Separate and Interactive Effects of Catechol-O-Methyltransferase and Tetrahydrocannabinol on Frontostriatal Dopamine Function



Katharina Stumpenhorst

St. Hilda's College

University of Oxford

A thesis submitted for the degree of

Doctor of Philosophy

Trinity Term 2017

Acknowledgements

I would like to thank all of the people who helped me so much during the course of my DPhil, and without whom getting to the finishing line would have been indefinitely more difficult.

To start with, my heartfelt thanks goes to my triumvirate of supervisors, Elizabeth Tunbridge, David Bannerman and Paul Harrison.

Liz, thank you for believing in me and for providing me with encouragement and guidance throughout my doctorate. David, your insights into animal behaviour, and your willingness to always make time for your students, has been a constant inspiration to me. Paul, thank you for your intellectual input and for pointing me towards Liz in the first place.

I would also like to extend my special thanks to Trevor Sharp for adopting me into his lab, Christmas dinners and BBQs at his house, as well as HPLC guidance.

This project would not have been possible without the Wellcome Trust, which not only funded me, but also provided me with a generous amount of research money – thank you very much.

The members of all the awesome labs that I have been lucky enough to be a part of have provided me with companionship, fun, and support during the highs and lows of scientific research.

Thanks to Mary Walker and Li Chen for helping out with the vast amounts of genotyping and brain slicing.

Thanks to Jill Betts for sharing HPLC frustrations.

Thanks to Ivi Antoniadou for entertaining evening conversations.

Thanks to Chris Barkus for taking Ivi's place when she left, with topics ranging from cats to politics.

Thanks to Florence, my favourite city.

Thanks to Judith Schweimer, for being a great friend and for sharing my passion for the Royal Opera House.

Special thanks to Mikko Käenmäki from the University of Helsinki without whom my start in the world of HPLC and microdialysis would have been a lot tougher.

Thank you to all of my friends who have made Oxford such an enjoyable experience.

Liliana Capitao, thanks for going through hell and back with me.

Thanks to Anna and Steve providing me with shelter and cheese. I will never forget our long evenings in the B.S.B. – work (and life in Oxford) just wouldn't have been the same without you.

Thanks to Konstantin Huber for providing the greatest intellectual input to my thesis. I hope you will soon grow out of your nappies.

Thanks to Felix Raulf, for always being understanding, patient, and unceasingly supportive. I wouldn't have come this far without you.

Most importantly, I would like to thank, and dedicate this work, to my family. To Miriam, for simply being the best sister I could wish for. And to Mum and Dad, I can't even start to thank you enough for your unceasing guidance, love, and support not only throughout this DPhil but throughout my life. You are the best!

Thank you.

Abstract

The frontostriatal dopamine system modulates brain function and is affected by both genetic and environmental factors. Dysfunction of this system is associated with many pathological states, including schizophrenia. The enzyme catechol-O-methyltransferase (COMT) metabolises dopamine and its gene contains a polymorphism ($Val^{158}Met$) that affects enzyme activity. Delta-9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis, has been suggested to interact with this polymorphism to increase the risk for psychosis and cognitive impairments. Dopaminergic mechanisms are a plausible candidate for mediating this interaction.

I used microdialysis coupled with high performance liquid chromatography (HPLC) to examine the effects of THC on extracellular dopamine and its metabolites in the nucleus accumbens, dorsal striatum and medial prefrontal cortex (mPFC) in freely moving mice. Following acute COMT inhibition with tolcapone, THC increased extracellular dopamine levels in the nucleus accumbens in tolcapone-, but not in vehicle-, treated mice. The introduction of the low activity Met allele into the COMT gene produced a highly specific, novel mouse model of the $Val^{158}Met$ polymorphism. In contrast to the effects of acute COMT inhibition, the Met allele protected against THC-induced changes in accumbal dopamine. No interactive neurochemical effects were observed in the dorsal striatum (pharmacological and genetic study) or in a preliminary study of the mPFC (genetic study only). On a progressive ratio task measuring motivational salience, the direction of the interactive effect between COMT genotype and THC differed between 2 independent cohorts and provided tentative leads that stress/arousal-dependent effects on COMT may have a confounding effect.

My data provide evidence that COMT activity modulates the effect of THC on accumbal dopamine function, and suggest the mechanism through which this interaction is mediated differs between acute and lifelong reduction in COMT activity. Through the interactive effect on the dopaminergic system, the data provide a potential mechanism for the reported interaction between COMT and cannabis/THC in determining psychosis risk and cognitive impairments.

Outline

Title page i

Acknowledgements ii

Abstract iv

Outline v

List of Abbreviations: viii

1. General Introduction 1
1.1 The dopaminergic system 1
1.1.1 Dopamine biosynthesis, release and inactivation 1
1.1.2 Dopaminergic projections 3
1.1.3 Dopamine function 4
1.2 Catechol-O-methyltransferase 15
1.2.1 Structure and expression 16
1.2.2 Cellular and subcellular location of COMT 17
1.2.3 Presence and importance of COMT across brain regions 19
1.2.4 COMT Val^{158}Met functional polymorphism 21
1.3 Cannabis 29
1.3.1 Mechanism and location of THC action 30
1.3.2 Effects of THC on the dopaminergic system 31
1.3.3 THC, the endocannabinoid system and behaviour 34
1.4 COMT, cannabis, and schizophrenia: a gene x environment interaction? . 40
1.5 Aims and objectives 44

2 Effect of acute COMT inhibition and THC on extracellular dopamine and metabolite levels 46
2.1 Introduction 46
2.1.1 Tolcapone 47
2.1.2 Tolcapone and cannabinoid receptor agonists 49
2.1.3 Choice of methodology 50
2.1.4 Hypotheses 53
2.2 Methods 54
2.2.1 Animals: 54
2.2.2 Stereotactic surgery: 54
2.2.3 Microdialysis: 54
2.2.4 Quantification of extracellular dopamine and dopamine metabolite concentrations: 56
2.2.5 Drug injections 56
2.2.6 Data analysis: 57
2.3 Results 58
2.3.1 Nucleus accumbens 58
2.3.2 Dorsal striatum 61
2.4 Discussion 64

3 Characterisation of COMT-Met transgenic mouse model 69
3.1 Introduction 69
3.1.1 Hypotheses 72
3.2 Methods 73
3.2.1 Animals 73
3.2.2 Microarrays 73
3.2.3 Tissue sample preparation and neurochemical analysis 75
3.3 Results 77
3.3.1 Effects of COMT-Met transgene on gene expression 77
3.3.2 Neurochemical changes in COMT-Met mice 80
3.4 Discussion: 82

4 Effect of THC on extracellular dopamine levels in COMT-Met and wild-type mice 87
4.1 Introduction 87
4.1.1 Hypothesis 90
4.2 Methods 91
4.2.1 Animals: 91
4.2.2 Stereotactic surgery: 91
4.2.3 Microdialysis: 91
4.2.4 Quantification of extracellular dopamine and dopamine metabolite concentrations: 92
4.2.5 Drug injections 93
4.2.6 Data analysis: 94
4.3 Results 96
4.3.1 Nucleus accumbens 96
4.3.2 Dorsal striatum 99
4.3.3 Medial prefrontal cortex 101
4.4 Discussion 105

5 Behaviour 116
5.1 Introduction 116
5.1.1 Progressive ratio 116
5.1.2 Sucrose Preference 119
5.1.3 Cognitive flexibility - Reversal learning 119
5.1.4 Sensorimotor gating 121
5.1.5 Locomotor activity 122
5.1.6 Hypotheses 123
5.2 Methods 124
5.2.1 Animals 124
5.2.2 Drug Injections 125
5.2.3 Behaviour tests 125
5.2.4 Statistical Analysis 133
5.3 Results 135
5.3.1 Progressive ratio 135
5.3.2 Reward Devaluation 138
5.3.3 Reversal learning 139
5.3.4 Sucrose preference 140
5.3.5 Locomotor activity task 141
5.3.6 Sensorimotor gating 143

5.4 Discussion 145
5.4.1 Progressive ratio 145
5.4.2 Reward devaluation 149
5.4.3 Reversal learning 149
5.4.4 Sucrose preference 150
5.4.5 Locomotor activity 150
5.4.6 Sensory motor gating 152
5.5 Conclusion 153

6 General Discussion 155
6.1 Summary of main findings 155
6.2 Implications of findings 162
6.2.1 Implication of findings regarding the proposed gene x environment interaction 162
6.2.1.1 Implication of findings regarding COMT's role in subcortical brain regions 163
6.3 Outstanding questions and areas of future research 164
6.3.1 What are the mechanisms that mediate the COMT genotype-dependent effects in the nucleus accumbens? 164
6.3.2 Effects of chronic THC exposure during neurodevelopment 166
6.3.3 COMT, cannabis and stress 167
6.4 Conclusion 168

7 Supplementary material: 170
7.1 Schematic overview of microdialysis probe locations 170
7.2 Summary of exclusion criterions and animals 171
7.3 Progressive ratio – number of active lever presses 172
7.4 Reversal learning – sex differences 173
7.5 Locomotor activity – sex differences 174
7.6 Startle response - sex differences 175

8 References 176

List of Abbreviations:

AD	*Aldehyde dehydrogenase*
CAPE	*Community Assessment of Psychic Experiences*
CB	*Cannabinoid*
CNVs	*Copy number variants*
COMT	*Catechol-O-methyltransferase*
DAT	*Dopamine transporter*
DβH	*Dopamine β-hydroxylase*
DDC	*DOPA-decarboxylase*
dHippo	*Dorsal hippocampus*
DHPA	*3,4-di-hydroxyphenylacetaldehyde*
DOPAC	*3,4-dihydroxy-phenylacetic acid*
dST	*Dorsal striatum*
EDTA	*Ethylenediamide-tetraacetic acid*
EMT	*Extra-neuronal monoamine transporter*
FCV	*Fast scan cyclic voltammetry*
FR	*Fixed ratio*
GABA	*Gamma-Aminobutyric acid*
HPLC	*High performance liquid chromatography*
HVA	*Homovanillic acid*
KO	*Knockout*
L-DOPA	*3,4-dihydrocyphenylalanine*
MAO	*Monoamine oxidase*
MAP	*Mitogen-activated protein*
MB-COMT	*Membrane-bound COMT*
MHPA	*3-methoxy-4-hydroxyphenylacet-aldehyde*
mPFC	*Medial prefrontal cortex*
NAc	*Nucleus accumbens*
NET	*Noradrenaline transporter*
PET	*Positron emission tomography*
PFC	*Prefrontal cortex*
PNMT	*Phenylethanolamine-N-methyltrasnferase*
PPI	*Prepulse inhibition*
PR	*Progressive ratio*
RMTg	*Rostromedial tegmental nucleus*
SAH	*S-adenosyl-L-homocysteine*
SAM	*S-adenosyl-L-methionine*
S-COMT	*Soluable COMT*
SNP	*Single nucleotide polymorphism*
SPECT	*Single-photon emission computer tomographic*
TH	*Tyrosine hydroxylase*
THC	*Delta-9-tetrahydrocannabinol*
vHippo	*Ventral hippocampus*
VMAT	*Vesicular monoamine transporter*
VTA	*Ventral tegmental area*
WCST	*Wisconsin Card Sorting Test*
3-MT	*3-methoxytyramine*
5-HIAA	*5-Hydroxyindoleacetic acid*

1. General Introduction

The primary psychoactive component of cannabis, delta-9-tetrahydrocannbinol (THC), may interact with a polymorphism in the catechol-*O*-methyltransferase (COMT) gene ($Val^{158}Met$) to increase the risk of psychosis and cognitive impairment. Dopaminergic mechanisms are an attractive candidate for mediating this interaction. This thesis therefore investigates the separate and interactive effects of COMT activity and THC on extracellular dopamine levels in three different brain regions, namely the dorsal and ventral striatum and the medial prefrontal cortex (mPFC), as well as their effect on behaviour. In this chapter I will first briefly introduce the dopaminergic system and the current understanding of its role in reward processing, cognitive function, and psychosis. I will then focus on COMT and cannabis/THC and how they may interact with the dopaminergic system, before presenting the objectives of the work summarised in this thesis.

1.1 The dopaminergic system

1.1.1 Dopamine biosynthesis, release and inactivation

Dopamine is a catecholamine, which can act as a neurotransmitter within the brain. Like all catecholamines, dopamine is synthesised from the amino acid precursor tyrosine (Figure 1).

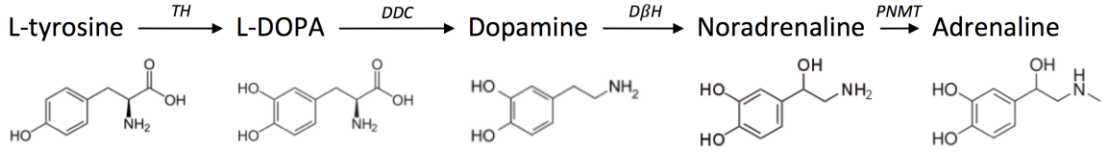


Figure 1: Catecholamine synthesis. *The catecholamines dopamine, noradrenaline and adrenaline are synthesised from the amino acid precursor L-tyrosine. The rate-limiting step in their formation is under the control of the enzyme tyrosine hydroxylase (TH). L-DOPA, 3,4-dihydroxyphenylalanine; DDC, DOPA-decarboxylase; DβH, dopamine β-hydroxylase; PNMT, phenylethanolamine-N-methyltrasnferase*

After synthesis, dopamine is transported into synaptic vesicles via the vesicular monoamine transporter (VMAT), where it is stored until its release via exocytosis. Once in the extracellular space, dopamine can act via 5 different subtypes of dopamine receptors (D1 – D5). These can be located either presynaptically (where they act as autoreceptors) or postsynaptically. All dopamine receptors are G-protein coupled, metabotropic receptors. They can be divided into 2 functional classes: the D1-like receptors (D1 and D5) are positively coupled to the adenylate cyclase signalling cascade and hence exhibit stimulatory effects, whilst the D2-like receptors (D2, D3 and D4) are negatively coupled to the adenylate cyclase signalling cascade and thus induce inhibitory effects. The D1- and D2-like receptors show distinct expression profiles; for example, D1-like receptors are particularly prominent in the PFC (Goldman-Rakic et al., 1990).

In order to terminate signalling, dopamine can be removed from the extracellular space by the dopamine transporter (DAT), the extra-neuronal monoamine transporter (EMT) or the noradrenaline transporter (NET), which also has a high affinity for dopamine (Morón et al., 2002; Jonker and Schinkel, 2004). Dopamine that enters the presynaptic side can be re-packaged into vesicles and recycled. Alternatively, it can be degraded via different enzymatic

routes, all of which involve the enzyme COMT (Figure 2). The relative importance of degradation versus reuptake varies between brain regions and will be discussed in more detail in chapter 1.2.3.

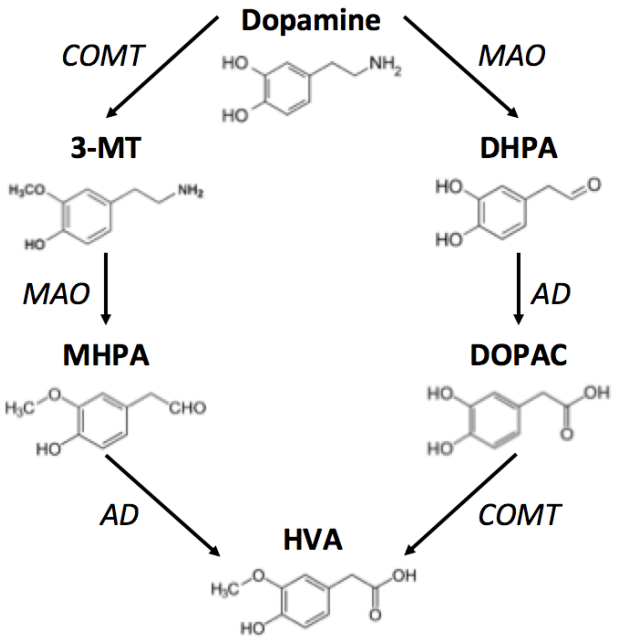


Figure 2: Dopamine catabolism. *Dopamine is degraded by the enzymes catechol-O-methyltransferase (COMT), monoamine oxidase (MAO), and aldehyde dehydrogenase (AD). The intermediate products are 3-methoxytyramine (3-MT), 3,4-di-hydroxyphenylacetaldehyde (DHPA), 3-methoxy-4-hydroxyphenylacet-aldehyde (MHPA), and 3,4-dihydroxy-phenylacetic acid (DOPAC). The end product is homovanillic acid (HVA).*

1.1.2 Dopaminergic projections

Generally speaking, there are four principal dopaminergic pathways in the brain: (1) The tuberinfundibular pathway projects from the hypothalamus to the pituitary gland and is involved in the secretion of the peptide hormones prolactin and somatotrophin. (2) The nigrostriatal pathway projects from the substantia nigra to the dorsal striatum and mediates motor control and cognitive functions. (3) The mesolimbic pathway projects primarily from the ventral tegmental area to the nucleus accumbens and the tuberculum olfactorium and mediates reward-related states, arousal and locomotor activity. (4) The mesocortical pathway projects primarily from the ventral tegmental area to a wide spectrum of structures including the prefrontal cortex, hippocampus and amygdala and is involved in higher cognitive function. However, these pathways

are not entirely independent from each other. This can be exemplified by the presence of frontostriatal circuits. These are topographical projections of different PFC regions onto functionally inter-related regions in the striatum (Chudasama and Robbins, 2006; Alexander et al., 1986). Studies as early as the 1980s showed that dopamine lesions in the PFC increase dopamine levels in the striatal regions (Pycock et al., 1980). Conversely, D2 receptor overexpression in the striatum increases dopamine levels and D1 receptor activation in the PFC (Kellendonk et al., 2006). In addition, there is emerging evidence for cross-talk and integration of information across these cortico-striatal loops (Draganski et al., 2008; Di Martino et al., 2008; Kehagia et al., 2010). The dopaminergic system is hence a very complex one, which targets a variety of different brain regions and is involved in a large number of brain functions, some of which will be discussed below.

1.1.3 Dopamine function

The dopaminergic system is involved in a number of brain functions including reward processing, cognition, motor control and pain. Here, I will focus on cognitive function, reward processing and psychosis, since these are the most relevant for the presented studies.

1.1.3.1 Dopamine and reward processing

Dopamine has long been known to play an important role in processes associated with reward and reinforcement. Though multiple brain regions are involved in different aspects of reward processing, the mesolimbic dopamine pathway has been the focus of many studies due to prominent dopaminergic reward responses observed in the nucleus accumbens. These dopamine

responses were initially believed to mediate hedonic pleasure (Wise et al., 1978). However, several subsequent findings such as the observation that dopamine release can precede the hedonic experience led to the development of alternative theories (Fibiger and Phillips, 1986).

One influential theory suggests that the firing of midbrain dopamine neurons represents a reward prediction error, a teaching signal used in reinforcement learning, which encodes the discrepancy between a reward (or more generally a stimulus) and its prediction (Schultz, 1997). Midbrain dopaminergic neurons can fire in two modes: 'tonic', low activity firing, which leads to steady baseline dopamine levels in the target area, and 'phasic' bursts of activity, which sharply increase dopamine levels. In brief, Schultz and colleagues showed that (1) the occurrence of an unexpected reward resulted in a phasic increase in dopamine activity, (2) the occurrence of an expected reward did not alter dopamine activity, and (3) the absence of an expected reward resulted in a decrease in the firing rate. These findings have consistently been replicated and, additionally, the dopamine signals appear sensitive to the quantity, quality, probability, and timing of the reward or cue that provides information about a future reward. (Schultz, 1997; Fiorillo et al., 2003; Satoh et al., 2003; Tobler et al., 2005; Roesch and Olson, 2007). Tonic activity has been suggested to modulate these phasic responses (Grace, 1991).

The incentive salience model, on the other hand, suggests that the role of dopamine in reward processing is the attribution of value to reward-predicting cues, thereby generating incentive salience or 'wanting' - a quality that makes the conditioned or unconditioned stimulus a desirable goal and hence captures

attention and can create motivation or drive towards this outcome (Robinson and Berridge, 1993; Berridge and Robinson, 1998; Berridge, 2006). This 'wanting' can clearly be dissociated from hedonic pleasure or 'liking' not only at a behavioural, but also at a neurochemical level. Contrary to the original hypothesis, hedonic reward responses appear to be relatively independent of dopamine. Although exposure to novel palatable foods increases extracellular dopamine in the nucleus accumbens, this neurochemical response habituates upon repeated presentation, while the hedonic ingestive reactions and consummatory behaviour remains the same (Bassareo et al., 2002; Segovia et al., 2011). Furthermore, elevation and depletion of accumbal dopamine levels through genetic- and pharmacological manipulation does not affect hedonic responses (Berridge and Robinson, 1998; Peciña et al., 2003; Smith et al., 2011). Instead the endocannabinoid and opioid system may be involved in mediating hedonic responses, which will be discussed in more detail in chapter 1.3.3.

In contrast to hedonic reward responses, incentive salience appears to be dopamine dependent. This is especially the case if the effort that has to be exerted in order to obtain the reward is high (Salamone et al., 2007). For example, suppression of dopamine transmission by dopamine depletion in the nucleus accumbens has been shown to reduce active lever pressing for food on a progressive ratio task (Salamone et al., 2001). Conversely, enhanced dopamine levels have been shown to enhance the drive to obtain a reward (Wyvell and Berridge, 2000; Peciña et al., 2003; Cagniard et al., 2006).

Though currently a matter of debate (Fiorillo, 2013), around 15% of dopamine neurons have been reported to respond to aversive (as well as appetitive) stimuli with increased activity (Brischoux et al., 2009; Matsumoto and Hikosaka, 2009; Cohen et al., 2012; Schultz, 2013) . These neurons have been proposed to contribute to motivational (rather than incentive) salience, a quality which refers to the extent to which a stimulus captures attention and drives goal-directed behaviour and which is high for rewarding and aversive, but low for behaviourally insignificant stimuli (Berridge and Robinson, 1998; Bromberg-Martin et al., 2010).

Recent evidence suggests that different sub-populations of dopamine neurons in the mesolimbic pathway may preferentially contribute to some of the different functions discussed above (Smith et al., 2011; Saddoris et al., 2015).

1.1.3.2 Dopamine and cognitive function

Research into the role of dopamine in cognitive function began after a landmark study in the 1970s demonstrated that catecholamine depletion in the PFC impaired spatial working memory function (Brozoski et al., 1979), a finding that has been well replicated (e.g. Roberts et al., 1994; Collins et al., 2000; Crofts et al., 2001). In the original study the role of dopamine was emphasised because animals with large noradrenaline and small dopamine lesions remained unimpaired. Although it has since become clear that noradrenaline also plays an important role in PFC function (reviewed in Arnsten, 2003), this introduction focuses on the role of dopamine since both COMT and THC are known to primarily impact on dopaminergic signalling (Tunbridge et al., 2004) (see also 1.3.2).

Following the finding by Brozoski and colleagues, several studies showed that both, D1 receptor agonists and antagonists, as well as dopamine depletions in the PFC have the capacity to impair working memory performance, (Zahrt et al., 1997; Sawaguchi and Goldman-Rakic, 1991; Sawaguchi, 2000). These findings suggest that PFC D1 receptor activity is finely tuned and follows an inverted-U-shaped dose-response curve in which intermediate levels of dopamine appear to be optimal for working memory performance (Figure 3).

In addition, it has been suggested that 'off peak' regions of the inverted-U dose-response curve proposed for working memory may well be optimal for other forms of cognitive function such as cognitive flexibility, though receptor activity for these other functions need not necessarily also follow inverted-U shaped dose-response curves (Floresco, 2013). Interestingly, several studies report that while PFC D1 receptor activation is optimal for working memory performance, a specific level of D2 receptor activation may be important for cognitive flexibility. For example, D2 receptor antagonists have been shown to cause perseverative errors on a set-shifting task in rodents and impair performance on a reversal task of response flexibility in monkeys (Floresco et al., 2006; Lee et al., 2007). Furthermore, D2 antagonists impaired task set-shifting but at the same time protected against negative effects of irrelevant distractors during a spatial working memory task in human (Mehta et al., 2004).

It has thus been proposed that D2 receptors, which are positioned in close proximity to the site of neurotransmitter release, attenuate inhibitory influences and hence allow the PFC to process multiple, and new stimuli, permitting flexible responses to change. In contrast, D1 receptors increase inhibitory influences,

thereby reducing the representation of weak stimuli and increasing the signal to noise ratio. This in turn supports the maintenance of existing representations and protects against distractors (Seamans and Yang, 2004; Floresco, 2013).

Interestingly, observations made in human with different COMT genotypes support the role of baseline dopamine levels and thus D1 receptor activation in cognitive flexibility (see chapter 1.2.4).

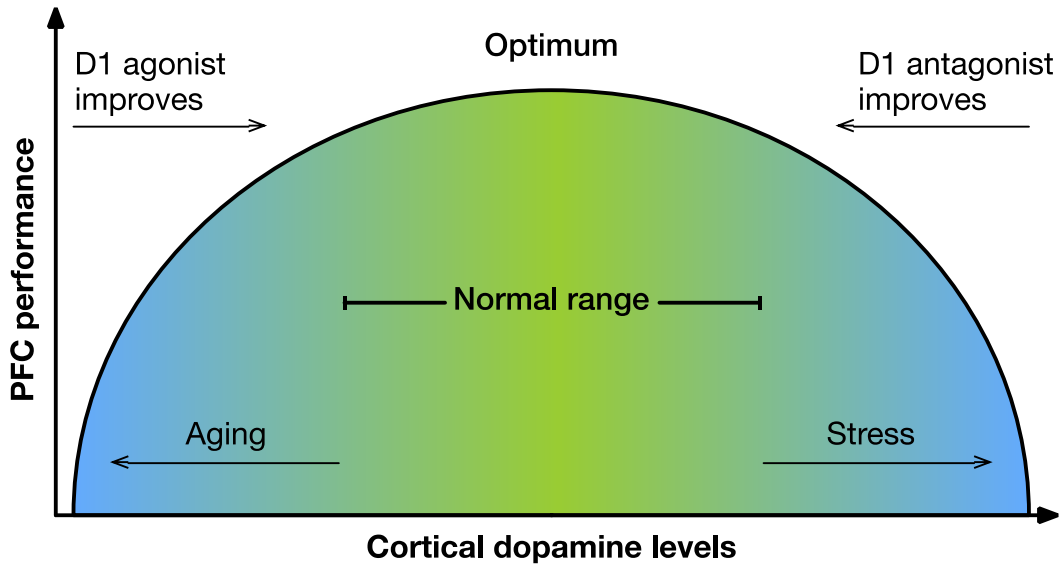


Figure 3: The inverted U-shape relationship between dopamine and PFC function. *PFC function is optimal at intermediate levels of dopamine (green area). Hypo- as well as hyper-dopaminergic stages (blue areas) impair PFC performance compared to the optimum. The precise level of dopamine that is optimal may differ between different task requirements. Pharmacological interventions like D1 receptor agonist or antagonist administration, life occurrences such as aging and stress, as well as genetic predispositions (discussed later on) can alter the individual's position on this curve. (adapted from Goldman-Rakic et al., 2000)*

1.1.3.3 Dopamine and schizophrenia

As described above, dopamine is involved in a multitude of processes in the brain and it is hence not surprising that dysfunction of the dopaminergic system has been associated with many pathological states including schizophrenia, obsessive-compulsive disorder, drug addiction, and cognitive decline with aging. The work presented here is based around the suggestion that COMT genotype

may affect the risk outcome of THC exposure on the development of schizophrenia. I will hence give a brief introduction into the pathophysiology and aetiology of schizophrenia focusing on the contribution of genetic and non-genetic risk factors as well as the role of the dopaminergic system. The contribution of other neurotransmitters such as glutamate, GABA, and 5HT is beyond the scope of this thesis but has been reviewed elsewhere (Moghaddam and Javitt, 2012; Nakazawa et al., 2012; Dean, 2003).

Schizophrenia is a complex illness, which affects about 1% of the population and usually first presents in adolescence or early adulthood. Patients exhibit a wide range of symptoms, which fall into 3 categories: positive, negative and cognitive. Positive symptoms describe the psychotic distortions of normal function including delusions, hallucinations, and disorganisation of thought and behaviour. Negative symptoms represent a loss of normal function and include blunted affect, anhedonia, avolition, and alogia. These negative and positive symptoms are central to current diagnostic criteria (Diagnostic and Statistical Manual of Mental Disorders (DSM-5), and International Statistical Classification of Disease and Related Health Problems (ICD-10)). However, in addition schizophrenic patients typically suffer from a range of cognitive dysfunctions most marked on tests of memory and attention (e.g. Bilder et al., 2000; Weickert et al., 2000).

1.1.3.4 Dopamine hypothesis of schizophrenia

Abnormalities in the dopaminergic system have been suggested as the final common pathway of schizophrenia (Howes and Kapur, 2009). According to the original dopamine hypothesis, a striatal hyper-dopaminergic state results in the

positive psychotic symptoms, whilst more recent formulations also postulate that a frontal hypo-dopaminergic state contributes to the negative and cognitive symptoms (Weinberger et al., 1992; Howes and Kapur, 2009).

Although a main characteristic, psychosis is not unique to schizophrenia and striatal dopamine elevation appears to relate more generally to psychosis and psychosis proneness (Howes and Kapur, 2009). Nonetheless, the first evidence for the role of dopamine originated from drug discoveries in schizophrenia treatment. Typical antipsychotics were found to increase dopamine metabolism and their efficacy correlated with D2 receptor affinity (Carlsson and Lindqvist, 1963; Creese et al., 1976).

In contrast drugs that increase striatal dopamine responses, such as amphetamine, were found to be capable of inducing psychosis in healthy people, and to worsen positive symptoms in schizophrenic patients (reviewed in Laruelle et al., 1996; Abi-Dargham et al., 1998). In addition it was found that dopamine occupancy of D2 receptors was also increased under baseline conditions in schizophrenia (Abi-Dargham et al., 2000). More recent evidence arising from positron emission tomographic (PET) and single-photon emission computer tomographic (SPECT) demonstrate elevated baseline synaptic dopamine levels and dopamine release in the striatum of schizophrenic patients as well as elevated presynaptic dopamine synthesis capacity, thus suggesting that the locus of the abnormality is presynaptic (Howes et al., 2012).

A theory that aims to explain how increased dopamine levels might contribute to the experience of psychosis is the motivational salience hypothesis (Kapur, 2003). As described in chapter 1.1.3 the mesolimbic dopamine system is a critical component in the attribution of salience to stimuli, thereby transforming neutral stimuli, which can be ignored, into attractive or aversive entities, which draw attention and influence goal-directed behaviour (Berridge and Robinson, 1998). In this context, increased striatal dopamine activity has been suggested to cause aberrant salience attribution in schizophrenic patients. Delusions have hence been proposed to be the cognitive explanation that the individual imposes upon the aberrant salience experiences in order to make sense of them (Kapur et al., 2005). This hypothesis is supported by studies gathering behavioural evidence that people with schizophrenia indeed show abnormalities in the assignment of salience to stimuli (Murray et al., 2008; Roiser et al., 2009). Conversely, at the physiological level there is evidence of an augmented brain response to stimuli that ought to be relatively neutral or non-salient in psychosis (Murray et al., 2008; Corlett et al., 2007; Jensen et al., 2008). These results are further strengthened by evidence that this phenomenon actually relates to the presence of unusual beliefs (Corlett et al., 2007).

In contrast to the striatal hyper-dopaminergic function, a hypo-dopaminergic state has been proposed to contribute to the negative and cognitive symptoms of schizophrenia. This hypothesis would suggest an inverse relationship between striatal and cortical dopamine levels. Support for such an inverse relationship originates from Pycock and colleagues in 1980 and will be discussed in more detail later on in this thesis (Pycock et al., 1980).

As described in section 1.1.3.2 comparatively low levels of dopamine can impair cognitive function. However, until recently, low cortical dopamine signalling in schizophrenia was equivocal and supported mainly by indirect evidence. While PFC D1 receptor binding was initially reported, to be decreased in schizophrenia (Okubo et al., 1997), other studies reported no change (Karlsson et al., 2002), or an increase (Abi-Dargham et al., 2002). Nonetheless, dopamine agonists were shown to improve working memory deficits in schizophrenic patients (Daniel et al., 1991; Barch, 2005; Barch and Ceaser, 2012), and post mortem studies revealed decreased tyrosine hydroxylase immunolabelling in the PFC of schizophrenic patients (Akil et al., 1999). Importantly, Slifstein and colleagues recently provided the first *in vivo* evidence for a deficit in the dopamine release capacity in the dorsolateral PFC, as well as a generalized blunting of dopamine responses across several other extra-striatal brain regions following amphetamine administration in schizophrenia patients (Slifstein et al., 2015). Though these results remain to be replicated, this study offers the first direct evidence for a hypo-dopaminergic state in non-striatal areas including the PFC.

Epidemiology and aetiology

Despite its complexity, schizophrenia has a strong heritable component, estimated around 80% (Sullivan et al., 2003). However, there are no single causal loci instead, numerous risk genes of small effect size act together in order to trigger disease (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Several non-genetic risk factors have been identified. For example, an increased risk for the development of schizophrenia has been associated with winter and spring births (Torrey et al., 1997), viral infections of

the central nervous system during the pre- and peri-natal period (Tsuang, 2000), and adverse life events (McDonald and Murray, 2000).

A theory that attempts to reconcile some of these risk factors, and relate them to key features of brain development, is the neurodevelopmental hypothesis of schizophrenia. In its simplest form this hypothesis proposes that schizophrenia is the behavioural outcome of an aberration in neurodevelopmental processes that begins long before the onset of clinical symptoms and is caused by a combination of environmental and genetic factors (Weinberger, 1987; Murray and Lewis, 1987). Several lines of evidence support the neurodevelopmental aspect of this hypothesis including the association of the disease with pre-natal malnutrition (Susser et al., 1998) and with neurodevelopmental genes such as neuregulin 1 (Harrison, 2003). Furthermore, for several of the published environmental variables associated with psychosis, a gene x environment interaction has been proposed. Table 1 shows a subset of these environmental variables for which at least one positive meta-analytic estimate exists and which have been associated with proxies of genetic variables. However, such associations have to be interpreted with caution and further research is necessary to verify the existence and to understand the nature of the proposed interactions. Of special interest for the work presented here is the risk factor of cannabis use and its possible interactive effects with a functional polymorphism in the COMT gene. The details of cannabis as an environmental risk factor are discussed in more detail below, following the introduction of COMT.

Table 1: *Subset of replicated environmental risk factors for psychosis, which act early during development and for which a gene x environment interaction has been suggested (van Winkel, 2010; van Os et al., 2008)*

Environmental Variable	**Proxy Genetic Variable**	**References**
Environmental variables with likely impact in fetal or early life:		
Maternal pregnancy complications esp. fetal hypoxia	Genes regulated by hypoxia increase risk of schizophrenia	(Cannon et al., 2002; Schmidt-Kastner et al., 2006; Nicodemus et al., 2008)
Increased paternal age	Increased mutations and epigenetic modifications during spermatogenesis	(Zammit et al., 2003; Perrin et al., 2007; Torrey et al., 2009; Miller et al., 2011)
Environmental variables with likely impact in late childhood and adolescence:		
Growing up in an urban environment	Positive family history and expression of genetically influenced psychometric psychosis liability	(van Os et al., 2003; Spauwen et al., 2006)
Cannabis use	Expression of genetically influenced psychometric psychosis liability and COMT genotype	(Henquet et al., 2005; Caspi et al., 2005)

1.2 Catechol-O-methyltransferase

The catechol-O-methyltransferase (COMT) enzyme is involved in the elimination of catechol-containing compounds by catalysing the transfer of a methyl group onto the catechol ring of its substrate. It was first identified and characterised by Axelrod and Tomchick in 1958 (Axelrod et al., 1958) and is present in vertebrates as well as invertebrates (Guldberg and Marsden, 1975). It is widely distributed throughout the mammalian body where it fulfils a variety of functions. For the purpose of the work presented here this introduction will focus on the role of COMT in the central nervous system and, more specifically, on its role in the breakdown of the catecholamine neurotransmitter dopamine

and its metabolites (see also Figure 2). For a comprehensive review of COMTs function in the periphery please refer to Myöhänen and Männistö, 2010.

1.2.1 Structure and expression

The protein structure of COMT consists of a central β-sheet surrounded by eight α-helices. The catechol substrate, as well as the methyl-group donor S-adenosyl-L-methionine can bind to the active site where the transfer of the methyl group is catalysed (Figure 4) (Vidgren et al., 1994; reviewed in Männistö and Kaakkola, 1999). The residues crucial for the active site are conserved across species suggesting similar substrates and reactions in both rodents and humans (Tenhunen et al., 1994; Vidgren et al., 1994; Lotta et al., 1995).

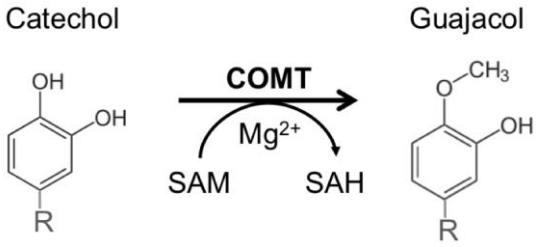


Figure 4: COMT catalyses the methyl-group transfer onto a catechol containing substrate. *In the presence of a magnesium ion, S-adenosyl-L-methionine (SAM) functions as a methyl-group donor and is thus transformed into S-adenosyl-L-homocysteine (SAH).*

In human the COMT gene is located on chromosome 22, band q11.2 (Grossman et al., 1992). It consists of six exons, of which the first two are non-coding (Figure 5). Exon three contains two AUG start codons from which two distinct COMT isoforms can be expressed: soluble (S-) and membrane-bound (MB-) COMT. Compared to the shorter, soluble form, MB-COMT contains additional hydrophobic amino acids that form a membrane-spanning domain (Bertocci et al., 1991; Lundström et al., 1991). Furthermore, though MB-COMT has a lower capacity, it has a 10 fold greater affinity for the catecholamine neurotransmitters dopamine and noradrenaline than S-COMT (Lotta et al., 1995). MB-COMT is therefore thought to play a predominant role in the termination of

catecholaminergic neurotransmission in the brain whilst S-COMT is believed to be mainly responsible for the elimination of toxic, primarily exogenous catechols (Roth, 1992; Männistö et al., 1992; Männistö and Kaakkola, 1999). Consistent with this, MB-COMT is the predominant form found in the brain (60-70%) while in peripheral tissue S-COMT tends to prevail (Tenhunen et al., 1994; Myöhänen and Männistö, 2010).

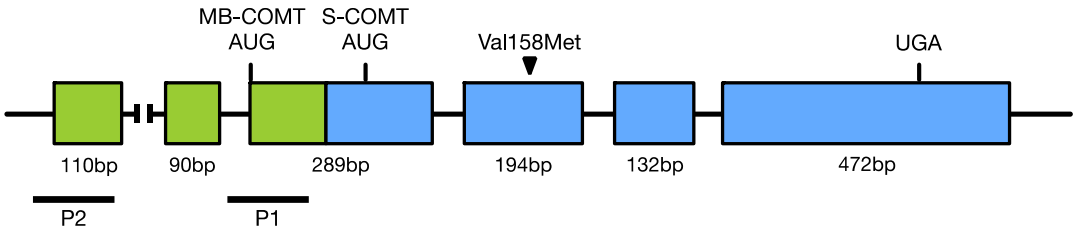


Figure 5: COMT gene structure. *Exons are depicted as boxes with their respective lengths in base pairs indicated below. Introns are shown as connecting lines. The promotors (P1 and P2), membrane-bound- and soluble COMT AUG translation start codons, UGA stop codon, as well as the location of the Val^{158}Met polymorphism are also depicted.*

1.2.2 Cellular and subcellular location of COMT

The cellular localisation of COMT in the CNS has long been a matter of debate: Some studies have reported COMT to be localised to glia cells (Kaplan et al., 1979; Reenilä et al., 1997), while Matsumoto and colleagues reported it to be mainly localised to neurons (Matsumoto et al., 2003). In most studies COMT was found in both, neurons and glia (Broch and Fonnum, 1972; Garbarg et al., 1975; Karhunen et al., 1994; Karhunen et al., 1995; Myöhänen et al., 2010). This discrepancy might, in part, be due to the lack of isoform-specific COMT antibodies, since it has been suggested that S-, and MB-COMT might be differentially localised to glia and neurons respectively (Rivett et al., 1983).

On a subcellular level the data to date is even less consistent. MB-COMT was initially suggested to be located in the plasma membrane possibly protruding into the synapse (Traiger and Calvert, 1969; Aprille and Malamud, 1975; Raxworthy et al., 1982), a suggestion supported by reports that COMT associates with postsynaptic dendritic spines (Kastner et al., 1994; Karhunen et al., 1995). However, exposure to the extracellular space has been proposed to significantly inhibit COMT function due to the presence of relatively high concentrations of calcium ions (Jeffery and Roth, 1984; Roth, 1992). Furthermore, sequence motives have been believed to indicate that MB-COMT is oriented to protrude into the cytoplasm (Lundström et al., 1995), though a more recent study using a different algorithm resulted in the opposite prediction (Chen et al., 2011). In addition, MB-COMT has also been suggested to be exclusively located on intracellular membranes including the membrane of the endoplasmatic reticulum (Ulmanen et al., 1997) and the outer mitochondrial membrane, which was supported by cell fraction studies (Grossman et al., 1985; Broch and Fonnum, 1972). In a recent study double labelling COMT and the plasma membrane specific marker Na-K-ATPase Myöhänen and colleagues report that COMT can be found in the vicinity of cell membranes but is not attached (Myöhänen and Männistö, 2010). However, weak co-localisation was observed and results could therefore be interpreted differently, as pointed out by Chen and colleagues (Chen et al., 2011). Chen et al. provided evidence for COMTs location in the plasma membrane and the protrusion of the catalytic domain into the extracellular space: Flow-cytometry detection of cells transfected with COMT tagged either on the C- or N-terminus of the transmembrane domain revealed that the catalytic domain was exposed to the extracellular space. Consistent with

this finding, high levels of COMT activity could be observed on MB-COMT transfected cells in which the cell impermeable coenzyme S-adenosyl-L-methionine was present exclusively extracellularly (Chen et al., 2011). These results suggest that at least part of the MB-COMT protein is located in the plasma membrane and positioned to fulfil its catalytic function independent of endocytosis or other cross-membrane transportation.

The presence of not only S- and MB-COMT, but further, differentially expressed splice variants (Tunbridge et al., 2007; Meloto et al., 2015), as well as reported dynamic changes of COMT distribution may contribute to the reported inconsistencies (Reenilä et al., 1997; Weisz et al., 2000; Hirose et al., 2007).

1.2.3 Presence and importance of COMT across brain regions

There is little difference in COMT protein quantity between brain regions. The highest levels of mRNA and protein expression have been observed in the spinal cord, cerebellum, medulla and cerebral cortex and comparatively low levels in hippocampus, amygdala and thalamus (Guldberg and Marsden, 1975; Roth, 1992; Tenhunen et al., 1994; Hong et al., 1998; Matsumoto et al., 2003; reviewed in Myöhänen and Männistö, 2010). For both human and rodents, levels of COMT mRNA are reportedly lower in the striatum compared to the PFC (Hong et al., 1998; Matsumoto et al., 2003), though protein levels do not seem to differ, at least in mice (Myöhänen and Männistö, 2010).

Though the differences in COMT expression between frontal and striatal regions appear to be small, the relative functional importance of COMT in these two regions may differ substantially due to factors other than COMT's abundance. Specifically, in striatal regions, high levels of DAT lead to rapid termination of

dopamine transmission independent of COMT. However, in the PFC the expression of DAT is relatively sparse (Sesack et al., 1998) and, although dopamine may also be taken up by NET and EMT (Morón et al., 2002; Martel and Azevedo, 2003), COMT appears to be important for the regulation of dopamine transmission in the PFC. Karoum and colleagues reported that 3-methoxytyramine (3-MT), which is a product formed by the O-methylation of dopamine by COMT, contributes to over 60% of total dopamine turnover in the frontal cortex, suggesting that this is the major degradation pathway in this region. In contrast, 3-MT formation contributes to only 15% of dopamine turnover in the striatum (Karoum et al., 1994). In rats, acute COMT inhibition elevated stimulated dopamine release in the PFC (Tunbridge et al., 2004; Lapish et al., 2009). Furthermore, COMT knockout induced a 2-3 fold increase of frontal dopamine in male mice (Gogos et al., 1998). Although this finding was not replicated in a different background strain (Huotari et al., 2002), Yavich and colleagues reported that the removal of dopamine was 2 fold slower in the PFC of COMT knock out mice compared to wild-types (Yavich et al., 2007).

Due to the high DAT levels present in the striatum most studies to date have focused on the PFC and there is very limited evidence that altered COMT activity affects dopamine function in the dorsal or ventral striatum (Garris and Wightman, 1995; Yavich et al., 2007; Forbes et al., 2007; Käenmäki et al., 2010), though one study observed an increase in dopamine release capacity in COMT overexpressing mice (Simpson et al., 2010). Budygin and colleagues reported no effect of acute COMT inhibition by itself, but increased striatal dopamine levels following the combined administration of COMT and dopamine reuptake

inhibitors compared to reuptake inhibitor only (Budygin et al., 1999). However, this combined effect was not observed in COMT knockout mice (Huotari et al., 2002). Independent of a direct effect of COMT on striatal dopamine function, there is evidence for an inverse relationship between PFC and striatal dopamine function (Pycock et al., 1980; Kellendonk et al., 2006), suggesting that alterations in COMT activity (and therefore PFC DA levels) might induce adaptive changes in the striatum downstream of COMT's primary effects in the PFC. One aim of the work presented here is to further determine the role of COMT in striatal and cortical regions.

1.2.4 COMT Val^{158}Met functional polymorphism

A single nucleotide polymorphism (SNP) consisting of a guanine to adenosine substitution in the coding sequence of the human COMT gene results in the functional valine^{158}methionine (Val^{158}Met, rs4680) polymorphism (Bertocci et al., 1991; Lundström et al., 1991). The Met form of the enzyme has lower thermostability than the Val isoform. As a consequence, the Val form is more active at body temperature, and Val homozygosity results in 40% higher enzyme activity in the CNS, compared to Met homozygosity (Lotta et al., 1995; Chen et al., 2004). Both alleles are codominant, leading to a trimodal distribution of high (Val/Val), intermediate (Val/Met), and low (Met/Met) COMT activity in human populations (Floderus et al., 1981; Lachman et al., 1996).

