2007). "Effects of acute tryptophan depletion on mood and facial emotion perception related brain activation and performance in healthy women with and without a family history of depression." Neuropsychopharmacology **32**(1):216-224.

Vaz-Serra, A., Palha, A., Figueira, M. L., Bessa-Peixoto, A., Brissos,S., Casquinha, P., Damas-Reis, F., Ferreira, L., Gago, J., Jara, J., Relvas, J., and J. Marques-Teixeira (2010). "Cognition, social cognition and functioning in schizophrenia." Acta Med Port **23**(6):1043-1058

Vitouch, O., Bauer, H., Gittler, G., Leodolter, M., and U. Leodolter (1997). "Cortical activity of good and poor spatial test performers during spatial and verbal processing studied with Slow Potential Topography." International Journal of Psychophysiology **27**(3):183-199.

Volavka, J., Kennedy, J. L., Ni, X., Czobor, P., Nolan,K., Sheitman, B., Lindenmayer, J. P. Citrome, L., McEvoy, J., and J. A. Lieberman (2004). "COMT158 polymorphism and hostility." American Journal of Medical Genetics **127B**(1):28-29.

Volkow, N. D., Ding, Y. S., Fowler, J. S., Wang, G J., Logan, J., Gatley, S. J., Hitzemann, R., Smith, G., Fields, S. D., and R. Gur (1996). "Dopamine transporters decrease with age." Journal of Nuclear Medicine **37**(4):554-559.

Volkow, N. D., Wang, C. J., Fowler, J. S., Logan, J., Gatley, S. J., MacGregor, R. R., Schlyer, D. J., Hizemann, R., and A. F. Wolf (1996). "Measuring age-related changes in dopamine D2 receptors with 11C-raclopride and 18F-N-methylspiroperidol." Psychiatry Research: Neuroimaging **67**(1):11-16.

Vrba, J. and S. E. Robinson (2001). "Signal processing in magnetoencephalography." Methods **25**(2):249-271.

Vuilleumier, P., Armony, J. L., Clarke, K., Husain, M., Driver, J., and R. J. Dolan (2002). "Neural response to emotional faces with and without awareness: event-related fMRI in a parietal patient with visual extinction and spatial neglect." Neuropsychologia **40**(12):2156-2166.

Weickert, T. W., Goldberg, T. E., Mishara, A., Apud, J. A., Kolachana, B. S., Egan, M. F., and D. R. Weinberger (2004). "Catechol-O-methyltransferase Val 108/158 Met genotype predicts working memory response to antipsychotic medications." Biological Psychiatry **56**(9):677-682.

Weinberger, D. R. (1987). "Implications of normal brain development for the pathogenesis of schizophrenia." Archives of General Psychiatry **44**(7):9.

Weinberger, D. R., Berman, K. F., and B. P. Illowsky (1988). "Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia: III. A new cohort and evidence for a monoaminergic mechanism." Archives of General Psychiatry **45**(7):609-615.

Weissman, D. H., Woldorff, M. G., Hazlett, C. J., and G. R. Mangun (2002). "Effects of practice on executive control investigated with fMRI." Brain research. Cognitive brain research **15**(1): 47-60.

Whalen, P. J., Rauch, S. L., Etcoff, N. L., McInerney, S. C., Lee, M. B., and M. A. Jenike (1998). "Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge." The Journal of Neuroscience **18**(1):411-418.

Whittaker, J.F., Deakin, J.F., and B. Tomenson (2001) "Face processing in schizophrenia: defining the deficit" Psychological medicine **31**(3):499-507

Wieser, M. J., Muhlberger, A., Alpers, G. W., Macht, M., Ellgring, H., and F. Pauli (2006). "Emotion processing in Parkinson's disease: dissociation between early neuronal processing and explicit ratings." Clinical Neurophysiology **117**(1):94-102.

Williams, G. V. and P. S. Goldman-Rakic (1995). "Modulation of memory fields by dopamine Dl receptors in prefrontal cortex." Nature **376**(6541):572-575.

Williams, M. A. and J. B. Mattingley (2006). "Do angry men get noticed?" Current Biology **16**(11):402-404

Willner, P., Hale, A.S., and S.J Argyrapoulos (2005) "Dopaminergic mechanism of antidepressant action in depressed patients. Journal of affective disorders **86**(1):37-45

Wilson, T. W., Wetzel, M. W., White, M. L., and N. L. Knott (2012). "Gamma-frequency neuronal activity is diminished in adults with attention-deficit/hyperactivity disorder: a pharmaco-MEG study." Journal of Psychopharmacology **26**(6):771-777.

Winterer, G., Coppola, R., Goldberg, T. E., Egan, M. F., Jones, D. W., Sanchez, C. E., and D. R. Weinberer (2004). "Prefrontal broadband noise, working memory, and genetic risk for schizophrenia." American Journal of Psychiatry **161**(3):490-500.