To date this polymorphism has not been reported in any other species, including primates. In mice and rats a leucine residue is found at the position of the human Val^{158}Met polymorphism. The rodent form of the enzyme has similar thermostability, and hence activity levels, as the human Val form (Lotta et al.,

1995; Chen et al., 2004). The high activity Val form of the enzyme is therefore the ancestral form (Palmatier et al., 1999).

Apart from Val^{158}Met, several other coding and non-coding SNPs have been identified in the COMT gene. However, to date the effect size and directionality of those SNPs is mostly unknown, although some haplotypes have been reported to affect the translation of COMT protein (Diatchenko et al., 2005; Nackley et al., 2006; Gothelf et al., 2014). Nonetheless, the Val^{158}Met polymorphism remains unambiguously functional and is the focus of this thesis not only due to the proposed interactive effect with cannabis use, but also because of its separate effects on behavioural performance.

1.2.4.1 COMT Val^{158}Met polymorphism and cognition

As described above, dopamine levels in the PFC are critical for modulating cognitive function and the COMT Val^{158}Met polymorphism has been shown to predict D1 receptor availability, and thus dopamine signalling in the PFC (Slifstein et al., 2008). Hence, the COMT genotype has been predicted to modulate cognitive function. However, the putative inverted-U shape relation between dopamine and PFC function implies that the relationship between COMT activity and cognitive function may be complex.

The first evidence that Val^{158}Met polymorphism may affect cognitive performance in humans originated from Egan and colleagues who demonstrated a allele dose-response relationship, whereby the Met allele was associated with better performance on the Wisconsin Card Sorting Test (WCST), compared with the Val allele (Egan et al., 2001).

While many studies replicated this initial finding (Joober et al., 2002; Malhotra et al., 2002; Rosa et al., 2004; Barnett et al., 2007), extended it to different cognitive tasks and observed genotype dependent brain activity levels during task performance (Goldberg et al., 2003; Farrell et al., 2012; Gallinat et al., 2003; Blasi et al., 2005; Bertolino et al., 2006; Bertolino et al., 2006; Dumontheil et al., 2011), others did not observe these effects (Ho et al., 2005; Barnett et al., 2008; Blanchard et al., 2011; Wardle et al., 2013). It is essential to note that not all cognitive tasks are sensitive to the Val^{158}Met polymorphism (Tunbridge et al., 2006). Furthermore, it has been suggested that age-related changes in dopamine may contribute to some of the discordant findings within this field (Wardle et al., 2013). Interestingly, a meta-analysis by Mier and colleagues not only confirmed the beneficial effect of the Met allele on brain activity patterns during executive cognitive function, but also revealed that the opposite was true for emotional paradigms: Val allele load was beneficial during tasks such as reward anticipation, and face matching (Mier et al., 2010).

COMT Val and Met genotype have been suggested to have differential effects on cognitive stability and flexibility. Nolan and colleagues demonstrated that, in a reversal learning task, Met homozygotes performed better at the acquisition stage but were outperformed by Val homozygotes at the reversal stage, suggesting that the Val allele hampers cognitive stability but enhances cognitive flexibility (Nolan et al., 2004). Furthermore, Val homozygotes outperform Met homozygotes on task switching performance (Colzato et al., 2010) and require greater PFC activation during the maintenance but lower transient medial lobe

activity during the updating phase of a working memory task (de Frias et al., 2010).

The mechanisms underlying these genotype-specific effects on cognitive stability may potentially be mediated through the effects of different dopamine receptor subtypes on signal to noise ratio (see also section 1.1.3.2) and / or in accordance with the tonic-phasic model of subcortical dopamine signalling. This model suggests that the increased dopamine levels in the PFC that can be found in Met carriers lead to increased tonic, but decreased phasic dopamine activity in the nucleus accumbens, thereby making new information for example about changed reward contingencies less salient (Bilder et al., 2004) (Figure 6).

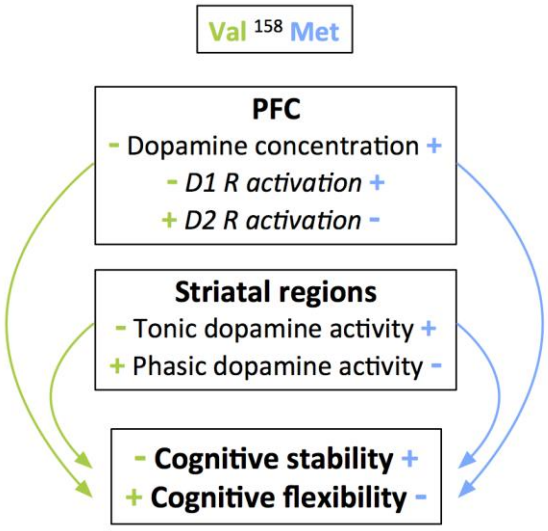


Figure 6: Potential mechanisms through which the COMT Val[158]Met polymorphism may influence cognitive stability and flexibility. *The Met allele (blue) has been associated with higher dopamine levels in the PFC, which leads to enhanced D1 receptor activation associated with higher cognitive stability. The Met allele has also been suggested to increase tonic and decrease phasic dopamine activity in striatal regions, which could make changes in contingencies less salient and could thus also contribute to cognitive stability. The opposite is true for the Val allele (green).*

Of particular interest are studies that investigate COMT genotype-dependent variability in cognitive function in response to pharmacological manipulations of the dopamine system (Mattay et al., 2003; Apud et al., 2007; Farrell et al., 2012; Kelm and Boettiger, 2013). For example it could be shown that an increase of dopamine signalling caused either by amphetamine administration (Mattay et al.,

2003) or COMT inhibition with tolcapone (Farrell et al., 2012) had opposite effects on working memory performances in Val and Met carriers in line with their predicted positions on the inverted-U relationship between dopamine and PFC performance on the tasks used (Figure 7).

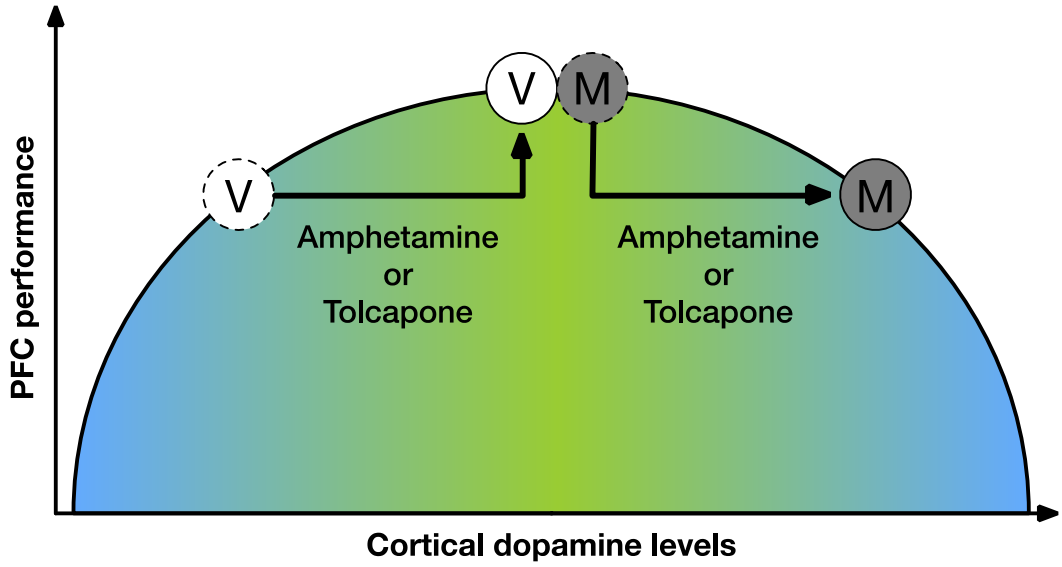


Figure 7: Catechol-O-methyltransferase genotype, drug manipulation, and the inverted-U. *COMT Val subjects (V) have higher COMT activity and thus lower dopamine levels compared to Met subjects. They therefore sit further to the left on the curve. An increase of extracellular dopamine by blocking the dopamine transporter with amphetamine or decreasing COMT activity with tolcapone shifts all subjects to the right leading to increase in Val- and a decrease in Met-subjects PFC performance (adapted from Goldman-Rakic et al., 2000).*

As mentioned above the Val^{158}Met polymorphism does not exist in other animals. However, the ancestral COMT form present in rodents shows levels of enzyme activity comparable with the Val allele in humans. COMT inhibition with tolcapone significantly improved performance on tasks of cognitive function in rodents (Tunbridge et al., 2004; Lapish et al., 2009). Furthermore, reduced COMT activity in COMT null mutant mice improved spatial working memory function while high COMT activity levels in COMT overexpressor- and humanised Val- versus Met-mice decreased working memory function, in line with the

human literature (Papaleo et al., 2008; Scheggia et al., 2013; Simpson et al., 2014).

1.2.4.2 COMT Val^{158}Met polymorphism and reward

In contrast to the abundant literature investigating the effects of COMT on cognition, the long prevailing view that COMT was insignificant for subcortical dopamine function meant that studies into the significance of COMT for reward processing are only beginning to emerge in recent years and require further investigation (Tunbridge et al., 2012).

One of the more consistent findings in humans is the association of the COMT Val^{158}Met polymorphism with ventral striatum activation during reward anticipation. In different kinds of gambling tasks the Met allele predicted greater activation in the ventral striatum during the anticipation of uncertain reward compared with the Val allele (Yacubian et al., 2007; Dreher et al., 2009). Furthermore, in a monetary incentive delay task in which the reward outcome is dependent on the participant's performance, the number of Met alleles also correlated with ventral striatum activation during reward anticipation, though only in trials in which the participants could lose money (Schmack et al., 2008). Despite their consistency, these results have to be considered with care since the behavioural correlates of the reported enhanced activation are not yet known.

Apart from reward anticipation, COMT has also been reported to affect ventral striatum activation during reward outcome. Compared to Met allele carriers, Val homozygotes showed a greater difference in ventral striatal activation between gain and loss trials during 'boosted' trials in a gambling task, in which the bet (normally low 5, or high 25) was replaced by 125. These results indicate that

COMT may modulate the relative activation to gains vs. losses in the ventral striatum (Camara et al., 2010). In a guessing task in which participants did not know that their reward was performance dependent, COMT genotype did not affect ventral striatum activation, though activation was modulated by a multilocus genetic profile including COMT (Forbes et al., 2007; Nikolova et al., 2011). The finding may thus suggest that COMT plays a less prominent role during tasks in which the actual reward is somewhat removed from the on-going performance. Furthermore, these results highlight the fact that effects of COMT may interact with polymorphisms in other dopaminergic genes to modulate dopamine activity.

It is important to note that in both reward anticipation and outcome, the genotype specific effects do not seem to be limited to the ventral striatum but also extend into reward-related cortical regions. Within these regions the PFC seems to play a key role in the stability of reward representations. As shown in Figure 6 the Met allele seems beneficial for the stability of reward representation, while the Val allele appears to facilitate flexibility under conditions in which reversal of reward contingencies is required (Frank et al., 2007; Doll et al., 2011; Krugel et al., 2009; Lancaster et al., 2015).

It has to be noted that only very few studies have focused on the subject of COMT and reward processing in animal studies. Lapish et al. reported that acute COMT inhibition with tolcapone enhanced PFC dopamine efflux during anticipation and consumption of food. Furthermore, male COMT knockout mice have been shown to consume significantly more ethanol than their wild-type littermates in a free choice self-administration paradigm (Lapish et al., 2009; Tammimäki et al.,

2008). However, more such studies will be essential in order to fully understand the involvement of COMT in reward processing and possibly addiction.

1.2.4.3 COMT and psychosis / schizophrenia

COMT has long been regarded as an attractive candidate gene for schizophrenia and psychosis not only because of its influence on dopamine function, but also because the COMT gene lies in a chromosomal region which according to genetic linkage studies is likely to contain schizophrenia susceptibility loci (Badner and Gershon, 2002; Lewis et al., 2003), but see also (DeLisi et al., 2014). More specifically, COMT resides at 22q11.2, where its hemizygous deletion along with a number of neighbouring genes results in velo-cardio-facial syndrome also known as DiGeorge syndrome, which is strongly associated with neuropsychiatric illness including schizophrenia, although psychiatric symptoms observed in 22q11.2 deletion syndrome have not been ascribed to a reduced copy number in any individual gene (Murphy et al., 1999; Karayiorgou et al., 1995; Bassett et al., 2003; Williams et al., 2006; International Schizophrenia Consortium, 2008).

To date, comparatively few studies have been published that have focused on a link between COMT activity levels and the aspect of psychosis / schizotypy in a healthy population. In the few published studies the Val allele appeared to be associated with schizotypy in male participants (Avramopoulos et al., 2002; Stefanis et al., 2004; Schürhoff et al., 2007). Far more studies have investigated the link between the COMT Val^{158}Met polymorphism and schizophrenia, albeit with mixed results. While some studies report the Val allele to be associated with schizophrenia (e.g. Egan et al., 2001; Shifman et al., 2002; meta-analysis Glatt et

al., 2003), others show an association with the Met allele (Ohmori et al., 1998; Sazci et al., 2004) and many, including meta-analysis, find no association (Munafò et al., 2005; Fan et al., 2005; Okochi et al., 2009). Interestingly, a more recent meta-analysis suggested that Val^{158}Met heterozygosity may have a protective effect (Costas et al., 2011). However, the effect sizes in most of these studies are small and the lack of robust association may well reflect the lack of a direct association. Alternatively, it may also reflect the complexity of the disorder in which COMT may only confer susceptibility to schizophrenia in combination with other genetic and / or environmental factors (Nicodemus et al., 2007; van Os et al., 2010). One of the factors proposed to potentially interact with COMT genotype is cannabis consumption. Cannabis, and its main psychoactive component THC, will be introduced in the following section.

1.3 Cannabis

Cannabis in the most commonly used illicit drug with an annual prevalence of 2.5% of the world's population (World Health Organisation, Sept. 2015, http://www.who.int/substance_abuse/facts/cannabis/en/). Although it is generally considered to be a safe drug, the effects of its consumption can extend from feelings of happiness and excitement, through dissociation of ideas, and errors in time and space perception, up to delusions, hallucinations and cognitive impairments (Morrison et al., 2009; Kaufmann et al., 2010; Mechoulam and Parker, 2013). Cannabis contains more than 70 different cannabinoid constituents, the composition of which can vary massively between cannabis plant (*Cannabis sativa*) varieties (Elsohly and Slade, 2005). However, the main psychoactive component is delta-9-tetrahydrocannabinol (THC) (Figure 8),

which was first isolated and synthesised in the 1960s (Gaoni and Mechoulam, 1964). Here, I will discuss how THC 'hijacks' the endocannabinoid system to elicit its effect, specifically with respect to the dopaminergic system. I will then summarise some of the effects of cannabis consumption on brain function, before discussing the current understanding of a potential link between cannabis consumption and the development of schizophrenia.

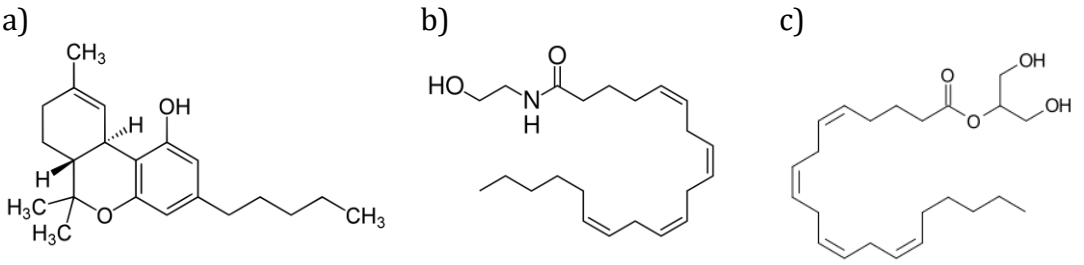


Figure 8: Exogenous and endogenous cannabinoids *a) delta-9-tetrahydrocannabinol b) anandamide c) 2-arachidonoyl glycerol*

1.3.1 Mechanism and location of THC action

THC exerts its effects via cannabinoid receptors. Under physiological conditions these receptors mediate the effects of endogenous cannabinoids such as anandamide and 2-arachidonoyl glycerol (Figure 8), which function as neuro- and immune-modulators. They act on two types of receptors: cannabinoid 1 (CB1), and cannabinoid 2 (CB2) receptors, though additional cannabinoid-sensitive receptors have been described as well (Matsuda et al., 1990; Munro et al., 1993; Pertwee, 2006). THC is a partial agonist of both CB1 and CB2 receptors but exhibits higher CB1 receptor efficacy (Pertwee, 2010). CB2 receptors are mainly expressed in immune cells and are hence of little relevance to the work presented here (Munro et al., 1993). I will therefore focus on CB1 receptors, which are predominantly located within the central and peripheral nervous system (Herkenham et al., 1990).

CB1 receptors are G_i / G_o protein coupled receptors that are linked to a variety of second messenger signalling cascades. In cell and tissue culture studies it has been shown that CB1 receptor activation leads to the inactivation of adenylyl cyclase and voltage gated calcium channels, thus inhibiting neurotransmitter release, and activation of potassium channels and mitogen-activated protein (MAP) kinase, via which pathways cannabinoids are able to affect synaptic plasticity (Bidaut-Russell et al., 1990; Twitchell et al., 1997; Henry and Chavkin, 1995; Bouaboula et al., 1995; reviewed in Howlett and Mukhopadhyay, 2000).

CB1 receptors are abundantly distributed throughout the brain with high densities in the basal ganglia, substantia nigra, globus pallidus, and hippocampus, as well as the frontal cortex and cerebellum (Herkenham et al., 1990). Cell-surface CB1 receptors are found predominantly on neurons within the presynaptic terminals (Tsou et al., 1998; Katona et al., 2000; Howlett et al., 2002) and are most common on GABergic and glutamatergic neurons, suggesting that they are involved in regulating GABA and glutamate neurotransmission (Howlett et al., 2002; Mechoulam and Parker, 2013). However, there is also abundant evidence that cannabinoids are also able to modulate dopaminergic activity, as described below.

1.3.2 Effects of THC on the dopaminergic system

As suggested by the presynaptic localisation of CB1 receptors, endocannabinoids can act as retrograde signalling molecules: Upon membrane depolarisation of the neuron, endocannabinoids are released from the postsynaptic side, which subsequently activate CB1 receptors on the presynaptic side and thus modulate future presynaptic neurotransmitter release (Alger, 2002; Riegel and Lupica,

2004). Importantly, midbrain dopamine neurons, especially within the ventral tegmental area VTA use this mechanism to regulate their glutamatergic and GABAergic input (Melis et al., 2004; Lupica and Riegel, 2005; Mátyás et al., 2008) (Figure 9). THC interferes with this feedback mechanism. By activating CB1 receptors independent of endocannabinoids, it inhibits glutamate and GABA neurotransmitter release and thus alters the firing pattern of VTA dopaminergic neurons (French, 1997). In addition, THC can also act on CB1 receptors in other brain areas. In the nucleus accumbens, for example, CB1 receptor activation reduces the activity of glutamatergic neurons, which, via GABAergic projections to the VTA, results in a disinhibition of VTA dopamine neurons (Mechoulam and Parker, 2013).

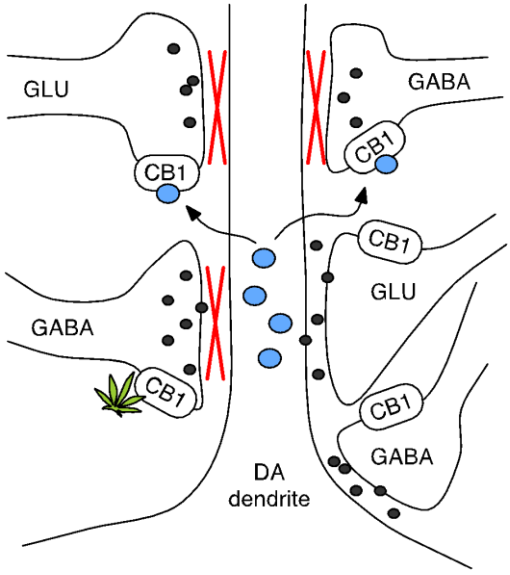


Figure 9: The convergence of dopamine and endo-, as well as exogenous, cannabinoids in the VTA. *Midbrain dopaminergic (DA) firing patterns depend on the glutamatergic and GABAergic inputs (both black dots) the neurons receive. Upon membrane depolarisation DA neurons release endocannabinoids (eCB, blue dots). Binding of the eCBs to CB1 receptors leads to an inhibition of neurotransmitter release. THC (indicated by cannabis leaf) acts as an agonist of CB1 receptors. Hence, in the presence of THC, the eCB feedback mechanism is disrupted due to exogenous inhibition of glutamate and GABA release, resulting in increased DA signalling. (adapted from Kuepper et al. 2010)*

Animal studies have shown that overall exogenous CB1 receptor activation by THC or similar CB1 receptor agonists results in increased firing rate and evoked burst firing in the VTA and substantia nigra (French et al., 1997; Diana et al.,

1998) and thus increased extracellular dopamine concentrations in target areas, such as the nucleus accumbens (Tanda, 1997; Cheer et al., 2004). Importantly, the increase in dopamine neuronal activity is attenuated by administration of rimonabant, a selective CB1 receptor antagonist, prior to THC exposure, confirming that this effect is indeed CB1-mediated (Gessa et al., 1998). Compared to the rodent literature, the human literature is more mixed. An initial case report suggested a 20% reduction in striatal D2 receptor binding following cannabis consumption in a medication-free schizophrenic patient (Voruganti et al., 2001). In another study acute, oral THC administration decreased D2/D3 receptor availability in the ventral striatum of healthy volunteers, again suggesting increased dopamine concentrations (Bossong et al., 2009). However, this finding was not replicated by Stokes and colleagues nor did Barkus and colleagues find any changes in dopamine concentration in the dorsal striatum following IV administration of THC (Stokes et al., 2009; Barkus et al., 2011). Interestingly, re-analysis of the combined data set from Bossong et al. and Stokes et al. revealed a 3.7% decrease in dopamine receptor availability in the ventral, but not dorsal striatum, following THC administration. Though sample sizes remain small, these results suggest an increase in ventral striatal dopamine levels similar to findings from microdialysis studies in rodents (Bossong et al., 2015; Tanda, 1997). Kuepper et al. reported a significant dopamine increase in the dorsal striatum of patients with psychosis and unrelated first degree relatives of individuals with psychotic disorder, but not unrelated healthy controls, following THC administration (Kuepper et al., 2013). These results indicate that there might be considerable variability in THC induced striatal dopamine responses possibly partly dependent upon the striatal sub-region

investigated, as well as familial liability. Furthermore, severe cannabis dependence has been associated with decreased striatal dopamine release (de Giessen et al., 2016).

Compared to the striatum, data on the effects of THC on cortical dopamine is scarce. However, acute THC intoxication has been shown to affect dopamine neurotransmission in the PFC. Microdialysis studies reveal that THC increases extracellular dopamine levels in the mPFC of rodents (Chen et al., 1990; Jentsch et al., 1997; Pistis et al., 2002). Furthermore, this effect is attenuated if the animals are pre-treated with a selective CB1 receptor antagonist (Pistis et al., 2002). In contrast, repeated exposure to THC has been shown to decrease PFC dopamine levels (Jentsch et al., 1998; Verrico et al., 2003). In human, Stokes and colleagues demonstrated an increase in dopamine levels in frontal cortical areas following acute THC intoxication (Stokes et al., 2010), though the low signal to noise ratios in [11C]-raclopride PET studies in cortical areas require cautious interpretation and hence replication.

1.3.3 THC, the endocannabinoid system and behaviour

Cannabinoid agonists such as THC are well known to produce a characteristic combination of four symptoms: hypoactivity, catalepsy, hypothermia and analgesia (Serrano and Parsons, 2011). However, more importantly for the work presented here, THC also influences reward related behaviours, cognition and psychosis.

1.3.3.1 THC and cognition

The first human studies investigating cognitive impairment following cannabis exposure date back to the 1970s and report deficits in several areas of cognitive

function including memory and attention (Miller et al., 1977; Hooker and Jones, 1987; Marks and MacAvoy, 1989). These results have largely been confirmed in more recent studies reporting transient impairments in learning, verbal and spatial working memory, episodic memory, distractibility, and executive function in response to acute THC intoxication in healthy volunteers (Curran et al., 2002; Ranganathan and D'Souza, 2006; D'Souza et al., 2008; Morrison et al., 2009). In addition, chronic cannabis users show cognitive impairments that outlast acute intoxication in a frequency-, duration-, and age of onset of cannabis use dependent manner (Battisti et al., 2010; Lundqvist, 2005; Solowij and Battisti, 2008).

These findings are supported by animal studies. Similar to the human literature, acute systemic THC intoxication has consistently been shown to induce deficits in cognitive function, including learning, reference and working memory in rodents (Jentsch et al., 1997; Hernandez-Tristan et al., 2000) and non-human primates (Wright et al., 2013). Pre-treatment with a CB1 receptor antagonist attenuated the cognitive deficits observed in mice following THC administration (Da Silva and Takahashi, 2002). These effects likely depend at least partly on drug effects in the mPFC, since local THC administration in this region has also been shown to impair spatial working memory (De Melo et al., 2005).

Animals models provide a great opportunity to investigate persistent effects of chronic cannabis exposure in a controlled environment. Quinn and colleagues could demonstrate, that deficits in working memory function could be observed 10 day after the last THC injection in rats treated chronically during adolescence, but not adult rats (Quinn et al., 2008). These findings are in line with other

studies in which long-lasting cognitive effects were observed in spatial working memory task following CB1 receptor agonist treatment in adolescent, but not adult rodents (Schneider and Koch, 2003; Realini et al., 2011; Abush and Akirav, 2012) and monkeys (Verrico et al., 2014). In addition, adult rats also showed impairment in cognitive flexibility on the attentional set-shifting task (Gomes et al., 2015). However, long lasting effects were less pronounced in aversive memory tasks or pure spatial memory tasks (Rubino et al., 2009; Higuera-Matas et al., 2009; Abush and Akirav, 2012). The animal data hence supports the notion that adolescence might be a particular vulnerable time for THC to induce long-term cognitive effects, especially in forms of memory and cognitive function involving the PFC.

1.3.3.2 THC and reward

As discussed in section 1.1.3.1 reward processing is a complex collective term that encompasses and influences a variety of different functions ranging from the experience of pleasure to learning. Here, I will focus on hedonic and appetitive aspects of THC. Acute administration of THC, or other CB1 receptor agonists is well known to increase consumption of freely accessible, palatable food and sweet solutions (Williams et al., 1998; Higgs et al., 2003), a phenomenon that has also been described as 'the munchies' in humans. Conversely, CB1 receptor antagonists induce anorectic effects in rodents (Arnone et al., 1997). Accordingly, the endocannabinoid system, together with the opioid system, has been shown to play an important role in the generation of pleasure. For example, THC administration has been shown to increases hedonic responses to sucrose, an effect that can be reversed by pre-treatment with a CB1 receptor antagonist (Jarrett et al., 2005; De Luca et al., 2012). A detailed discussion of the

endocannabinoid system and its overlap with the opioid system, especially in areas of the nucleus accumbens shell to build 'hedonic hotspots' is beyond the scope of this thesis, but is discussed in Mahler et al., 2007, Berridge et al., 2009, and Castro and Berridge, 2014.

THC has also been proposed to interfere with the motivational aspect of reward processing. Evidence for the contribution of exogenous and endogenous cannabinoids to appetitive behaviour is discussed in more detail in chapter 5.1. In short CB1 receptor agonists like THC have been shown to increase the motivation of animals to work in order to obtain a reward and this effect seems to be at least partly mediated via increased accumbal dopamine signalling (Solinas and Goldberg, 2005; Higgs et al., 2005; Trujillo-Pisanty et al., 2011).

Although not the focus of this thesis, THC has rewarding properties in itself, as demonstrated in conditioned place preference, and self-administration tests, though the results have been less consistent than with other drugs of abuse (reviewed in Maldonado et al., 2011 and Vlachou and Panagis, 2014).

1.3.3.3 THC and psychosis / schizophrenia

Acute THC intoxication is well known to induce positive and negative psychotic symptoms, as well as perceptual alterations in healthy subjects, as measured by different kinds of self- and clinician-assessed symptom scales (D'Souza et al., 2004; Morrison et al., 2009; Kaufmann et al., 2010; Liem-Moolenaar et al., 2010). These effects are transient, dose dependent, and distinct from sedation effects (D'Souza et al., 2004; Morrison and Stone, 2011). The extent to which these effects are mediated by dopamine D2 receptor signalling is currently not clear. While one study reports an attenuation of THC induced psychotomimetic effects

in response to the D2-receptor antagonist haloperidol (Liem-Moolenaar et al., 2010), D'Souza and colleagues did not observe this effect (D'Souza et al., 2008).

Given these acute psychotomimetic effects of THC it is not surprising that cannabis use has been proposed as a risk factor for the development of schizophrenia. Patients suffering from schizophrenia have been reported to have at least a two-fold increased rate of cannabis use disorder (Arseneault, 2004; Moore et al., 2007; Volkow, 2009). Furthermore, acute cannabis intoxication has been associated with symptom exacerbation in laboratory based studies in schizophrenic patients (D'Souza et al., 2005) as well as an overall poorer treatment response and increased risk of relapse (Linszen et al., 1994; Krystal et al., 1999; Zammit et al., 2008; Henquet et al., 2010; Schoeler et al., 2016; Schoeler et al., 2016), although Zammit and colleagues have cautioned the reported associations might not be specific to cannabis consumption.

The most compelling evidence for an association between cannabis consumption and psychosis arises from longitudinal studies. Of five systematic reviews published between 2004 and 2007 that examined the cumulative evidence for an association between cannabis and psychosis, four found a consistent association (Arseneault, 2004; Henquet et al., 2005; Semple, 2005; Moore et al., 2007), but (Macleod et al., 2004) did not. Moore and colleagues for example reported that after adjustment for confounding factors any cannabis use was associated with a 2 fold increased risk of developing psychotic disorder. In line with one of the first longitudinal studies, Moore et al. also reported evidence for a dose-response effect: high frequency users showed the highest risk for a psychotic outcome (Andréasson et al., 1987; Moore et al., 2007). This was further supported by

more recent evidence in which cannabis use, especially of high potency skunk-type cannabis, was associated with a higher risk of early-onset psychosis (Di Forti et al., 2009; Di Forti et al., 2014; Di Forti et al., 2015). In addition, two recent meta-analysis studies confirmed an association between cannabis use and age at onset of psychosis, as well as a dose response between cannabis use and psychosis (Large et al., 2011; Marconi et al., 2016).

Whether cannabis use is causal or whether emerging psychotic symptoms may lead to cannabis use (referred to as self-medication hypothesis) is currently still a matter of debate. While some longitudinal studies found no evidence for self-medication (Fergusson et al., 2003; Henquet et al., 2005; Kuepper et al., 2011), others found a bidirectional association between cannabis use and psychotic symptoms (Ferdinand et al., 2005; McGrath et al., 2010). However, given the robust association across different samples, the evidence for a dose-response relationship, and the temporal relationship in which cannabis use precedes psychotic symptoms (Kuepper et al., 2011), most investigators assume cannabis use to be causal, but in itself neither necessary nor sufficient to induce schizophrenia (van Winkel and Kuepper, 2014; Radhakrishnan et al., 2014). This suggests that other genetic (and possibly environmental) factors may need to act together to influence vulnerability. For example genetic variation in the AKT1 and DRD2 gene have been shown to have interactive effects with cannabis use (van Winkel et al., 2011; Di Forti et al., 2012; Colizzi et al., 2015; Morgan et al., 2016). Furthermore, as discussed in section 1.2.4.3, COMT is a well-studied candidate gene in this respect and several studies have investigated the potential association between COMT genotype, cannabis use and schizophrenia.

1.4 COMT, cannabis, and schizophrenia: a gene x environment interaction?

The COMT Val^{158}Met polymorphism was first suggested to moderate cannabis induced risk for psychotic illness by Caspi and colleagues in 2005. In a longitudinal study they demonstrated that adolescent cannabis use increased the risk for psychotic disorder at age 26 in those homozygous for the Val- (odds ratio (OR) = 10.9) but not Met- allele (OR = 1.1), with heterozygous individuals showing only a slightly increased risk (OR = 2.5). However, several subsequent studies have failed to replicate this initial finding (Zammit et al., 2007; Kantrowitz et al., 2009; van Winkel, 2010; Zammit et al., 2011), found only a weak trend in a subpopulation of patients (Gutiérrez et al., 2009), or even reported an increased risk for homozygous Met allele carriers (Costas et al., 2011). Although a study by Estrada and colleagues did not support an association between COMT genotype, cannabis use and the risk for psychotic disorder itself, they reported that the age of onset of psychosis following adolescent cannabis use was mediated by the COMT polymorphism, with the average age of onset being 15.4 years in Val- compared to 18.8 years in Met-homozygotes and 17.1 years in heterozygotes (Estrada et al., 2011). Pelayo-Teran et al. reported a similar interactive effect with age of onset of psychosis, however in their study the COMT genotype effect was only present in the non-cannabis using group (Pelayo-Terán et al., 2010). Furthermore, an emerging literature suggests that a history of childhood abuse may interact with cannabis consume to confer risk of psychosis (Houston et al., 2008; Harley et al., 2010; Konings et al., 2012), but (Kuepper et al., 2011). Interestingly, Vinkers and colleagues recently reported a three-way interaction in which Val homozygotes

were more likely than Met homozygotes to experience psychotic episodes when they had a history of cannabis use and childhood abuse (Vinkers et al., 2013). Similar results have since been reported by (Alemany et al., 2014), suggesting that a putative interaction between COMT genotype and cannabis use on psychosis may depend on additional environmental (and possibly genetic) factors.

In the mouse animal model, O'Tuathaigh et al. reported that a knockout (KO) of the COMT gene modulated the effects of subchronic adolescent administration of THC or the synthetic cannabinoid WIN 55,212 on phenotypes relevant to the positive, negative and cognitive symptoms associated with schizophrenia later on during adulthood (O'Tuathaigh et al., 2010; O'Tuathaigh et al., 2011). Specifically, COMT KO mice showed increased exploratory activity, but disrupted sensorimotor gating following adolescent cannabinoid treatment. In addition, cannabinoid treatment disrupted social functioning and spatial working memory more in COMT KO than wild-type mice, hence supporting a putative COMT x cannabis exposure interaction on symptoms related to schizophrenia. These genotype specific effects were mostly observed in male mice and were absent in animals that had been exposed to cannabinoids during adulthood instead of adolescence. Analysis of morphological changes in these mice following adolescent THC exposure revealed that COMT deletion induced a reduction in dopaminergic cell size in the ventral tegmental area and increased CB1 receptor intensity in the frontal cortex (Behan et al., 2012). Recent studies investigating possible interactive effects of chronic cannabis use and COMT genotype on structural and functional effects in humans provide tentative evidence for

volume effects in the caudate nucleus, hippocampal and amygdala (Solowji et al., 2012; Batalla et al., 2014).

Studies investigating the acute effect of cannabis on psychotic experiences are more consistent, and convergent with the initial report by Caspi et al. Using a structured diary technique in patients with psychotic disorder and healthy controls, carriers of the Val allele were shown to experience an increase in hallucinations after cannabis consumption compared to Met homozygotes. This effect was conditional to prior evidence of psychometric psychosis liability as measured by CAPE (Henquet et al., 2009). Similarly, in an experimental, double-blind, placebo-controlled, cross-over study with volunteers who had a history of repeated cannabis consumption, carriers of the Val allele were more sensitive to THC induced acute psychotic experiences than Met homozygotes in the group of subjects with a prior liability to psychosis (Henquet et al., 2006). In the same study acute THC administration also affected cognitive function in a COMT genotype dependent manner: verbal memory and attention were impaired in Val- but not Met homozygous participants. This effect was independent of prior psychosis liability. Further support for an interactive effect between COMT genotype and acute THC exposure originates from a study of young cannabis users by Verdejo-Garcia et al., in which cannabis users homozygous for the Val allele exhibited lower accuracy of sustained attention than Val/Val non-users and committed more monitoring/shifting errors than cannabis users homozygous for the Met allele (Verdejo-García et al., 2013). In addition, a recent double-blind, placebo controlled study in our laboratory investigated interactive effects of COMT genotype and acute THC administration on non-clinical

volunteers prone to paranoia. We did not observe a COMT genotype specific effect on psychotic experiences measured by CAPE positive dimension score, but found that THC impaired working memory performance on a Digit Span Backwards task in Val homozygotes but not Met carriers (Tunbridge et al., 2015). Interestingly, a study investigating the effects of acute THC administration on dopamine release reported increased frontal cortex dopamine release in Val- compared to Met- homozygotes. Though sample sizes were very small (n=3-6 per genotype) and require replication, this study suggests that at least part of the observed interactive effects between cannabis consumption and COMT could be mediated by dopamine (Stokes et al., 2010)

Although still few in number these studies suggest that cannabis exposure and COMT genotype might have interactive effects on aspects of cognitive function and possibly, though the literature is more complex and less consistent, psychosis. However, the neurobiological mechanisms underlying this putative gene x environment interaction are only beginning to be investigated.

1.5 Aims and objectives

As described above, some studies have (albeit inconsistently) separately implicated the COMT Val^{158}Met polymorphism and cannabis use/THC in modulating the risk of psychosis and cognitive impairment. Furthermore, emerging evidence suggests a gene x environment interaction between these two factors. Based on the neurobiological substrates that COMT and THC act upon, the dopaminergic system is a plausible candidate for mediating this interaction. However, little is known about the interactive effects of COMT and THC on dopamine function.

In addition to human studies, the complex functional genetic architecture of COMT, as well as the possible modulatory effect of environmental factors, make animal models essential to conduct research under controlled conditions. The aim of this thesis was therefore to investigate the separate and interactive neurochemical effects of altered COMT function and THC exposure in the mouse model. Based on the dopamine hypothesis of schizophrenia, the brain regions of particular interest were the mPFC and the striatal regions. The latter is of special interest because COMT was believed to be of importance mainly in cortical regions, and although it has long been suggested that COMT-induced effects on PFC dopamine may have indirect effects in the striatum, studies investigating this proposed effect are only beginning to emerge.

As a proof of principle, chapter 2 investigates the neurochemical effects of acute COMT inhibition and THC administration in the striatum. However, acute COMT inhibition cannot model the effect of the COMT Val^{158}Met polymorphism, since the latter has lifelong effects and might induce developmental and compensatory

changes. The following experiments were therefore carried out in a novel mouse model in which the human Met allele was introduced into the mouse COMT gene. This mouse model is introduced in chapter 3, together with a microarray study investigating the effects of this genetic manipulation on overall gene expression as well as a study investigating neurochemical changes at tissue level. In Chapter 4, the effect of THC on extracellular dopamine and metabolite levels in the awake, transgenic mouse are described. Based on the results obtained in this chapter, the interactive effects of THC and COMT genotype on behavioural aspects such as locomotor activity, reward processing and sensorimotor gating were determined in chapter 5. Finally, in chapter 6, I discuss the findings of this thesis and propose future experiments to further this research.

2 Effect of acute COMT inhibition and THC on extracellular dopamine and metabolite levels

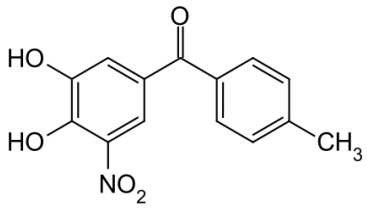
2.1 Introduction

As outlined in chapter 1 COMT activity levels and cannabis (or THC) have been proposed to have interactive effects on cognitive function and psychosis (Caspi et al., 2005; Henquet et al., 2006; Henquet et al., 2009; Estrada et al., 2011; Verdejo-García et al., 2013; Tunbridge et al., 2015). Although the dopaminergic system is a plausible candidate for mediating these effects, studies have almost exclusively focused on the behavioural consequences and little is known about the underlying neurochemical mechanisms. Furthermore, despite early evidence for a role of COMT in subcortical regions under specific conditions (chapter 1.2.3), most studies investigating the modulatory effect of COMT on brain function have focused on the PFC, due to the predominance of DAT in clearing released dopamine in striatal regions (Sesack et al., 1998). However, changes in PFC dopamine can modulate dopamine function in the striatum (Pycock et al., 1980). In addition, suggestions of a putative role of COMT activity levels in reward processing and addiction have sparked an interest in COMT's effects on subcortical dopamine (Tunbridge et al., 2012). The aim of this chapter was thus to investigate the effects of acute COMT inhibition and THC administration on dopamine and metabolite levels in the ventral and dorsal striatum.

2.1.1 Tolcapone

2.1.1.1 The use of tolcapone

Although the COMT gene is conserved across species, the Val^{158}Met polymorphism appears to be human specific. The naturally occurring COMT enzyme in rodent has an activity level similar (Lotta et al., 1995; Risbrough et al., 2014), or higher (Chen et al., 2004), than that of the human ancestral Val form. The lower activity level of the Met allele can thus be modelled by the administration of COMT inhibitors. One of the commonly used drugs is tolcapone (Ro 40-7592) (Figure 10). It is a selective, brain-penetrant COMT inhibitor, which binds competitively to the active site of the enzyme (Zürcher et al., 1990; Ceravolo et al., 2002). Tolcapone has been licensed for the clinic and, despite a link with hepatotoxicity leading to a temporary withdrawal from the European market, is used as an adjunct to Parkinson's disease treatment, as it increases central L-DOPA availability (Männistö and Kaakkola, 1999; Marsala et al., 2012). In addition, COMT inhibition has been proposed as a treatment strategy for several other disorders including pathological gambling, and marijuana and nicotine dependence (Weinstein and Gorelick, 2011; Grant et al., 2013; Herman et al., 2013), though these indications are currently still speculative.



Tolcapone

Figure 10: Tolcapone structure and function. *Tolcapone is a highly selective, second-generation COMT inhibitor, which competitively binds to COMT's active site (Borges et al., 1997). The nitrocatechol structure determines the selectivity and action duration. The negatively charged nitrite group (-NO_2) at the 5th position of the catechol ring stabilises the catechol-COMT complex. Tolcapone is thus poorly methylated and very slowly released from the COMT active site (Ovaska and Yliniemelä, 1998).*

2.1.1.2 Tolcapone's effects on dopamine levels

A number of studies have investigated the levels of dopamine and metabolites in homogenised tissue preparations, following systemic COMT inhibition. Most of the studies were conducted in the 1990s and focused on the dorsal striatum, where tolcapone produces robust increases in DOPAC and decreases in HVA, as would be anticipated after COMT inhibition (Figure 2). Despite these robust effects on dopamine metabolites, tolcapone administration has no effect on striatal tissue dopamine levels (Maj et al., 1990; Budygin et al., 1999), a result that has more recently been confirmed in our lab (Laatikainen et al., 2013). Furthermore, tolcapone does not affect dopamine levels in striatal tissue homogenates when combined with other dopamine altering drugs such as L-DOPA or the DAT blocker GBR 12909 (Männistö et al., 1992; Budygin et al., 1999).

In line with the findings from tissue preparations, *in vivo* microdialysis studies confirmed that tolcapone (3 – 30 mg/kg) administration increases extracellular DOPAC and decreases HVA levels in the dorsal striatum. However, systemic tolcapone administration alone does not alter extracellular dopamine levels (Li et al., 1998; Napolitano et al., 2003), although local administration may increase dopamine (Huotari et al., 2001). In addition, several studies have investigated the combined effects of tolcapone and other drugs on extracellular dopamine levels in the dorsal striatum. When administered together with the dopamine precursor L-DOPA, tolcapone potentiates its dopamine-increasing effects (Acquas et al., 1992; Napolitano et al., 1995; Napolitano et al., 2003; Huotari et al., 1999). Similarly tolcapone further increases extracellular dopamine levels in the presence of dopamine reuptake blockade (Kaakkola and Wurtman, 1992;

Budygin et al., 1999; Huotari et al., 1999). Fast-scan cyclic voltammetry did not reveal any changes in electrically-evoked dopamine release after the combination of these drugs (Budygin et al., 1999). Together, these results indicate that COMT might affect striatal dopamine under conditions of dopamine uptake blockade and/or increased dopamine transmission. However, tolcapone does not enhance the increase in extracellular dopamine levels in the dorsal striatum elicited by amphetamine or the D2 receptor antagonist haloperidol (Törnwall et al., 1993; Tuomainen et al., 1996; Huotari et al., 1999).

The effects of tolcapone on dopamine and metabolite levels in brain regions other than the dorsal striatum have been less well studied. In the mPFC, tolcapone has no effect on basal dopamine levels, but potentiates increased dopamine levels following pharmacological challenge and food reward (Tunbridge et al., 2004; Lapish et al., 2009). Apart from an early study by Li et al., which reported increased DOPAC and decreased HVA, but no change in extracellular dopamine levels (Li et al., 1998), the question of whether acute COMT inhibition is functionally significant for the ventral striatum remains largely unexplored.

2.1.2 Tolcapone and cannabinoid receptor agonists

To my knowledge, this is the first study to investigate the interactive effects of acute tolcapone and THC administration on extracellular dopamine levels. However, similar to THC, the acute administration of other CB receptor agonists, such as CP 55940 and WIN 55212, has been shown to increase dopamine neuron activity (Gessa et al., 1998). A recent study investigated the interactive effects of acute COMT inhibition by tolcapone and CP 55940 or WIN 55212 on psychosis-

related animal phenotypes, including prepulse inhibition and habituation of acoustic startle response. Whilst tolcapone pre-treatment did not alter the effects of acute WIN 551212 on either startle measure, it prevented the effect of acute CP 55940 administration on startle responsivity and increased prepulse inhibition (O'Tuathaigh et al., 2011). The authors of this study argued that the specific behavioural effects observed with the cannabinoid agonist CP 55940 (which is more selective than WIN 55212) may depend upon frontal cortex dopamine levels. These results suggest that under certain circumstances acute COMT inhibition and THC administration can have interactive behavioural effects. However, in contrast to the data presented here, this study did not investigate neurochemical effects.

2.1.3 Choice of methodology

2.1.3.1 Microdialysis coupled with electrochemical detection

The effects of drugs on catecholamine and metabolite levels could conceivably be measured in a number of different ways. A commonly-used method, which allows for quick collection of datasets from different brain regions within one animal, is to analyse the content of homogenised tissue preparations. However, this technique does not support within-subject treatment, or time course comparisons, since only one data point can be collected per animal. In addition, dopamine signalling may be altered without changes in overall dopamine tissue quantity, and para-, and post-mortem effects may influence the results. Analysis of neurochemical tissue levels therefore provides a quick but rather crude measurement for examining pharmacological effects.

In contrast, microdiaysis and fast scan cyclic voltammetry (FCV) can be applied *in vivo* in the awake animal, and thus allow repeated measures to be taken in the same animal. Although voltammetry offers a much higher temporal resolution compared to microdialysis it is currently not possible to reliably monitor several neurochemicals - like dopamine and its metabolites – simultaneously. In addition, FCV measures phasic dopamine responses against background current, potentially masking changes in overall extracellular dopamine levels, which might be expected following pharmacological manipulation of dopamine degradation. Hence, microdialysis was chosen to investigate the effect of COMT inhibition and THC administration on striatal neurochemistry.

Intracerebral microdialysis is a well-established technique, which involves stereotactic implantation of a microdialysis probe containing a semi-permeable membrane. Molecules from the extracellular space can diffuse across this membrane into the probe, allowing them to be collected and quantified in the perfusate (for details see Sharp and Zetterstrom, 1992). Although microdialysis probes are considerably bigger than FCV electrodes, it has been demonstrated that the neurotransmitter levels measured in the microdialysis perfusate are sensitive to sodium channel blockade and calcium removal, and hence do not originate primarily from damaged neurons but, rather, reflect neuronal activity (Sharp and Zetterstrom, 1992).

To separate, identify and quantify different neurochemical compounds contained in the microdialysis perfusate samples, high-performance liquid chromatography (HPLC) coupled with electrochemical detection was used. This technique makes use of the different chemical and physical interactions between a solid,

adsorbent material packed in a column and the sample compounds that are washed through it. Following this separation process, compounds elute from the column and are detected and quantified by the amount of current generated by their oxidation in an electrochemical detector.

2.1.3.2 Drug dose

Oral or i.p. administration of 10 – 30 mg/kg tolcapone significantly inhibits peripheral and central COMT activity in rodents and humans. In mice, oral administration of 30 mg/kg tolcapone results in central COMT inhibition levels of 99% at 1 h, and 86% at 6 h post administration (Learmonth et al., 2004). Similarly, i.p. injection of 30 mg/kg tolcapone in rats leads to maximal COMT inhibition approximately 2 h after administration (Tunbridge et al., 2004).

Cannabis contains more than 70 different cannabinoids. By using a single cannabinoid - the main psychoactive component THC - it is possible to exclude interactive effects between different cannabinoids as previously reported for THC and cannabidiol (C Arnold et al., 2012). A rise in THC content, and thus cannabis potency has been observed over the past years (Cascini et al., 2012). In addition, Henquet and colleagues showed interactive effects of THC and COMT in a laboratory based study (Henquet et al., 2006). For the current study a THC dose of 3 mg/kg i.p. was chosen, since this dose and route of administration resulted in THC mouse blood plasma levels around 50 ng/ml 30 min after injection (Laatikainen and Tunbridge, unpublished data), levels which are similar to those observed in humans after smoking cannabis (Drummer et al., 2003).

Based on the time course of tolcapone and THC, the effects of COMT inhibition (or vehicle) were observed for 1h following stable baseline levels, before THC (or vehicle) was administered to investigate interactive effects.

2.1.4 Hypotheses

I hypothesised

1) That THC would increase dopamine in the striatum.
2) That acute COMT inhibition would alter dopamine metabolite levels, but not dopamine levels (specifically an increase DOPAC and a decrease in HVA was anticipated).
3) That COMT and THC might have interactive effects on striatal dopamine. More specifically, if COMT inhibition and THC have acute local effects, dopamine levels are expected to be higher in animals with reduced COMT activity.

2.2 Methods

2.2.1 Animals:

Male C57Bl/6J wild-type mice (Charles River, Margate, UK, total number: n=73), aged 7-11 weeks, were group-housed at 21-23˚C under 12 h light/dark cycle with *ad libitum* food and water. All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated Home Office Guidelines.

2.2.2 Stereotactic surgery:

Stereotactic surgery was performed under isoflurane general anaesthesia (4% induction, 1-2% maintenance, as required), and local lidocaine analgesia, with buprenorphine (0.1 mg/kg, s.c.) administered for pre- and post-surgery analgesia. A guide cannula (MAB-4.9.1C, Royem Scientific Limited, Luton, UK; made by Microbiotech/se AB, Stockholm, Sweden) was inserted above the region of interest (see coordinates below) and cemented in place. Animals were allowed to recover for 5-7 days in individual home-made recovery cages. During this period they were weight and handled daily in the holding room but not habituated to probe insertion.

2.2.3 Microdialysis:

Mice were awake and freely moving throughout the microdialysis procedure. At least 24h prior to testing mice were transferred into individual plastic test cages (33 cm x 26 cm x 25 cm, length x width x height). On the test day animals were transferred into the experimental procedure room, food and water were removed and a microdialysis probe (1mm membrane length: MAB-4.9.1PES, Royem Scientific Limited, Luton, UK; made by Microbiotech/se AB, Stockholm,

Sweden; membrane length 1mm, outer diameter 0.2mm) was inserted into the guide cannula of the restrained animal (final co-ordinates: A/P = +0.6, L/M = +1.8, D/V = -2.5 from brain surface for dorsal striatum; A/P = +1.4, L/M = +0.5, D/V = -4.2 from brain surface for nucleus accumbens), according to the mouse brain atlas by Franklin and Paxinos (Paxinos and Franklin, 2004). A schematic overview of the probe locations can be found in the supplementary material (Supplementary material 7.1). The probe was continuously perfused at a flow rate of 2µl/min with artificial CSF (140mM NaCl, Fisher Chemical, Loughborough, UK; 3mM KCl, VWR, Leuven, Belgium; 1.2mM Na2HPO4*2H2O Sigma-Aldrich, Steinheim, Germany; 0.27mM NaH2PO4*H2O VWR, Poole, UK; 1mM MgCl2 VWR, Leuven, Belgium; 2.4mM CaCl2 VWR, Leuven, Belgium; 7.2mM D(+)-Glucose VWR, Poole, UK) using a Harvard Apparatus CMA/100 Microinjection Pump (CMA Microdialysis, Solna, Sweden). Samples were collected every 20 min. Drugs were administered once dopamine levels stopped dropping and a stable baseline was acquired (usually approximately 2-3 hours after probe implantation). At the end of the experiment the animals were sacrificed and the brains frozen rapidly. The positions of the probes were verified histologically from post mortem brain slices.

2.2.4 Quantification of extracellular dopamine and dopamine metabolite concentrations:

Dialysate samples were analysed immediately after collection and dopamine, DOPAC, HVA and 5-HIAA concentrations were determined using a HPLC system coupled with electrochemical detection. Aliquots of 35 µl dialysate were injected into a mobile phase consisting of 120.0 mM $NaH_2PO_4*H_2O$ (VWR, Poole, UK), 1.66 mM 1-Octanesulphonic acid sodium salt (VWR, Leuven, Belgium), 2mM NaCl (Fisher Chemical, Loughborough, UK), 100mM EDTA, 13.4% MeOH (VWR, Leuven, Belgium) and separated via a Spherisorb OSD2 column (3 µm, 4.6x100mm; Waters, Milford, MA, USA). The flow rate was kept at 1 ml/min using a Jasco PU-2085 PLUS pump (Jasco, Essex, UK) equipped with a pulse damper (LO-Pulse Damper, Arc Sciences, Alton, UK). Electrochemical detection (DECADE II, Antec, Leyden, Zoeterwoude, The Netherlands) was performed at a potential of +600 mV against an ISAAC reference electrode. The chromatograms were processed using ChromPass chromatography data system software (Jasco, Essex, UK). The detection limit for dopamine was approximately 5 fmol.

2.2.5 Drug injections

After a baseline of three to four samples was acquired, during which dopamine levels no longer decreased, tolcapone (Roche, Basel, Switzerland; 30 mg/kg, i.p., suspended in 20% β-cyclodextrin, 0.9% saline) or its vehicle was administered. THC (Tocris, Bristol, UK; 3 mg/kg, i.p., dissolved in 5% ethanol, 5% Tween-80 and 90% 0.9% w/v saline) or its vehicle was administered 60 min after tolcapone.

2.2.6 Data analysis:

For statistical analysis, neurochemical levels following drug administration were averaged and expressed as a percentage of their baseline levels. One-way ANOVAS were carried out to compare baseline levels between the four drug groups. The effects of tolcapone and THC were examined using repeated-measures analysis of variance (ANOVA) with time (comparing the average of the 3 samples following administration of tolcapone or its vehicle ['post-Tol'] with the average of the 3 samples following administration of THC or its vehicle ['post-THC']) as a within-subjects factor, and tolcapone (vs. vehicle) and THC (vs. vehicle) as between-subject factors. Significant interactions were explored using LSD post-hoc tests. Due to technical issues DOPAC and HVA levels could not be measured in two animals (NAc: vehicle-vehicle, dST: vehicle-THC), and were thus not included in the analysis. Statistics were computed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) and results were considered significant at $P < 0.05$.

2.3 Results

2.3.1 Nucleus accumbens

2.3.1.1 Effects of tolcapone and THC on dopamine

In the nucleus accumbens, the average dopamine levels prior to drug administration were 18.1 ± 0.8 fmol/35µl (mean ± SEM) and a one-way ANOVA revealed no differences between the four drug groups ($F_{3,35}$=0.32, p=0.81) (Figure 11a). Analysis of the baseline-normalised drug effects at the post-Tol and post-THC time points (Figure 11b) revealed an interaction between tolcapone*time ($F_{1,35}$=4.16, p=0.049), as well as a THC*tolcapone*time interaction ($F_{1,35}$=4.47, p=0.042) but no further main or interactive effects (F's <0.83, p's>0.36). Post-hoc analysis revealed that dopamine levels did not differ significantly between the four treatment groups at the post-Tol time point (all p's>0.22) or the post-THC time point (all p's>0.10).

However, analysis of the tolcapone*time interaction and the tolcapone*THC*time interaction revealed that there was a trend increase in dopamine levels from the post-Tol to the post-THC time point in the tolcapone-treated (p=0.069), but not in the vehicle treated animals (p=0.31), and that this interaction resulted from an increase in dopamine levels after THC administration in the Tol-THC group (p=0.008,) but not any of the other drug groups (p's>0.18).

2.3.1.2 Effects of tolcapone and THC on DOPAC and HVA

Baseline DOPAC levels prior to any drug administration were 2653 ± 309 fmol/35µl (mean ± SEM) and did not differ between drug groups ($F_{3,34}$=0.24, p=0.87) (Figure 11c). Statistical analysis of the effect of tolcapone and THC on

baseline normalised DOPAC levels revealed main effects of tolcapone ($F_{1,34}$=86.2, p<0.001) and time ($F_{1,34}$=75.9, p<0.001), as well as a time*tolcapone interaction ($F_{1,34}$=75.6, p<0.001) but no other main or interactive effects (F's<1.92, p's>0.17) (Figure 11d). Post-hoc analysis revealed normalised DOPAC levels were higher in tolcapone compared to vehicle treated animals at the post-Tol time point (mean difference 50.3%, p<0.001). In addition, DOPAC levels further increased between the post-Tol and the post-THC time point in the tolcapone (p<0.001) but not in the vehicle treated animals (p=0.99), resulting in an even bigger difference at the post-THC time point (p<0.001). However, this effect was independent of THC (THC*tolcapone*time interaction $F_{1,34}$=0.63, p=0.43).

Pre-drug HVA levels were 3048 ± 215 fmol/35µl (mean ± SEM) and no differences between the 4 drug groups could be observed ($F_{3,34}$=0.72, p=0.55) (Figure 11e). There were main effects of tolcapone ($F_{1,34}$=649.3, p<0.001) and time ($F_{1,34}$=187.4, p<0.001), as well as a time*tolcapone interaction ($F_{1,34=233.1,}$ p<0.001), but no further main or interactive effects (F's<2.2, p's>0.15) (Figure 11f). HVA levels were lower in tolcapone-, compared to vehicle-, treated animals at the post-Tol time point (p<0.001). Between the post-Tol and post-THC time point HVA further decreased in the tolcapone- (p<0.001) but not in the vehicle-treated animals (p=0.29). Accordingly, at the post-THC time point normalised HVA levels were significantly lower in tolcapone compared to vehicle treated animals (p<0.001). However, these results were independent of THC administration (THC*tolcapone*time interaction $F_{1,34}$=0.19, p=0.67).

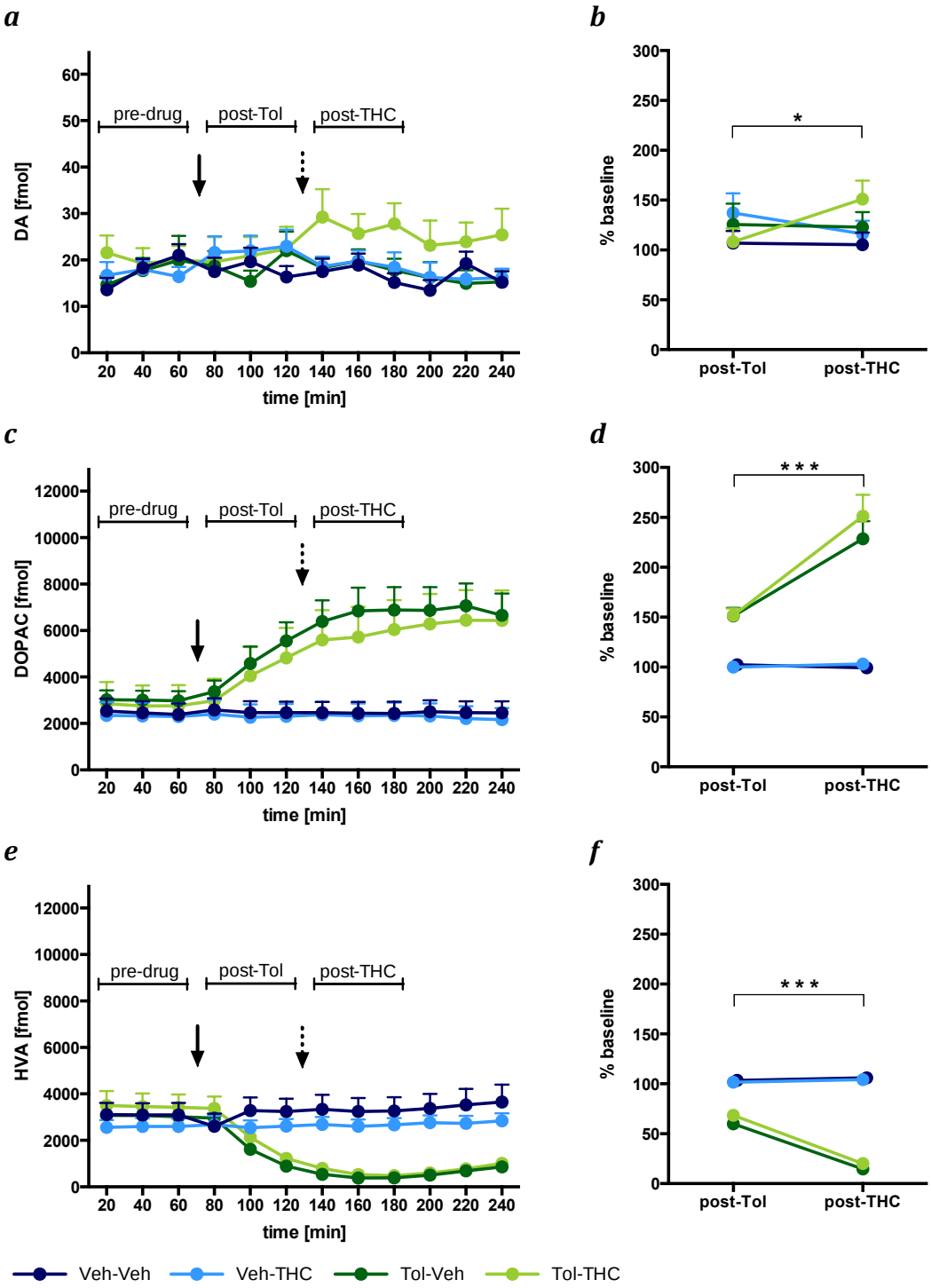


Figure 11: Nucleus accumbens - Separate and interactive effects of tolcapone and THC on extracellular dopamine (a,b), DOPAC (c,d), and HVA (e,f) levels. *Microdialysis samples were taken every 20 min (a,c,e). The solid and dotted arrows indicate tolcapone (30mg/kg)/vehicle and THC (3mg/kg)/vehicle injections, respectively. For statistical analysis, data was normalised to pre-drug baseline levels and averaged across the post-Tol and post-THC time points (b,d,f). Statistical annotation refers to time*treatment interactions only (please refer to text for additional statistical comparisons). *P < 0.05, due to a significant increase between post-Tol and post-THC time points in the Tol-THC group only. ***P < 0.001, due to a significant change between post-Tol and post-THC time points in tolcapone treated animals. Values are mean ± SEM from n=9 animals (n=8 for metabolites) in the Veh-Veh group, and n=10 animals in all other groups.*

2.3.2 Dorsal striatum

2.3.2.1 Effects of tolcapone and THC on dopamine

In the dorsal striatum the average dopamine level prior to drug administration was 27.1 ± 2.2 fmol/35µl (mean ± SEM) and no group differences were observed ($F_{3,30}$=0.25, p=0.86) (Figure 12a). There were no main or interactive effects (time: $F_{1,30}$=0.01, p=0.99; tolcapone $F_{1,30}$=2.81, p=0.10; THC $F_{1,30}$=0.34, p=0.57; time*THC: $F_{1,30}$=0.01, p=0.94; time*tolcapone*THC:$F_{1,30}$=0.01, p=0.91) other than a trend interaction between time*tolcapone ($F_{1,30}$=3.15, p=0.086) (Figure 12b). This interaction was driven by a difference between tolcapone- and vehicle-treated animals at the post-THC (p=0.012), but not the post-tolcapone (p=0.85), time point.

2.3.2.2 Effects of tolcapone and THC on DOPAC and HVA

Pre-drug baseline DOPAC levels were 5167.3 ± 586.6 fmol/35µl (mean ± SEM) and no group differences could be observed ($F_{3,29}$=0.97, p=0.42) (Figure 12c). There were main effects of tolcapone ($F_{1,29}$=44.5, p<0.001) and time ($F_{1,29}$=55.4, p<0.001), as well as a time*tolcapone interaction ($F_{1,29}$=71.8, p<0.001) (Figure 12d). Post-hoc analysis revealed that, at the post-Tol time point, DOPAC levels were higher in tolcapone compared to vehicle treated animals (mean difference 31.9%, p<0.001). DOPAC levels further increased from the post-Tol to the post-THC time point in tolcapone (p<0.001) but not vehicle treated animals (p=0.49), resulting in an even higher difference at the post-THC time point (mean difference 79.6%, p<0.001). However, there were no main effects or interactions involving THC, (F's<0.73; p's>0.39).

Baseline HVA levels were 5331 ± 614 fmol/35µl (mean ± SEM) and no differences between the four drug groups could be observed ($F_{3,29}$=0.52, p=0.67) (Figure 12e). There were main effects of tolcapone ($F_{1,29}$=180.6, p<0.001) and time ($F_{1,29}$=271.4, p<0.001), as well as a time*tolcapone interaction ($F_{1,29}$=291.9, p<0.001) (Figure 12f). Post-hoc analysis showed that at the post-Tol time point HVA levels were lower in tolcapone than vehicle treated animals (mean difference 32.2%, p<0.001). HVA decreased further in the tolcapone (p<0.001) but not vehicle treated animals (p=0.68) from the post-Tol to the post-THC time points, resulting in an even higher difference between vehicle and tolcapone treated animals at the post-THC time point (mean difference 81.1%, p<0.001). However, there were no main or interactive effects involving THC (F's<2.5, p's>0.13).

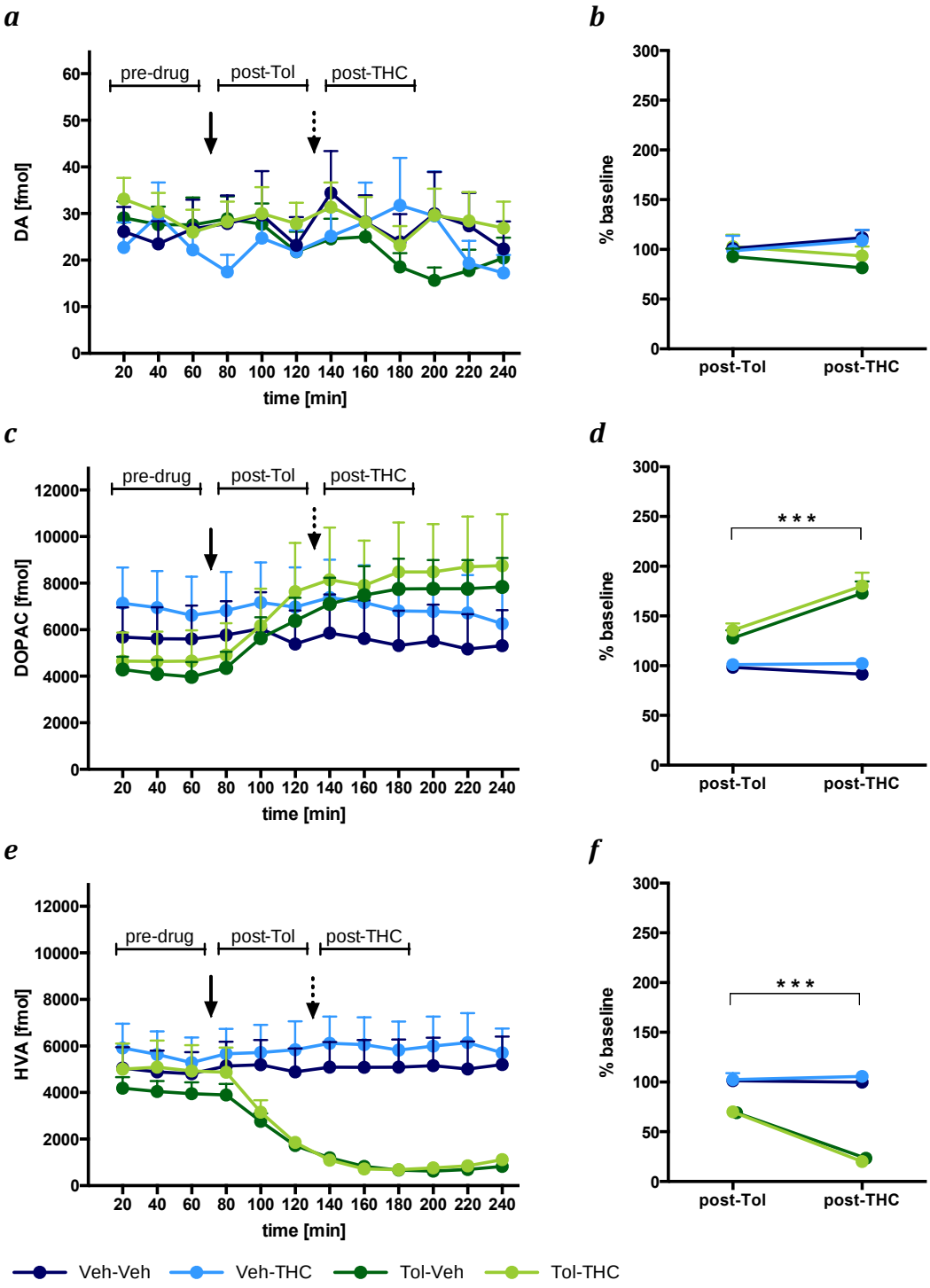


Figure 12: Dorsal striatum - Separate and interactive effects of tolcapone and THC on extracellular dopamine (a,b), DOPAC (c,d), and HVA (e,f) levels. *Microdialysis samples were taken every 20 min (a,c,e). The solid and dotted arrows indicate tolcapone (Tol)(30mg/kg)/vehicle (Veh) and THC (3mg/kg)/Veh injections, respectively. For statistical analysis data was normalised to pre-drug levels and averaged across the post-Tol and post-THC time points (b,d,f). Statistical annotation refers to time*treatment interactions only (please refer to text for additional statistical comparisons). *P < 0.05, due to a significant increase between post-Tol and post-THC time points in the Tol-THC group only. ***P < 0.001, due to a significant change between post-Tol and post-THC time points in tolcapone treated animals. Values are mean ± SEM from Veh-Veh n=9, Veh-THC n=7 (n=6 for metabolites), Tol-Veh n=10, Tol-THC n=8 animals.*

2.4 Discussion

The study presented above was designed to investigate the separate and interactive effects of acute COMT inhibition and THC on extracellular dopamine and dopamine metabolite levels. When administered alone, neither the COMT inhibitor tolcapone, nor THC, affected extracellular dopamine levels in the dorsal striatum or the nucleus accumbens. However, THC increased extracellular dopamine levels in the nucleus accumbens, but not the dorsal striatum, in animals pre-treated with tolcapone. This study is, to my knowledge, the first to specifically report an interactive effect between an acute alteration in COMT activity and THC at a neurochemical level.

Effects of tolcapone on basal dopamine and metabolites

The basal dopamine and metabolite levels found in this study lie within the range of those previously reported in mice (Fadda et al., 2005; Huotari et al., 2004). Tolcapone significantly inhibited COMT in both striatal regions, as indexed by the increase in DOPAC and decrease of HVA. However, despite this marked change in metabolism, extracellular dopamine levels remained unchanged throughout the experiment. As well as replicating results from dorsal striatal and prefrontal cortex studies (Li et al., 1998; Napolitano et al., 2003; Tunbridge et al., 2004), these results show that, consistent with other brain regions, an acute alteration of COMT activity does not affect extracellular dopamine levels in the nucleus accumbens under baseline conditions. The abundance of dopamine transporters, which is the primary way of terminating the dopamine signal in the striatum, is likely a significant contributor to this lack of effect (Sesack et al., 1998; Cass et al., 1993; Eisenhofer et al., 2004).

Effects of THC on basal dopamine

In the present study THC alone did not affect dopamine or metabolite levels in either of the examined brain regions. Our microdialysis results thus show inconsistency with those discovered by Robledo et al., 2007 and Chen et al., 1990, which both showed that a single THC administration (0.3-1.0 mg/kg) increased accumbal dopamine in mice and rats, respectively. However, in another microdialysis study by Castañeda et al., 1991 a single oral dose of 1-10mg/kg THC failed to increase extracellular dopamine in the dorsal striatum or nucleus accumbens. Several factors might contribute to the mixed results found in microdialysis studies: First of all, Tanda and colleagues reported that THC selectively increased dopamine levels in the shell, but not the core of the nucleus accumbens (Tanda, 1997), a fact that earlier studies (e.g. Chen et al., 1990; Castañeda et al., 1991) could not take into account as they did not differentiate between accumbal subregions. In addition, the observation that lower (< 3mg/kg) THC doses generally seem to elevate accumbal dopamine, whilst higher do not, might indicate a non-linear, biphasic dose-response curve for THC, similar to that previously seen in locomotor activity and intracranial self-stimulation experiments (Sañudo-Peña et al., 2000; Katsidoni et al., 2013). Furthermore, strain-specific effects of THC on accumbal dopamine have previously been reported in rats (Chen et al., 1991; Cadoni et al., 2015). Both the mouse strain and THC dose in previous microdialysis studies differed from our experiment. It is thus possible that discrepancies between the results are caused by strain-specific genetic factors affecting the individual response to THC in mice. In fact, based on the results obtained in this study, it is possible to

speculate that strain differences in COMT activity levels in mice (Grice et al., 2007; Kember et al., 2010) and rats (Goldstein et al., 1980) may well be one of the factors contributing to strain specific variability in response to THC.

Interactive effects of COMT and THC on dopamine

In the present study THC increased extracellular dopamine levels in the nucleus accumbens in mice with transiently lowered COMT activity, but not in animals with unaltered enzyme activity. As described earlier, the dopamine system is a key modulator of psychosis and cognitive function and is regulated by complex interactions between cortical and subcortical regions (Pycock et al., 1980). These findings therefore support the hypothesis that interactive effects between COMT and THC on accumbal dopamine might contribute to the suggested gene x environment interaction and provide a possible neurochemical mechanism mediating this effect.

However, based on the first study reporting a gene x environment interaction (Caspi et al., 2005), as well as studies investigating the acute effect of cannabis on psychotic experiences and cognitive function in human Met and Val allele carriers (Henquet et al., 2006; Tunbridge et al., 2015), lower enzyme activity would be expected to protect from THC effects. In contrast, only mice with lowered COMT activity showed an effect of THC on accumbal dopamine. Based on the present study it is difficult to determine the cause for this discrepancy. However, in contrast to the physiological condition in which genetically determined differences in COMT activity exist throughout life and may result in developmental and/or adaptive changes, COMT activity was only lowered acutely in the present study. It is thus of interest to compare these results with

the effects of THC in an animal model of constantly lowered COMT activity. Such studies are included later in this thesis (see chapter 4).

It has previously been suggested that effects of altered COMT activity on subcortical and, more specifically striatal regions, could result from an inverse relationship between PFC and striatal dopamine function (Akil et al., 2003). Although this possibility cannot be ruled out, the direction of the dopamine effect in the nucleus accumbens would suggest a direct rather than an indirect effect of COMT on dopamine levels following acute tolcapone and THC treatment. However, it is possible that indirect effects might not be able to manifest following a single incidence of COMT inhibition.

These data thus add to the growing body of evidence suggesting that COMT is of relevance to dopamine function outside of the prefrontal cortex (Laatikainen et al., 2012). Specifically, they identify the nucleus accumbens as a region in which COMT modulates dopamine function, not under baseline conditions but under certain conditions of increased dopamine metabolism. In this respect our data are consistent with those found in S-COMT deficient mice, which showed elevated dopamine levels in the nucleus accumbens following the administration of carbidopa/L-DOPA compared to wild-type littermates (Tammimäki et al., 2010). Consistent with the results obtained in the present study, this effect was absent in the dorsal striatum. The data could thus indicate that COMT is of less relevance for dopamine function in the dorsal striatum compared to the nucleus accumbens. However, despite studies showing that THC affects neuronal firing in the substantia nigra as well as in the VTA (French et al., 1997), previous microdialysis studies investigating the effect of THC on dopamine in the dorsal

striatum gave mixed results (Castañeda et al., 1991; Malone and Taylor, 1999). Given that in the present study THC did not affect extracellular dopamine levels in the dorsal striatum, the lack of a COMT modulatory effect in this case may well reflect the lack of a THC induced dopamine challenge that COMT could act upon.

Behavioural effects

The present study did not aim or expect to observe behavioural effects, especially since experiments were carried out during the light phase, which mice mostly spend resting and inactive. Personal observation during sample collection generally confirmed this behaviour (or lack of it) in all animals independent of drug treatment. However, it is worth noting that while vehicle treated animals rested in a crouched or curled up positions typical for mice, THC treated animals tended to spread themselves out and almost lie flat on their stomach, possibly reflecting the effects of THC on muscle tone (Varvel et al., 2005), which will be discussed in more detail in chapter 5.

Conclusion

The results demonstrate that THC elevates accumbal, but not dorsal striatal, extracellular dopamine levels only in mice pre-treated with the COMT inhibitor tolcapone. By implication, these data suggest that the neurochemical response to THC / cannabis is modulated by the activity level of the COMT enzyme and therefore provide a plausible neurochemical mechanism for the suggested gene x environment interaction between COMT genotype and cannabis use.

3 Characterisation of COMT-Met transgenic mouse model

3.1 Introduction

In chapter 2, pharmacologically lowered COMT activity in combination with THC administration revealed an interactive effect on extracellular dopamine levels in the nucleus accumbens. This provides evidence for an interactive effect of acutely lowered COMT activity levels and THC at a neurochemical level. However, in humans, differences in COMT activity levels also occur as the consequence of common genetic variability. Long-term, genetically-encoded differences in enzyme activity may result in adaptive, or possibly even developmental changes in catecholamine metabolism, which may differ from the acute effects modelled in chapter 2. In order to further address interactive effects between consistently lowered COMT activity and THC a novel mouse model was used, which will be introduced in this chapter.

In COMT-Met mice the native COMT amino acid (Leu^{148}) at the locus equivalent to the human $Val^{158}Met$ position was replaced by methionine (Barkus et al., 2016) (Figure 13). These mice modelling the human $Val^{158}Met$ polymorphism were generated using a PCR-based strategy at the National Institute of Mental Health, US.

Genetic manipulations bear the potential for off-target effects, which can affect the phenotype of mouse models by altering the expression of neighbouring genes (Olson et al., 1996). Off-target effects are of special relevance in the case of COMT, because the gene is embedded within the critical deletion region of the

22q11 Deletion Syndrome (22q11DS) in human. Hemizygous deletion of this region is associated with cognitive impairment and a significantly increased risk for developing schizophrenia. The mouse genome contains an equivalent region (16qA13) with orthologues of most of the genes implicated in the pathogenesis of 22q11DS (Karayiorgou et al., 2010). Consistent with the human data, multiple genes contribute to the cognitive changes observed in 22q11DS mouse model. In addition, epistatic interactions have been reported. For example interactive effects between COMT and proline dehydrogenase (PRODH) seem to underlie spatial working deficits in this model (Paterlini et al., 2005; Drew et al., 2011). To examine potentially confounding changes in gene expression especially within the 22q11 critical region, a microarray study was carried out, the results of which will be presented in the first part of this chapter.

A basic behavioural characterisation of the COMT-Met mouse model revealed no significant differences between COMT-Met mice and their wild-type littermates on anxiety related tasks and only subtle differences in learning and memory at baseline, as summarised in Table 2 (Barkus et al., 2016).

Basic neurochemical characterisation was carried out by analysing tissue levels of dopamine, DOPAC, and HVA in the regions of focus (i.e. mPFC, nucleus accumbens and dorsal striatum). Since our lab had previously reported COMT activity to also play a role in the hippocampus (Laatikainen et al., 2012), dorsal and ventral hippocampi were included in the study. The results will be presented in the second part of this chapter.

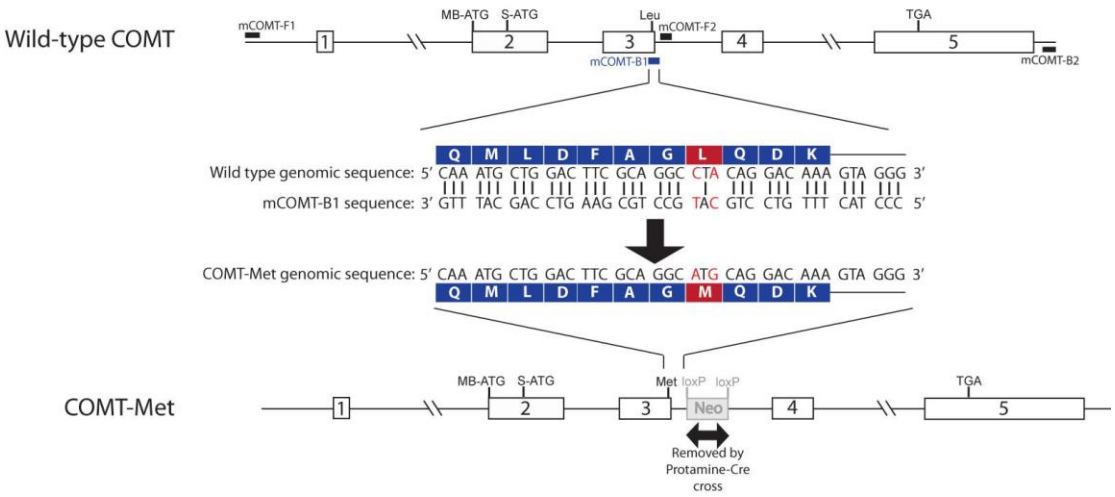


Figure 13: Generation of COMT-Met mice: *The Met allele was knocked into the mouse COMT gene using a PCR-based strategy. The mCOMT-B1 primer introduces the Met allele into the mouse COMT gene (mismatched bases are highlighted in red). The resulting genomic DNA fragments were cloned into a pLoxP vector containing a neo selection cassette. Mouse embryonic stem (ES) cells were transfected with the final transgene containing the mouse COMT gene (Amplified region: chr 16:18, 407,548-18,415,235, according to the Mouse Genome December 2011 GRCm38/mm10 Assembly) with the Met allele, as well as a floxed PGK-neo selection cassette in the intron between exons 3 and 4. ES cells were screened for homologous recombination at the COMT locus by long range PCR and used for blastocyst injection. The selection cassette was subsequently removed by crossing the COMT-Met mice with a Cre recombinase-expressing line. (Barkus et al., 2016)*

Table 2: Basic behavioural summary of COMT-Met versus wild-type mice on learning and memory, as well as anxiety related tasks

Behavioural Task	**Result: COMT-Met vs. Wt**	
Feeding behaviour		
Body weight	n.s.	(p's>0.05 in several behavioural cohorts across different ages and backcrosses)
Anxiety related tasks		
Elevated plus maze	n.s.	(time spend in open arm: $F_{1,56}$=0.97, p=0.33)
Light-dark box	n.s.	(F's<1.8, p's>0.2)
Novelty suppressed feeding task	n.s.	($F_{1,56}$=0.53, p=0.47)
Anxiogenic open field	n.s.	(latency to enter central region: $F_{1,56}$=2.8, p=0.47; time spent in central region: $F_{1,56}$=0.80, p=0.39)
Learning and memory		
Spatial novelty preference	Subtle increase in COMT-Met	(t=-1.86; p=0.033)
Discrete trial spontaneous alteration	n.s.	($F_{1,35}$=0.2, p=0.65)
Novel object recognition	n.s.	($F_{1,35}$=1.0, p=0.32)
Acquisition of appetitively-motivated reference Y memory task	n.s.	($F_{1,38}$=0.59, p=0.45)
Morris water maze	n.s.	(F's<1.6; p's>0.11)

n.s. : no significant genotype differences; (Barkus et al., 2016)

3.1.1 Hypotheses

I hypothesised

1) That by targeting a single amino acid, COMT-Met mice will not show off-target differences in the expression of neighbouring genes. However, the genetic manipulation may result in adaptive changes in the expression of other genes related to dopamine metabolism.
2) That COMT-Met mice show increased DOPAC and decreased HVA levels compared to wild-type animals. However, in the absence of off-target effects or compensatory mechanisms, COMT-Met mice are not expected to show alterations in dopamine, 5HT or 5HIAA at baseline.

3.2 Methods

3.2.1 Animals

The novel COMT-Met mouse was backcrossed onto a C57Bl/6J background for 8-10 generations. Mice were bred in individually ventilated cages. Mice were genotyped using a restriction fragment length polymorphism assay (as detailed in Barkus et al., 2016) at approximately 7 weeks of age, prior to their transfer into open cages. Mice used for neurochemical analysis were 7 months of age and had completed behavioural testing at least 3 weeks prior to sample collection. Behavioural tasks included elevated plus maze, open field, light/dark box, hyponeophagia, reference memory Y-maze, and water maze. Mice used for microarray analysis were naïve and 8-10 weeks of age. All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated Home Office Guidelines.

3.2.2 Microarrays

COMT-Met mice and their wild-type littermates were sacrificed and their brains were rapidly removed and snap-frozen in isopentane and kept at -80°C. Brain sections (approx. 150-180 μm thick) were cut on a cryostat. Tissue punches were collected from frontal cortex (between bregma +3.1 and +1.7), dorsolateral striatum (between bregma +0.7 and +0.2) and nucleus accumbens (shell, between bregma +1.5 and +0.9 according to Paxinos and Franklin, 2004) from 6 COMT-Met and 6 wild-type male mice, and were kept frozen throughout. RNA was extracted using the Qiagen RNeasy Micro Kit (Qiagen Ltd, Manchester, UK) according to the manufacturer's instructions. Microarrays were run and analysed by the OXION Microarray Facility, in collaboration with Sheena Lee

(Department of Physiology, Anatomy and Genetics, Oxford). All samples were processed at the same time. RNA quality was assessed using Agilent 2100 Bioanalyser Nano-Chips (Agilent Technologies Inc, CA, USA). Only replicates with RNA Integrity Numbers (RIN) of 6 or above were used to ensure RNA was of sufficient quality, giving final n's of 5 COMT-Met mice (6 for nucleus accumbens) and 5 wild-types (although similar results were obtained if all mice were included). 1.5ng of RNA was reverse transcribed and amplified using the NuGen Ovation Pico WTA System V2 (NuGen Technologies Inc, CA, USA). Briefly, amplification is started at the 3' end and at sites throughout the transcript thus reducing 3' bias. The RNA was reverse transcribed to synthesise a cDNA with a SPIA® RNA tag sequence at the 5' end of the cDNA. The cDNA is then amplified using the SPIA® process. Amplified, double-stranded cDNA was transformed into single strand sense. The sense cDNA was chemically and enzymatically fragmented to produce strands of 50-100bp in length using the Encore Biotin Module V2 (NuGen Technologies Inc.). The fragmented sample was run on an Agilent Bioanalyser NanoChip (Agilent Technologies Inc) to confirm successful fragmentation. The strands were then labelled with biotin. Fragmented, labelled, single-strand sense cDNA was hybridised overnight at 45°C to the Affymetrix GeneChip Mouse 2.0 ST Array chip (Affymetrix UK Ltd, High Wycombe, UK). Using the GeneChip Hybridization, Wash and Stain Kit (Affymetrix UK Ltd) the chips were washed and stained with streptavidin-phycoerythrin. The hybridised chips were scanned on the Affymetrix Gene Chip Scanner. Signals were computed using the GeneChip® Command Console® Software (Affymetrix UK Ltd). Quality control by the Command Console™ 4.1.2 (Affymetrix UK Ltd) indicated that all samples were of sufficient quality for inclusion in the

subsequent analysis. Data processing was performed using GeneSpring GX 12.6.1. The CEL files for individual brain regions were normalised in GeneSpring using Probe Logarithmic Intensity Error (PLIER). The gene expression values were compared between COMT-Met and wild-type mice for each region using Linear Models for Microarray Data (LIMMA). Genotype group differences were assessed using moderated t-tests with Benjamini-Hochberg correction for multiple testing, although genes falling within the 22q11DS critical deletion region were also examined at a highly-lenient uncorrected threshold, to check for any suggestion of genotype differences.

3.2.3 Tissue sample preparation and neurochemical analysis

12 COMT-Met and 8 wild-type male mice were sacrificed. Their brains were rapidly removed and snap-frozen in isopentane. Brain sections (approx. 150-180 μm thick) were cut on a cryostat. Tissue punches were collected according to the Franklin and Paxinos brain atlas (Paxinos and Franklin, 2004) from frontal cortex (approx. between +3.1 and +1.7), nucleus accumbens (approx. between +1.5 and +0.9), dorsolateral striatum (approx. between +0.7 and +0.2), dorsal and ventral hippocampus (approx. between -2.7 and -3.8) under a dissection microscope while the slide rested on a stage maintained at approx. -10°C. Tissue samples were homogenised in 0.06M perchloric acid (Sigma-Aldrich, Dorset, UK) and centrifuged for 2 min. The supernatant was used to determine dopamine, DOPAC, HVA, 5HT and 5HIAA by high-performance liquid chromatography (HPLC) using the same assay as previously described (chapter 2), except for changes in mobile phase, which for this assay contained 120mM NaH2PO4, 2mM NACl, 0.1 mM EDTA, 2mM OSA and 15% methanol, pH 3.71. To account for potential variability in tissue punch collection, the amount of protein present in

each tissue punch sample was determined using the Bradford method and results are expressed as fmol/mg protein.

In the ventral hippocampus tissue was lost from one wild-type mouse. The dopamine peak in one wild-type and one COMT-Met mouse, and the DOPAC peak in one COMT-Met mice, was masked and had to be excluded from analysis. For 10 of the 12 COMT-Met mice (but none of the wild-types), dorsal hippocampal HVA was undetectable, these values were encoded as zero. Because the data were non-normally distributed, the effect of genotype was examined using Mann-Whitney U tests.

3.3 Results

3.3.1 Effects of COMT-Met transgene on gene expression

Comparison of the gene expression profiles showed clear differences between brain regions (Figure 14). However, after correction for multiple comparisons, no genes showed differential expression between genotype groups. Given the position of COMT within the 22q11DS critical deletion region, the expression of loci within this region was examined more closely, using a very lenient, uncorrected threshold. In all brain regions COMT expression was nominally reduced in COMT-Met mice compared to wild-types (fold changes: dorsal striatum and nucleus accumbens -1.4, frontal cortex -1.6; uncorrected p's <0.01; corrected p's>0.8). Importantly, only 3 more nominally significant changes were found in the expression of genes within the 22q11 critical deletion region (one per region in 3 different loci) (Table 3). In the nucleus accumbens the expression of Dgcr14, a gene possibly encoding a component of splicosomes, was nominally increased in COMT-Met mice (fold change +1.2, p=0.049). In the dorsal striatum the expression of reticulon 4 receptor (Rtn4r) mediating axonal growth inhibition was nominally decreased (fold change -1.2, p=0.034) and in the frontal cortex tRNA methyltransferase 2 homolog A was nominally increased (fold change +1.4, p=0.011) in COMT-Met mice compared to wild-type littermates. However, for all 3 loci showing nominal genotype differences this very lenient, uncorrected statistical analysis, the direction of change differed across regions (i.e. expression was increased in at least one region and decreased in at least one other region). Furthermore, the number of nominally significant differences equates to 4.2% of the comparisons made and thus falls within the predicted rate

of Type 1 errors for α=0.05. Therefore, the results strongly indicate that the nominally significant differences observed in these 3 loci reflect Type 1 errors. Thus the COMT-Met transgene does not appear to have notable off-target effects on the expression of genes within the 22q11DS critical deletion region.