Winterer, G., Egan, M. F., Kolachana, B. S., Goldberg, T. E., Coppola, R., and D. R. Weinberger (2006). "Prefrontal electrophysiologic "noise" and catechol-O-methyltransferase genotype in schizophrenia." Biological Psychiatry **60**(6):578-584.

Winterer, G., Musso, F., Vucurevic, G., Stoeter, P, Konrad, A., Seker, B., Gallinat, J., Dahmen, N., and D. R. Weinberger (2006). "COMT genotype predicts BOLD signal and noise characteristics in prefrontal circuits." Neuroimage **32**(4):1722-1732.

Winterer, G. and D. R. Weinberger (2003). "Cortical signal-to-noise ratio: insight into the pathophysiology and genetics of schizophrenia." Clinical Neuroscience Research **3**:55-66.

Winterer, G. and D. R. Weinberger (2004b). "Genes, dopamine and cortical signal-to-noise ratio in schizophrenia." Trends in Neurosciences **27**(11):683-690.

Wise, R. A. (2004). "Dopamine, learning and motivation." Nature Reviews Neuroscience **5**(6):483-494.

Woo, J.-M., Yoon, K. S., Choi, Y. H., Oh, K. S., Lee, Y. S., and B. H. Yu (2004). "The association between panic disorder and the L/L genotype of catechol-O-methyltransferase." Journal of Psychiatric Research **38**(4):365-370.

Wronka, E. and W. Walentowska (2011). "Attention modulates emotional expression processing." Psychophysiology **48**(8):1047-1056.

Yacubian, J., Sommer, T., Schroeder, K., Glascher, J., Kalisch, R., Leuenberger, B., Braus, D. F., and C. Buchel (2007). "Gene-gene interaction associated with neural reward sensitivity." Proceedings of the National Academy of Sciences of the United States of America **104**(19): 8125-8130.

Yamamoto, M., Yokochi, M., Kuno, S., Hattori, Y., Tsukamoto, Y., Narabayashi, H., Tohgi, H., Mizuno, Y., Kowa, H., Yanagisawa, N. and I. Kanazawa(1997). "Effects of tolcapone, a catechol-O-methyltransferase inhibitor, on motor symptoms and pharmacokinetics of levodopa in patients with Parkinson's disease." Journal of Neural Transmission **104**(2):229-236.

Yang, C. and J. Seamans (1996). "Dopamine D1 receptor actions in layers V-VI rat prefrontal cortex neurons in vitro: modulation of dendritic-somatic signal integration." The Journal of Neuroscience **16**(5):1922-1935.

Yang, T. T., Menon, V., Eliez, S., Blasey, C. D., Reid, A. J., Gotlib, I. H., and A. L. Reiss (2002). "Amygdalar activation associated with positive and negative facial expressions." NeuroReport **13**(14):1737-1741.

Yavich, L., Forsberg, M. M., Karayiorgou, M., Gogos, J. A., and P. T. Mannisto (2007). "Site-specific role of catechol-O-methyltransferase in dopamine overflow within prefrontal cortex and dorsal striatum." Journal of Neuroscience **27**(38): 10196-10209.

Yu, C., Wu T., Deng W.Wong, G. and X. Sun, (2009) “Comparison of visual evoked-related potentials in healthy young adults of different catechol-O-methyltransferase genotypes in a continuous 3-back task.” NeuroReport **20**(5): 521-4

Yu, R., X. N. Zhang, Huang, X. X., Ding, S. F., and J. C. Li (2007). "Association analysis of COMT polymorphisms and schizophrenia in a Chinese Han population: A case-control study." American Journal of Medical Genetics Part B-Neuropsychiatric Genetics **144B**(4):570-573.

Zahrt, J., Taylor, J. R., Mathew, R. G., and A. F. Arnsten (1997). "Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance." Journal of Neuroscience **17**(21): 8528-8535.

Zanto, T. P., Rubens, M. T., Thangavel, A., and A. Gazzaley (2011). "Causal role of the prefrontal cortex in top-down modulation of visual processing and working memory." Nature Neuroscience **14**(5):656-661

Zheng, P., Zhang, X. X., Bunney, B. S., and W. X. Shi (1999). "Opposite modulation of cortical N-methyl-d-aspartate receptor-mediated responses by low and high concentrations of dopamine." Neuroscience **91**(2):527-535.

Zubieta, J. K., Heitzeg, M. M., Smith, Y. R., Bueller, J. A., Xu, K., Xu, Y., Koeppe, R. A., Stohler, C. S., and D. Goldman (2003). "COMT Val158Met genotype affects mu-opioid neurotransmitter responses to a pain stressor." Science **299**(5610): 1240-1243.