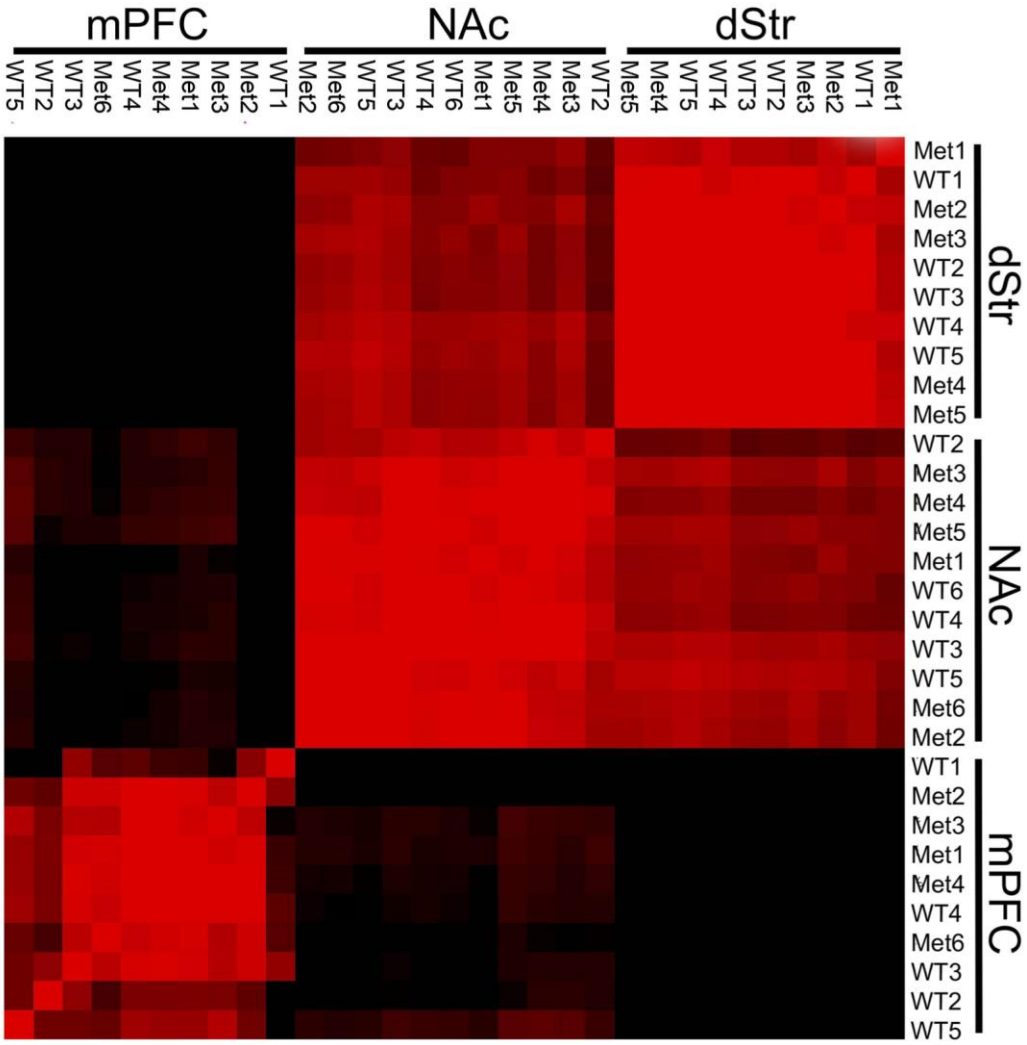


Figure 14: Heatmap correlation plot of microarray result across individual samples. *Red colouring indicates samples with similar expression patterns, while black indicates samples with significantly different expression patterns. Samples within one brain region show comparatively strong correlations independent of genotype. Furthermore, samples from the nucleus accumbens (NAc) and the dorsal striatum (dStr) show moderately high correlation coefficients, while correlations between these regions and the medial prefrontal cortex (mPFC) are weak.*

Table 3: Expression of genes in the 22q11DS critical deletion region in COMT-Met vs wild-type mice. *Red shading indicates a nominal fold change greater than 1.0; blue shading indicates a nominal fold change of less than 1.0. Nominally significant changes in expression are indicated with asterisks. (Barkus et al., 2016)*

	Frontal cortex		Dorsal striatum		Nucleus accumbens	
Gene locus	Fold change	P (uncorr)	Fold change	P (uncorr)	Fold change	P (uncorr)
Dgcr2	1.0	0.673	-1.1	0.495	1.0	0.549
Tssk1	+1.1	0.320	+1.1	0.596	-1.2	0.140
Dgcr14	-1.2	0.119	1.0	0.627	+1.2	0.049*
Gsc2	+1.1	0.534	1.0	0.712	1.0	0.894
Slc25a1	-1.2	0.204	-1.1	0.555	-1.1	0.261
Vpreb2	1.0	0.577	1.0	0.684	-1.1	0.482
Dgcr6	1.0	0.807	1.0	0.666	1.0	0.872
Prodh	-1.1	0.171	-1.2	0.076	+1.1	0.299
Rtn4r	-1.2	0.114	-1.2	0.034*	+1.2	0.349
Zdhhc8	1.0	0.896	-1.2	0.081	1.0	0.705
Ranbp1	1.0	0.840	1.0	0.832	1.0	0.962
Trmt2a	+1.4	0.011*	1.0	0.982	-1.1	0.327
Dgcr8	1.0	0.564	1.0	0.798	1.0	0.703
Tango2	1.0	0.851	-1.1	0.106	+1.1	0.273
Arvcf	-1.1	0.402	1.0	0.591	1.0	0.663
Comt	**-1.6**	**0.001***	**-1.4**	**0.009***	**-1.4**	**0.001***
Txnrd2	-1.1	0.338	1.0	0.794	-1.2	0.227
Gnb1l	1.0	0.830	1.0	0.668	+1.2	0.053
Tbx1	1.0	0.944	-1.1	0.243	1.0	0.556
Gp1bb/ Sept5	-1.1	0.196	1.0	0.956	-1.1	0.384
Cldn5	-1.1	0.516	+1.1	0.552	+1.1	0.387
Cdc45	+1.2	0.216	1.0	0.843	-1.1	0.330
Ufd1l	1.0	0.725	1.0	0.918	1.0	0.612
Mrpl40	-1.1	0.495	-1.1	0.269	+1.1	0.427
Hira	+1.1	0.481	1.0	0.912	1.0	0.766

3.3.2 Neurochemical changes in COMT-Met mice

Genetic manipulation of COMT activity in the COMT-Met mice resulted in increased tissue DOPAC levels in all regions examined, compared to wild-type mice (dorsal striatum $U=6$, $p=0.001$; nucleus accumbens $U=14$, $p=0.013$; frontal cortex $U=7$, $p=0.002$; dorsal hippocampus $U=0$, $p<0.001$, ventral hippocampus $U=10$, $p=0.007$) (Figure 15). Furthermore, COMT-Met mice showed significantly lower HVA tissue levels in the dorsal striatum ($U=9$, $p=0.003$), nucleus accumbens ($U=0$, $p<0.001$), and dorsal hippocampus ($U=17$, $p=0.012$), as well as a trend level decrease in medial frontal cortex ($U=23$, $p=0.054$). Tissue HVA levels were unchanged in ventral hippocampus ($U=40$, $p=0.90$). No genotype differences in tissue levels of dopamine were observed in any of the examined brain regions (all p's>0.40). In addition, no genotype differences in 5-hydroxytryptamine or its metabolite 5-hydroxyindoleacetic acid could be observed (Table 4).

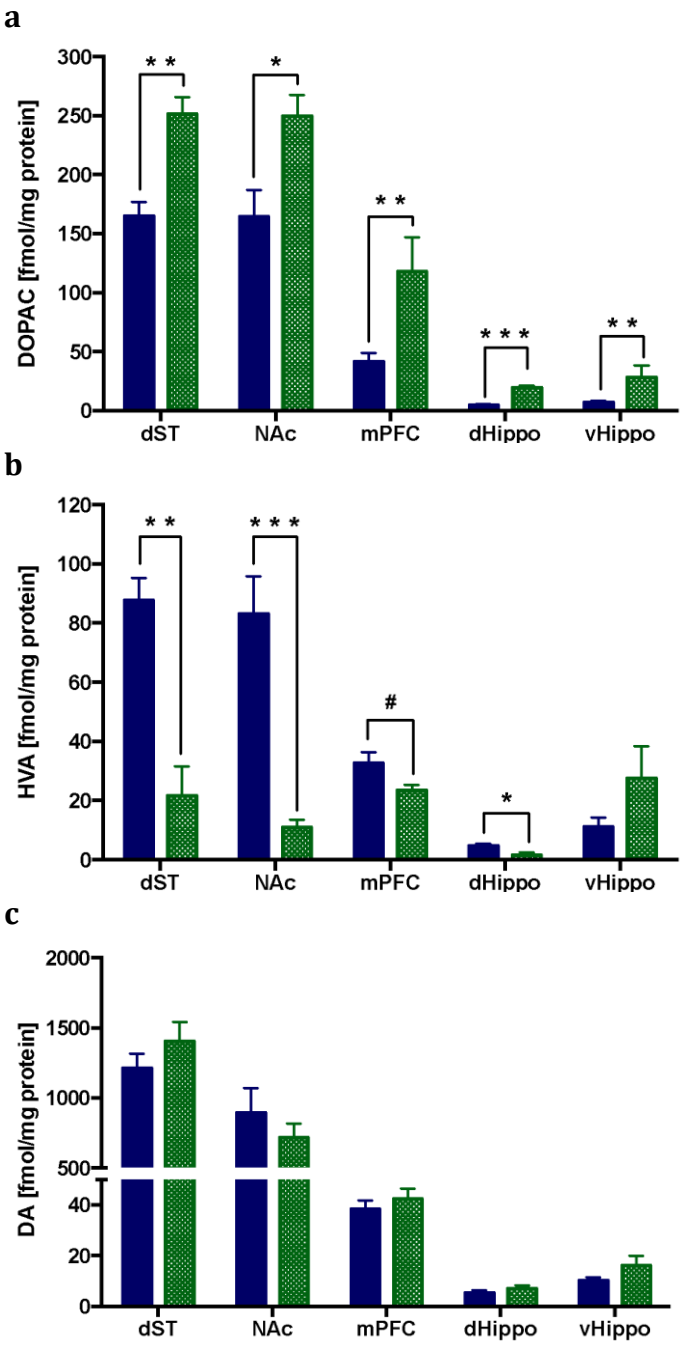


Figure 15: Tissue levels of dopamine metabolites and dopamine in COMT-Met and wild-type mice. *(a) DOPAC is increased in dorsal striatum (dST), nucleus accumbens (NAc), medial prefrontal cortex (mPFC), dorsal hippocampus (dHippo) and ventral hippocampus (vHippo) in COMT-Met (dotted green bars) compared to wild-type mice (solid blue bars). (b) HVA is decreased in all brain regions except ventral hippocampus in COMT-Met compared to wild-type mice. (c) Tissue dopamine levels do not differ between COMT-Met and wild-type mice in any of the examined brain regions. Wild-type n=8, COMT-Met n=12, (see 3.2.3 for exception) *** P<0.001; **P<0.01; *P<0.05; # P<0.08*

Table 4: Tissue levels of 5-hydroxytryptamine and 5- hydroxyindoleacetic acid in wild-type and COMT-Met mice. *Data are presented as means ± SEMs and were compared using Mann Whitney-U tests.*

Region	5-hydroxytryptamine			5- hydroxyindoleacetic acid		
	Wild-type	COMT-Met	*p*	Wild-type	COMT-Met	*p*
Medial prefrontal cortex	76.1 ± 14.5	72.8 ± 7.7	*0.99*	28.7 ± 5.4	40.8 ± 5.0	*0.18*
Nucleus accumbens	79.3 ± 15.7	69.6 ± 12.2	*0.62*	26.0 ± 5.0	28.8 ± 5.25	*0.99*
Dorsal striatum	31.0 ± 3.4	24.7 ± 3.7	*0.18*	8.6 ± 2.0	12.1 ± 2.0	*0.21*
Dorsal hippocampus	48.8 ± 6.4	47.4 ± 5.1	*0.99*	32.9 ± 3.3	31.5 ± 3.1	*0.91*
Ventral hippocampus	108.7 ± 12.4	151.8 ± 37.5	*0.90*	60.9 ± 6.8	112.5 ± 36.0	*0.99*

3.4 Discussion:

The results presented above were part of a set of studies designed to validate the novel COMT-Met mouse model. The COMT gene, and especially the Val^{158}Met polymorphism, is among the most extensively studied in neuropsychiatry. However, the presence of other functional variants within the human COMT gene as well as gene x gene and gene x environment interactions make transgenic mouse models an important tool to investigate the effect of differences in COMT enzyme activity in a controlled environment and the relative absence of genetic variability. As shown in this chapter, COMT-Met mice provide a novel, highly specific model of reduced COMT activity. The introduction of the human Met^{158} allele into the native COMT gene, results in partial reduction of COMT expression and dopamine metabolism without altering the expression of other genes.

Effects of COMT-Met transgene on gene expression

COMT-Met mice were generated by targeting a single amino acid within the mouse COMT gene and thus only show a very small alteration from the native genome. Consistent with this, the molecular phenotype was highly specific: Whilst COMT's abundance was slightly reduced in COMT-Met mice compared with wild-types, the expression of other genes was unaltered. Most critically, the expression of genes within the equivalent of the human 22q11DS deletion region was unaltered. Although subsequent alterations in the translation process are conceivable and require further investigation at protein level, the genetic manipulation used to create COMT-Met mice appears highly specific and does not cause any off-target effects. Despite the importance of gene expression levels within the 22q11DS critical deletion region on the effect of altered COMT activity

(Paterlini et al., 2005), the COMT-Met mouse is the first COMT model in which global gene expression has been reported. In order to reach conclusive information from the comparison between COMT transgenic mouse models (some of which will be discussed in more detail below), it will therefore be important to examine the expression of 22q11DS orthologues in all existing models.

Effects of COMT-Met transgene on dopamine metabolism

Dopamine metabolism was reduced in all examined brain areas in COMT-Met, compared to wild-type, mice. As indicated by the increase in DOPAC and decrease in HVA levels, introduction of the COMT Met allele into the native mouse COMT gene resulted in a reduction in COMT enzyme activity. This result has since been confirmed in an enzyme activity assay, which showed a reduction of enzyme activity by approximately 40% in the frontal cortex of COMT Met mice, similar to the effect observed in humans carrying only the Met allele (Chen et al., 2004; Barkus et al., 2016).

COMT enzyme activity can increase during postnatal development (Stanton et al., 1975; Brust et al., 2004). However, most of these studies focused on adolescent development and a study investigating COMT activity in the human PFC did not reveal changes between adult and aged individuals (Tunbridge et al., 2007). The relatively old age of the animals used in this study is thus unlikely to have biased the results.

My data are consistent with previous findings in a different COMT mouse model: mice in which the COMT gene has been knocked out also show a significant increase in DOPAC, and a decrease in HVA tissue levels, compared to wild-types (Gogos et al., 1998; Huotari et al., 2002; Käenmäki et al., 2009; O'Tuathaigh et al., 2011).

In the present study the decrease in COMT activity did not affect the amount of dopamine present in the examined tissue samples. This result is consistent with similar studies in COMT knockout mice, in which no differences in striatal dopamine levels was observed. However, results obtained from cortical regions differ between studies. While two studies reported increased dopamine levels in frontal cortex tissue samples in COMT knockout mice (Gogos et al., 1998; O'Tuathaigh et al., 2011) two other studies did not observe this effect (Huotari et al., 2002; Käenmäki et al., 2009). Compared to the knockout model, COMT-Met mice only show an incomplete reduction in enzyme activity. Given the inconsistency in the knockout literature it is thus not surprising that no effect on dopamine tissue level could be observed in this model.

Comparison of COMT-Met mice with other models of reduced COMT activity

As mentioned above a number of COMT mouse models have been developed including a COMT knockout (Gogos et al., 1998), a humanised COMT Val^{158}Met (Risbrough et al., 2014), a COMT overexpressor (Simpson et al., 2014), a COMT-Val transgene overexpressor (Papaleo et al., 2008), and a soluble COMT deficient (Käenmäki et al., 2009) mouse model. Like the novel COMT-Met mouse model, two of these models aim to reduce COMT activity in order to closely mimic the human Val^{158}Met polymorphism, based on the fact that the ancestral COMT form

has high enzyme activity. The COMT knockout mouse contains a disrupted COMT gene, while the humanised Val^{158}Met mouse contains the human Val- or Met-COMT open reading frame on a COMT-null background (Gogos et al., 1998; Risbrough et al., 2014). Thus the COMT locus – in contrast to the COMT-Met mouse model - is deleted in both of these models thereby increasing the potential for yet unexplored off-target effects. Although the risk is slightly lower in the humanised mice since the native COMT gene is deleted in both genotypes, and off-target effects should thus not affect genotype comparisons, the possibility of off-target effects remains to be explored. Thus, differences in COMT activity and their behavioural impact may be magnified by off-target effects in the other COMT mouse models developed to date (Paterlini et al., 2005).

Compared to COMT knockout mice, COMT-Met mice show very little behavioural phenotype in tasks of cognitive function at baseline (Babovic et al., 2008; Papaleo et al., 2008; Papaleo et al., 2012; Scheggia et al., 2013; Barkus et al., 2016). Since COMT activity is completely absent in COMT knockout mice, these differences may be in part due to differences in the magnitude of the effect of the transgene. However, some cognitive changes have also been observed in heterozygous knockout mice (Papaleo et al., 2012), as well as in humanised COMT mice (Risbrough et al., 2014), which show a reduction in enzyme activity closer to the levels observed in COMT-Met mice.

Compared to the humanised COMT mice, the reduction in enzyme activity in COMT-Met mice results from a single base change in the native COMT sequence and thus does not represent all of the polymorphic complexities found in the human COMT gene (Nackley et al., 2006). However, the insertion of the human

COMT sequence into the mouse genomic environment is also unlikely to fully recapitulate the human situation.

Ultimately, comparisons between different COMT mouse models, their areas of convergence as well as the search for explanations of possible discrepancies are most likely to successfully further our understanding of COMTs impact on brain function.

Conclusion:

As demonstrated in this chapter the novel COMT-Met mouse is a highly specific model of altered COMT activity. Any behavioural and neurochemical phenotype observed in this model can be expected to result from specific changes in COMT activity. Ultimately, the COMT-Met mouse model – in combination with other models of altered COMT activity - can help to further understand the impact of COMT genotype not only on behavioural aspects such as cognition and anxiety but also on related gene-gene and gene x environment interactions.

4 Effect of THC on extracellular dopamine levels in COMT-Met and wild-type mice

4.1 Introduction

Chapter 2 revealed an interactive effect between acutely lowered COMT activity and THC on dopamine. It thus provided evidence for the occurrence of interactive effects between COMT and THC at a neurochemical level, which may contribute to the proposed gene x environment interaction on cognitive function and psychosis (Caspi et al., 2005; Henquet et al., 2006; Henquet et al., 2009; Estrada et al., 2011; Verdejo-García et al., 2013; Tunbridge et al., 2015), as well as demonstrating that COMT can affect striatal dopamine under certain conditions. However, the acute effects modelled in chapter 2 differ from the human situation in which long-term genetically encoded differences in enzyme activity may result in adaptive changes in catecholamine metabolism (Pycock et al., 1980). The aim of this chapter was thus to investigate the interactive effects between consistently lowered COMT activity and THC on extracellular dopamine and metabolite levels in the COMT-Met mouse model.

In addition to the striatal regions, the mPFC was included in the present study. Dopamine levels in the PFC play an important role in cortical function and a hypo-dopaminergic state contributes to the cognitive deficits observed in schizophrenia (see chapter 1.1.3.4). Furthermore, it has been suggested that subcortical effects of COMT may be mediated via an inverse relationship between cortical and striatal dopamine levels (see chapter 1.1.2). The relatively low dopamine levels present in the mPFC make microdialysis experiments very

challenging. By setting up a new HPLC assay I was able to include this brain region in the present study to investigate the effect of consistently lowered COMT activity on PFC DA metabolism in the novel mouse model and to gain insight, whether striatal effects could be mediated by cortical effects.

To date, only very few studies have investigated the effect of altered COMT activity on extracellular dopamine function in any of the existing COMT mice models (Huotari et al., 2002; Huotari et al., 2002; Huotari et al., 2004; Yavich et al., 2007; Tammimäki et al., 2010; Simpson et al., 2014; Tammimaki et al., 2016). In COMT knockout mice microdialysis studies focused on the dorsal striatum and were conducted in anaesthetised animals. Under baseline conditions the knockout mice consistently showed increased DOPAC and decreased HVA levels (Huotari et al., 2002; Huotari et al., 2002) but no difference in dopamine (Huotari et al., 2004). Tissue studies investigating DA levels in dST and PFC following L-DOPA and carbidopa administration in KO mice produced inconsistent results (Huotari et al., 2002; Käenmäki et al., 2009) and treatment with amphetamine or the DAT inhibitor GBR12909 increased extracellular dopamine levels independent of genotype (Huotari et al., 2002; Huotari et al., 2004). However, in a pharmacological study in rats, the brain-penetrant COMT inhibitor tolcapone significantly potentiated the increase in extracellular dopamine induced by the administration of the depolarising agent potassium chloride (Tunbridge et al., 2004). Furthermore, Yavich and colleagues investigated extracellular dopamine dynamics following stimulation of the medial forebrain bundle in COMT knockout mice using constant potential amperometry combined with fast scan cyclic voltammetry for specificity. The lack of COMT did not affect dopamine

overflow or decline in the dorsal striatum, but induced a twofold reduced decline rate in the mPFC. In addition, COMT-deficient mice showed an increase in evoked dopamine release in the mPFC, but not the dorsal striatum (Yavich et al., 2007). COMT overexpressor mice on the other hand showed an increase in evoked dopamine release in the dorsal striatum, while the effect of COMT overexpression on cortical regions still needs to be investigated (Simpson et al., 2014). Studies investigating the lack of the membrane bound form of COMT did not reveal altered dopamine levels in the mPFC or dorsal striatum under baseline conditions. Following L-DOPA and carbidopa administration MB-COMT deficient male mice showed higher extracellular dopamine levels than their wild-type littermates in the dorsal striatum, but not the mPFC (Tammimaki et al., 2016).

These findings already indicate that under certain conditions genetically altered COMT activity may affect dopamine transmission. However, the neurochemical effects following THC administration have not been investigated in any of these models despite the proposed gene x environment interaction between COMT genotype and cannabis consumption in cognitive function and psychosis.

4.1.1 Hypothesis

Based on the available literature as well as the results presented in chapter 2 I hypothesised

1) That COMT genotype would alter extracellular dopamine metabolite levels. Specifically that it would increase DOPAC and decrease HVA levels in all examined brain regions.
2) That COMT genotype would not affect basal dopamine levels.
3) That in COMT wild-type mice THC might not affect dopamine levels in the dorsal or ventral striatum (based on the findings described in Chapter 2), but might increase dopamine in the mPFC.
4) That COMT genotype and THC will have interactive effects. Specifically, that if the effects are mediated locally reduced COMT activity increases dopamine levels following THC administration in the nucleus accumbens and mPFC, but based on the results obtained in chapter 2, not the dorsal striatum.

4.2 Methods

4.2.1 Animals:

Male COMT-Met mice and their wild-type littermates (backcrossed onto C57/BL6 background for 10 generations) were group-housed in individually ventilated cages at 21-23˚C under 12 h light/dark cycle with ad libitum food and water. Surgery was conducted aged 9-12 weeks. All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated Home Office Guidelines.

4.2.2 Stereotactic surgery:

Stereotactic surgery was performed as described in chapter 2. Deviating from the previously described protocol the guide cannula used for the mPFC differed in size: They were 7 instead of 9 mm long and designed for a 2mm instead of a 1mm long membrane at the tip of the probe (MAB-4.7.2C, Royem Scientific Limited, Luton, UK; made by Microbiotech/se AB, Stockholm, Sweden). Furthermore, following surgery the animals were housed individually in ventilated cages (floor area 542cm^2) for 5-7 days prior to testing, which were transferred daily into the procedure room to handle and weigh the animals.

4.2.3 Microdialysis:

For the microdialysis experiment the ventilated home cages in which the animals had stayed following surgery were transferred into the procedure room, the lid was lifted and food and water removed for the duration of the experiment. Microdialysis in the nucleus accumbens and dorsal striatum was carried out as previously described in chapter 2. The protocol for the mPFC deviated in that probes with a membrane length of 2mm were used. Final coordinates for the

mPFC were A/P = +1.9, L/M = +0.3 (if danger of damaging the sinus during cannula insertion was too big, +0.5 and an angle of 1-2 degrees was used resulting in the tip of the probe being located close to +0.3), D/V = -2.8 from brain surface, according to the mouse brain atlas by Franklin and Paxinos (Paxinos and Franklin, 2004). A schematic overview of the probe locations can be found in the supplementary material (Supplementary material 7.1).

4.2.4 Quantification of extracellular dopamine and dopamine metabolite concentrations:

Due to the comparatively low dopamine levels present in the mPFC, microdialysis samples obtained from this region required a higher dopamine sensitivity assay and were thus analysed differently from striatal samples.

Dorsal striatum and nucleus accumbens samples:

Dialysate samples were analysed immediately after collection and DA, DOPAC and HVA concentrations were determined using a HPLC system coupled with electrochemical detection. Using an autosampler (Alexys AS 110, Antec, Netherlands) aliquots of 35µl dialysate were injected into a mobile phase consisting of 50mM phosphoric acid, 50mM citric acid, 0.1mM Ethylenediamide-tetraacetic acid (EDTA) (all Fisher Scientific, Loughborough, UK), 17.5% methanol, 600mg/l 1-Octanesulphonic acid sodium salt (both VWR, Leuven, Belgium), pH 3.0, and separated by a NeuroSep 115 column (150x1.0mm, 3µm, C18, Antec, Leyden, Netherlands). The flow rate was kept at 50µl/min using a LC 110S pump (Antec, Leyden, Netherlands) equipped with a pulse damper (LO-Pulse Damper, Arc Sciences, Alton, UK). Electrochemical detection (DECADE II, Antec, Leyden, Netherlands) was performed at 35°C and a potential of 0.65V

against a salt bridge Ag/AgCl reference electrode. The chromatograms were processed using Clarity chromatography software (Antec, Leyden, Netherlands).

mPFC samples:

To detect the low levels of dopamine in the mPFC, microdialysis samples were split and dopamine content was analysed separately from DOPAC and HVA on two HPLC assays. For dopamine detection, an autosampler (Alexys AS 110, Antec, Netherlands) injected dialysate aliquots of 20 µl into a mobile phase containing 50mM phosphoric acid, 0.1mM Ethylenediamide-tetraacetic acid (EDTA) (both Fisher Scientific, Loughborough, UK), 17.5% methanol, 400mg/l 1-Octanesulphonic acid sodium salt (both VWR, Leuven, Belgium), pH 6.0, and separated by a NeuroSep 105 column (50x1.0mm, 3µm, C18, Antec, Leyden, Netherlands). The flow rate was kept at 50µl/min using a LC 110S pump (Antec, Leyden, Netherlands) equipped with a pulse damper (LO-Pulse Damper, Arc Sciences, Alton, UK). Electrochemical detection (DECADE II, Antec, Leyden, Netherlands) was performed at 35°C and a potential of 0.46V against a salt bridge Ag/AgCl reference electrode. The chromatograms were processed using Clarity chromatography software (Antec, Leyden, Netherlands). DOPAC and HVA content were measured from the remaining 20µl dialysate sample using the HPLC assay previously described in chapter 2.

4.2.5 Drug injections

After a baseline of three samples was acquired, THC (Tocris, Bristol, UK; 3 mg/kg, i.p., dissolved in 5% ethanol, 5% Tween-80 and 90% 0.9% w/v saline) or its vehicle was administered.

4.2.6 Data analysis:

For statistical analysis data was grouped into pre-drug and post-THC time points. To investigate baseline genotype differences prior to any drug injections, the mean dopamine and metabolite levels were calculated across the three pre-drug time points and compared between COMT-Met and wild-type mice using a 2-tailed, independent samples t-test. Following drug administration data was averaged across the three post-THC time points, normalised to the average 'pre-drug' baseline levels of each animal and expressed as percentage baseline. Drug effects were analysed using Univariate ANOVA with genotype and THC as between subject factors. In the mPFC an unstable baseline and apparent time dependent injection effects caused additional, exploratory analysis in which post-THC data points were normalised against the last baseline point only (60 min) and analysed individually using repeated measures ANOVA. Significant interactions were explored using LSD post-hoc tests. Statistics were computed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) and results were considered significant at $P < 0.05$.

Only data from mice with accurate probe placements, as verified histologically from post mortem brain slices, were included in data analysis, giving the following final total of mice: NAc n=36, dST n=37, mPFC n=31. In COMT-Met mice a total of four animals had to be excluded from HVA analysis (NAc n=1, dST n=1, PFC n=2) because no HVA peak could be measured in the chromatogram. In addition, 9 of the remaining 17 COMT-Met animals in the NAc group, 8 of the remaining 18 COMT-Met animals in the dST group, and 5 of the remaining 17 PFC had HVA levels in which the average pre-drug baseline levels lay below the limit of detection (LOD, i.e. below 2:1 signal to noise ratio; LOD for HVA in

striatal HPLC assay: 12.8 fmol, LOD for HVA in mPFC HPLC assay: 80 fmol). For analysis of baseline HVA levels, values that lay below the LOD were replaced with the LOD. The percentage baseline HVA levels were calculated on the raw data, irrespective of LOD.

4.3 Results

4.3.1 Nucleus accumbens

4.3.1.1 Effects of COMT genotype and THC on dopamine

In the nucleus accumbens, dopamine levels were 20.43 ± 2.66 fmol/35µl (mean ± SEM) in wild-type and 23.42 ± 2.92 fmol/35µl (mean ± SEM) in COMT-Met mice prior to drug administration. Hence extracellular dopamine levels did not differ significantly between genotypes at baseline (two-tailed, independent samples t-test: $t(34)=0.76$, $p=0.46$) (Figure 16a,b).

Analysis of the baseline normalised data revealed no main effect of THC ($F_{1,32}=2.11$, $p=0.16$) or genotype ($F_{1,32}=0.28$, $p=0.60$), but a significant genotype*THC interaction ($F_{1,32}=5.04$, $p=0.032$) (Figure 16c). Post-hoc tests showed that this resulted from a difference between vehicle and THC treated animals in the wild-type ($p=0.014$), but not the COMT-Met mice ($p=0.58$). In addition there was a trend genotype difference between the THC ($p=0.052$), but not the vehicle treated animals ($p=0.25$).

4.3.1.2 Effects of COMT genotype and THC on dopamine metabolites

In contrast to dopamine, extracellular DOPAC and HVA levels showed genotype specific differences at the pre-drug baseline. The average DOPAC level in wild-type mice was 3172 ± 293 fmol/35µl (mean ± SEM), which was significantly lower than the 9099 ± 750 fmol/35µl (mean ± SEM) observed in COMT-Met mice (two-tailed, independent samples t-test: $t(34)=7.4$, $p<0.001$) (Figure 16d,e). The average HVA level in wild-type mice was 2861 ± 182 fmol/35µl (mean ± SEM), which was significantly higher than 17.2 ± 2.1 fmol/35µl (mean ± SEM) observed in COMT-Met mice (two-tailed, independent samples t-test: $t(33)=15.2$, $p<0.001$)

(Figure 16g,h). The transgenic mice hence showed increased DOPAC and decreased HVA levels prior to drug injections. However, statistical analysis of the baseline normalise drug effect revealed on main or interactive effects of THC and genotype on DOPAC or HVA (F's<1 p's>0.6) (Figure 16f,i).

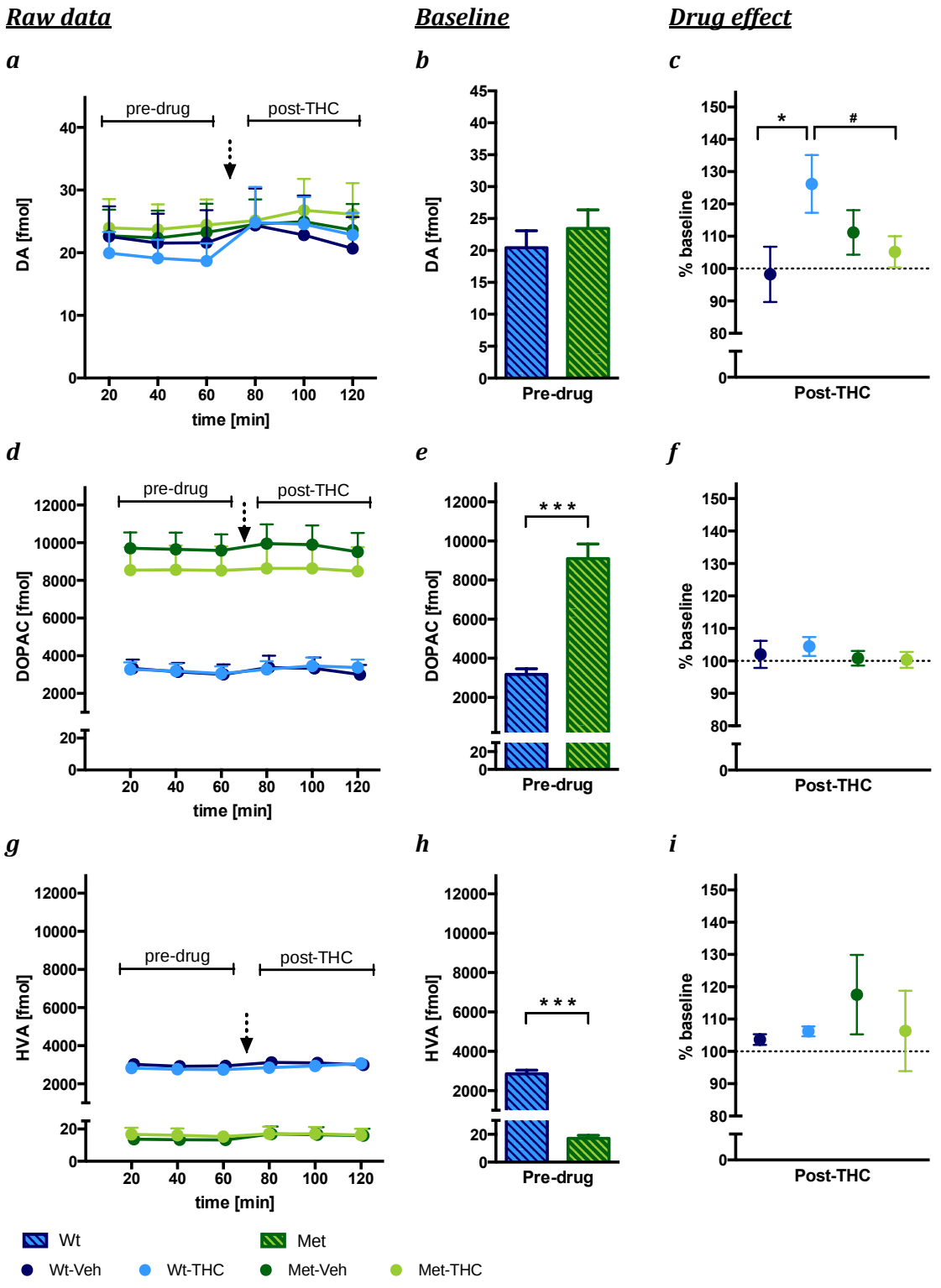


Figure 16: Nucleus accumbens - Separate and interactive effects of COMT genotype and THC on extracellular dopamine (a,b,c), DOPAC (d,e,f), and HVA (g,h,i) levels. *Microdialysis samples were taken every 20 min (a,d,g). The dotted arrow indicates a THC (3mg/kg) or vehicle injection. For statistical analysis of baseline levels, raw data were averaged across the 3 pre-drug time points and grouped by genotype (b,e,h). For statistical analysis of a drug effect, data were normalised to pre-drug levels and averaged across the 3 post-THC time points (c,f,i). *** P < 0.001, **P < 0.01, *P < 0.05, # P <0.09. Values are mean ± SEM from n=8-10 animals per group.*

4.3.2 Dorsal striatum

4.3.2.1 Effects of COMT genotype and THC on dopamine

In the dorsal striatum dopamine levels prior to drug administration were 26.08 ± 5.48 fmol/35µl (mean ± SEM) in wild-type and 35.14 ± 7.72 fmol/35µl (mean ± SEM) in COMT-Met mice (Figure 17a,b). Extracellular dopamine levels did not differ between genotypes at baseline (two-tailed, independent samples t-test: $t(35)=0.95$, $p=0.35$). Following drug injections, analysis of the baseline normalised data revealed no main or interactive effects of THC and COMT genotype ($F's<1$, $p's>0.3$) (Figure 17c).

4.3.2.2 Effects of COMT genotype and THC on dopamine metabolites

As expected, extracellular DOPAC and HVA levels in the dorsal striatum showed genotype specific differences at baseline. DOPAC levels in wild-type mice were 8020 ± 724 fmol/35µl (mean ± SEM) and thus were significantly lower than the 18893 ± 1584 fmol/35µl (mean ± SEM) levels observed in COMT-Met mice (two-tailed, independent samples t-test: $t(35)=6.1$, $p<0.001$) (Figure 17d,e). With an average 6617 ± 562 fmol/35µl (mean ± SEM) HVA levels in wild-type mice were higher than the 18.8 ± 1.9 fmol/35µl (mean ± SEM) observed in COMT-Met mice (two-tailed, independent samples t-test: $t(33)=11.4$, $p<0.001$) (Figure 17g,h).

Following drug injections analysis of baseline normalised DOPAC revealed no main or interactive effects of THC and genotype ($F's<1$, $p's>0.25$) (Figure 17f). Baseline normalised HVA levels showed a trend genotype difference ($F_{1,31}=3.76$, $p=0.06$), but this was independent of THC (main effect of THC $F_{1,31}=0.08$, $p=0.78$, THC*genotype interaction $F_{1,31}=01.88$, $p=0.18$) (Figure 17i).

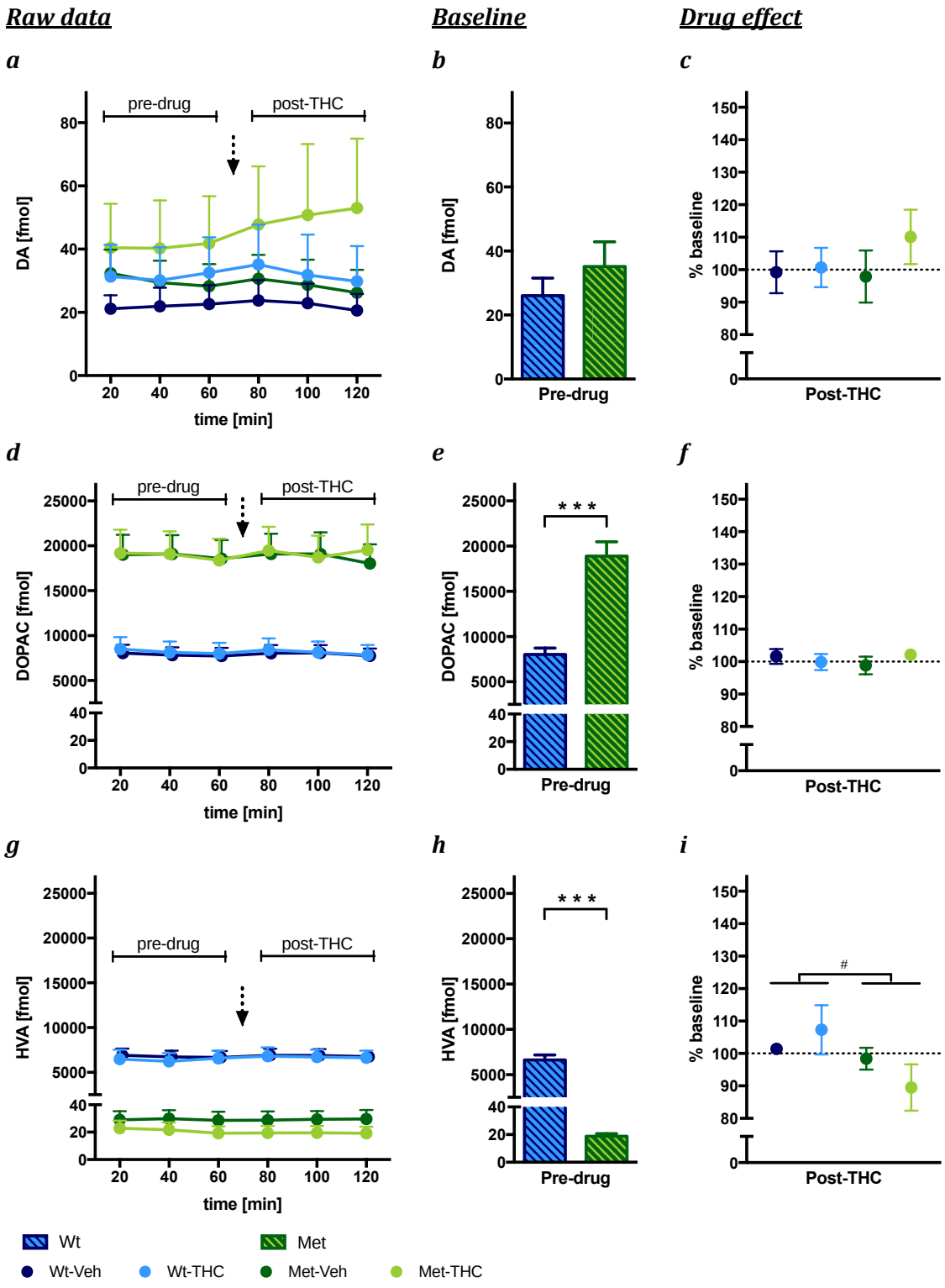


Figure 17: Dorsal striatum - Separate and interactive effects of COMT genotype and THC on extracellular dopamine (a,b,c), DOPAC (d,e,f), and HVA (g,h,i) levels. *Microdialysis samples were taken every 20 min (a,d,g). The dotted arrow indicates a THC (3mg/kg) or vehicle injection. For statistical analysis of baseline levels, raw data were averaged across the 3 pre-drug time points and grouped by genotype (b,e,h). For statistical analysis of a drug effect, data were normalised to pre-drug levels and averaged across the 3 post-THC time points (c,f,i). *** P < 0.001, **P < 0.01, *P < 0.05, # P <0.09. Values are mean ± SEM from n=8-10 animals per group.*

4.3.3 Medial prefrontal cortex

4.3.3.1 Effects of COMT genotype and THC on dopamine

The average dopamine level in the PFC of wild-type mice was 3.03 ± 0.45 fmol/20µl (mean ± SEM). In COMT-Met mice the average dopamine level was 2.48 ± 0.33 fmol/20µl (mean ± SEM) and thus did not differ significantly from wild-type animals (two-tailed, independent samples t-test: $t(29)=0.96$, $p=0.34$) (Figure 18 a,b). When normalised against the mean baseline level, there was no main effect of THC ($F_{3,27}=2.96$, $p=0.10$), genotype ($F_{3,27}=0.09$, $p=0.77$), or interactive effect ($F_{3,27}=0.65$, $p=0.43$) observed (Figure 18c). However, while the statistical analysis is consistent with the one used in the striatal brain regions, three factors prompted an additional, exploratory analysis in the mPFC: (1) The dopamine baseline in the mPFC was not stable prior to drug injections (main effect of time, $F_{2,54}=3.30$, $p=0.045$). (2) The lack of a main drug effect was unexpected and inconsistent with the available literature. (3) The plotted raw data suggests a time dependent effect following drug administration (i.e. a dopamine increase following injections independent of substance), which cannot be accounted for using univariate analysis but may mask drug effects later on. In an additional analysis each post-THC data point was thus normalised against the last pre-drug data point only and analysed by repeated measures ANOVA. This exploratory analysis revealed a significant main effect of time ($F_{3,81}=7.9$, $p<0.001$) and THC ($F_{1,27}=7.43$, $p=0.011$) as well as a significant time*drug interaction ($F_{3,81}=3.70$, $p=0.015$) (Figure 19). According to post-hoc analysis THC treated mice only show a trend towards higher dopamine levels compared to vehicle treated animals during the initial 20 min following drug injections ($p=0.064$). However, this effect reaches significance level in the two following

samples (p=0.010 and p=0.018 respectively). Despite the observed effect of THC on dopamine levels in this analysis no main or interactive effects with genotype were observed (F's<0.9, p's>0.47).

4.3.3.2 Effects of COMT genotype and THC on dopamine metabolites

DOPAC levels were 206 ± 32 fmol/20µl in wild-type and 1250 ± 124 fmol/20µl (mean ± SEM) in COMT-Met mice (Figure 18d,e). HVA levels were 456 ± 65 fmol/20µl and 125 ± 14 fmol/20µl (mean ± SEM) in wild-type and COMT-Met mice, respectively (Figure 18g,h). Both metabolite levels thus differed between genotypes (two-tailed, independent samples t-test: DOPAC $t(29)=16.3$, $p<0.001$; HVA $t(29)=10.4$, $p<0.001$).

Following drug administration, there was a main effect of genotype on DOPAC ($F_{1,29}=27.1$, $p<0.001$) but no effect of THC ($F_{1,29}=0.84$, $p=0.37$), nor a THC*genotype interaction ($F_{1,29}=1.49$, $p=0.23$) (Figure 18f). Although baseline levels were not stable in COMT-Met homozygous mice (RM-ANOVA: time*genotype interaction ($F_{2,54}=9.68$, $p=0.002$) driven by a decrease in COMT-Met (all p's<0.005) but not wild-type mice (all p's>0.55)) normalisation of the drug data against the last pre-drug point only, did not alter these results. There was no main or interactive effect of THC and genotype on HVA levels observed (F's<1, p's>0.6) (Figure 18i).

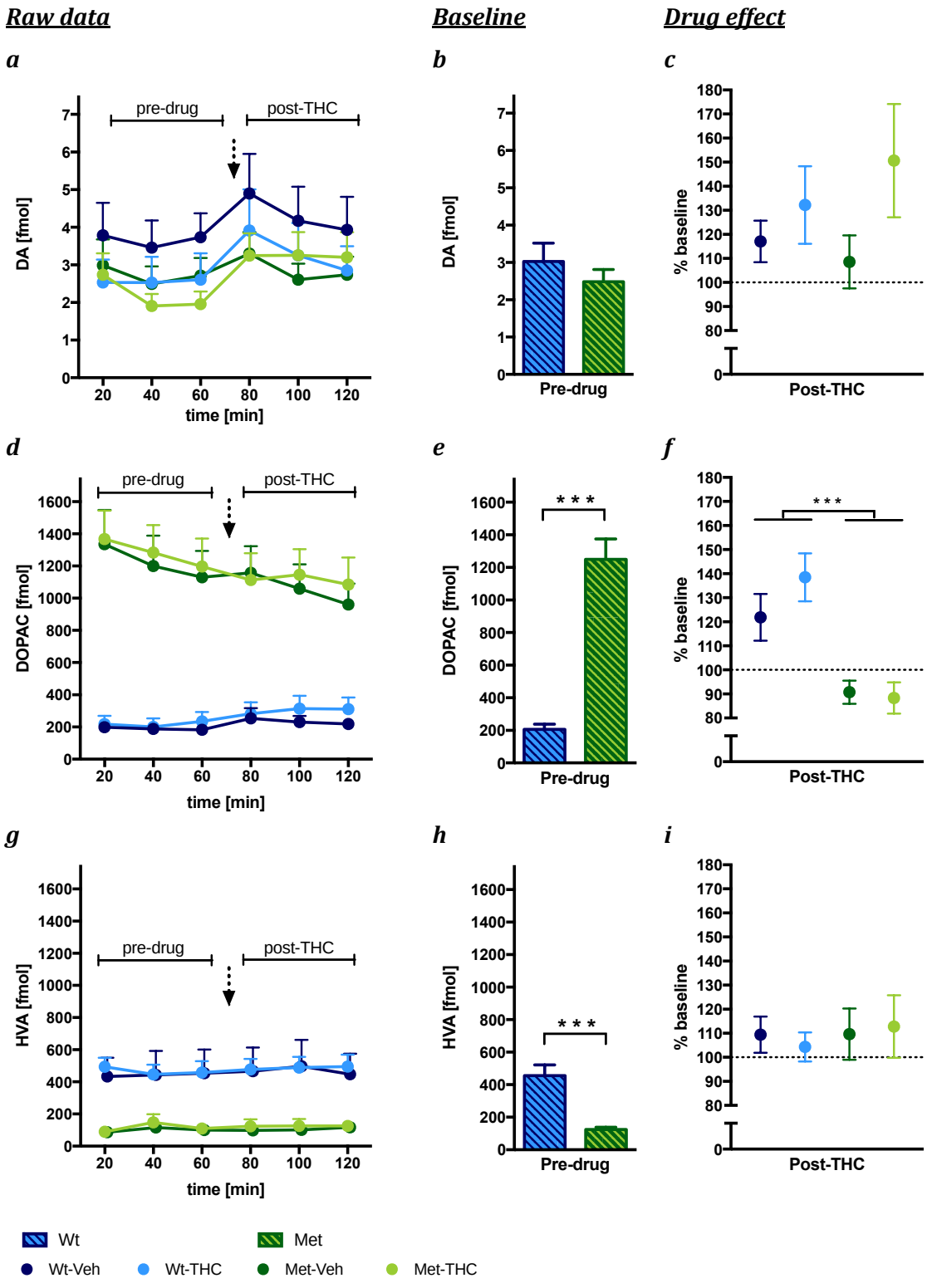


Figure 18: Medial prefrontal cortex - Separate and interactive effects of COMT genotype and THC on extracellular dopamine (a,b,c), DOPAC (d,e,f), and HVA (g,h,i) levels. *Microdialysis samples were taken every 20 min (a,d,g). The dotted arrow indicates THC (3mg/kg) or vehicle injection. For statistical analysis of baseline levels, raw data were averaged across the 3 pre-drug time points and grouped by genotype (b,e,h). For statistical analysis of a drug effect, data were normalised to pre-drug levels and averaged across the 3 post-THC time points (c,f,i). *** P < 0.001, **P < 0.01, *P < 0.05, # P <0.09. Values are mean ± SEM from n=6-9 animals per group.*

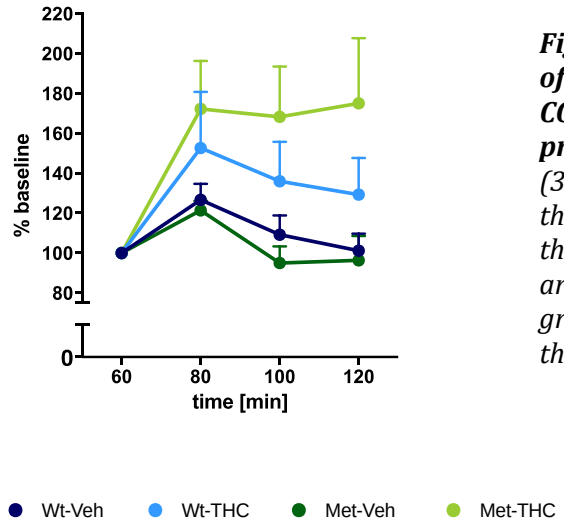


Figure 19: Additional, explorative analysis of the separate and interactive effects of COMT genotype and THC on medial prefrontal dopamine. *Following THC (3mg/kg) or vehicle injections indicated by the dotted arrow, raw data was normalised to the last pre-drug time point at 60min. Values are mean ± SEM from n=6-9 animals per group. For statistical analysis please refer to the text in Chapter 4.3.3.1*

4.4 Discussion

The present study not only assessed extracellular dopamine and metabolite levels in a novel mouse model of the COMT Val^{158}Met polymorphism, but also addressed the question of interactive effects with THC at a neurochemical level. These experiments suggest interactive effects of COMT and THC in the nucleus accumbens, but not the dorsal striatum or mPFC. Specifically, I found that THC increased dopamine levels in the nucleus accumbens in wild-type but not COMT-Met mice. Consistent with the results obtained during acute COMT inhibition in chapter 2, the experiment revealed COMT specific effects on dopamine metabolites as well as brain region specific interactive effects.

COMT genotype effects on basal dopamine and metabolites

The basal dopamine and metabolite levels found in this study lie within the range of those previously reported in wild-type mice (Fadda et al., 2005; Huotari et al., 2004; Tammimaki et al., 2016). Across all brain regions, COMT-Met transgenic mice showed increased DOPAC and decreased HVA levels at baseline, thus indicating altered dopamine metabolism. In fact, HVA levels lay below the limit of detection in some COMT-Met mice (see also section 4.2.6). However, basal dopamine levels did not differ between genotypes. COMT-Met mice thus not only have unaltered tissue dopamine levels (chapter 3) but also do not show altered extracellular dopamine levels under baseline conditions. Consistent with previous studies in COMT knockout and MB-COMT deficient mice these results support the notion that COMT activity levels do not affect dopamine transmission in rodent animal models under baseline conditions in the dorsal

striatum and mPFC (Huotari et al., 2004; Tammimaki et al., 2016), and extends the finding to the nucleus accumbens in the COMT-Met mice model.

Effects of THC in the COMT-Met mouse model

Nucleus accumbens

In the nucleus accumbens THC increased extracellular dopamine levels in a genotype dependent manner. Thus, these results further support the importance of COMT activity levels for dopamine signalling in the nucleus accumbens under specific conditions, consistent with the tolcapone-THC study discussed in chapter 2.

However, in contrast to the findings outlined in Chapter 2, THC increased accumbal dopamine in wild-type, but not COMT-Met mice. The direction of the effect is thus opposite to what would be predicted by reduced dopamine metabolism. These results also stand in contrast to the data obtained in the pharmacological study (chapter 2) in which mice with acutely lowered enzyme activity showed higher dopamine levels following THC administration. However, they are consistent with studies investigating the acute effect of cannabis on psychotic experience and cognitive function in human Met and Val allele carriers, according to which lower enzyme activity would be expected to protect from THC effects (Henquet et al., 2006; Tunbridge et al., 2015).

COMT-Met mice were backcrossed onto a C57Bl/6J background, which is the same strain that was used in the pharmacological study. Nonetheless wild-type mice that had not been treated with tolcapone in the pharmacological study did not show a significant increase in dopamine following THC administration, while

wild-type mice in the present study did. Although it is only possible to speculate, several factors might have contributed to these diverging results. First of all the animals obtained for the two studies were born and raised in different environments (Charles River vs. our own breeding facility). In addition, the two studies lay more than 18 months apart during which the animal housing facilities as well as the procedure rooms in which the experiments took place were refurbished. These changes resulted in a number of unavoidable alterations to the procedures during the recovery phase as well as the experimental set-up itself including different test-cages (special test cage vs. home cage), and different light intensity and background noise levels in the procedure rooms used during the microdialysis experiment. From behavioural studies it is well established that environmental conditions both during animal development and in the test situation can have major effects on the obtained results (Crabbe, 1999). Furthermore, factors such as stress levels, which can be affected by environmental conditions, are able to affect the dopaminergic system (Belujon and Grace, 2015). Only few studies have investigated direct effects between THC and environmental conditions at a neurochemical level; however, a very early study reported no change in dopaminergic neurotransmission in rats housed under normal conditions, while increased dopamine uptake was observed in rats that had been kept under stressful housing conditions (i.e. rehoused, isolated and food deprived) for 24h prior to THC administration (MacLean and Littleton, 1977). The given circumstances thus make it difficult to compare results across the two microdialysis studies.

Under physiological conditions in which genetically determined differences in COMT activity exist throughout life, the direction of the observed effect in the nucleus accumbens of COMT-Met mice would suggest it to be an indirect rather than a direct effect of COMT on extracellular dopamine. Although it is difficult to determine the cause of this effect based on neurochemical studies alone, it is plausible that the results could reflect developmental and/or adaptive changes that may occur in response to constantly lowered COMT activity. These could either target components of the dopaminergic system or, if the results prove to be specific for THC, they could target the endocannabinoid system. For example COMT has already been shown to affect CB1 receptor intensity in the PFC and hippocampus of COMT knockout mice (Behan et al., 2012).

While the present study provides neurochemical evidence for a gene x environment interaction between COMT genotype and THC, consistent with the pharmacological study presented in chapter 2, the direction and thus mechanisms through which this effect is mediated seem to differ and require further investigations.

Dorsal striatum

In the present study THC did not affect dopamine or metabolite levels in the dorsal striatum of either COMT-Met mice or their wild-type littermates. Previous studies investigating the effect of THC on dopamine in the dorsal striatum provided mixed results, possibly depending on factors such as drug dose, route of administration and genetic background (Ng Cheong Ton et al., 1988; Castañeda et al., 1991; Malone and Taylor, 1999). In the present study the drug dose was chosen to induce THC blood plasma levels similar to those observed in

humans following cannabis consumption (Drummer et al., 2003). Under these conditions THC did not affect dorsal striatal dopamine levels at all. It is thus possible that the lack of a COMT modulatory effect reflects the lack of a THC induced dopamine challenge that COMT could act upon. In a recent study mice deficient of membrane bound COMT showed higher dopamine levels than their wild-type littermates in the dorsal striatum following L-DOPA-carbidopa treatment (Tammimaki et al., 2016). However, this result was unexpected, since a previous tissue study in COMT knockout mice had not revealed increased dopamine levels following L-DOPA-carbidopa treatment (Huotari et al., 2002). Furthermore COMT knockout mice did not show genotype specific alterations in extracellular dopamine levels following the administration of a DAT inhibitor or amphetamine, despite increased extracellular dopamine levels (Huotari et al., 2002; Huotari et al., 2004). The authors argue, that in contrast to MB-COMT deficient mice, which do not show a decrease in total COMT activity, full COMT knockout mice may have developed compensatory mechanisms that prevent the accumulation of dopamine (Tammimaki et al., 2016). COMT-Met mice COMT activity is reduced by approximately 40% compared to wild-type littermates (Barkus et al., 2016), similar to the variation in enzyme activity associated with the Val^{158}Met polymorphism in humans (Chen et al., 2004). They may thus prove very useful to further investigate a potential role of COMT genotype on dorsal striatal dopamine signalling following L-DOPA-carbidopa or DAT inhibitor administration in the future.

However, the present study was designed to investigate potential interactive effects between the COMT Val^{158}Met polymorphism and cannabis consumption at a neurochemical level. The mouse model, drug, and drug dose were chosen to model the human situation as closely as possible. Based on this experimental set up the results do not provide evidence for interactive effects between COMT genotype and THC on extracellular dorsal striatal dopamine levels that might contribute to the proposed interactive effect between COMT genotype and cannabis consumption in the development of psychotic symptoms. This study thus seems to contrast with previous findings in human which demonstrated that the Val allele was associated with increased psychotic symptoms such as hallucinations and attention impairments following acute administration of cannabis or THC, compared with the Met allele (Henquet et al., 2006; Henquet et al., 2009). However, both of these studies found COMT genotype-dependent effects of THC only in participants with high liability to psychosis (defined as > 50th or > 75th percentile of the Community Assessment of Psychic Experiences (CAPE)-positive dimension score). In line with the lack of a dorsal striatal neurochemical effect in the present study, COMT genotype did not affect the psychotic response to THC in non-clinical individuals in a more recent study (Tunbridge et al., 2015). Although difficult in animal models it may thus be of interest to include behavioural outcomes of individual animals in phenotypes relevant for psychosis (such as sensory motor gating, latent inhibition, sociability and anxiety related behaviours (O'Tuathaigh et al., 2011)) in the analysis and interpretation of future experiments. In addition, future studies will have to investigate whether chronic or juvenile THC administration mimicking human cannabis consumption patterns and adolescent cannabis exposure respectively,

may have long-term effects on the dorsal striatum not represented in the present study.

Prefrontal cortex

In the present study THC did not affect dopamine or metabolite levels in the mPFC of either COMT-Met mice or their wild-type littermates, when the data was normalised to mean baseline levels. However, one of the limitations in the mPFC study, which only became obvious following data analysis, was that dopamine - and in the case of the COMT Met homozygous mice DOPAC - levels had not fully stabilised by the time recording started (i.e. at least 2.5h after microdialysis probe insertion), thus resulting in an unstable baseline. The insertion of a microdialysis probe is known to result in excessive neurotransmitter release. However, in contrast to the mPFC stable dopamine levels in striatal regions can be reliably measured 2h after probe insertion. The results thus highlight the differences in dopamine metabolism that exist between those brain regions and the relative importance of COMT in the prefrontal cortex.

In order to circumvent this issue, an exploratory analysis was carried out in which drug data points were normalised to the last pre-drug data point only. This analysis revealed a significant effect of drug according to which dopamine levels were significantly higher in animals treated with THC compared to vehicle treated animals. Although interpretation of this data is complicated in the absence of a stable baseline across 3 data points, the increase of mPFC dopamine following THC administration is consistent with previous studies in rats (Chen et al., 1990; Jentsch et al., 1997; Pistis et al., 2002). In these studies THC administration (1-2 mg/kg THC i.p, or i.v.) resulted in up to 60% increased

extracellular dopamine levels. Dopamine levels peaked between 40 and 80min post injection and the effect could be blocked by previous administration of a cannabinoid receptor agonist (Chen et al., 1990; Pistis et al., 2002).

In the present study the highest dopamine levels in THC treated mice were observed within the first 20 min following drug administration. At first, this may seem surprising, especially since the difference to vehicle treated animals only reached trend level significance at this stage. However, it is well known that acute stressors such as the restraint and mild pain associated with an injection can trigger dopamine release in the PFC (Abercrombie et al., 1989; Enrico et al., 1998; Feenstra et al., 2000; Butts and Phillips, 2013). The initial nominal increase in dopamine by at least 20% that was observed in all groups 20 min post drug injection as well as the subsequent decrease most likely represents this effect.

There were no main or interactive effects of genotype observed in the present study. However, it has to be noted, that several limitations of the study may reduce the informative value of this finding. First of all dopamine levels in the mPFC are extremely low and lay close to the detection limit of the HPLC system, thus increasing its susceptibility to error. Baseline issues set aside the system was able to pick up alterations in dopamine levels over time. However, genotype effects may be far more subtle than the observed drug effect and may thus be lost in the relatively high variability of the data obtained in the prefrontal cortex.

In addition, the study included a high potassium challenge, which was given 1h after drug injections to further spark neurotransmitter release. However, the effect of potassium challenge was highly variable, inconclusive and was therefore

not included in the thesis. As a result, the effects of THC were only measured for an hour following drug injection. This is worth noting because while all other groups show nominal decreases in dopamine following the initial increase in the first 20 min following drug administration, dopamine levels remain consistently high in THC treated COMT-Met homozygous mice, indicating that with time a significant difference between COMT-Met homozygous and wild-type mice may emerge.

COMT activity is known to affect cortical dopamine levels under certain conditions. A study in rats showed that treatment with tolcapone did not affect baseline dopamine levels but elevated extracellular dopamine levels following potassium induced stimulated dopamine release (Tunbridge et al., 2004). It has to be noted, however, that in contrast to the present study COMT activity was acutely lowered to levels that far exceeds the reduction observed in COMT-Met mice. Similarly, COMT knockout mice have been suggested to show an increase in stimulated dopamine release as well as decreased dopamine decay in the prefrontal cortex, although the amperometry method used in the study did not allow to differentiate between noradrenaline and dopamine (Yavich et al., 2007). In contrast, a recent study in MB-COMT deficient mice did not report altered extracellular dopamine levels in the medial prefrontal cortex following carbidopa / L-DOPA treatment.

THC treatment did not affect metabolite levels. Although a significant genotype effect on DOPAC was observed in the baseline normalised data, this is likely to be caused by the constantly declining baseline in COMT-Met mice.

In summary, while it was possible to measure extremely low dopamine levels with the HPLC assay specifically developed for the cortical study, several limitations of the study make it difficult to draw comprehensive conclusions from the data. In the future the use of mass spectrometry and electrical stimulation of the prefrontal cortex, as well as a longer observation period following drug administration may prove useful to comprehensively answer the question of whether COMT genotype and THC have interactive effects on cortical dopamine in COMT-Met mice.

Behavioural effects

As previously described in chapter 2 personal observations revealed that the animals appeared to rest throughout the experimental procedure and only the resting position differed between THC and vehicle treated animals.

Conclusion

These results demonstrate that COMT genotype and THC have interactive effects on extracellular dopamine levels in the nucleus accumbens but not the dorsal striatum of COMT-Met mice. The direction of the effect (i.e. reduced dopamine in Met homozygous compared to wild-type animals) indicates it to be indirect. Although the mechanisms and brain regions through which this indirect effect is mediated remain unknown, the results are consistent with human studies according to which lower enzyme activity would be expected to protect from THC effects. The results therefore provide a plausible neurochemical mechanism through which the suggested gene x environment interaction might be mediated. Although no interactive effects between COMT genotype and THC were observed

in the mPFC, further investigations are required to comprehensively answer this question due to the technical limitations encountered in the present study.

5 Behaviour

5.1 Introduction

In the previous chapter an interactive effect between COMT genotype and THC on extracellular dopamine levels in the nucleus accumbens was reported in the COMT-Met mouse model. The aim of the present chapter was to investigate the extent to which these neurochemical findings translate into behavioural differences between COMT Met and wild-type mice with regard to dopamine dependent processes such as reward processing, cognitive flexibility and sensorimotor gating.

5.1.1 Progressive ratio

As outlined in chapter 1.1.3, accumbal dopamine plays an important role in reward processing and more specifically is believed to confer motivational salience ('wanting') within the mesocorticolimbic pathway. One of the tasks commonly used to measure the motivation of an animal to work for a certain reward is a ratio schedule of reinforcement operant task. In this task the subject is required to emit a certain amount of operant responses to obtain a reward (Hodos, 1961). This schedule can either be fixed (i.e. the same number of responses is required for each successive reward), or progressive (i.e. the number increases with each successive reward). The last reward obtained before the subject stops responding is generally known as the breakpoint. Although other variables may contribute, the breakpoint is highly sensitive to the reward magnitude and the motivational state of the animal and is thus widely accepted as a measure of the incentive value of the reward.

Several studies suggest dopaminergic signalling within the nucleus accumbens plays an essential role in this task: accumbal dopamine depletion as well as dopamine antagonists have consistently been reported to reduce the breakpoint. However, this effect was schedule dependent: accumbal dopamine manipulations affected the breakpoint mainly in high, rather than low effort operant schedules (Caul and Brindle, 2001; Salamone et al., 2001; Ishiwari et al., 2004), indicating that dopamine contributes to effort-related decision making in this task (reviewed in Salamone et al., 2007). Furthermore, Cheer and colleagues demonstrated, that D1 receptor signalling in the nucleus accumbens shell critically participates in the initiation of lever presses linked to the stimulation of reward mediating pathways (Cheer et al., 2007).

The cannabinoid system is known to affect dopaminergic signalling within the nucleus accumbens (chapter 1.3.2) and manipulations of the endocannabinoid system have been shown to alter progressive ratio performance. For example mice in which one of the endocannabinoid receptors, CB1, has been knocked out, show reduced sensitivity to motivational properties of food as indicated by lower breakpoints on the progressive ratio task, though the authors argue that part of this effect might reflect consummatory rather than motivational aspect (Sanchis-Segura et al., 2004). However, in addition to genetic manipulations, a variety of CB1 receptor antagonists have consistently been shown to decrease breakpoints in food rewarded fixed and progressive ratio tasks in rodents (Freedland et al., 2000; McLaughlin et al., 2003; Ward and Dykstra, 2005; Maccioni et al., 2008; Randall et al., 2014). Furthermore, a microdialysis study by Trujillo-Pisanty et al. demonstrated that CB1 receptor blockade attenuated

dopamine release in the nucleus accumbens in response to rewarding medial forebrain bundle stimulation, while at the same time decreasing performance for the electrical reward (Trujillo-Pisanty et al., 2011). Conversely, THC as a CB1 receptor agonist has been shown to increase progressive ratio performance (Solinas and Goldberg, 2005; Higgs et al., 2005; Olarte-Sánchez et al., 2015).

In contrast to the cannabinoid system, information on the effect of COMT on reward related behaviours is still limited. Though there is emerging evidence that COMT genotype may play a role in reward processing (reviewed in Tunbridge et al., 2012), to our knowledge this is the first study investigating the effects of the COMT Val^{158}Met polymorphism on progressive ratio performance for food reward.

Reward Devaluation

Instrumental conditioning paradigms can result in habit formation in which case the animal's behaviour (in this case lever pressing) is no longer goal directed, but habitual. The COMT Val158Met polymorphism has previously been associated with Obsessive Compulsive Disorder (OCD), which has been characterised as an imbalance between goal-directed and habit-learning, though this characterisation may not be exclusive to OCD (Taylor, 2013; Gillan et al., 2016). Habits can be revealed by the insensitivity of behaviour to outcome devaluation treatments (Yin et al., 2004; Yin and Knowlton, 2006). Reward devaluation was thus run at the end of the progressive ratio task to ensure that independent of genotype the behaviour of all animals was still goal directed.

5.1.2 Sucrose Preference

In contrast to the 'wanting' or motivation for food believed to be measured by the progressive ratio task described above, the 'liking' or hedonic value of freely accessible food itself seems to be independent of accumbal dopamine signals (reviewed in Berridge, 2006; Berridge et al., 2009). As well as increasing eating motivation via interactions with the mesolimbic dopamine pathway, THC has been known to alter food palatability, including hedonic responses to sucrose solutions (Higgs et al., 2003; De Luca et al., 2012), and transiently regulates orexigenic and anorectic mediators to induce appetite (reviewed in Di Marzo and Matias, 2005; Bermudez-Silva et al., 2010).

The effects of COMT genotype on hedonia are only beginning to be studied and little is known about COMT's influence on food consumption (Galvão et al., 2012). In an attempt to control for possible differences between COMT genotypes in the hedonic value or palatability of the reward used in the progressive ratio task, a sucrose preference test was also carried out as an indication of reward palatability.

5.1.3 Cognitive flexibility - Reversal learning

COMT has been suggested to play an important role in cognitive function including cognitive stability and flexibility. As discussed in chapter 1.2 the Met allele - due to lowered COMT activity - is thought to increase tonic, and decrease phasic dopamine transmission in the subcortical regions, as well as increase dopamine in cortical regions, thereby leading to increased extrasynaptic D1 and decreased D2 transmission in the PFC which has been associated with increased stability of working memory functions (reviewed in Bilder et al., 2004; Winterer

and Weinberger, 2004; Cools and D'Esposito, 2011). Both, decreased striatal phasic dopamine signalling, necessary to encode prediction errors, and increased network stability make it more difficult to update networks that represent ongoing behavioural programs, when flexible adaptations to a changing environment become necessary. Though somewhat inconsistent (Rosa et al., 2010), recent studies investigating the effect of COMT genotype on cognitive flexibility in humans start to support this hypothesis: In a task-switching paradigm Met carriers showed significantly higher switch costs as measured by an increase in reaction time between task repetition and alteration (Colzato et al., 2010). Furthermore, Met homozygouts showed greater deficits on a task requiring the shift from an imitation to a reversal rule (Nolan et al., 2004), as well as on a probabilistic reversal learning task (Krugel et al., 2009).

In contrast to the proposed effects of COMT genotype on dopamine signalling believed to underlie these effects, we did not observe any genotype specific changes in cortical (or accumbal) baseline dopamine levels between COMT Met and wild-type mice in microdialysis studies, as discussed in chapter 4. However, preliminary data obtained from COMT Met and wild-type male mice (n=5 per group) revealed that following reversal of cue-reward contingencies during a Pavlovian conditioning task Met mice were slower to reduce responding to the no longer rewarded cue (Anna Huber, unpublished data). Hence, in the present study COMT Met and wild-type mice that had completed the progressive ratio task were tested on their ability to adapt their behaviour after the location of the active and inactive lever was reversed in a fixed ratio of reinforcement operant task.

5.1.4 Sensorimotor gating

Prepulse inhibition (PPI) is a measure of sensorimotor gating, a mechanism which is believed to filter out irrelevant sensory information at an early processing stage. Specifically, PPI refers to the ability to decrease the startle reflex to a stimulus, if it is preceded by a non-startling stimulus. Neural substrates known to contribute to PPI belong to the cortico-striato-pallido-pontine circuitry and include the mPFC as well as the nucleus accumbens (reviewed in Swerdlow et al., 2001; Fendt et al., 2001). Briefly, it is thought that increased accumbal dopamine signalling, via reduced activity of GABAergic projections to the ventral pallidum, regulates activity in the pedunculopontine nucleus, which is involved in mediating PPI. Evidence supporting the role of dopaminergic mechanisms in regulating PPI come from pharmacological manipulation studies and particularly D2 receptor agonists have been shown to disrupt PPI (Mansbach et al., 1988; Völter et al., 2012).

Deficits in PPI are widely accepted as an endophenotype of schizophrenia (reviewed in Braff et al., 2001; Swerdlow et al., 2008). Furthermore, in human studies the COMT Val^{158}Met polymorphism has been associated with differences in PPI performance: People homozygous for the Val allele showed lower PPI than Met homozygotes (Roussos et al., 2008; Giakoumaki et al., 2008; Quednow et al., 2009). Interestingly, the COMT inhibitor tolcapone increased PPI significantly in the Val, but not the Met, homozygous group, indicating that PPI performance might relate to dopamine levels according to an inverted U-shaped function (Giakoumaki et al., 2008).

Despite consistent findings in human studies, murine models of COMT gene variations have so far failed to show this effect (Gogos et al., 1998; O'Tuathaigh et al., 2011). However, consistent with a trend difference in baseline startle responses between COMT Val and Met genotypes in human (Giakoumaki et al., 2008), COMT knockout increased, while Val overexpression decreased baseline startle responses to a 120dB acoustic stimuli in mice (Papaleo et al., 2008).

To date, studies investigating the effect of the cannabinoid system on PPI in mice vary substantially in their methodology as well as their results: While some observed impairment of PPI following treatment with a variety of cannabinoid receptor agonists (Schneider and Koch, 2002; Martin et al.; Nagai et al., 2006), others did not find this effect (Bortolato et al., 2004), or only observed it in socially isolated rats (Malone and Taylor, 2006). Conversely, a study investigating the effects of acute THC administration on adult c57Bl/6JArc mice reported a significant increase in PPI (Long et al., 2010). A study investigating the effect of chronic adolescent exposure to the cannabinoid agonist WIN55212 in COMT knockout mice did not find any main or interactive effects on PPI (O'Tuathaigh et al., 2011).

5.1.5 Locomotor activity

THC has previously been reported to affect locomotor activity (Varvel et al., 2005; Monory et al., 2007; Long et al., 2010). A pilot study investigating the effects of 3mg/kg THC in COMT heterozygous mice revealed a very severe decrease in locomotor activity in drug compared to vehicle treated animals (K. Stumpenhorst, unpublished data). Accordingly THC doses were reduced to 1mg/kg for most behavioural tasks presented here and locomotor activity

following 1mg/kg THC or vehicle administration was tested to control for changes in locomotor activity as a confounding factor.

5.1.6 Hypotheses

In general the aim of the current chapter was to investigate, whether THC induces genotype-specific behavioural differences might reflect the neurochemical differences in dopamine metabolism observed in chapter 3.

I hypothesised

1) That THC administration would increase progressive ratio performance in a genotype dependent manner.
2) That THC administration would increase sucrose consumption in the sucrose preference task.
3) That - based on the preliminary behavioural data - Met homozygous mice may show less cognitive flexibility on a reversal learning task (although the neurochemical data obtained so far would argue against this prediction).
4) That COMT genotype does not affect locomotor activity, but that THC may reduce movement.
5) That COMT genotype does not affect sensorimotor gating in the COMT-Met mouse model, given mixed reports in the literature.

5.2 Methods

5.2.1 Animals

The following behavioural tasks were conducted in two cohorts (Co1 and Co2) of COMT-Met mice (described in chapter 3) and their wild-type littermates, generated from the same breeding trios and tested approximately 6 weeks apart (Table 5). In total 64 mice underwent behavioural testing, which started at 2-3 months, and ended at 7-8 months, of age.

Animals were housed in groups of 2-5 in standard open cages enriched with a white cardboard house, a toilet roll refuge and shredded brown paper as nesting material. They were kept on a 12-hour light-dark cycle (lights on at 07:00 and off at 19:00). All behavioural testing was conducted during the light phase. Animals were kept on a restricted feeding schedule at no less than 85% of their free-feeding body weight. The weight was age-weight-curve adjusted over time (C57/Bl6 curve obtained from Charles River, UK) to allow for growth with age.

All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986 and associated Home Office guidelines.

Table 5: Animal numbers per group and cohort

	Male - Wt	**Male - Met**	**Female - Wt**	**Female - Met**
Cohort 1	11	9	10	10
Cohort 2	7	9	4	4
Total	18	18	14	14

5.2.2 Drug Injections

THC (and vehicle) solutions were prepared and administered as described previously (chapter 2.2.5). The progressive ratio task was designed as a within-subject design during which each animal received four different drug conditions (vehicle, 0.3, 1, and 3 mg/kg THC). All other behavioural tasks were run in a between-subject design to avoid habituation confounds and excessive THC exposure. In addition to the progressive ratio task, mice received either one or two further THC injections (1mg/kg), separated by a minimum of 14 days. Drug groups were counterbalanced across genotypes, sex, and, as far as possible cohorts.

5.2.3 Behaviour tests

All behaviour tests were performed in the order outlined below.

5.2.3.1 Operant test battery

The progressive ratio, reward devaluation, and reversal learning tasks were conducted in operant test chambers (15.9 x 14 cm, Med Associates, USA). An individual animal was always tested in the same chamber, and specific testing boxes were assigned to a single sex. Test chambers were installed in sound attenuating boxes, with fans providing air circulation and a constant low-level background noise. Each chamber was equipped with a receptacle for liquid reward delivery, two retractable levers (one on each side of the receptacle), a light above the food receptacle indicating reward delivery, and an upwards facing house light on the opposite side (Figure 20). The apparatus was controlled by a computer system (SmartControl-Interface and MedPC software; Med Associates, USA). Liquid reward (60µl, 10% sucrose solution) was delivered via

syringe pumps (PHM-100A; Med Associates; USA). Onset of the house light and lever extension signalled the beginning of a training or test session, and lever retraction and a switched off house light marked the end. Each mouse received one session per day and animals were tested 5-6 days per week unless stated otherwise.

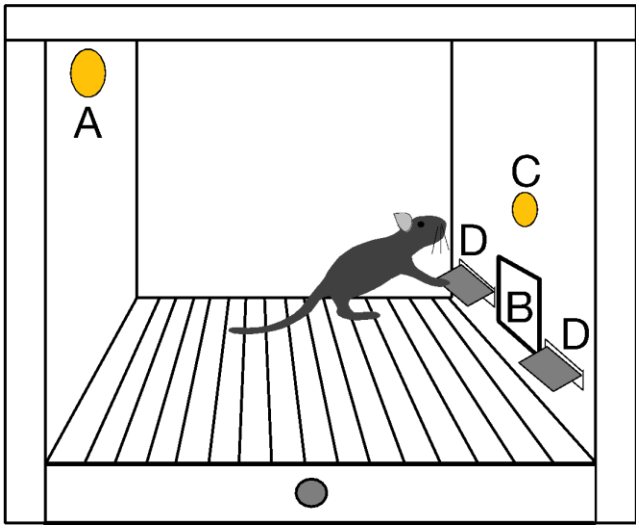


Figure 20: Operant test chamber

Operant chambers were equipped with a house light (A), a receptacle for liquid reward delivery (B), a receptacle light indicating reward delivery (C), as well as an active and inactive retractable lever (D), the position of which was counter-balanced across groups.

5.2.3.2 Progressive Ratio

Training

Mice were handled and exposed to 10% sucrose solution in their home cages overnight prior to training. Following magazine training, during which animals were habituated to reward delivery in the operant chamber for 5 days, each mouse was assigned one active and one inactive lever, the position of which was counterbalanced across groups. Active lever responses resulted in reward delivery and a timeout period (together 5s, signalled by sound of syringe pump and receptacle light) during which lever responses were recorded, but did not count towards reward delivery. Inactive lever responses were recorded as a

parameter of nonspecific activity. All mice were first trained on a fixed ratio (FR) 1 schedule of reinforcement (1 active lever response = 1 reward) for 7 days (Table 6). Next, mice were trained for up to 7 days on a FR 2 schedule followed by up to 5 days of FR 3 and FR 5 training.

Mice which on two consecutive days had an active / inactive lever response ratio ≥ 3 at the time they received the 15^{th} reward fulfilled the criteria and were rested on the next training day. Each session terminated after either the maximum number of rewards or session length was reached, whichever came first (Table 6).

Following FR training all mice received 5 days of progressive ratio (PR) schedule training according to the following algorithm (rounded to the nearest integer) as per Richardson and Roberts, 1996:

$$Number\ of\ responses\ required = [5e^{(R*0.16)}] - 5$$

where R = number of rewards already earned +1. The number of responses required to obtain a single reward thus followed the order 1, 2, 3, 4, 6, 8, 10, 13, 16, 20, 24, 29, 35, 42, 50, 60, 71, 84, 100, and so on. Sessions terminated either after 60 min or when the mouse stopped responding and there was no lever response for 5 min.

Following 5 days of PR training, the effect of injection stress on PR performance was tested. On two consecutive days mice received either a saline injection (0.9% NaCl, 10 µl/g, i.p., Sigma-Aldrich, UK) or no injection in a counterbalanced manner 20 min prior to the PR test start. Exposure to an initial saline injection

was also hoped to minimise changes in injection stress responses throughout the following drug testing.

Table 6: Progressive ratio training schedule

Training day	Schedule	Injection	Max. session length	Max. rewards	Training sessions per animal	Criteria	
						Rewards	Ratio active / inactive lever responses
1-7	FR 1	/	30 min	20	Up to 7 *)	n/a	n/a
8-14	FR 2	/	30 min	20	Up to 7 *)	≥ 15	≥ 3 / 1
15-19	FR 3	/	30 min	20	Up to 5 *)	≥ 15	≥ 3 / 1
20-24	FR 5	/	30 min	25	Up to 5 *)	≥ 15	≥ 3 / 1
25-29	PR	/	60 min	n/a	5	n/a	n/a
30-31	PR	saline or no inj.	60 min	n/a	2	n/a	n/a

*FR: Fixed ratio, PR: Progressive ratio, inj.: Injection, *)Animals that fulfilled both criteria on two consecutive training sessions, were excluded from the next training session*

Drug testing

The effect of THC injections (0, 0.3, 1.0 and 3.0 mg/kg, i.p.) 20 min prior to testing on PR performance in COMT-Met mice and their wild-type littermates was then assessed using a within-subject latin square design. Testing followed the 7-day cycle depicted in table 7, which was repeated 4 times.

Table 7: Progressive ratio drug testing schedule

Test day	Schedule	Injection	Max. session length	Comment
1	PR	n.a.	60	Baseline performance
2	PR	0.3, 1, 3 mg/kg THC or Veh	60	Tests effects of THC on PR performance
3-6	n.a.	n.a.	n.a.	4 day wash out period, no testing
7	FR 5	n.a.	30	Training
8	Restart at test day 1			

FR: Fixed ratio, PR: Progressive ratio, n.a.: not applicable

Analysis

PR data are commonly analysed and presented as breakpoint data, which describes the last completed ratio (= total number of rewards received).

However, this is not a particularly sensitive readout because as an example in the present set up the difference between obtaining 19 or 20 rewards can vary between 1 and 256 active lever presses. Hence, the total number of active lever presses was used as a more sensitive readout. Data obtained during the drug sessions was normalised against the average baseline performance by calculating the difference score in order to control for between-subject variability.

5.2.3.3 Reward devaluation

Sensitivity to the reward outcome was tested using an outcome-specific pre-feeding procedure. At least 10 days after the last drug injection, animals received one day of FR 5 training, followed by two days of PR, to re-establish baseline performance.

For reward devaluation each animal was allowed *ad lib* access to lab chow or 10% sucrose solution for 2 hours immediately prior to testing. Using the same experimental set up previously described for the progressive ratio task, active and inactive lever presses were then recorded for 6 min while undergoing extinction (i.e. no reward delivery). The order of sucrose or lab chow pre-exposure was counterbalanced across two test days separated by a standard PR test session to re-establish baseline performance.

5.2.3.4 Reversal

Reversal learning was tested on 10 consecutive days under a FR 3 schedule. Test sessions lasted 20 min. Consistent with the progressive ratio task, onset of the house light and lever extension signalled the beginning of a session, and lever retraction and a switched off house light marked the end. Active lever responses

resulted in reward delivery and a timeout period (together 5s, signalled by the sound of a syringe pump and a receptacle light) during which lever responses were recorded, but did not count towards reward delivery. On the first 3 test days active levers remained the same as previously used for PR and reward devaluation. From day 4 onwards the assignment of the active and inactive levers was reversed. Testing under reversed conditions was carried out for a total of 7 days. The percentage of active lever presses was calculated and the average of the first three sessions was used as baseline reference.

5.2.3.5 Sucrose Preference

Sucrose preference testing was conducted in cages (perspex, 30*16*20cm l*w*h) containing a thin layer (<0.5cm) of wood chips, and a cardboard house incl. a small amount of shredded brown paper on one side. Two egg cups (metal, diameter 8cm, rim height 2.5cm), one containing water (no mark), the other containing 10% sucrose solution (blue mark around the rim) were placed on opposite sides of the cage (Figure 21). To calculate consumption egg cups were weighed immediately before and after testing.

Each mouse was assigned its own test cage and was allowed 2 hours of habituation to the test set up on 2 consecutive days prior to the test day. During the habituation days the sucrose solution was once placed on the left and once on the right side of the cage and consumption was recorded (data not shown).

On the test day the location of the sucrose solution was assigned in a pseudorandom order. The mice were placed into their test cages 5 min after THC (1mg/kg, i.p.) or Veh administration. Sucrose and water consumption were

measured after 2 hours and the difference score was calculated (difference score = sucrose consumption – water consumption).

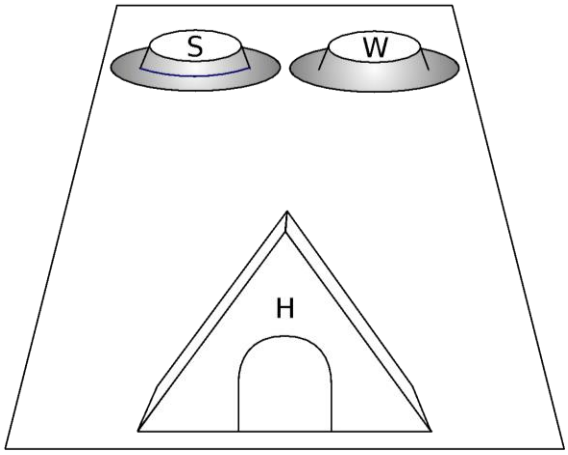


Figure 21: Sucrose preference test chamber

Each mouse was individually placed in a test chamber and allowed to move freely for 2 hours. Eggcups containing either 10% sucrose solution or water (position counterbalanced across groups) were weighed immediately before and after each test session to calculate consumption. S: egg cup containing 10% sucrose solution, W: egg cup containing water, H: cardboard house

5.2.3.6 Locomotor activity

Locomotor activity was measured using the PAS-Home Cage setup (San Diego Instruments, USA). Recording was started immediately after each animal was placed into its test chamber and the number of central and peripheral beam breaks was recorded for 60 min. Animals then received THC (1mg/kg, i.p.) or Veh in a between-subjects design and locomotor activity was recorded for an additional 120 min. Data were binned into 20 min bins for analysis. The average number of beam breaks as well as the percentage of central beam breaks was calculated for the initial 60 min baseline activity. Post-injection data were expressed and analysed as a percentage of baseline data.

5.2.3.7 Sensorimotor gating

Acoustic startle responses were measured using the SR-Lab System (San Diego Instruments, USA). Briefly, each sound-attenuating, ventilated test chamber contained a house light (switched on during the test phase), a high-frequency loudspeaker producing continuous white noise at 65dB, as well as white noise

stimuli at various intensities, and a clear Plexiglass cylinder resting on a platform. Mice were placed individually into each cylinder and vibrations caused by whole-body startle responses to acoustic stimuli were measured using an accelerometer attached to the underside of the platform. The average (baseline-normalised) vibration measured over 60ms post startle pulse onset was calculated and used for analysis. Sound volume and vibration detection were calibrated prior to testing.

Each animal received either THC (1mg/kg, i.p.) or Veh in a between-subjects design 20 min prior to testing. Sessions began with a 5-min acclimation period (65dB, white noise as background noise was presented throughout the test session). Following acclimation, three blocks of trials were presented. Blocks one and three consisted of 5 120dB startle pulse presentations (40ms, white noise), which were used to calculate habituation to acoustic stimuli across the test session (Equation 1). Block two consisted of trials in which an acoustic stimuli was either presented on its own (65, 69, 73, 77, 81, or 120dB, 40ms, white noise) or in which a 120dB startle pulse was preceded by a prepulse (69, 73, 77, or 81dB, 20ms, white noise). Each trial type was presented 10 times and trials were interleaved in a pseudorandom order with a variable ITI of 10-20s (average 15s). Baseline acoustic startle responses for each sound intensity were calculated as the mean startle response to trials without prepulse during block 2. Percentage prepulse inhibition was calculated according to Equation 2:

Equation 1:

$$Percent\ habituation = 100 * \frac{mean\ block\ 1 - \ mean\ block\ 3}{mean\ block\ 1}$$

Equation 2:

$$Percent\ prepulse\ inhibition = 100 * \frac{S - PPi_S}{S}$$

S: Mean startle response to 120dB pulse alone during block 2
PPi_S: Mean startle response to 120dB pulse preceded by prepulse

5.2.4 Statistical Analysis

Data were plotted using Graphpad Prism 6 (Graphpad Software Inc, USA) and analysed using SPSS v. 22 (SPSS Inc, USA).

Data was analysed using univariate or repeated measures ANOVA, as appropriate, with LSD post-hoc tests, with genotype, drug, sex and cohort as between-subject factors and time or session as a within-subjects factor (Table 8). Greenhouse-Geisser corrections were used where data failed the assumption of sphericity. Animals that had to be excluded from statistical analysis as well as the reasons for exclusion are summarised in supplementary material 7.2.

Table 8: Summary of statistical tests used

Task		Statistical Analysis	Factors
Operant behaviour	Progressive Ratio		
	Reward devaluation	RM-ANOVA	Genotype, sex, cohort[1)], drug session, order of drug sessions as covariate
	Reversal learning	RM-ANOVA	Genotype, sex, cohort[1)], session
Sucrose preference		Univariate ANOVA	Genotype, drug, sex, cohort[1)]
Locomotor activity	Baseline activity	Univariate ANOVA	Genotype, sex, cohort[1),]
	THC effect	RM-ANOVA	Genotype, drug, sex, cohort[1)], time
Sensorimotor gating	Startle amplitude	RM-ANOVA	Genotype, drug, sex, cohort[1)], volume intensities
	Pre-pulse inhibition	RM-ANOVA	Genotype, drug, sex, cohort[1)], prepulse volume intensities
	Habituation	Univariate ANOVA	Genotype, drug, sex, cohort[1)]

[1)] Cohort was included as a factor. To avoid small sample sizes in the between-subject design of the experiment, only pre-specified effects that were of a priori interest based on the progressive ratio data (main effect of cohort and interactive effects with genotype and/or drug) were analysed.

5.3 Results

5.3.1 Progressive ratio

There were no genotype differences in acquisition of the PR task, nor the response to an initial saline injection (F's<2.0, p's>0.17; data not shown). Analysis of the active and inactive lever responses on each of the four pre-drug days revealed stable baseline performance throughout the experiment. Thus, day, genotype and cohort did not affect the number of active lever responses during baseline performance (F's<1.2; p's>0.25; data not shown).

Analysis of the effects of THC on PR baseline normalised performance revealed a main effect of drug ($F_{2.3,108.9}$=35.24, p<0.001). 0.3, and especially 1mg/kg THC significantly increased, while 3.0 mg/kg significantly decreased, the number of active lever responses compared to Veh injections (all p's <0.005) (Figure 22). Post hoc analysis of the sex*drug interaction ($F_{2.3,108.9}$=35.24, p<0.001) revealed that in females only 1mg/kg THC significantly altered performance levels compared to Veh injections (p=0.02; 0.3mg/kg p=0.06; 3mg/kg p=0.3), while in male animals all drug doses induced significant changes in response levels (p's<0.001).

Although there was no genotype*drug interaction ($F_{2.3,108.9}$=0.53, p=0.62), there was a significant drug*genotype*cohort interaction ($F_{2.3,108.9}$=2.97, p=0.048). Cohort 1 wild-type animals showed a bigger increase in active lever presses in response to 1mg/kg THC than COMT-Met mice (p=0.042) (Figure 22a). Analysis of the significant drug*genotype*cohort*sex interaction ($F_{2.3,108.9}$=3.19, p=0.038) revealed that male animals from both cohorts showed significant genotype differences in response to 1mg/kg THC, albeit in opposite directions: in cohort 1

wild-type mice showed a bigger increase in active lever responses than COMT-Met mice ($p=0.034$), while this genotype difference was reversed in cohort 2 male mice ($p=0.039$). In addition, the cohorts differed in the impact of vehicle administration condition on performance in male animals: active lever responses were reduced in male COMT-Met mice, compared to wild-types in cohort 2 ($p=0.020$) but not in cohort 1 ($p=0.13$). Female mice did not show any significant genotype or cohort differences in response to THC. These findings remained the same when the total number of active lever presses without baseline normalisation was analysed (Supplementary material 7.3).

Following extensive training the number of inactive lever presses is often regarded as a measurement of general activity (Brunzell et al., 2010). In contrast to active lever responses, inactive lever responses were decreased following not only 3.0 but also 1.0mg/kg THC (p's<0.001, main effect of drug $F_{2.3,109.3}=25.9$, $p<0.001$) (Figure 22b), suggesting that these higher doses of THC suppress general activity. Administration of 0.3mg/kg THC did not significantly alter inactive lever responses compared to vehicle.

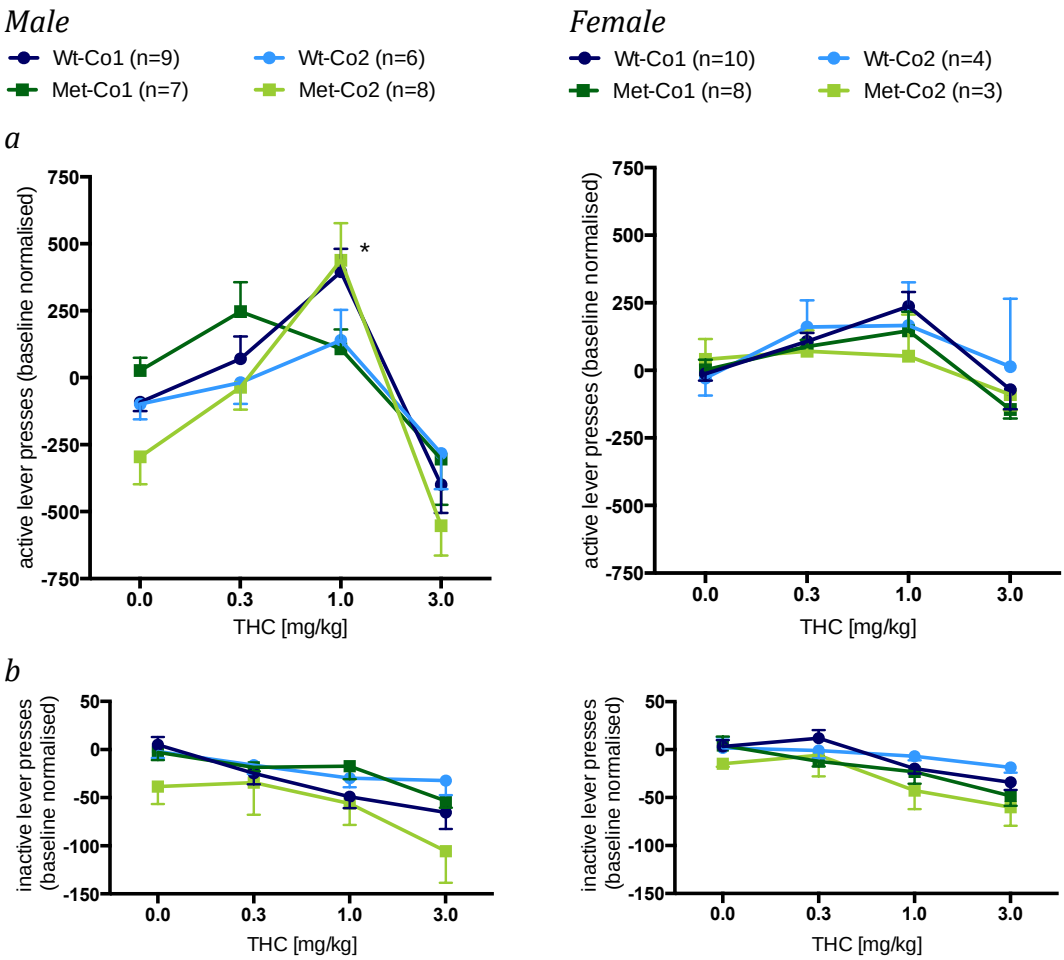


Figure 22: Effect of THC administration on progressive ratio performance in COMT-Met mice and their wild-type littermates in male (left column) and female (right column) animals. *Following drug administration (Veh, 0.3, 1.0, and 3.0 mg/kg THC, within-subject design) the number of active (a) and inactive (b) lever presses was recorded and normalised against pre-injection day baseline performance levels. Male mice showed a significant genotype x drug x cohort interaction. Post-hoc tests revealed genotype differences following 1mg/kg THC within each cohort but in opposite directions: * P<0.05 Values are mean ± SEM. Please refer to text for more detailed statistical analysis.*

5.3.2 Reward Devaluation

Baseline performance on an FR3 ratio was stable prior to and in between devaluation days ($F_{2,94}$=1.60, p=0.21, data not shown). There were no main or interactive effects of genotype on active lever presses during the devaluation paradigm (F's<2.44, p's>0.13). Furthermore, no main or interactive effects of sex and cohort with genotype could be observed (F's<2.84, p's>0.10). The data is therefore represented pooled across these variables (Figure 23). Devaluation of the outcome variable by pre-exposure with either 10% sucrose solution or lab chow decreased the number of active lever responses compared to baseline (main effect of day: $F_{1.7,67.4}$=19.75, p<0.001) in a substrate (sucrose vs chow) dependent manner (all p's <0.001) (Figure 23). Analysis of the total number of lever presses, including the previously inactive lever, did not alter the result (data not shown).

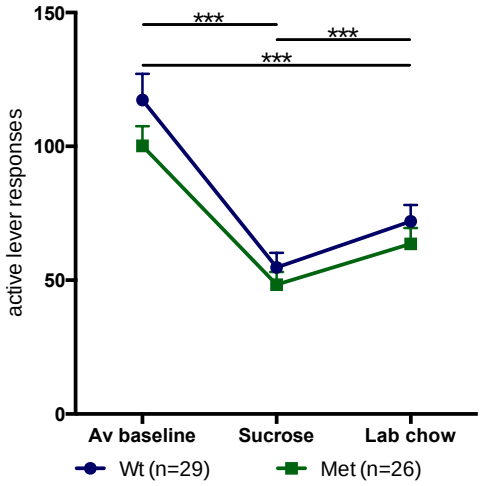


Figure 23: Effect of reward devaluation on active lever responses in COMT-Met mice and their wild-type littermates. *There was no main or interactive effect of genotype, but pre-exposure with either sucrose solution or lab-chow reduced lever responses in a pre-exposure-substrate dependent manner. *** P<0.001, values are mean ± SEM*

5.3.3 Reversal learning

Reversal of the active and inactive lever significantly decreased percent active lever responses during reversal session 1 compared to average baseline performance ($p<0.001$; main effect of session: $F_{3.4,132.8}=216.9$, $p<0.001$) (Figure 24). During each of the following reversal sessions, performance improved significantly ($p's<0.05$) until it started to stabilise from reversal session 5 onwards. However, there were no main or interactive effects of genotype ($F's<2.3$, $p's>0.14$). Nor were there any main or interactive effects of cohort ($F's<2.3$, $p's>0.14$). There was a significant main effect of sex and a sex*session interaction which are presented in full in supplementary 7.4.

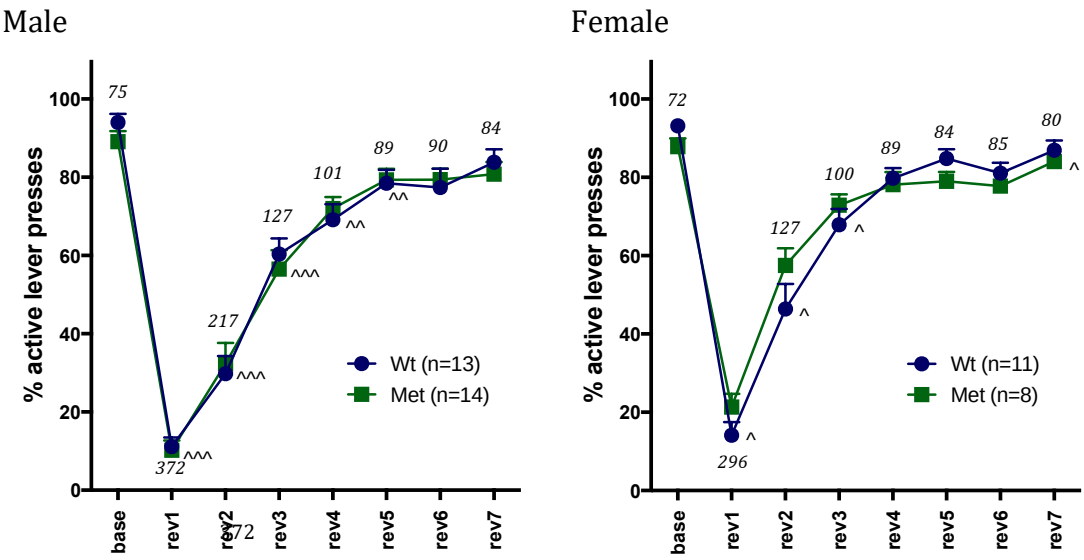


Figure 24: Reversal learning. *COMT-Met mice and their wild-type littermates learned the reversal of the active and inactive lever independent of genotype. Data is depicted as the percentage of active lever presses. The total number of active and inactive lever presses averaged across all mice (in the absence of main or interactive effects with genotype: F's<0.54, p>0.53) is indicated by the number above or below each data point. Base: average baseline (sessions 1-3), rev 1-7: reversal sessions 1-7, ^^^ P<0.001, ^^ P<0.01, ^ P<0.05 compared to preceding session, values are mean ± SEM*

5.3.4 Sucrose preference

On the drug test day analysis of the raw data revealed that all mice consumed significantly more sucrose solution than water (mean difference 3.5g, $F_{1,41}$=706.4, p<0.001, data not shown). The difference score used as a measurement of preference of sucrose over water consumption was not affected by genotype ($F_{1,41}$=0.76, p=0.39), THC (1mg/kg, i.p.; $F_{1,41}$<0.001, p=1.0) or cohort ($F_{1,41}$=2.10, p=0.16) (Figure 25), nor were there any interactive effects (F's≤2.6, p≥0.11). Males had a trend higher difference score than females (mean difference 0.54g, $F_{1,41}$=3.84, p=0.06) based on higher sucrose but not water consumption ($F_{1,41}$=6.1, p=0.02 and $F_{1,48}$=0.08, p=0.92 respectively, data not shown).

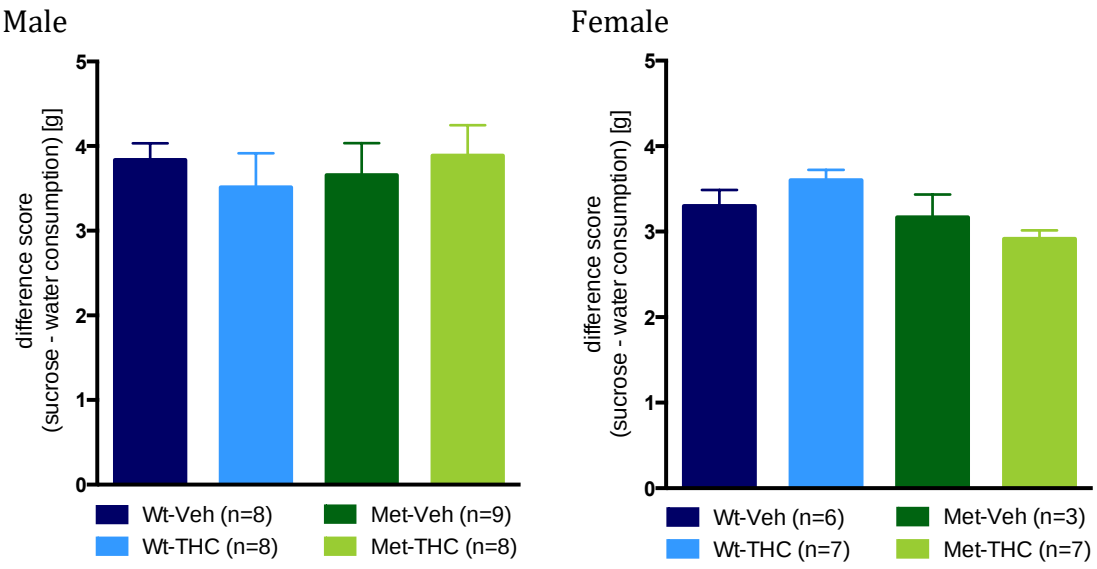


Figure 25: Sucrose preference. *COMT genotype and THC (1mg/kg) had no effect on difference score between sucrose and water consumption. Values are mean ± SEM*

5.3.5 Locomotor activity task

Baseline locomotor activity did not differ between COMT-Met mice and their wild-type littermates ($F_{1,53}$=2.06, p=0.16), nor were any interactive effects observed (F's<0.77, p's>0.38) (Figure 26a). A trend main effect of sex indicated that females were slightly more active than males ($F_{1,53}$=3.57, p=0.06). Furthermore, genotype and sex did not affect central beam breaks during baseline ($F_{1,53}$=0.02, p=0.89, $F_{1,53}$=2.33, p=0.13 respectively). However, there was a trend effect of cohort indicating that cohort 2 moved slightly less in the central area compared to cohort 1 ($F_{1,53}$=3.00, p=0.089)(Figure 26b).

Following drug injections there was a significant effect of time ($F_{3.9,173.3}$=81.3, p<0.001). As animals were still habituating to the test environment locomotor activity consistently decreased within the first 80min following drug injections (p's<0.001). Furthermore, there was a significant main effect of drug ($F_{1,45}$=7.9, p=0.007) as well as a drug*time interaction ($F_{3.9,173.3}$=11.2, p<0.001). More specifically, while THC treated mice exhibited trend-level higher locomotor activity during the initial 20min post injection time bin compared to vehicle treated mice (p=0.059), they showed significantly decreased activity from 60min onwards (p's≤0.02) (Figure 26c). There were no main or interactive effects of genotype (F's≤2.8, p's≥0.1). There was no main effect of cohort ($F_{1,45}$=0.33, p=0.57) nor were interactive effects with THC or genotype observed (F's≤2.8, p≥0.1). However, there was a main effect of sex ($F_{1,45}$=26.2, p<0.001) as well as a sex*time interaction ($F_{3.9,173.3}$=11.2, p<0.001, details in supplementary 7.5).

THC significantly increased the percentage of activity in the centre of the cage (main effect of drug: $F_{1,45}$=5.4, p=0.024) (Figure 26d). No further main or interactive effects were observed (F's<2.1, p's>0.12).

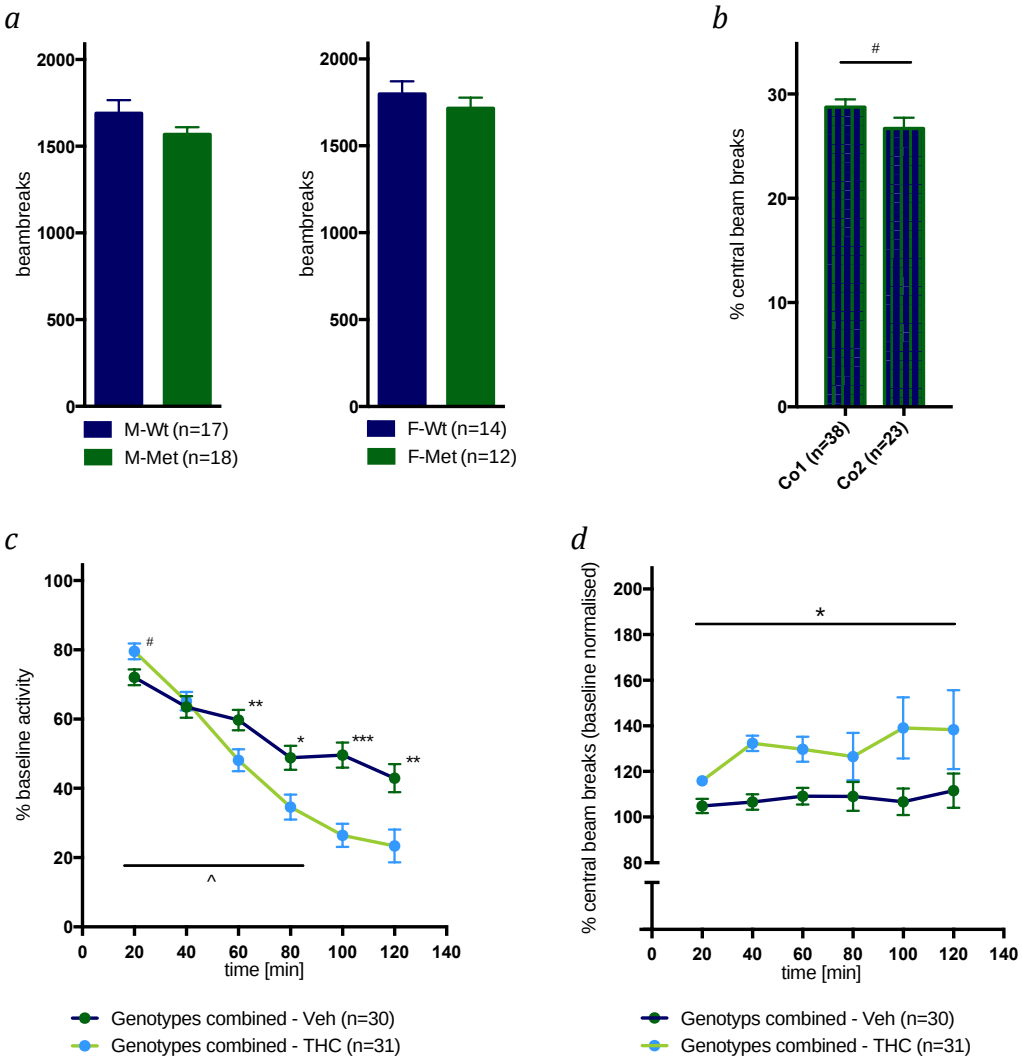


Figure 26: Locomotor activity (LMA) at baseline (a,b) and following THC (1mg/kg) administration (c,d). *LMA was measured by number of beam breaks recorded in 20min time blocks. Baseline LMA was averaged across initial 60 min time period (a). During baseline cohort 2 animals showed a trend towards less central activity (b). 60min post drug injections, THC treated animal started to show decreased LMA (c). THC treated animals spend more time in the cage centre (d). # P≤0.09, * P<0.05, **P<0.01, ***P<0.001, significant effect of time: ^ P<0.05 compared to preceding time bin. Values are mean ± SEM*

5.3.6 Sensorimotor gating

5.3.6.1 Acoustic startle amplitude

As expected, increasing the sound intensity of the acoustic stimulus increased startle responses in all animals ($F_{2.0,80.0}$=136.5, p<0.001). However, there were no significant main or interactive effects of either drug, or genotype on startle responses (F's<1.92, p>0.16) (Figure 27a). The two cohorts did not differ in their startle responses ($F_{1,39}$=0.03 p=0.86) and there were no interactive effects with drug and/or genotype observed (F's<0.06, p's>0.8). There was a significant main effect of sex ($F_{1,39}$=22.0, p<0.001) as well as a sex*volume interaction ($F_{2.0,80.0}$=12.3, p<0.001).

5.3.6.2 Prepulse inhibition

The volume of the prepulse had a significant effect on percent PPI. In general mice exhibited higher prepulse inhibition following louder prepulses compared to quieter ones ($F_{1.5,58.8}$=44.2, p<0.001). This difference was significant between all pre-pulse intensities (p's<0.005), except for the difference between 73 and 77dB (p=0.13) (Figure 27b). There was a trend effect of genotype according to which COMT-Met mice showed lower prepulse inhibition compared to their wild-type littermates ($F_{1,39}$=3.1, p=0.084). No further main or interactive effects could be observed (F's<1.4, p's>0.25).

5.3.6.3 Habituation

Analysis of habituation to the startle stimulus revealed no main effects of genotype ($F_{1,39}$=2.8, p=0.10), drug ($F_{1,39}$<0.001, p=0.996), sex ($F_{1,39}$=0.90, p=0.25), or cohort ($F_{1,39}$=1.98, p=0.17). Furthermore, there were no interactive effects observed (F's<1.17, p's>0.28) (Figure 27c).

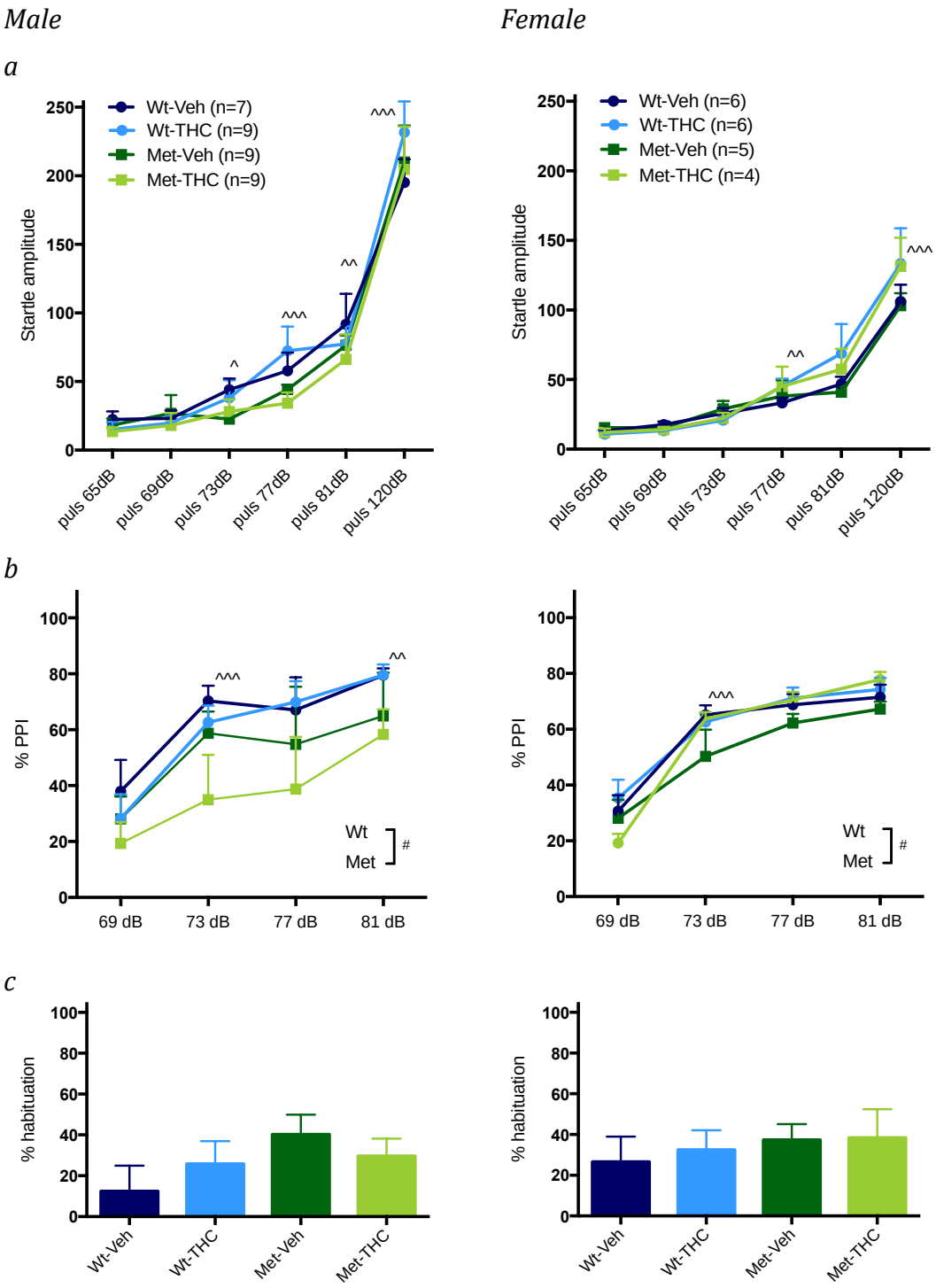


Figure 27: Sensorimotor gating. *In a prepulse inhibition task the effect of COMT genotype and THC (1mg/kg) on startle amplitudes (a), percent prepulse inhibition (PPI)(b), and percent habituation (c) were measured. Significant effect of pulse or pre-pulse sound intensity: ^ P<0.05, ^^ P<0.01, ^^^ P<0.001 compared to preceding sound intensity. Trend main effect of genotype: # P≤0.09. Values are mean ± SEM*

5.4 Discussion

5.4.1 Progressive ratio

THC affected progressive ratio performance in a dose dependent manner. 0.3 and especially 1mg/kg THC increased progressive ratio performance compared to vehicle injections. These findings are consistent with previous studies, which showed increased breakpoints following the treatment with CB1 receptor agonists including THC (Drews et al., 2005; Solinas and Goldberg, 2005; Higgs et al., 2005). In contrast, administration of 3mg/kg THC resulted in decreased progressive ratio performance. This decrease is most likely the result of decreased locomotor activity / increased catalepsy, which is well known to occur following high THC doses and which I observed in heterozygous COMT-Met mice treated with 3mg/kg THC (K. Stumpenhorst, unpublished data).

Interestingly, a cohort dependent genotype*drug interaction was observed, which was prominent in male animals. Sex differences in relation with COMT genotype are a common (albeit not always statistically robust) phenomenon, which is usually ascribed to oestrogenic regulation of COMT transcription, although other factors may contribute (Tunbridge and Harrison, 2011). Furthermore, the effects of cannabinoids have frequently been reported to be sex dependent, though which sex is more vulnerable appears to vary with the outcome variable examined (Craft, 2005; Fattore and Fratta, 2010). In the present study the behavioural performance of females appeared to be less affected by the different THC doses. Several mechanisms including differences in THC metabolism and the endocannabinoid system as well as hormonal

influences like estradiol, which is known to down-regulate CB1 receptors, may contribute to the observed effect (Craft et al., 2013; Wagner, 2016).

The differences between the two cohorts observed in the male animals were unexpected, especially since no major cohort differences in other behavioural tasks could be observed, that could explain these observations. It is thus possible that the results represent a chance finding, as the combined data from both cohorts would suggest. However, the neurochemical data obtained during the microdialysis study (chapter 4) showed interactive effects of COMT genotype and THC in the nucleus accumbens. Consistent with the neurochemical data in which Met homozygous mice were less affected by THC administration, they also showed lower breakpoints following THC administration in cohort 1 male mice. In contrast, in the second cohort the exact opposite effect was observed in male mice following 1mg/kg THC administration. Furthermore, cohort differences were also observed in response to vehicle injections: In cohort 2 male COMT-Met homozygous mice showed significantly reduced active lever responses compared to their wild-type littermates. This genotype difference was absent in cohort 1.

It is worth noting, that similar to these cohort differences observed on the progressive ratio task, neurochemical responses to THC differed between the pharmacological and transgenic study in wild-type mice (chapter 2 and 4 respectively). In all these cases (the two microdialysis studies and both behavioural cohorts) significant interactive effects between THC and COMT activity levels were observed.

Although it is not possible to rule out that these results are false positive findings, the fact that significant interactive effects were consistently found in

the nucleus accumbens and accumbal related behaviour, but not anywhere else makes it less likely. However, the differences between the datasets strongly suggest the influence of additional factors, which interact with COMT and THC at a neurochemical and behavioural level.

One of the potential modulatory environmental factors that has been emerging in the human COMT literature is stress. Stress has long been known to affect the dopaminergic system (Abercrombie et al., 1989; Enrico et al., 1998; Kalivas and Duffy, 1995) and several studies show that the COMT Val^{158}Met polymorphism can affect cognitive, neural and physiological responses to acute stress (Qin et al., 2012; Buckert et al., 2012; Hernaus et al., 2013). In these studies the Met allele has frequently been associated with a more negative impact of acute stress on cognitive performance (consistent with their predicted position on the 'inverted-U', see chapter 1.1.3), and perhaps also with generally increased stress responses, which in turn can affect effort-related decision making processes involved in the progressive ratio task (Shafiei et al., 2012; Bryce and Floresco, 2016).

None of the tasks in the present study was specifically designed to examine stress levels or indeed any other factor possibly contributing to the unexpected cohort differences. It is therefore not surprising that no significant cohort differences were found. However, it is worth noting, that in the locomotor activity paradigm, a trend towards slightly higher anxiety levels in cohort 2 compared to cohort 1 was observed when measuring central activity on the locomotor activity paradigm (chapter 5.3.5). These results, though the effect size

is very small, could be an indication for higher stress levels in cohort 2 compared to cohort 1 animals.

The restraint and pain associated with i.p. injection can be regarded as an acute stressor in the present study. The lack of a genotype or cohort effect on progressive ratio performance in response to the initial saline injections (saline vs. no injection) would thus argue against stress (at least in its acute form) as a factor contributing to the observed results. However, in contrast to the initial saline injection, genotype specific cohort differences were observed in response to vehicle injections. In addition to saline, vehicle injections contained small amounts of the excipient Tween-80 and the THC solvent ethanol, which potentially increased the injection associated discomfort. In cohort 2 male homozygous mice showed significantly lower lever responses following vehicle injections compared to their wild-type littermates, a genotype difference that was not observed in cohort 1.

Since the Met-allele is frequently associated with more pronounced stress responses, this finding may provide another indication of increased stress responsiveness in cohort 2, which may affect accumbal dopamine and thus contribute to the observed cohort differences in the progressive ratio following THC administration, though at present this remains speculative.

Taken together, it is thus not possible to determine the cause of the observed cohort differences. However, future studies investigating interactive effects between THC and stress levels on PR performance in COMT-Met mice may hold the answer to the observed discrepancies and may add to an emerging literature reporting interactive effects between COMT genotype and stress as well as

cannabis use and stress on psychosis and cognitive function (Stefanis et al., 2007; Collip et al., 2011; Ursini et al., 2011; Konings et al., 2012).

5.4.2 Reward devaluation

In the present study COMT-Met mice as well as their wild-type littermates showed sensitivity to outcome specific devaluation indicating that independent of their genotype the behaviour of all animals remained goal directed throughout the progressive ratio task.

5.4.3 Reversal learning

COMT-Met and wild-type mice did not differ in their performance on the reversal-learning task, a task commonly used to test cognitive flexibility (Izquierdo et al., 2017). At first glance, these data stand in contrast to the emerging human literature in which (albeit somewhat controversial Rosa et al., 2010), Met carriers have been suggested to outperform Val carriers on tasks requiring flexible performance (Nolan et al., 2004; Colzato et al., 2010; Witte and Flöel, 2012). However, increased cognitive flexibility has been hypothesised to be mediated through increased dopamine signalling in the PFC. In this respect the results obtained in the present study are consistent with the neurochemical data in which cortical dopamine levels in COMT-Met mice did not differ significantly from wild-type littermates under baseline conditions (chapter 4). Interestingly, and again in line with the neurochemical data, COMT-Met mice also do not perform differently in simple working memory tasks such as the Y- or T-maze, compared to wild-type littermates (Barkus et al., 2016, K. Stumpenhorst unpublished data).

To date, other existing animal models of altered COMT activity have not been tested on an operant reversal-learning task. However, in a more complex attentional set shifting task, which mimics the human WCST test more closely and allows to test cognitive flexibility by implementing intra- as well as extra-dimensional shifts, COMT-KO mice outperformed their wild-type littermates, while COMT-Val-transgenic mice performed worse than their controls on the extradimensional shift (Scheggia et al., 2013; Papaleo et al., 2008). COMT-Met mice only show a moderate alteration of COMT activity, and the effect on extradimensional set shifting remains to be examined.

5.4.4 Sucrose preference

In the present study sucrose preference was unaffected by COMT genotype and THC administration. This finding was somewhat unexpected since considerable evidence supports a role of the endocannabinoid system in the control of food intake (Brown et al., 1977; Williams et al., 1998; Di Marzo and Matias, 2005; Bermudez-Silva et al., 2010) and 1mg/kg THC has been shown to increase the consumption of sucrose solution in rats (Higgs et al., 2003). It is thus possible that a ceiling effect was reached. However, since the consumption during the sucrose preference task lay well above of the consumption observed during the progressive ratio task and since no genotype (or cohort) specific differences were observed, sucrose preference is unlikely to have affected the data obtained in the progressive ratio task.

5.4.5 Locomotor activity

COMT genotype did not affect baseline locomotor activity in the present study consistent with the data obtained from other murine COMT models including the

COMT knockout and humanised Val-Met mouse (Gogos et al., 1998; Papaleo et al., 2008; Simpson et al., 2014; Risbrough et al., 2014; Tammimaki et al., 2016). Furthermore, COMT genotype did not affect the percentage of central beam breaks, a measurement, which can be used as an approximate indication of anxiety.

No interactive effects of COMT genotype and THC on locomotor activity or anxiety measures were observed. However, THC administration had an anxiolytic-like effect, and decreased locomotor activity from 40 min post injection onwards. THC has previously been shown to have a biphasic effect on both anxiety and locomotor activity: While low doses up to 1mg/kg tend to be anxiolytic, higher doses are often anxiogenic (Moreira and Wotjak, 2009; Long et al., 2010). Similarly, very low doses of THC or other CB1 receptor agonists have been shown to increase locomotor activity while higher doses result in decreased activity and ultimately catalepsy (Varvel et al., 2005; Drews et al., 2005; Monory et al., 2007; Katsidoni et al., 2013). This biphasic action may also be responsible for the initial trend towards higher levels of locomotor activity observed during the initial 20 min following drug injection.

Although 1mg/kg THC decreased locomotor activity in an open field, the progressive ratio data showed that mice were able to overcome this effect and perform tasks involving lever pressing better following THC than following vehicle administration. Decreased locomotor activity is hence unlikely to have altered the behavioural results of other tasks included in this study, especially since the locomotor effect was independent of genotype.

5.4.6 Sensory motor gating

Previous studies investigating startle responses in murine COMT models have been inconsistent. COMT knockout mice show increased startle responses while COMT-Val transgenic mice show decreased responses following a 120dB stimulus compared to wild-type controls. However, in the humanised COMT model, Val homozygous animals showed increased startle responses following 120dB stimuli. In the present study, no genotype or drug effects on startle reactivity were observed.

Furthermore, neither THC nor COMT genotype significantly influenced prepulse inhibition in the COMT-Met mouse model. However, a trend genotype effect was observed according to which COMT Met mice showed lower levels of prepulse inhibition compared to their wild-type littermates. This trend was somewhat unexpected and likely represents a 'false positive', since in two previous studies in which COMT-Met mice obtained from backcross 5 and 8 were tested for prepulse inhibition (in the absence of drug) no such differences were observed (K. Stumpenhorst, unpublished data: main effect of genotype: Bx5: $F_{1,15}$=1.84, p=0.20; Bx8 $F_{1,37}$=0.33, p=0.57). Furthermore, most of the other mouse models of altered COMT activity do not exhibit altered prepulse inhibition, despite many of them showing more severe alterations on COMT activity levels than COMT-Met mice (Gogos et al., 1998; Papaleo et al., 2008; Simpson et al., 2014; Risbrough et al., 2014).

In contrast COMT genotype has consistently been shown to affect PPI performance in humans (Giakoumaki et al., 2008; Quednow et al., 2009; Roussos et al., 2009). The more prominent role of monoamine oxidase compared to COMT

in the mouse has been suggested to contribute to these species differences (Powell et al., 2009). However, MB-COMT deficient male mice showed reduced PPI compared to wild-type animals in a recent study (Tammimaki et al., 2016). While generally speaking the Val allele has been associated with decreased levels of PPI in the human literature (Giakoumaki et al., 2008; Roussos et al., 2008; Quednow et al., 2009), Giakoumaki and colleagues showed that enhancement of PFC dopamine signalling through treatment with the COMT inhibitor tolcapone, increased PPI performance in Val homozygous participants while a trend decrease was observed in the Met homozygous group (Giakoumaki et al., 2008). Somewhat paralleling these findings, it is possible that the trend genotype effect in the present study is a result of increased cortical dopamine signalling as a response to the injection stress, which was absent in my previous studies.

5.5 Conclusion

In the transgenic COMT-Met mouse model the Met allele does not affect the behavioural outcome on tasks measuring locomotor activity, sucrose preference, reversal learning, or sensorimotor gating, either on its own or following vehicle injections. The lack of a genotype specific effect on these tasks is in line with the neurochemical data obtained in the COMT-Met mouse model, showing no effect of COMT alone on extracellular dopamine. Behavioural tasks, which have more reliably been shown to depend on COMT genotype alone, such as attentional set shifting, still remain to be investigated in the COMT-Met mouse model.

In contrast, significant interactive effects between THC and COMT were observed on a task that specifically depends on accumbal dopamine. However, these effects interacted with cohort, complicating their interpretation. Although the

study was not designed to show cohort differences or identify additional factors contributing to genotype effects, and thus this finding should be regarded with caution, two independent findings suggest that stress may be a contributing factor. Firstly, data obtained during the locomotor activity task suggests that cohort 2 animals may be slightly more anxious, in line with personal observations during the handling of the animals. Secondly, the animals showed genotype specific cohort differences in response to vehicle injections. However, these cohort differences were unexpected and future studies in which stress levels are actively modulated prior to testing will help to shed more light on the proposed interactive effects between COMT genotype, THC and stress levels.

6 General Discussion

Rarely, a single genetic polymorphism may result in the development of a psychiatric illness; however, most only act to alter risk and therefore interact with other genetic and environmental factors. The human-specific COMT Val^{158}Met polymorphism has been suggested to moderate cannabis-induced risk for psychotic illness. However, at the time of this thesis, human studies had provided mixed results and the neurochemical basis for the proposed gene x environment interaction had not been investigated directly. The aim of this thesis was therefore to investigate potential interactive effects between the main psychoactive component of cannabis (THC) and COMT at the neurochemical and behavioural levels in the mouse model. Given the importance of dopamine in mediating psychosis, the work focused on dopamine levels in the striatum and medial prefrontal cortex, as well as related behavioural phenotypes.

In this final chapter the main findings and limitations of the experiments are first summarised and discussed in the context of the current literature. Potential future extensions to the work presented in this thesis are then proposed.

6.1 Summary of main findings

In chapters 2 and 4 I demonstrate that COMT and THC have interactive effects on accumbal dopamine; however, the direction of these effects differ between acute pharmacological manipulation of COMT, and (lifelong) genetically-mediated COMT activity differences. In chapter 3, I provide a basic characterisation of the COMT genetic mouse model used here, and demonstrate its specificity. In chapter 5, I describe the effects of THC and COMT on the performance of several behavioural tasks. Whilst there were no clear interactive effects of these factors

on the reversal learning and sensorimotor gating tasks, I did observe cohort-dependent interactive effects on progressive ratio performance that hint that the relationship between COMT and THC may be confounded by stress/arousal state.

Prior to these studies, several questions remained about the neurochemical effects of altered COMT activity on striatal brain regions. While COMT has been shown to be a major route of dopamine metabolism in the mPFC, the abundance of dopamine transporter in the striatum meant that little attention had been paid to its potential role in this area (outside of studies focused on COMT's influence on the effects of L-DOPA). Despite the proposed reciprocal relationship between PFC and striatal dopamine, the few studies investing the neurochemical effect of altered COMT activity found elevated cortical but unaltered striatal dopamine levels in COMT knockout mice. Nonetheless, based on the dopamine hypothesis of schizophrenia, I hypothesised that if an interactive effect between COMT genotype and cannabis consumption on psychosis existed, it was likely to be mediated in part through interactive effects in the striatum. Thus the experiments in chapters 2 and 4 were designed to investigate - as a proof of principle - whether the effect of THC on extracellular dopamine could be altered by COMT activity levels.

As outlined in chapter 2, COMT activity was first modulated pharmacologically by systemic administration of the COMT inhibitor tolcapone. Using microdialysis in awake mice, I demonstrated that tolcapone significantly inhibits COMT in mouse dorsal and ventral striatum, as indexed by an accumulation of DOPAC and a depletion of HVA. Despite this dramatic change in dopamine metabolism,

extracellular dopamine levels were not altered by tolcapone alone. Furthermore, administration of THC did not alter dopamine levels in the dorsal striatum. However, in the nucleus accumbens, tolcapone-, but not vehicle-, treated animals showed a significant increase in dopamine following THC administration.

To my knowledge these data provide the first neurochemical evidence for interactive effects between THC and COMT activity. The implication of this finding will be discussed in more detail in section 6.2. In addition, they provide evidence for a plausible neurochemical mechanism, which may contribute to the proposed gene x environment interaction between COMT genotype and cannabis use in the development of schizophrenia.

However, this study has several limitations, which have to be taken into account when considering the significance of these findings. First of all, the functional significance of the effects observed in chapter 2 remains unknown. Furthermore, inhibition of COMT activity by tolcapone can only ever be a very crude model of the human Val^{158}Met polymorphism, which is of a smaller magnitude (Learmonth et al., 2004; Chen et al., 2004), and affects COMT activity throughout life.

In order to better model the Val^{158}Met polymorphism, and also detect neurochemical or behavioural changes arising from secondary or compensatory effects of altered COMT activity, all subsequent studies were conducted in COMT-Met mice and their wild-type littermates. As outlined in chapter 3 these mice model the human specific Val^{158}Met polymorphism through the replacement of the native amino acid leucine at the relevant amino acid position with methionine, resulting in reduced enzyme activity in COMT-Met mice compared

with wild-types. The studies presented in chapter 3 were part of a set of studies designed to validate this novel model. Because the COMT gene is embedded within the 22q11DS critical deletion region, which is associated with cognitive impairment and increased risk for developing schizophrenia, a microarray study was carried out to discover potential off-target effects of the transgene within this region. Importantly, the COMT-Met transgene had no notable off-target effects on gene expression in the dorsal striatum, nucleus accumbens, or the mPFC. The molecular phenotype of COMT-Met mice is thus highly specific, and any observed genotype differences can be expected to result from lowered COMT activity induced by the COMT-Met transgene.

A basic neurochemical characterisation investigating dopamine and metabolite at tissue levels was also conducted. Tissue dopamine levels did not differ between genotypes in any of the analysed brain regions. However, COMT Met homozygous animals showed increased DOPAC and decreased HVA levels, compared with wild-types. Consistent with this, an enzyme activity assay demonstrated that the COMT-Met mice show a reduction in activity by approximately 40%, compared with wild-types, which is of a similar magnitude to that observed in Met homozygous people, compared with Val homozygotes (Chen et al., 2004).

The results outlined in chapter 3 show that COMT-Met mice are a highly specific animal model of the human Val^{158}Met polymorphism, which can help understand the neurochemical and behavioural effects of this polymorphism alone as well as in combination with other genetic or environmental factors.

The studies described in chapter 4 were designed to investigate the separate and interactive neurochemical effects of THC and genetically altered COMT activity in the dorsal and ventral striatum, and mPFC, of COMT-Met mice. Microdialysis data revealed that the Met allele significantly reduced HVA and increased DOPAC levels but did not affect extracellular dopamine in any of the three regions of interest. Following THC (3mg/kg) administration, interactive effects were observed in the nucleus accumbens. Specifically, THC increased extracellular dopamine levels in wild-type but not Met homozygous mice. The direction of the observed effect thus differs from that observed after acute COMT inhibition. Importantly, these findings are consistent with studies investigating the acute effects of cannabis or THC in humans, which have demonstrated protective effects of the Met allele on psychotic experience and cognitive function (Henquet et al., 2006; Tunbridge et al., 2015). However, further studies will be needed to establish the mechanism underlying this effect, a point which will be discussed in more detail in chapter 6.3. Similar to the mPFC data, and in line with the tolcapone study (chapter 2) no interactive effects between COMT genotype and THC were observed in the dorsal striatum.

The studies outlined in chapter 4 provide neurochemical evidence for a gene x environment interaction between COMT and THC under physiological conditions in which genetically determined differences in COMT activity exist throughout life. The implications of this findings and the fact that interactive effects were only observed in the nucleus accumbens will be discussed in more detail below (chapter 6.2).

The aim of chapter 5 was to investigate the extent to which the neurochemical findings seen in chapter 4 translate into behavioural differences between COMT Met and wild-type mice. I focused on aspects of motivational salience, cognitive flexibility and sensorimotor gating. Two independent cohorts of COMT-Met mice and their wild-type littermates were tested. COMT genotype alone did not affect cognitive flexibility on a simple reversal learning task nor did it modulate locomotor activity or sensorimotor gating on its own or following THC (1mg/kg) administration.

In contrast, significant interactive effects between COMT genotype and THC were observed on a progressive ratio task, however, these effects also interacted with cohort, complicating their interpretation. Differential effects in the response to vehicle injections in Met homozygous mice provide tentative leads that the results may be confounded by stress/arousal-dependent effects on COMT.

One of the limitations of studies in this thesis, which has not been discussed thus far, is the difference in the THC dose used in the behavioural and neurochemical studies, which compromises their direct comparison. The dose of 3mg/kg THC (used for neurochemical studies) was chosen because it elicited THC blood plasma levels that resembled those induced in humans by cannabis use. However, during the course of the neurochemical studies I realised, that locomotor activity was reduced following 3mg/kg THC, making behavioural tasks using this dose unfeasible. Therefore, a lower drug dose had to be chosen for the behavioural tasks.

The use of an animal model was essential to these studies, since the direct measurement of dopamine and its metabolites is not possible *in vivo* in humans. The mouse was specifically chosen as a model organism because of the available tools that allow its relatively easy genetic manipulation. However, the use of a model organism obviously has limitations as well. Although conserved in its general structure and function, there are differences between the rodent and human dopaminergic systems, which need to be borne in mind when extrapolating findings obtained in rodents to humans. Furthermore, and more specific to the COMT-Met mouse model, a single base change will never be able to represent all the polymorphic complexities seen in the human COMT gene that may well interact with each other (Nackley et al., 2006).

Human laboratory studies using THC/cannabis are never carried out in drug naive participants for ethical reasons. Similarly, the studies, which reported interactive effects of COMT and cannabis consumption on the development of schizophrenia typically include individuals who have consumed cannabis for extended periods of time, often starting during adolescence.

In the present study, I only used the main psychoactive component of cannabis, THC, in order to avoid confounding influences by the numerous other cannabinoids present in cannabis. This is of particular importance because cannabidiol can have opposing effects to THC (C Arnold et al., 2012). In fact, some recent studies suggest that the rise in THC and decrease in cannabidiol content observed over time may result in more first onset psychosis cases being attributed to cannabis use (Di Forti et al., 2015). Mouse models therefore

provide an opportunity to study the impact of initial exposure to THC on neurochemical and behavioural parameters.

Finally, it has to be noted that the COMT-Met mouse was not designed as a model organism for schizophrenia or psychosis, nor did the tasks used in this thesis aim to investigate behaviours believed to relate to these human conditions. Further studies should investigate the separate and interactive effects of COMT and THC on more complex cognitive tasks, such as attentional set shifting.

6.2 Implications of findings

6.2.1 Implication of findings regarding the proposed gene x environment interaction

In the studies presented here, interactive effects of COMT and THC on dopamine were only observed in the nucleus accumbens, not the dorsal striatum or the mPFC (although data obtained from the mPFC should be regarded as preliminary due to the technical difficulties discussed in detail in chapter 4). Since THC was only given acutely, the studies do not rule out interactive effects on the dopaminergic system in the dorsal striatum or mPFC following chronic or sub-chronic THC administration.

The data presented in this thesis for the first time provides direct neurochemical evidence for a plausible mechanism through which interactive effects between THC and COMT genotype might contribute to behavioural interaction observed in human studies. Namely, interactive effects of COMT and THC on dopamine levels may result in genotype-dependent differences in the attribution of salience to environmental stimuli, a process which has been proposed to drive psychosis (Kapur, 2003; Winton-Brown et al., 2014).

6.2.1.1 Implication of findings regarding COMT's role in subcortical brain regions

The results outlined in this thesis have implications for our understanding of COMT's role in subcortical brain regions. Until recently, the prevailing view was that COMT genotype had no significant impact on striatal dopamine signalling due to the predominance of uptake mechanisms in this region. Tissue and microdialysis studies investigating the effect of lowered COMT activity under baseline conditions, but also following pharmacologically enhanced dopamine activity in the dorsal striatum of COMT knockout mice seemed to confirm this hypothesis, as extracellular dopamine levels did not differ between genotypes (Gogos et al., 1998; Huotari et al., 2002; Huotari et al., 2004). However, studies looking at the effects of acute, pharmacological COMT inhibition on dopamine in the dorsal striatum have always produced more mixed results (Maj et al., 1990; Acquas et al., 1992; Törnwall et al., 1993; Li et al., 1998), but (Männistö et al., 1992; Napolitano et al., 1995; Budygin et al., 1999; Huotari et al., 1999).

The studies presented in this thesis provide evidence, for the first time, that COMT modulates dopamine levels in the nucleus accumbens following THC administration but not under baseline conditions. Future studies are required to determine whether the observed effects are specific to THC, or whether they generalise under all conditions in which dopamine efflux is increased. This latter possibility is particularly interesting, because COMT is emerging as a candidate gene for modulating numerous reward-related phenotypes. For example, COMT has been shown to modulate activity in the ventral striatum during reward anticipation and delivery (Yacubian et al., 2007; Dreher et al., 2009), reviewed in (Tunbridge et al., 2012). Further studies are required to confirm these results

and establish the underlying neurobiological mechanisms. However, the data presented in this thesis adds to the growing body of evidence suggesting that, at least under certain circumstances, COMT can modulate dopamine signalling in subcortical areas, specifically the nucleus accumbens.

6.3 Outstanding questions and areas of future research

There are many outstanding questions regarding the role and function of the COMT Val^{158}Met polymorphism, both on its own but also in association with other genetic and environmental factors. Below I outline some of these future research areas.

6.3.1 What are the mechanisms that mediate the COMT genotype-dependent effects in the nucleus accumbens?

One of the main issues raised by my findings is the need to determine the neurobiological mechanism by which the observed interactive effect in the nucleus accumbens is mediated. This is especially interesting because the direction of the effect appears to differ between acute pharmacological and chronic (i.e. genetically lowered) modulations of COMT activity. Acute COMT inhibition led to an increase in dopamine levels following THC administration, suggesting a direct effect of COMT in this region. In contrast, under conditions in which genetically-determined differences in COMT activity exist throughout life, the low activity Met allele prevented an increase in extracellular dopamine following THC administration, a change which is likely to result from secondary changes in brain function resulting from the COMT Met transgene, rather than a direct, local effect of genotype-dependent differences in COMT activity.

One plausible mechanism through which such an indirect effect might be mediated is the reciprocal relationship between PFC and striatal dopamine (Pycock et al., 1980). In this case, THC administration would be expected to result in higher extracellular dopamine levels in the cortex of Met homozygous, compared to wild-type mice, which in turn would decrease striatal dopamine responses. Arguing against this, in the neurochemical study no genotype-dependent neurochemical responses to THC could be observed in the mPFC of COMT-Met mice. The results thus do not seem to support this mechanism. (Although, as outlined in chapter 4, the measurement of mPFC dopamine in mice was technically challenging, making it difficult to draw comprehensive conclusions from this data).

If the observed neurochemical effect proved to be specific to THC, investigations would have to focus more specifically on THC signalling pathways. For example, a plausible mechanism might involve reduced CB1 receptor expression in the rostromedial tegmental nucleus (RMTg) in COMT-Met mice. The RMTg provides inhibitory input into the VTA that is reduced by CB1 agonists, resulting in disinhibition of VTA dopamine activity (Lecca et al., 2012). Therefore, a reduction of CB1 receptor in this region in the COMT-Met mice would be predicted to make them insensitive to the effects of THC on accumbal dopamine.

Further investigations are required to clarify the mechanisms underlying the interactive neurochemical effect of COMT and THC observed here, and thus help to understand the biological basis for the proposed gene x environment interaction.

6.3.2 Effects of chronic THC exposure during neurodevelopment

The studies detailed in this thesis provide the first direct evidence of interactive effects between THC and COMT genotype at the neurochemical level. They therefore provide a plausible neurochemical mechanism for the observed gene x environment interaction between COMT and cannabis/THC. Notably, for ethical reasons, and in contrast to the studies presented here, human studies reporting interactive effects were carried out in individuals who were not naïve to cannabis use; in many cases their use started during adolescence (Caspi et al., 2005; Estrada et al., 2011). This is relevant not only because repeated cannabis exposure may lead to secondary changes to the dopamine and / or endo-cannabinoid system, but also because according to the neurodevelopmental hypothesis of schizophrenia events occurring early in development may at least in part contribute to later symptoms (Weinberger, 1986; Murray and Lewis, 1987; Owen et al., 2011). A study investigating the effect of CB1 receptor agonist exposure reported functional downregulation of GABAergic transmission in the PFC of adult rats following exposure in early (P35-40) and mid (P40-45) adolescence, but not in late adolescence or adulthood (Cass et al., 2014). Furthermore, a study investigating the effects of chronic adolescent CB1 receptor agonist exposure in COMT knockout mice reported interactive effects on sensorimotor gating, sociability and anxiety-related behaviours, but did not measure neurotransmitter levels (O'Tuathaigh et al., 2011). Therefore, additional studies investigating the effects of sub-chronic THC exposure during specific developmental stages are required to model the effects of human (and possibly adolescent) cannabis consumption on neurochemical indices.

6.3.3 COMT, cannabis and stress

As outlined in chapter 1.3, cannabis use is a risk factor for the development of schizophrenia. Similarly, psychosocial stressors have been shown to increase the risk of developing schizophrenia (McGrath et al., 2004; Cantor-Graae and Selten, 2005; Varese et al., 2012) and the effects of both have been suggested to be modulated by COMT genotype (Caspi et al., 2005; Stefanis et al., 2007; Ira et al., 2014). It may thus not be surprising that recent studies have focused on the combined effect of cannabis use, stressful life events, and COMT genotype on psychotic symptoms. For example, Vinkers and colleagues reported a significant three-way interaction in which Val homozygous participants displayed increased psychotic experiences if they had been exposed to both cannabis use and childhood maltreatment (Vinkers et al., 2013). Consistent with these results, Alemany and colleagues observed significantly higher psychotic experience scores in Val compared to Met homozygous individuals following cannabis exposure and childhood abuse, while COMT genotype appeared to have no effect on psychotic experiences in the absence of childhood abuse. Met homozygous participants on the other hand appeared to be specifically vulnerable to the effect of childhood abuse on positive psychotic experiences, an effect, which appeared to be reduced by cannabis consumption (Alemany et al., 2014).

It is interesting to note that animal, as well as human studies have shown that glucocorticoids, which mediate the classic central endocrine response to prolonged stress, cause an increase in endocannabinoid levels (Malcher-Lopes et al., 2006; Dlugos et al., 2012). Although the evidence regarding the link between stress responses, and the cannabinoid system is currently still limited, especially with regard to their relationship with psychosis (reviewed in Appiah-Kusi et al.,

2016), these studies provide further insight into possible mechanisms through which cannabis, stress and COMT genotype may interact.

Notably, we and others have observed apparent stress-dependent effects of COMT (Laatikainen et al., 2012; Scheggia et al., 2013). Some of the studies presented in this thesis hint at stress/arousal-dependent effects of COMT as a possible confounding factor. Here, these manifest as tentative evidence of different THC-dependent effects between different animal cohorts. Although the studies were not designed in a way that allowed to determine stress as a factor contributing to these discrepancies, and it remains possibly that the trend-level and anecdotal effects seen here represent false-positive errors, the COMT-Met model may prove a useful tool to further investigate the proposed environment x gene x environment (i.e. cannabis x COMT x stress) interaction at a neurochemical and behavioural level.

6.4 Conclusion

The experiments in this thesis demonstrate that COMT activity and THC have interactive effects on extracellular dopamine levels in the nucleus accumbens and that the nature of (and therefore probably the mechanisms underlying) these effects differs between acute, pharmacological, and genetic (i.e. chronic) reductions in COMT activity. The results provide evidence that COMT activity modulates dopamine signalling in the nucleus accumbens following THC administration, adding to the growing body of evidence that COMT is of relevance in subcortical regions despite the abundance of dopamine transporter. Furthermore, they provide evidence for a plausible neurochemical mechanism for the suggested gene x environment interaction between COMT genotype and

cannabis use. In contrast, no interactive effects of COMT genotype and THC on dopamine were observed in the dorsal striatum or the mPFC, although the technical difficulties caused by the extremely low dopamine levels in the mPFC make further investigations in this brain region necessary. Last but not least the studies establish the novel COMT-Met mouse as a good model organism to study the effect of the human specific Val^{158}Met polymorphism in a relatively genetically- and environmentally-controlled background.

The studies were designed to increase understanding of separate and interactive effects of COMT and THC at a neurochemical and behavioural level. The results have clearly provided new insights into both of these aspects. However as outlined in this and previous chapters, the interactive effects of COMT and THC on dopamine activity in different brain regions is complex and may well be further complicated by additional factors such as stress levels and endogenous differences in the endocannabinoid system. Further investigations are therefore essential to obtain a fuller understanding of the processes underlying the proposed gene x environment interaction(s), and their contribution to symptoms of schizophrenia.

7 Supplementary material:

7.1 Schematic overview of microdialysis probe locations

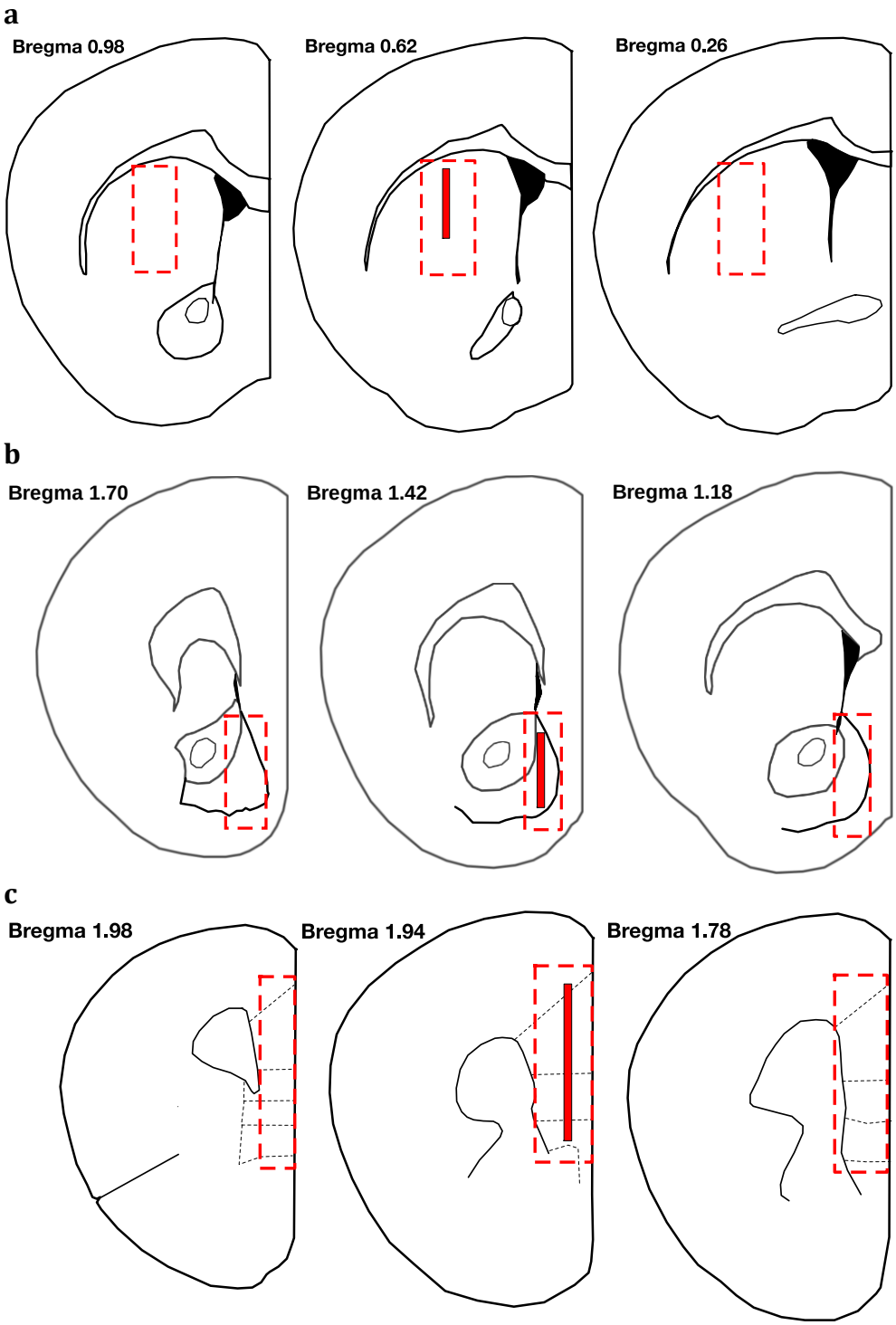


Figure 28: Schematic overview of microdialysis probe locations. *Solid red rectangles show the size of the microdialysis probes in their target locations for a) dorsal striatum, b) nucleus accumbens, and c) medial prefrontal cortex. Data were included if at least 80% of the probe was located in the targeted brain region and all included probes were located within the areas marked by the dotted red lines.*

7.2 Summary of exclusion criterions and animals

Table 2: Summary table of the number of animals that were excluded from statistical analysis of specific tasks and the reason for exclusion

		M-Wt	*M-Met*	*F-Wt*	*F-Met*
Total number of animals		*18*	*18*	*14*	*14*
Task	***Reason for exclusion***	***Number of animals excluded***			
All tasks	*Wrong injection during one of the drug test days resulted in different cumulative THC doses*	*1*	*/*	*/*	*2*
PR, Reward Devaluation, Reversal	*Baseline performance on pre-drug days did not reach criterion; active / inactive lever response ratio < 3*	*2*	*3*	*/*	*1*
Reversal	*Technical issues during 7th day of reversal [including data up to day 7 did not alter results]*	*2*	*1*	*3*	*3*
Sucrose Preference	*Sucrose consumption < 0.5g (difference> 2 standard deviations from mean sucrose consumption on test day)*	*1*	*/*	*1*	*2*
	Moved egg cup and caused spillage during testing	*/*	*1*	*/*	*/*
Locomotor activity	*No task specific exclusion*	*/*	*/*	*/*	*/*
Sensorimotor gating	*Animals were run on a PPI protocol that differed from all others*	*/*	*/*	*2*	*3*
	Mean PPI responses differed >2 standard deviations in all of the prepulse sound intensities	*1*	*/*	*/*	*/*

7.3 Progressive ratio – number of active lever presses

The statistical findings were mainly the same when raw data was analysed:

- Main effect of drug ($F_{2.3,108.9}$=35.2, p<0.001); 0.3 and 1mg/kg increased while 3mg/decreased active lever responses (all p's <0.001)
- No drug*genotype interaction ($F_{2.3,108.9}$=0.53, p=0.62)
- Drug*genotype*cohort interaction ($F_{2.3,108.9}$=2.97, p=0.048)
- Drug*genotype*cohort*sex interaction ($F_{2.3,108.9}$=2.32, p=0.038); in male animals both cohorts showed genotype differences in response to 1mg/kg THC (p's<0.042), albeit in opposite directions; in addition male Met mice showed cohort differences in response to Veh injections (p=0.005)
- In addition to a main effect of sex and a sex*drug interaction present in the raw and normalised data (F's>4.3, p's<0.044), a significant sex*cohort interaction was observed in the raw data ($F_{1,47}$=5.28, p=0.026), driven by sex differences in cohort 1 (p<0.001) but not cohort 2 (p=0.76).

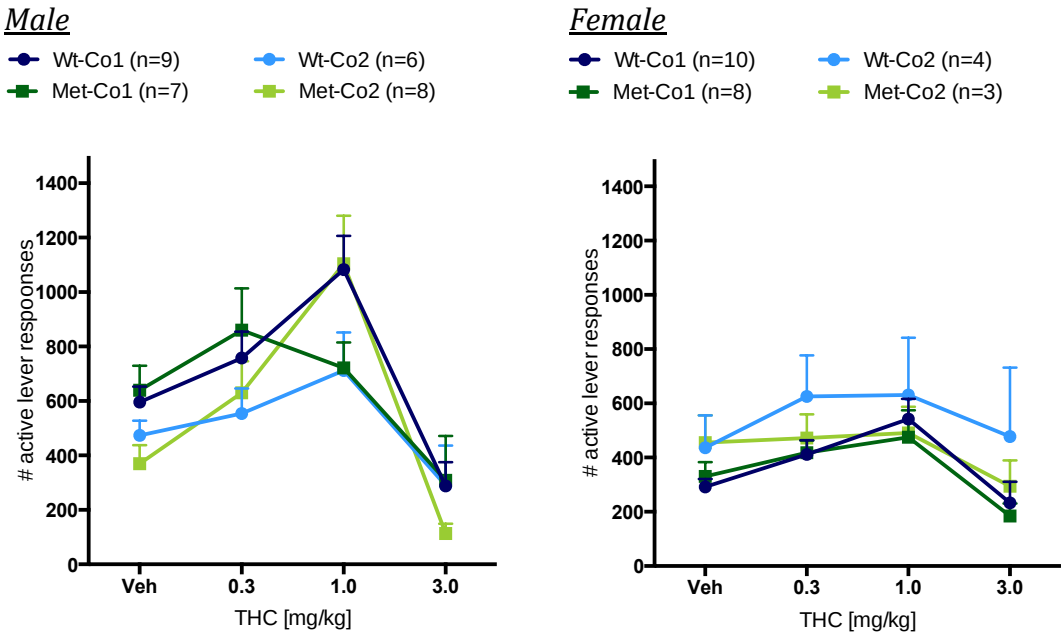


Figure 29: Progressive Ratio performance. *Number of active lever presses (raw data).*

7.4 Reversal learning – sex differences

Females exhibited a higher percentage of active lever presses compared to males ($F_{1,38}$=11.0, p=0.002), an effect that was mainly driven by females performing significantly better during the first 4 reversal sessions (reversal session 1, 2, 3, 4: p=0.030, p=0.001, p=0.011, p=0.020 respectively; session*sex: $F_{3.5,132.8}$=3.98, p=0.006).

7.5 Locomotor activity – sex differences

Following drug injections (at time = 0min) all mice decreased baseline normalised locomotor activity (main effect of time $F_{4.2,224.7}$=5.7, p<0.001) (Figure 30a). This reduction was more pronounced in male mice (main effect of sex: $F_{1,53}$=22.3, p<0.001, mean difference 13.0%) and sex differences were significant from 40 min post injection onwards (time*sex $F_{4.2,224.7}$=6.76, p<0.001).

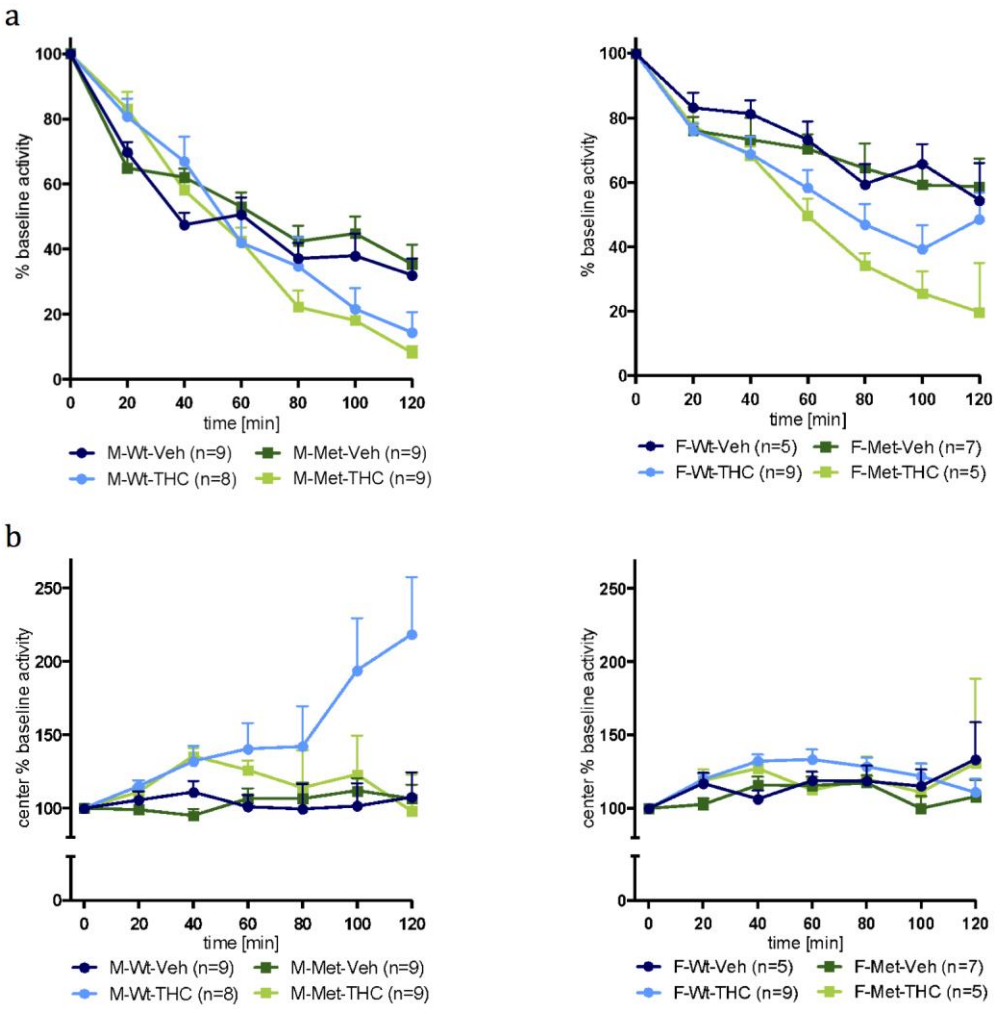


Figure 30: Locomotor activity and percentage of activity conducted in central cage area following THC (1mg/kg, i.p.) or vehicle injections.

7.6 Startle response - sex differences

In general startle responses were lower in female mice compared to male mice (mean difference 24.8, $F_{1,48}$=27.4, p<0.001) and there was a significant volume*sex interaction ($F_{1.8,86.6}$=9.52, p<0.001) indicating that this sex effect was mainly driven by differences in response to 81 and 120dB stimuli (M>F, p=0.024 and p<0.001, respectively).

8 References

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

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R.M., Seibyl, J.P., Bowers, M., et al. (1998) Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am J Psychiatry* 155: 761-767. doi:10.1176/ajp.155.6.761

Abi-Dargham, A., Mawlawi, O., Lombardo, I., Gil, R., Martinez, D., Huang, Y., et al. (2002) Prefrontal dopamine D1 receptors and working memory in schizophrenia. *J Neurosci* 22: 3708-3719. doi:20026302

Abi-Dargham, A., Rodenhiser, J., Printz, D., Zea-Ponce, Y., Gil, R., Kegeles, L.S., et al. (2000) Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proceedings of the National Academy of Sciences* 97: 8104-8109. doi:10.1073/pnas.97.14.8104

Abush, H., and Akirav, I. (2012) Short- and long-term cognitive effects of chronic cannabinoids administration in late-adolescence rats. *PLoS One* 7: e31731. doi:10.1371/journal.pone.0031731

Acquas, E., Carboni, E., de Ree, R.H., Da Prada, M., and Di Chiara, G. (1992) Extracellular concentrations of dopamine and metabolites in the rat caudate after oral administration of a novel catechol-O-methyltransferase inhibitor Ro 40-7592. *J Neurochem* 59: 326-330.

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

Akil, M., Pierri, J.N., Whitehead, R.E., Edgar, C.L., Mohila, C., Sampson, A.R., and Lewis, D.A. (1999) Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. *Am J Psychiatry* 156: 1580-1589.

Alemany, S., Arias, B., Fatjó-Vilas, M., Villa, H., Moya, J., Ibáñez, M.I., et al. (2014) Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes. *Acta Psychiatr Scand* 129: 54-62. doi:10.1111/acps.12108

Alexander, G.E., DeLong, M.R., and Strick, P.L. (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci* 9: 357-381. doi:10.1146/annurev.ne.09.030186.002041

Alger, B.E. (2002) Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog Neurobiol* 68: 247-286.

Andréasson, S., Allebeck, P., Engström, A., and Rydberg, U. (1987) Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet* 2: 1483-1486.

Appiah-Kusi, E., Leyden, E., Parmar, S., Mondelli, V., McGuire, P., and Bhattacharyya, S. (2016) Abnormalities in neuroendocrine stress response in psychosis: the role of endocannabinoids. *Psychological Medicine* 46: 27-45. doi:10.1017/S0033291715001786

Aprille, J.R., and Malamud, D.F. (1975) Catechol-O-methyl transferase in mouse liver plasma membranes. *Biochem Biophys Res Commun* 64: 1293-1302.

Apud, J.A., Mattay, V., Chen, J., Kolachana, B.S., Callicott, J.H., Rasetti, R., et al. (2007) Tolcapone improves cognition and cortical information processing in normal human subjects. *Neuropsychopharmacology* 32: 1011-1020. doi:10.1038/sj.npp.1301227

Arnone, M., Maruani, J., Chaperon, F., Thiébot, M.H., Poncelet, M., Soubrié, P., and Le Fur, G. (1997) Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology (Berl)* 132: 104-106.

Arnsten, A.F.T. (2003) Adrenergic targets for the treatment of cognitive deficits in schizophrenia. *Psychopharmacology* 174: 25-31. doi:10.1007/s00213-003-1724-3

Arseneault, L. (2004) Causal association between cannabis and psychosis: examination of the evidence. *The British Journal of Psychiatry* 184: 110-117. doi:10.1192/bjp.184.2.110

Avramopoulos, D., Stefanis, N.C., Hantoumi, I., Smyrnis, N., Evdokimidis, I., and Stefanis, C.N. (2002) Higher scores of self reported schizotypy in healthy young males carrying the COMT high activity allele. *Mol Psychiatry* 7: 706-711. doi:10.1038/sj.mp.4001070

Axelrod, J., Senoh, S., and Witkop, B. (1958) O-Methylation of catechol amines in vivo. *Journal of Biological Chemistry* 233: 697-701.

Babovic, D., O'Tuathaigh, C.M., O'Connor, A.M., O'Sullivan, G.J., Tighe, O., Croke, D.T., et al. (2008) Phenotypic characterization of cognition and social behavior in mice with heterozygous versus homozygous deletion of catechol-O-methyltransferase. *Neuroscience* 155: 1021-1029. doi:10.1016/j.neuroscience.2008.07.006

Badner, J.A., and Gershon, E.S. (2002) Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 7: 405-411. doi:10.1038/sj.mp.4001012

Barch, D.M. (2005) The cognitive neuroscience of schizophrenia. *Annu Rev Clin Psychol* 1: 321-353. doi:10.1146/annurev.clinpsy.1.102803.143959

Barch, D.M., and Ceaser, A. (2012) Cognition in schizophrenia: core psychological and neural mechanisms. *Trends Cogn Sci* 16: 27-34. doi:10.1016/j.tics.2011.11.015

Barkus, C., Korn, C., Stumpenhorst, K., Laatikainen, L.M., Ballard, D., Lee, S., et al. (2016) Genotype-Dependent Effects of COMT Inhibition on Cognitive Function in a Highly Specific, Novel Mouse Model of Altered COMT Activity. *Neuropsychopharmacology* . doi:10.1038/npp.2016.119

Barkus, E., Morrison, P.D., Vuletic, D., Dickson, J.C., Ell, P.J., Pilowsky, L.S., et al. (2011) Does intravenous Δ9-tetrahydrocannabinol increase dopamine release? A SPET study. *J Psychopharmacol* 25: 1462-1468. doi:10.1177/0269881110382465

Barnett, J.H., Jones, P.B., Robbins, T.W., and Müller, U. (2007) Effects of the catechol-O-methyltransferase Val158Met polymorphism on executive function: a meta-analysis of the Wisconsin Card Sort Test in schizophrenia and healthy controls. *Mol Psychiatry* 12: 502-509. doi:10.1038/sj.mp.4001973

Barnett, J.H., Scoriels, L., and Munafò, M.R. (2008) Meta-analysis of the cognitive effects of the catechol-O-methyltransferase gene Val158/108Met polymorphism. *Biol Psychiatry* 64: 137-144. doi:10.1016/j.biopsych.2008.01.005

Bassareo, V., De Luca, M.A., and Di Chiara, G. (2002) Differential Expression of Motivational Stimulus Properties by Dopamine in Nucleus Accumbens Shell versus Core and Prefrontal Cortex. *J Neurosci* 22: 4709-4719. doi:20026445

Bassett, A.S., Chow, E.W., AbdelMalik, P., Gheorghiu, M., Husted, J., and Weksberg, R. (2003) The schizophrenia phenotype in 22q11 deletion syndrome. *Am J Psychiatry* 160: 1580-1586.

Batalla, A., Soriano-Mas, C., López-Solà, M., Torrens, M., Crippa, J.A., Bhattacharyya, S., et al. (2014) Modulation of brain structure by catechol-O-methyltransferase Val(158) Met polymorphism in chronic cannabis users. *Addict Biol* 19: 722-732. doi:10.1111/adb.12027

Battisti, R.A., Roodenrys, S., Johnstone, S.J., Respondek, C., Hermens, D.F., and Solowij, N. (2010) Chronic use of cannabis and poor neural efficiency in verbal memory ability. *Psychopharmacology* 209: 319-330. doi:10.1007/s00213-010-1800-4

Behan, A.T., Hryniewiecka, M., O'Tuathaigh, C.M., Kinsella, A., Cannon, M., Karayiorgou, M., et al. (2012) Chronic Adolescent Exposure to Delta-9-Tetrahydrocannabinol in COMT Mutant Mice: Impact on Indices of Dopaminergic, Endocannabinoid and GABAergic Pathways. *Neuropsychopharmacology* 37: 1773-1783. doi:10.1038/npp.2012.24

Belujon, P., and Grace, A.A. (2015) Regulation of dopamine system responsivity and its adaptive and pathological response to stress. *Proc Biol Sci* 282. doi:10.1098/rspb.2014.2516

Bermudez-Silva, F.J., Viveros, M.P., McPartland, J.M., and Rodriguez de Fonseca, F. (2010) The endocannabinoid system, eating behavior and energy homeostasis: the end or a new beginning? *Pharmacol Biochem Behav* 95: 375-382. doi:10.1016/j.pbb.2010.03.012

Berridge, K.C. (2006) The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology* 191: 391-431. doi:10.1007/s00213-006-0578-x

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

Berridge, K.C., Robinson, T.E., and Aldridge, J.W. (2009) Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol* 9: 65-73. doi:10.1016/j.coph.2008.12.014

Bertocci, B., Miggiano, V., Da Prada, M., Dembic, Z., Lahm, H.W., and Malherbe, P. (1991) Human catechol-O-methyltransferase: cloning and expression of the membrane-associated form. *Proc Natl Acad Sci U S A* 88: 1416-1420.

Bertolino, A., Blasi, G., Latorre, V., Rubino, V., Rampino, A., Sinibaldi, L., et al. (2006) Additive effects of genetic variation in dopamine regulating genes on working memory cortical activity in human brain. *J Neurosci* 26: 3918-3922. doi:10.1523/JNEUROSCI.4975-05.2006

Bertolino, A., Caforio, G., Petruzzella, V., Latorre, V., Rubino, V., Dimalta, S., et al. (2006) Prefrontal dysfunction in schizophrenia controlling for COMT Val158Met genotype and working memory performance. *Psychiatry Res* 147: 221-226. doi:10.1016/j.pscychresns.2006.04.001

Bidaut-Russell, M., Devane, W.A., and Howlett, A.C. (1990) Cannabinoid receptors and modulation of cyclic AMP accumulation in the rat brain. *J Neurochem* 55: 21-26.

Bilder, R.M., Goldman, R.S., Robinson, D., Reiter, G., Bell, L., Bates, J.A., et al. (2000) Neuropsychology of first-episode schizophrenia: initial characterization and clinical correlates. *Am J Psychiatry* 157: 549-559. doi:10.1176/appi.ajp.157.4.549

Bilder, R.M., Volavka, J., Lachman, H.M., and Grace, A.A. (2004) The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology* 29: 1943-1961. doi:10.1038/sj.npp.1300542

Blanchard, M.M., Chamberlain, S.R., Roiser, J., Robbins, T.W., and Müller, U. (2011) Effects of two dopamine-modulating genes (DAT1 9/10 and COMT Val/Met) on n-back working memory performance in healthy volunteers. *Psychol Med* 41: 611-618. doi:10.1017/S003329171000098X

Blasi, G., Mattay, V.S., Bertolino, A., Elvevåg, B., Callicott, J.H., Das, S., et al. (2005) Effect of catechol-O-methyltransferase val158met genotype on attentional control. *J Neurosci* 25: 5038-5045. doi:10.1523/JNEUROSCI.0476-05.2005

Borges, N., Vieira-Coelho, M.A., Parada, A., and Soares-Da-Silva, P. (1997) Studies on the tight-binding nature of tolcapone inhibition of soluble and membrane-bound rat brain catechol-O-methyltransferase. *Journal of Pharmacology and Experimental Therapeutics* 282: 812-817.

Bortolato, M., Aru, G.N., Frau, R., Orrù, M., Luckey, G.C., Boi, G., and Gessa, G.L. (2004) The CB receptor agonist WIN 55,212-2 fails to elicit disruption of prepulse inhibition of the startle in Sprague-Dawley rats. *Psychopharmacology* 177: 264-271. doi:10.1007/s00213-004-1941-4

Bossong, M.G., Mehta, M.A., van Berckel, B.N., Howes, O.D., Kahn, R.S., and Stokes, P.R. (2015) Further human evidence for striatal dopamine release induced by administration of Δ9-tetrahydrocannabinol (THC): selectivity to limbic striatum. *Psychopharmacology (Berl)* 232: 2723-2729. doi:10.1007/s00213-015-3915-0

Bossong, M.G., van Berckel, B.N., Boellaard, R., Zuurman, L., Schuit, R.C., Windhorst, A.D., et al. (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology* 34: 759-766. doi:10.1038/npp.2008.138

Bouaboula, M., Poinot-Chazel, C., Bourrié, B., Canat, X., Calandra, B., Rinaldi-Carmona, M., et al. (1995) Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J* 312 (Pt 2): 637-641.

Braff, D.L., Geyer, M.A., and Swerdlow, N.R. (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology* 156: 234-258. doi:10.1007/s002130100810

Brischoux, F., Chakraborty, S., Brierley, D.I., and Ungless, M.A. (2009) Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. *Proc Natl Acad Sci U S A* 106: 4894-4899. doi:10.1073/pnas.0811507106

Broch, O.J., and Fonnum, F. (1972) The regional and subcellular distribution of catechol-O-methyl transferase in the rat brain. *J Neurochem* 19: 2049-2055.

Bromberg-Martin, E.S., Matsumoto, M., and Hikosaka, O. (2010) Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron* 68: 815-834. doi:10.1016/j.neuron.2010.11.022

Brown, J.E., Kassouny, M., and Cross, J.K. (1977) Kinetic studies of food intake and sucrose solution preference by rats treated with low doses of Δ 9-tetrahydrocannabinol. *Behavioral biology* 20: 104-110.

Brozoski, T.J., Brown, R.M., Rosvold, H.E., and Goldman, P.S. (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science* 205: 929-932. doi:10.1126/science.112679

Brunzell, D.H., Boschen, K.E., Hendrick, E.S., Beardsley, P.M., and McIntosh, J.M. (2010) Alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors in the nucleus accumbens shell regulate progressive ratio responding maintained by nicotine. *Neuropsychopharmacology* 35: 665-673. doi:10.1038/npp.2009.171

Brust, P., Walter, B., Hinz, R., Füchtner, F., Müller, M., Steinbach, J., and Bauer, R. (2004) Developmental changes in the activities of aromatic amino acid decarboxylase and catechol-O-methyl transferase in the porcine brain: a positron emission tomography study. *Neuroscience letters* 364: 159-163.

Bryce, C.A., and Floresco, S.B. (2016) Perturbations in Effort-Related Decision-Making Driven by Acute Stress and Corticotropin-Releasing Factor. *Neuropsychopharmacology* 41: 2147-2159. doi:10.1038/npp.2016.15

Buckert, M., Kudielka, B.M., Reuter, M., and Fiebach, C.J. (2012) The COMT Val158Met polymorphism modulates working memory performance under acute stress. *Psychoneuroendocrinology* 37: 1810-1821. doi:10.1016/j.psyneuen.2012.03.014

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

Butts, K.A., and Phillips, A.G. (2013) Glucocorticoid receptors in the prefrontal cortex regulate dopamine efflux to stress via descending glutamatergic feedback to the ventral tegmental area. *Int J Neuropsychopharmacol* 16: 1799-1807. doi:10.1017/S1461145713000187

Cadoni, C., Simola, N., Espa, E., Fenu, S., and Di Chiara, G. (2015) Strain dependence of adolescent Cannabis influence on heroin reward and mesolimbic dopamine transmission in adult Lewis and Fischer 344 rats. *Addict Biol* 20: 132-142. doi:10.1111/adb.12085

Cagniard, B., Balsam, P.D., Brunner, D., and Zhuang, X. (2006) Mice with chronically elevated dopamine exhibit enhanced motivation, but not learning, for a food reward. *Neuropsychopharmacology* 31: 1362-1370. doi:10.1038/sj.npp.1300966

Camara, E., Krämer, U.M., Cunillera, T., Marco-Pallarés, J., Cucurell, D., Nager, W., et al. (2010) The effects of COMT (Val108/158Met) and DRD4 (SNP -521) dopamine genotypes on brain activations related to valence and magnitude of rewards. *Cereb Cortex* 20: 1985-1996. doi:10.1093/cercor/bhp263

Cannon, M., Jones, P.B., and Murray, R.M. (2002) Obstetric complications and schizophrenia: historical and meta-analytic review. *Am J Psychiatry* 159: 1080-1092. doi:10.1176/appi.ajp.159.7.1080

Cantor-Graae, E., and Selten, J.P. (2005) Schizophrenia and migration: a meta-analysis and review. *Am J Psychiatry* 162: 12-24. doi:10.1176/appi.ajp.162.1.12

Carlsson, A., and Lindqvist, M. (1963) Effect of Chlorpromazine or Haloperidol on Formation of 3-Methoxytyramine and Normetanephrine in Mouse Brain. *Acta Pharmacol Toxicol (Copenh)* 20: 140-144.

C Arnold, J., A Boucher, A., and Karl, T. (2012) The Yin and Yang of Cannabis-induced Psychosis: the Actions of 9-Tetrahydrocannabinol and Cannabidiol in Rodent Models of Schizophrenia. *Current pharmaceutical design* 18: 5113-5130.

Cascini, F., Aiello, C., and Di Tanna, G. (2012) Increasing Delta-9-Tetrahydrocannabinol (Δ-9-THC) Content in Herbal Cannabis Over Time: Systematic Review and Meta-Analysis. *Current Drug Abuse Reviews* 5: 32-40.

Caspi, A., Moffitt, T.E., Cannon, M., McClay, J., Murray, R., Harrington, H., et al. (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry* 57: 1117-1127. doi:10.1016/j.biopsych.2005.01.026

Cass, D.K., Flores-Barrera, E., Thomases, D.R., Vital, W.F., Caballero, A., and Tseng, K.Y. (2014) CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex. *Molecular Psychiatry* 19: 536. doi:10.1038/mp.2014.14

Cass, W.A., Zahniser, N.R., Flach, K.A., and Gerhardt, G.A. (1993) Clearance of exogenous dopamine in rat dorsal striatum and nucleus accumbens: role of metabolism and effects of locally applied uptake inhibitors. *J Neurochem* 61: 2269-2278.

Castañeda, E., Moss, M., Oddie, S.D., and Whishaw, I.Q. (1991) THC does not affect striatal dopamine release: Microdialysis in freely moving rats. *Pharmacology Biochemistry and Behavior* 40: 587 - 591. doi:10.1016/0091-3057(91)90367-B

Castro, D.C., and Berridge, K.C. (2014) Advances in the neurobiological bases for food 'liking' versus 'wanting'. *Physiol Behav* 136: 22-30. doi:10.1016/j.physbeh.2014.05.022

Caul, W.F., and Brindle, N.A. (2001) Schedule-dependent effects of haloperidol and amphetamine: multiple-schedule task shows within-subject effects. *Pharmacol Biochem Behav* 68: 53-63.

Ceravolo, R., Piccini, P., Bailey, D.L., Jorga, K.M., Bryson, H., and Brooks, D.J. (2002) 18F-dopa PET evidence that tolcapone acts as a central COMT inhibitor in Parkinson's disease. *Synapse* 43: 201-207. doi:10.1002/syn.10034

Cheer, J.F., Aragona, B.J., Heien, M.L., Seipel, A.T., Carelli, R.M., and Wightman, R.M. (2007) Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. *Neuron* 54: 237-244. doi:10.1016/j.neuron.2007.03.021

Cheer, J.F., Wassum, K.M., Heien, M.L., Phillips, P.E., and Wightman, R.M. (2004) Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *J Neurosci* 24: 4393-4400. doi:10.1523/JNEUROSCI.0529-04.2004

Chen, J., Lipska, B.K., Halim, N., Ma, Q.D., Matsumoto, M., Melhem, S., et al. (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet* 75: 807-821. doi:10.1086/425589

Chen, J., Paredes, W., Lowinson, J.H., and Gardner, E.L. (1990) [Delta] 9-Tetrahydroeannabinol enhances presynaptic dopamine efflux In medial prefrontal cortex. *European journal of pharmacology* 190: 259-262.

Chen, J., Song, J., Yuan, P., Tian, Q., Ji, Y., Ren-Patterson, R., et al. (2011) Orientation and cellular distribution of membrane-bound catechol-O-methyltransferase in cortical neurons: implications for drug development. *J Biol Chem* 286: 34752-34760. doi:10.1074/jbc.M111.262790

Chen, J.P., Paredes, W., Li, J., Smith, D., Lowinson, J., and Gardner, E.L. (1990) Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. *Psychopharmacology (Berl)* 102: 156-162.

Chen, J.P., Paredes, W., Lowinson, J.H., and Gardner, E.L. (1991) Strain-specific facilitation of dopamine efflux by delta 9-tetrahydrocannabinol in the nucleus accumbens of rat: an in vivo microdialysis study. *Neurosci Lett* 129: 136-180.

Chudasama, Y., and Robbins, T.W. (2006) Functions of frontostriatal systems in cognition: comparative neuropsychopharmacological studies in rats, monkeys and humans. *Biol Psychol* 73: 19-38. doi:10.1016/j.biopsycho.2006.01.005

Cohen, J.Y., Haesler, S., Vong, L., Lowell, B.B., and Uchida, N. (2012) Neuron-type-specific signals for reward and punishment in the ventral tegmental area. *Nature* 482: 85-88. doi:10.1038/nature10754

Colizzi, M., Iyegbe, C., Powell, J., Blasi, G., Bertolino, A., Murray, R.M., and Di Forti, M. (2015) Interaction between DRD2 and AKT1 genetic variations on risk of psychosis in cannabis users: a case-control study. *NPJ Schizophr* 1: 15025. doi:10.1038/npjschz.2015.25

Collins, P., Wilkinson, L.S., Everitt, B.J., Robbins, T.W., and Roberts, A.C. (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: 3-17. doi:10.1037//0735-7044.114.1.3

Collip, D., van Winkel, R., Peerbooms, O., Lataster, T., Thewissen, V., Lardinois, M., et al. (2011) COMT Val158Met-stress interaction in psychosis: role of background psychosis risk. *CNS Neurosci Ther* 17: 612-619. doi:10.1111/j.1755-5949.2010.00213.x

Colzato, L.S., Waszak, F., Nieuwenhuis, S., Posthuma, D., and Hommel, B. (2010) The flexible mind is associated with the catechol-O-methyltransferase (COMT) Val158Met polymorphism: evidence for a role of dopamine in the control of task-switching. *Neuropsychologia* 48: 2764-2768. doi:10.1016/j.neuropsychologia.2010.04.023

Cools, R., and D'Esposito, M. (2011) Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol Psychiatry* 69: e113-e125. doi:10.1016/j.biopsych.2011.03.028

Corlett, P.R., Murray, G.K., Honey, G.D., Aitken, M.R., Shanks, D.R., Robbins, T.W., et al. (2007) Disrupted prediction-error signal in psychosis: evidence for an associative account of delusions. *Brain* 130: 2387-2400. doi:10.1093/brain/awm173

Costas, J., Sanjuán, J., Ramos-Ríos, R., Paz, E., Agra, S., Ivorra, J.L., et al. (2011) Heterozygosity at catechol-O-methyltransferase Val158Met and schizophrenia: new data and meta-analysis. *J Psychiatr Res* 45: 7-14. doi:10.1016/j.jpsychires.2010.04.021

Costas, J., Sanjuán, J., Ramos-Ríos, R., Paz, E., Agra, S., Tolosa, A., et al. (2011) Interaction between COMT haplotypes and cannabis in schizophrenia: a case-only study in two samples from Spain. *Schizophr Res* 127: 22-27. doi:10.1016/j.schres.2011.01.014

Crabbe, J.C. (1999) Genetics of Mouse Behavior: Interactions with Laboratory Environment. *Science* 284: 1670-1672. doi:10.1126/science.284.5420.1670

Craft, R.M. (2005) Sex differences in behavioral effects of cannabinoids. *Life Sci* 77: 2471-2478. doi:10.1016/j.lfs.2005.04.019

Craft, R.M., Marusich, J.A., and Wiley, J.L. (2013) Sex differences in cannabinoid pharmacology: a reflection of differences in the endocannabinoid system? *Life Sci* 92: 476-481. doi:10.1016/j.lfs.2012.06.009

Creese, I., Burt, D.R., and Snyder, S.H. (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192: 481-483. doi:10.1126/science.3854

Crofts, H.S., Dalley, J.W., Collins, P., Van Denderen, J.C.M., Everitt, B.J., Robbins, T.W., and Roberts, A.C. (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: 1015-1026. doi:10.1093/cercor/11.11.1015

Curran, H.V., Brignell, C., Fletcher, S., Middleton, P., and Henry, J. (2002) Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology (Berl)* 164: 61-70. doi:10.1007/s00213-002-1169-0

Daniel, D.G., Goldberg, T.E., Gibbons, R.D., and Weinberger, D.R. (1991) Lack of a bimodal distribution of ventricular size in schizophrenia: a Gaussian mixture analysis of 1056 cases and controls. *Biol Psychiatry* 30: 887-903.

Da Silva, G.E., and Takahashi, R.N. (2002) SR 141716A prevents Δ< sup> 9-tetrahydrocannabinol-induced spatial learning deficit in a Morris-type water maze in mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 26: 321-325.

Dean, B. (2003) The cortical serotonin2A receptor and the pathology of schizophrenia: a likely accomplice. *Journal of Neurochemistry* 85: 1-13. doi:10.1046/j.1471-4159.2003.01693.x

DeLisi, L.E., Shaw, S.H., Crow, T.J., Shields, G., Smith, A.B., Larach, V.W., et al. (2014) A genome-wide scan for linkage to chromosomal regions in 382 sibling pairs with schizophrenia or schizoaffective disorder. *American Journal of Psychiatry* .

Diana, M., Melis, M., and Gessa, G.L. (1998) Increase in meso-prefrontal dopaminergic activity after stimulation of CB1 receptors by cannabinoids. *Eur J Neurosci* 10: 2825-2830.

Diatchenko, L., Slade, G.D., Nackley, A.G., Bhalang, K., Sigurdsson, A., Belfer, I., et al. (2005) Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum Mol Genet* 14: 135-143. doi:10.1093/hmg/ddi013

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, M.A., Paparelli, A., et al. (2012) Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biol Psychiatry* 72: 811-816. doi:10.1016/j.biopsych.2012.06.020

Di Forti, M., Marconi, A., Carra, E., Fraietta, S., Trotta, A., Bonomo, M., et al. (2015) Proportion of patients in south London with first-episode psychosis attributable to use of high potency cannabis: a case-control study. *Lancet Psychiatry* 2: 233-238. doi:10.1016/S2215-0366(14)00117-5

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C., Mondelli, V., Marques, T.R., et al. (2009) High-potency cannabis and the risk of psychosis. *Br J Psychiatry* 195: 488-491. doi:10.1192/bjp.bp.109.064220

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S.A., et al. (2014) Daily Use, Especially of High-Potency Cannabis, Drives the Earlier Onset of Psychosis in Cannabis Users. *Schizophrenia Bulletin* 40: 1509-1517. doi:10.1093/schbul/sbt181

Di Martino, A., Scheres, A., Margulies, D.S., Kelly, A.M., Uddin, L.Q., Shehzad, Z., et al. (2008) Functional connectivity of human striatum: a resting state FMRI study. *Cereb Cortex* 18: 2735-2747. doi:10.1093/cercor/bhn041

Di Marzo, V., and Matias, I. (2005) Endocannabinoid control of food intake and energy balance. *Nat Neurosci* 8: 585-589. doi:10.1038/nn1457

Dlugos, A., Childs, E., Stuhr, K.L., Hillard, C.J., and de Wit, H. (2012) Acute stress increases circulating anandamide and other N-acylethanolamines in healthy humans. *Neuropsychopharmacology* 37: 2416-2427. doi:10.1038/npp.2012.100

Doll, B.B., Hutchison, K.E., and Frank, M.J. (2011) Dopaminergic genes predict individual differences in susceptibility to confirmation bias. *J Neurosci* 31: 6188-6198. doi:10.1523/JNEUROSCI.6486-10.2011

Draganski, B., Kherif, F., Klöppel, S., Cook, P.A., Alexander, D.C., Parker, G.J., et al. (2008) Evidence for segregated and integrative connectivity patterns in the human Basal Ganglia. *J Neurosci* 28: 7143-7152. doi:10.1523/JNEUROSCI.1486-08.2008

Dreher, J.C., Kohn, P., Kolachana, B., Weinberger, D.R., and Berman, K.F. (2009) Variation in dopamine genes influences responsivity of the human reward system. *Proc Natl Acad Sci U S A* 106: 617-622. doi:10.1073/pnas.0805517106

Drew, L.J., Crabtree, G.W., Markx, S., Stark, K.L., Chaverneff, F., Xu, B., et al. (2011) The 22q11.2 microdeletion: fifteen years of insights into the genetic and neural complexity of psychiatric disorders. *Int J Dev Neurosci* 29: 259-281. doi:10.1016/j.ijdevneu.2010.09.007

Drews, E., Schneider, M., and Koch, M. (2005) Effects of the cannabinoid receptor agonist WIN 55,212-2 on operant behavior and locomotor activity in rats. *Pharmacol Biochem Behav* 80: 145-150. doi:10.1016/j.pbb.2004.10.023

Drummer, O.H., Gerostamoulos, J., Batziris, H., Chu, M., Caplehorn, J.R., Robertson, M.D., and Swann, P. (2003) The incidence of drugs in drivers killed in Australian road traffic crashes. *Forensic Science International* 134: 154-162. doi:10.1016/s0379-0738(03)00134-8

D'Souza, D.C., Abi-Saab, W.M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., et al. (2005) Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biol Psychiatry* 57: 594-608. doi:10.1016/j.biopsych.2004.12.006

D'Souza, D.C., Braley, G., Blaise, R., Vendetti, M., Oliver, S., Pittman, B., et al. (2008) Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of Delta-9-tetrahydrocannabinol in humans. *Psychopharmacology (Berl)* 198: 587-603. doi:10.1007/s00213-007-1042-2

D'Souza, D.C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y.T., et al. (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* 29: 1558-1572. doi: 10.1038/sj.npp.1300496

Dumontheil, I., Roggeman, C., Ziermans, T., Peyrard-Janvid, M., Matsson, H., Kere, J., and Klingberg, T. (2011) Influence of the COMT genotype on working memory and brain activity changes during development. *Biol Psychiatry* 70: 222-229. doi:10.1016/j.biopsych.2011.02.027

Egan, M.F., Goldberg, T.E., Kolachana, B.S., Callicott, J.H., Mazzanti, C.M., Straub, R.E., et al. (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 98: 6917-6922. doi:10.1073/pnas.111134598

Eisenhofer, G., Kopin, I.J., and Goldstein, D.S. (2004) Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol Rev* 56: 331-349. doi:10.1124/pr.56.3.1

Elsohly, M.A., and Slade, D. (2005) Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sci* 78: 539-548. doi:10.1016/j.lfs.2005.09.011

Enrico, P., Bouma, M., De Vries, J.B., and Westerink, B.H. (1998) The role of afferents to the ventral tegmental area in the handling stress-induced increase in the release of dopamine in the medial prefrontal cortex: a dual-probe microdialysis study in the rat brain. *Brain research* 779: 205-213.

Estrada, G., Fatjó-Vilas, M., Muñoz, M.J., Pulido, G., Miñano, M.J., Toledo, E., et al. (2011) Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. *Acta Psychiatr Scand* 123: 485-492. doi:10.1111/j.1600-0447.2010.01665.x

Fadda, P., Scherma, M., Fresu, A., Collu, M., and Fratta, W. (2005) Dopamine and serotonin release in dorsal striatum and nucleus accumbens is differentially modulated by morphine in DBA/2J and C57BL/6J mice. *Synapse* 56: 29-38. doi:10.1002/syn.20122

Fan, J.B., Zhang, C.S., Gu, N.F., Li, X.W., Sun, W.W., Wang, H.Y., et al. (2005) Catechol-O-methyltransferase gene Val/Met functional polymorphism and risk of schizophrenia: a large-scale association study plus meta-analysis. *Biol Psychiatry* 57: 139-144. doi:10.1016/j.biopsych.2004.10.018

Farrell, S.M., Tunbridge, E.M., Braeutigam, S., and Harrison, P.J. (2012) COMT Val(158)Met genotype determines the direction of cognitive effects produced by catechol-O-methyltransferase inhibition. *Biol Psychiatry* 71: 538-544. doi:10.1016/j.biopsych.2011.12.023

Fattore, L., and Fratta, W. (2010) How important are sex differences in cannabinoid action? *Br J Pharmacol* 160: 544-548. doi:10.1111/j.1476-5381.2010.00776.x

Feenstra, M.G.P., Botterblom, M.H.A., and Mastenbroek, S. (2000) Dopamine and noradrenaline efflux in the prefrontal cortex in the light and dark period: effects of novelty and handling and comparison to the nucleus accumbens. *Neuroscience* 100: 741-748. doi:10.1016/s0306-4522(00)00319-5

Fendt, M., Li, L., and Yeomans, J.S. (2001) Brain stem circuits mediating prepulse inhibition of the startle reflex. *Psychopharmacology* 156: 216-224. doi:10.1007/s002130100794

Ferdinand, R.F., Sondeijker, F., van der Ende, J., Selten, J.P., Huizink, A., and Verhulst, F.C. (2005) Cannabis use predicts future psychotic symptoms, and vice versa. *Addiction* 100: 612-618. doi:10.1111/j.1360-0443.2005.01070.x

Fergusson, D.M., Horwood, L.J., and Swain-Campbell, N.R. (2003) Cannabis dependence and psychotic symptoms in young people. *Psychol Med* 33: 15-21.

Fibiger, H.C., and Phillips, A.G. (1986) Reward, motivation, cognition: psychobiology of mesotelencephalic dopamine systems. *Comprehensive Physiology* .

Fiorillo, C.D. (2013) Two dimensions of value: dopamine neurons represent reward but not aversiveness. *Science* 341: 546-549. doi:10.1126/science.1238699

Fiorillo, C.D., Tobler, P.N., and Schultz, W. (2003) Discrete coding of reward probability and uncertainty by dopamine neurons. *Science* 299: 1898-1902. doi:10.1126/science.1077349

Floderus, Y., Ross, S.B., and Wetterberg, L. (1981) Erythrocyte catechol-O-methyltransferase activity in a Swedish population. *Clin Genet* 19: 389-392.

Floresco, S.B. (2013) Prefrontal dopamine and behavioral flexibility: shifting from an "inverted-U" toward a family of functions. *Front Neurosci* 7: 62. doi:10.3389/fnins.2013.00062

Floresco, S.B., Magyar, O., Ghods-Sharifi, S., Vexelman, C., and Tse, M.T. (2006) Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. *Neuropsychopharmacology* 31: 297-309. doi:10.1038/sj.npp.1300825

Forbes, E.E., Brown, S.M., Kimak, M., Ferrell, R.E., Manuck, S.B., and Hariri, A.R. (2007) Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity. *Molecular Psychiatry* 14: 60-70. doi:10.1038/sj.mp.4002086

Frank, M.J., Moustafa, A.A., Haughey, H.M., Curran, T., and Hutchison, K.E. (2007) Genetic triple dissociation reveals multiple roles for dopamine in reinforcement learning. *Proc Natl Acad Sci U S A* 104: 16311-16316. doi:10.1073/pnas.0706111104

Freedland, C.S., Poston, J.S., and Porrino, L.J. (2000) Effects of SR141716A, a central cannabinoid receptor antagonist, on food-maintained responding. *Pharmacol Biochem Behav* 67: 265-270.

French, E.D. (1997) delta9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. *Neurosci Lett* 226: 159-162.

French, E.D., Dillon, K., and Wu, X. (1997) Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *NeuroReport* 8: 649.

de Frias, C.M., Marklund, P., Eriksson, E., Larsson, A., Oman, L., Annerbrink, K., et al. (2010) Influence of COMT gene polymorphism on fMRI-assessed sustained and transient activity during a working memory task. *J Cogn Neurosci* 22: 1614-1622. doi:10.1162/jocn.2009.21318

Gallinat, J., Bajbouj, M., Sander, T., Schlattmann, P., Xu, K., Ferro, E.F., et al. (2003) Association of the G1947A COMT (Val108/158Met) gene polymorphism with prefrontal P300 during information processing. *Biological Psychiatry* 54: 40-48. doi:10.1016/s0006-3223(02)01973-x

Galvão, A.C., Krüger, R.C., Campagnolo, P.D., Mattevi, V.S., Vitolo, M.R., and Almeida, S. (2012) Association of MAOA and COMT gene polymorphisms with palatable food intake in children. *J Nutr Biochem* 23: 272-277. doi:10.1016/j.jnutbio.2010.12.004

Gaoni, Y., and Mechoulam, R. (1964) Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American chemical society* 86: 1646-1647.

Garbarg, M., Baudry, M., Benda, P., and Schwartz, J.C. (1975) Simultaneous presence of histamine-N-methyltransferase and catechol-O-methyltransferase in neuronal and glial cells in culture. *Brain Res* 83: 538-541.

Gessa, G.L., Melis, M., Muntoni, A.L., and Diana, M. (1998) Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *Eur J Pharmacol* 341: 39-44.

Giakoumaki, S.G., Roussos, P., and Bitsios, P. (2008) Improvement of prepulse inhibition and executive function by the COMT inhibitor tolcapone depends on COMT Val158Met polymorphism. *Neuropsychopharmacology* 33: 3058-3068. doi:10.1038/npp.2008.82

de Giessen, E.V., Weinstein, J.J., Cassidy, C.M., Haney, M., Dong, Z., Ghazzaoui, R., et al. (2016) Deficits in striatal dopamine release in cannabis dependence. *Molecular Psychiatry* 22: 68. doi:10.1038/mp.2016.21

Gillan, C.M., Robbins, T.W., Sahakian, B.J., van den Heuvel, O.A., and van Wingen, G. (2016) The role of habit in compulsivity. *Eur Neuropsychopharmacol* 26: 828-840. doi:10.1016/j.euroneuro.2015.12.033

Glatt, S.J., Faraone, S.V., and Tsuang, M.T. (2003) Association between a functional catechol O-methyltransferase gene polymorphism and schizophrenia: meta-analysis of case-control and family-based studies. *Am J Psychiatry* 160: 469-476.

Gogos, J.A., Morgan, M., Luine, V., Santha, M., Ogawa, S., Pfaff, D., and Karayiorgou, M. (1998) Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc Natl Acad Sci U S A* 95: 9991-9996.

Goldberg, T.E., Egan, M.F., Gscheidle, T., Coppola, R., Weickert, T., Kolachana, B.S., et al. (2003) Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. *Arch Gen Psychiatry* 60: 889-896. doi:10.1001/archpsyc.60.9.889

Goldman-Rakic, P.S., Lidow, M.S., and Gallager, D.W. (1990) Overlap of dopaminergic, adrenergic, and serotoninergic receptors and complementarity of their subtypes in primate prefrontal cortex. *J Neurosci* 10: 2125-2138.

Goldman-Rakic, P.S., Muly III, E.C., and Williams, G.V. (2000) D 1 receptors in prefrontal cells and circuits. *Brain Research Reviews* 31: 295-301.

Goldstein, D.J., Weinshilboum, R.M., Dunnette, J.H., and Creveling, C.R. (1980) Developmental Patterns of Catechol-O-Methyltransferase in Genetically Different Rat Strains: Enzymatic and Immunochemical Studies. *Journal of neurochemistry* 34: 153-162.

Gomes, F.V., Guimarães, F.S., and Grace, A.A. (2015) Effects of pubertal cannabinoid administration on attentional set-shifting and dopaminergic hyper-responsivity in a developmental disruption model of schizophrenia. *Int J Neuropsychopharmacol* 18. doi:10.1093/ijnp/pyu018

Gothelf, D., Law, A.J., Frisch, A., Chen, J., Zarchi, O., Michaelovsky, E., et al. (2014) Biological effects of COMT haplotypes and psychosis risk in 22q11.2 deletion syndrome. *Biol Psychiatry* 75: 406-413. doi:10.1016/j.biopsych.2013.07.021

Grace, A.A. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience* 41: 1-24.

Grant, J.E., Odlaug, B.L., Chamberlain, S.R., Hampshire, A., Schreiber, L.R., and Kim, S.W. (2013) A proof of concept study of tolcapone for pathological gambling: relationships with COMT genotype and brain activation. *Eur Neuropsychopharmacol* 23: 1587-1596. doi:10.1016/j.euroneuro.2013.07.008

Grice, D.E., Reenilä, I., Männistö, P.T., Brooks, A.I., Smith, G.G., Golden, G.T., et al. (2007) Transcriptional profiling of C57 and DBA strains of mice in the absence and presence of morphine. *BMC Genomics* 8: 76. doi:10.1186/1471-2164-8-76

Grossman, M.H., Creveling, C.R., Rybczynski, R., Braverman, M., Isersky, C., and Breakefield, X.O. (1985) Soluble and particulate forms of rat catechol-O-methyltransferase distinguished by gel electrophoresis and immune fixation. *J Neurochem* 44: 421-432.

Grossman, M.H., Emanuel, B.S., and Budarf, M.L. (1992) Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11.1->q11.2. *Genomics* 12: 822-825.

Guldberg, H.C., and Marsden, C.A. (1975) Catechol-O-methyl transferase: pharmacological aspects and physiological role. *Pharmacol Rev* 27: 135-206.

Gutiérrez, B., Rivera, M., Obel, L., McKenney, K., Martínez-Leal, R., Molina, E., et al. (2009) Variability in the COMT gene and modification of the risk of schizophrenia conferred by cannabis consumption. *Rev Psiquiatr Salud Ment* 2: 89-94. doi:10.1016/S1888-9891(09)72250-5

Harley, M., Kelleher, I., Clarke, M., Lynch, F., Arseneault, L., Connor, D., et al. (2010) Cannabis use and childhood trauma interact additively to increase the risk of psychotic symptoms in adolescence. *Psychol Med* 40: 1627-1634. doi:10.1017/S0033291709991966

Harrison, P.J. (2003) Genes for schizophrenia? Recent findings and their pathophysiological implications. *Lancet* 361: 417-419.

Henquet, C., Krabbendam, L., Spauwen, J., Kaplan, C., Lieb, R., Wittchen, H.U., and van Os, J. (2005) Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *BMJ* 330: 11. doi:10.1136/bmj.38267.664086.63

Henquet, C., Murray, R., Linszen, D., and van Os, J. (2005) The environment and schizophrenia: the role of cannabis use. *Schizophr Bull* 31: 608-612. doi:10.1093/schbul/sbi027

Henquet, C., Rosa, A., Delespaul, P., Papiol, S., Faňanás, L., van Os, J., and Myin-Germeys, I. (2009) COMT Val158Met moderation of cannabis-induced psychosis: a momentary assessment study of 'switching on' hallucinations in the flow of daily life. *Acta Psychiatrica Scandinavica* 119: 156-160. doi:10.1111/j.1600-0447.2008.01265.x

Henquet, C., Rosa, A., Krabbendam, L., Papiol, S., Fananás, L., Drukker, M., et al. (2006) An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology* 31: 2748-2757. doi:10.1038/sj.npp.1301197

Henquet, C., van Os, J., Kuepper, R., Delespaul, P., Smits, M., Campo, J.A., and Myin-Germeys, I. (2010) Psychosis reactivity to cannabis use in daily life: an experience sampling study. *Br J Psychiatry* 196: 447-453. doi:10.1192/bjp.bp.109.072249

Henry, D.J., and Chavkin, C. (1995) Activation of inwardly rectifying potassium channels (GIRK1) by co-expressed rat brain cannabinoid receptors in *Xenopus oocytes. Neurosci Lett* 186: 91-94.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., De Costa, B.R., and Rice, K.C. (1990) Cannabinoid receptor localization in brain. *Proceedings of the national Academy of sciences* 87: 1932-1936.

Herman, A.I., Jatlow, P.I., Gelernter, J., Listman, J.B., and Sofuoglu, M. (2013) COMT Val158Met modulates subjective responses to intravenous nicotine and cognitive performance in abstinent smokers. *Pharmacogenomics J* 13: 490-497. doi:10.1038/tpj.2013.1

Hernandez-Tristan, R., Arevalo, C., Canals, S., and Leret, M.L. (2000) The effects of acute treatment with Δ 9-THC on exploratory behaviour and memory in the rat. *Journal of physiology and biochemistry* 56: 17-24.

Hernaus, D., Collip, D., Lataster, J., Ceccarini, J., Kenis, G., Booij, L., et al. (2013) COMT Val158Met genotype selectively alters prefrontal [18F]fallypride displacement and subjective feelings of stress in response to a psychosocial stress challenge. *PLoS One* 8: e65662. doi:10.1371/journal.pone.0065662

Higgs, S., Barber, D.J., Cooper, A.J., and Terry, P. (2005) Differential effects of two cannabinoid receptor agonists on progressive ratio responding for food and free-feeding in rats. *Behavioural pharmacology* 16: 389-393.

Higgs, S., Williams, C.M., and Kirkham, T.C. (2003) Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after delta(9)-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. *Psychopharmacology (Berl)* 165: 370-377. doi:10.1007/s00213-002-1263-3

Higuera-Matas, A., Botreau, F., Miguéns, M., Del Olmo, N., Borcel, E., Pérez-Alvarez, L., et al. (2009) Chronic periadolescent cannabinoid treatment enhances adult hippocampal PSA-NCAM expression in male Wistar rats but only has marginal effects on anxiety, learning and memory. *Pharmacol Biochem Behav* 93: 482-490. doi:10.1016/j.pbb.2009.06.013

Hirose, Y., Tsutsui, T.W., Ohno, M., Barrett, J.C., and Tsutsui, T. (2007) Effects of a catechol-O-methyltransferase inhibitor on catechol estrogen-induced cellular transformation, chromosome aberrations and apoptosis in Syrian hamster embryo cells. *Int J Cancer* 120: 1627-1633. doi:10.1002/ijc.22398

Ho, B.C., Wassink, T.H., O'Leary, D.S., Sheffield, V.C., and Andreasen, N.C. (2005) Catechol-O-methyl transferase Val158Met gene polymorphism in schizophrenia: working memory, frontal lobe MRI morphology and frontal cerebral blood flow. *Mol Psychiatry* 10: 229, 287-229, 298. doi:10.1038/sj.mp.4001616

Hodos, W. (1961) Progressive ratio as a measure of reward strength. *Science* 134: 943-944.

Hong, J., Shu-Leong, H., Tao, X., and Lap-Ping, Y. (1998) Distribution of catechol-O-methyltransferase expression in human central nervous system. *Neuroreport* 9: 2861-2864.

Hooker, W.D., and Jones, R.T. (1987) Increased susceptibility to memory intrusions and the Stroop interference effect during acute marijuana intoxication. *Psychopharmacology (Berl)* 91: 20-24.

Houston, J.E., Murphy, J., Adamson, G., Stringer, M., and Shevlin, M. (2008) Childhood sexual abuse, early cannabis use, and psychosis: testing an interaction model based on the National Comorbidity Survey. *Schizophr Bull* 34: 580-585. doi:10.1093/schbul/sbm127

Howes, O.D., and Kapur, S. (2009) The dopamine hypothesis of schizophrenia: version III-the final common pathway. *Schizophr Bull* 35: 549-562. doi:10.1093/schbul/sbp006

Howes, O.D., Kambeitz, J., Kim, E., Stahl, D., Slifstein, M., Abi-Dargham, A., and Kapur, S. (2012) The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry* 69: 776-786. doi:10.1001/archgenpsychiatry.2012.169

Howlett, A.C., and Mukhopadhyay, S. (2000) Cellular signal transduction by anandamide and 2-arachidonoylglycerol. *Chem Phys Lipids* 108: 53-70.

Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., et al. (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological reviews* 54: 161-202.

Huotari, M., Gainetdinov, R., and Männistö, P.T. (1999) Microdialysis studies on the action of tolcapone on pharmacologically-elevated extracellular dopamine levels in conscious rats. *Pharmacol Toxicol* 85: 233-238.

Huotari, M., García-Horsman, J.A., Karayiorgou, M., Gogos, J.A., and Männistö, P.T. (2004) D-amphetamine responses in catechol-O-methyltransferase (COMT) disrupted mice. *Psychopharmacology (Berl)* 172: 1-10. doi:10.1007/s00213-003-1627-3

Huotari, M., Gogos, J.A., Karayiorgou, M., Koponen, O., Forsberg, M., Raasmaja, A., et al. (2002) Brain catecholamine metabolism in catechol-O-methyltransferase (COMT)-deficient mice. *Eur J Neurosci* 15: 246-256.

Huotari, M., Passlin, M., Nordberg, H.-L., Forsberg, M., Kotisaari, S., Tuomisto, L., et al. (2001) Effect of intracerebral 6-nitronoradrenaline, an endogenous catechol-O-methyltransferase (COMT) inhibitor, on striatal dopamine metabolism in anaesthetised rats. *Journal of neuroscience methods* 109: 47-52.

Huotari, M., Santha, M., Lucas, L.R., Karayiorgou, M., Gogos, J.A., and Männistö, P.T. (2002) Effect of dopamine uptake inhibition on brain catecholamine levels and locomotion in catechol-O-methyltransferase-disrupted mice. *J Pharmacol Exp Ther* 303: 1309-1316. doi:10.1124/jpet.102.043042

Ira, E., Santi, K.D., Lasalvia, A., Bonetto, C., Zanatta, G., Cristofalo, D., et al. (2014) Positive symptoms in first-episode psychosis patients experiencing low maternal care and stressful life events: a pilot study to explore the role of the COMT gene. *Stress* . doi:10.3109/10253890.2014.948841

Ishiwari, K., Weber, S.M., Mingote, S., Correa, M., and Salamone, J.D. (2004) Accumbens dopamine and the regulation of effort in food-seeking behavior: modulation of work output by different ratio or force requirements. *Behav Brain Res* 151: 83-91. doi:10.1016/j.bbr.2003.08.007

Izquierdo, A., Brigman, J.L., Radke, A.K., Rudebeck, P.H., and Holmes, A. (2017) The neural basis of reversal learning: An updated perspective. *Neuroscience* 345: 12-26. doi:10.1016/j.neuroscience.2016.03.021

Jarrett, M.M., Limebeer, C.L., and Parker, L.A. (2005) Effect of Delta9-tetrahydrocannabinol on sucrose palatability as measured by the taste reactivity test. *Physiol Behav* 86: 475-479. doi:10.1016/j.physbeh.2005.08.033

Jeffery, D.R., and Roth, J.A. (1984) Characterization of membrane-bound and soluble catechol-O-methyltransferase from human frontal cortex. *J Neurochem* 42: 826-832.

Jensen, J., Willeit, M., Zipursky, R.B., Savina, I., Smith, A.J., Menon, M., et al. (2008) The formation of abnormal associations in schizophrenia: neural and behavioral evidence. *Neuropsychopharmacology* 33: 473-479. doi:10.1038/sj.npp.1301437

Jentsch, J.D., Andrusiak, E., Tran, A., Bowers, M.B., and Roth, R.H. (1997) Delta 9-tetrahydrocannabinol increases prefrontal cortical catecholaminergic utilization and impairs spatial working memory in the rat: blockade of dopaminergic effects with HA966. *Neuropsychopharmacology* 16: 426-432. doi:10.1016/S0893-133X(97)00018-3

Jentsch, J.D., Verrico, C.D., Le, D., and Roth, R.H. (1998) Repeated exposure to delta 9-tetrahydrocannabinol reduces prefrontal cortical dopamine metabolism in the rat. *Neurosci Lett* 246: 169-172.

Jonker, J.W., and Schinkel, A.H. (2004) Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). *J Pharmacol Exp Ther* 308: 2-9. doi:10.1124/jpet.103.053298

Joober, R., Gauthier, J., Lal, S., Bloom, D., Lalonde, P., Rouleau, G., et al. (2002) Catechol-O-methyltransferase Val-108/158-Met gene variants associated with performance on the Wisconsin Card Sorting Test. *Archives of General Psychiatry* 59: 662-663.

Kaakkola, S., and Wurtman, R.J. (1992) Effects of COMT inhibitors on striatal dopamine metabolism: a microdialysis study. *Brain research* 587: 241-249.

Kalivas, P.W., and Duffy, P. (1995) Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. *Brain Res* 675: 325-328.

Kantrowitz, J.T., Nolan, K.A., Sen, S., Simen, A.A., Lachman, H.M., and Bowers, M.B. (2009) Adolescent cannabis use, psychosis and catechol-O-methyltransferase genotype in African Americans and Caucasians. *Psychiatr Q* 80: 213-218. doi:10.1007/s11126-009-9108-4

Kaplan, G.P., Hartman, B.K., and Creveling, C.R. (1979) Immunohistochemical demonstration of catechol-o-methyltransferase in mammalian brain. *Brain Res* 167: 241-250.

Kapur, S. (2003) Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. *Am J Psychiatry* 160: 13-23.

Kapur, S., Mizrahi, R., and Li, M. (2005) From dopamine to salience to psychosis--linking biology, pharmacology and phenomenology of psychosis. *Schizophr Res* 79: 59-68. doi:10.1016/j.schres.2005.01.003

Karayiorgou, M., Morris, M.A., Morrow, B., Shprintzen, R.J., Goldberg, R., Borrow, J., et al. (1995) Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. *Proc Natl Acad Sci U S A* 92: 7612-7616.

Karayiorgou, M., Simon, T.J., and Gogos, J.A. (2010) 22q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. *Nat Rev Neurosci* 11: 402-416. doi:10.1038/nrn2841

Karhunen, T., Tilgmann, C., Ulmanen, I., and Panula, P. (1995) Catechol-O-methyltransferase (COMT) in rat brain: immunoelectron microscopic study with an antiserum against rat recombinant COMT protein. *Neurosci Lett* 187: 57-60.

Karhunen, T., Tilgmann, C., Ulmanen, I., Julkunen, I., and Panula, P. (1994) Distribution of catechol-O-methyltransferase enzyme in rat tissues. *J Histochem Cytochem* 42: 1079-1090. doi:10.1177/42.8.8027527

Karlsson, P., Farde, L., Halldin, C., and Sedvall, G. (2002) PET study of D(1) dopamine receptor binding in neuroleptic-naive patients with schizophrenia. *Am J Psychiatry* 159: 761-767. doi:10.1176/appi.ajp.159.5.761

Karoum, F., Chrapusta, S.J., and Egan, M.F. (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. *J Neurochem* 63: 972-979.

Kastner, A., Anglade, P., Bounaix, C., Damier, P., Javoy-Agid, F., Bromet, N., et al. (1994) Immunohistochemical study of catechol-O-methyltransferase in the human mesostriatal system. *Neuroscience* 62: 449-457.

Katona, I., Sperlágh, B., Maglóczky, Z., Sántha, E., Köfalvi, A., Czirják, S., et al. (2000) GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neuroscience* 100: 797-804.

Katsidoni, V., Kastellakis, A., and Panagis, G. (2013) Biphasic effects of Δ9-tetrahydrocannabinol on brain stimulation reward and motor activity. *Int J Neuropsychopharmacol* 16: 2273-2284. doi:10.1017/S1461145713000709

Kaufmann, R.M., Kraft, B., Frey, R., Winkler, D., Weiszenbichler, S., Bäcker, C., et al. (2010) Acute psychotropic effects of oral cannabis extract with a defined content of Delta9-tetrahydrocannabinol (THC) in healthy volunteers. *Pharmacopsychiatry* 43: 24-32. doi:10.1055/s-0029-1237397

Käenmäki, M., Tammimäki, A., Garcia-Horsman, J.A., Myöhänen, T., Schendzielorz, N., Karayiorgou, M., et al. (2009) Importance of membrane-bound catechol-O-methyltransferase in L-DOPA metabolism: a pharmacokinetic study in two types of Comt gene modified mice. *Br J Pharmacol* 158: 1884-1894. doi:10.1111/j.1476-5381.2009.00494.x

Käenmäki, M., Tammimäki, A., Myöhänen, T., Pakarinen, K., Amberg, C., Karayiorgou, M., et al. (2010) Quantitative role of COMT in dopamine clearance in the prefrontal cortex of freely moving mice. *J Neurochem* 114: 1745-1755. doi:10.1111/j.1471-4159.2010.06889.x

Kehagia, A.A., Murray, G.K., and Robbins, T.W. (2010) Learning and cognitive flexibility: frontostriatal function and monoaminergic modulation. *Curr Opin Neurobiol* 20: 199-204. doi:10.1016/j.conb.2010.01.007

Kellendonk, C., Simpson, E.H., Polan, H.J., Malleret, G., Vronskaya, S., Winiger, V., et al. (2006) Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. *Neuron* 49: 603-615. doi:10.1016/j.neuron.2006.01.023

Kelm, M.K., and Boettiger, C.A. (2013) Effects of acute dopamine precusor depletion on immediate reward selection bias and working memory depend on catechol-O-methyltransferase genotype. *J Cogn Neurosci* 25: 2061-2071. doi:10.1162/jocn_a_00464

Kember, R.L., Fernandes, C., Tunbridge, E.M., Liu, L., Payá-Cano, J.L., Parsons, M.J., and Schalkwyk, L.C. (2010) A B2 SINE insertion in the Comt1 gene (Comt1(B2i)) results in an overexpressing, behavior modifying allele present in classical inbred mouse strains. *Genes Brain Behav* 9: 925-932. doi:10.1111/j.1601-183X.2010.00614.x

Konings, M., Stefanis, N., Kuepper, R., de Graaf, R., ten Have, M., van Os, J., et al. (2012) Replication in two independent population-based samples that childhood maltreatment and cannabis use synergistically impact on psychosis risk. *Psychol Med* 42: 149-159. doi:10.1017/S0033291711000973

Krugel, L.K., Biele, G., Mohr, P.N., Li, S.C., and Heekeren, H.R. (2009) Genetic variation in dopaminergic neuromodulation influences the ability to rapidly and flexibly adapt decisions. *Proc Natl Acad Sci U S A* 106: 17951-17956. doi:10.1073/pnas.0905191106

Krystal, J.H., D'Souza, D.C., Madonick, S., and Petrakis, I.L. (1999) Toward a rational pharmacotherapy of comorbid substance abuse in schizophrenic patients. *Schizophr Res* 35 Suppl: S35-S49.

Kuepper, R., Ceccarini, J., Lataster, J., van Os, J., van Kroonenburgh, M., van Gerven, J.M., et al. (2013) Delta-9-tetrahydrocannabinol-induced dopamine release as a function of psychosis risk: 18F-fallypride positron emission tomography study. *PLoS One* 8: e70378. doi:10.1371/journal.pone.0070378

Kuepper, R., Henquet, C., Lieb, R., Wittchen, H.-U., and van Os, J. (2011) Non-replication of interaction between cannabis use and trauma in predicting psychosis. *Schizophrenia research* 131: 262-263.

Kuepper, R., van Os, J., Lieb, R., Wittchen, H.U., Höfler, M., and Henquet, C. (2011) Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. *BMJ* 342: d738.

Laatikainen, L., Sharp, T., Bannerman, D., Harrison, P., and Tunbridge, E. (2012) Modulation of hippocampal dopamine metabolism and hippocampal-dependent cognitive function by catechol-O-methyltransferase inhibition. *J Psychopharmacol* . doi:10.1177/0269881112454228

Laatikainen, L.M., Sharp, T., Harrison, P.J., and Tunbridge, E.M. (2013) Sexually dimorphic effects of catechol-O-methyltransferase (COMT) inhibition on dopamine metabolism in multiple brain regions. *PLoS One* 8: e61839. doi:10.1371/journal.pone.0061839

Lachman, H.M., Papolos, D.F., Saito, T., Yu, Y.-M., Szumlanski, C.L., and Weinshilboum, R.M. (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics and Genomics* 6: 243.

Lancaster, T.M., Heerey, E.A., Mantripragada, K., and Linden, D.E.J. (2015) Replication study implicates COMT val158met polymorphism as a modulator of probabilistic reward learning . *Genes, Brain and Behavior* 14: 486-492. doi:10.1111/gbb.12228

Lapish, C.C., Ahn, S., Evangelista, L.M., So, K., Seamans, J.K., and Phillips, A.G. (2009) Tolcapone enhances food-evoked dopamine efflux and executive memory processes mediated by the rat prefrontal cortex. *Psychopharmacology (Berl)* 202: 521-530. doi:10.1007/s00213-008-1342-1

Large, M., Sharma, S., Compton, M.T., Slade, T., and Nielssen, O. (2011) Cannabis use and earlier onset of psychosis: a systematic meta-analysis. *Arch Gen Psychiatry* 68: 555-561. doi:10.1001/archgenpsychiatry.2011.5

Laruelle, M., Abi-Dargham, A., Dyck, C.H.V., Gil, R., D'Souza, C.D., Erdos, J., et al. (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proceedings of the National Academy of Sciences* 93: 9235-9240.

Learmonth, D.A., Palma, P.N., Vieira-Coelho, M.A., and Soares-da-Silva, P. (2004) Synthesis, biological evaluation, and molecular modeling studies of a novel, peripherally selective inhibitor of catechol-O-methyltransferase. *J Med Chem* 47: 6207-6217. doi:10.1021/jm040848o

Lecca, S., Melis, M., Luchicchi, A., Muntoni, A.L., and Pistis, M. (2012) Inhibitory inputs from rostromedial tegmental neurons regulate spontaneous activity of midbrain dopamine cells and their responses to drugs of abuse. *Neuropsychopharmacology* 37: 1164-1176. doi:10.1038/npp.2011.302

Lee, B., Groman, S., London, E.D., and Jentsch, J.D. (2007) Dopamine D2/D3 receptors play a specific role in the reversal of a learned visual discrimination in monkeys. *Neuropsychopharmacology* 32: 2125-2134. doi:10.1038/sj.npp.1301337

Lewis, C.M., Levinson, D.F., Wise, L.H., DeLisi, L.E., Straub, R.E., Hovatta, I., et al. (2003) Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73: 34-48. doi:10.1086/376549

Li, Y.H., Wirth, T., Huotari, M., Laitinen, K., MacDonald, E., and Männistö, P.T. (1998) No change of brain extracellular catecholamine levels after acute catechol-O-methyltransferase inhibition: a microdialysis study in anaesthetized rats. *Eur J Pharmacol* 356: 127-137.

Liem-Moolenaar, M., te Beek, E.T., de Kam, M.L., Franson, K.L., Kahn, R.S., Hijman, R., et al. (2010) Central nervous system effects of haloperidol on THC in healthy male volunteers. *J Psychopharmacol* 24: 1697-1708. doi:10.1177/0269881109358200

Linszen, D.H., Dingemans, P.M., and Lenior, M.E. (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. *Arch Gen Psychiatry* 51: 273-279.

Long, L.E., Chesworth, R., Huang, X.F., McGregor, I.S., Arnold, J.C., and Karl, T. (2010) A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *Int J Neuropsychopharmacol* 13: 861-876. doi:10.1017/S1461145709990605

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melén, K., Julkunen, I., and Taskinen, J. (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: 4202-4210.

De Luca, M.A., Solinas, M., Bimpisidis, Z., Goldberg, S.R., and Di Chiara, G. (2012) Cannabinoid facilitation of behavioral and biochemical hedonic taste responses. *Neuropharmacology* 63: 161-168.

Lundqvist, T. (2005) Cognitive consequences of cannabis use: comparison with abuse of stimulants and heroin with regard to attention, memory and executive functions. *Pharmacol Biochem Behav* 81: 319-330. doi:10.1016/j.pbb.2005.02.017

Lundström, K., Salminen, M., Jalanko, A., Savolainen, R., and Ulmanen, I. (1991) Cloning and characterization of human placental catechol-O-methyltransferase cDNA. *DNA Cell Biol* 10: 181-189.

Lundström, K., Tenhunen, J., Tilgmann, C., Karhunen, T., Panula, P., and Ulmanen, I. (1995) Cloning, expression and structure of catechol-O-methyltransferase. *Biochim Biophys Acta* 1251: 1-10.

Lupica, C.R., and Riegel, A.C. (2005) Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. *Neuropharmacology* 48: 1105-1116. doi:10.1016/j.neuropharm.2005.03.016

Maccioni, P., Pes, D., Carai, M.A., Gessa, G.L., and Colombo, G. (2008) Suppression by the cannabinoid CB1 receptor antagonist, rimonabant, of the reinforcing and motivational properties of a chocolate-flavoured beverage in rats. *Behav Pharmacol* 19: 197-209. doi:10.1097/FBP.0b013e3282fe8888

MacLean, K.I., and Littleton, J.M. (1977) Environmental stress as a factor in the response of rat brain catecholamine metabolism to delta8-tetrahydrocannabinol. *European journal of pharmacology* 41: 171-182. doi:10.1016/0014-2999(77)90206-0

Macleod, J., Oakes, R., Copello, A., Crome, I., Egger, M., Hickman, M., et al. (2004) Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. *Lancet* 363: 1579-1588. doi:10.1016/S0140-6736(04)16200-4

Mahler, S.V., Smith, K.S., and Berridge, K.C. (2007) Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. *Neuropsychopharmacology* 32: 2267-2278. doi:10.1038/sj.npp.1301376

Maj, J., Rogóż, Z., Skuza, G., Sowińska, H., and Superata, J. (1990) Behavioural and neurochemical effects of Ro 40-7592, a new COMT inhibitor with a potential therapeutic activity in Parkinson's disease. *Journal of Neural Transmission-Parkinson's Disease and Dementia Section* 2: 101-112. doi:10.1007/BF02260898

Malcher-Lopes, R., Di, S., Marcheselli, V.S., Weng, F.-J., Stuart, C.T., Bazan, N.G., and Tasker, J.G. (2006) Opposing Crosstalk between Leptin and Glucocorticoids Rapidly Modulates Synaptic Excitation via Endocannabinoid Release. *Journal of Neuroscience* 26: 6643-6650. doi:10.1523/JNEUROSCI.5126-05.2006

Maldonado, R., Berrendero, F., Ozaita, A., and Robledo, P. (2011) Neurochemical basis of cannabis addiction. *Neuroscience* 181: 1-17. doi:10.1016/j.neuroscience.2011.02.035

Malhotra, A.K., Kestler, L.J., Mazzanti, C., Bates, J.A., Goldberg, T., and Goldman, D. (2002) A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. *Am J Psychiatry* 159: 652-654.

Malone, D.T., and Taylor, D.A. (1999) Modulation by fluoxetine of striatal dopamine release following Delta9-tetrahydrocannabinol: a microdialysis study in conscious rats. *Br J Pharmacol* 128: 21-26. doi:10.1038/sj.bjp.0702753

Malone, D.T., and Taylor, D.A. (2006) The effect of Delta9-tetrahydrocannabinol on sensorimotor gating in socially isolated rats. *Behav Brain Res* 166: 101-109. doi:10.1016/j.bbr.2005.07.009

Mansbach, R.S., Geyer, M.A., and Braff, D.L. (1988) Dopaminergic stimulation disrupts sensorimotor gating in the rat. *Psychopharmacology (Berl)* 94: 507-514.

Marconi, A., Di Forti, M., Lewis, C.M., Murray, R.M., and Vassos, E. (2016) Meta-analysis of the Association Between the Level of Cannabis Use and Risk of Psychosis. *Schizophr Bull* 42: 1262-1269. doi:10.1093/schbul/sbw003

Marks, D.F., and MacAvoy, M.G. (1989) Divided attention performance in cannabis users and non-users following alcohol and cannabis separately and in combination. *Psychopharmacology (Berl)* 99: 397-401.

Marsala, S.Z., Gioulis, M., Ceravolo, R., and Tinazzi, M. (2012) A Systematic Review of Catechol-O-Methyltransferase Inhibitors: Efficacy and Safety in Clinical Practice. *Clinical Neuropharmacology* 35: 185. doi:10.1097/WNF.0b013e31825c034a

Martel, F., and Azevedo, I. (2003) An Update on the Extraneuronal Monoamine Transporter (EMT): Characteristics, Distribution and Regulation. *Current Drug Metabolism* 4: 313-318. doi:10.2174/1389200033489433

Martin, R.S., Secchi, R.L., Sung, E., Lemaire, M., Bonhaus, D.W., Hedley, L.R., and Lowe, D.A. Effects of cannabinoid receptor ligands on psychosis-relevant behavior models in the rat. *Psychopharmacology* 165: 128-135. doi:10.1007/s00213-002-1240-x

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., and Bonner, T.I. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346: 561-564.

Matsumoto, M., and Hikosaka, O. (2009) Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature* 459: 837-841. doi:10.1038/nature08028

Matsumoto, M., Weickert, C.S., Akil, M., Lipska, B.K., Hyde, T.M., Herman, M.M., et al. (2003) Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. *Neuroscience* 116: 127-137.

Mattay, V.S., Goldberg, T.E., Fera, F., Hariri, A.R., Tessitore, A., Egan, M.F., et al. (2003) Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc Natl Acad Sci U S A* 100: 6186-6191. doi:10.1073/pnas.0931309100

Mátyás, F., Urbán, G.M., Watanabe, M., Mackie, K., Zimmer, A., Freund, T.F., and Katona, I. (2008) Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. *Neuropharmacology* 54: 95-107. doi:10.1016/j.neuropharm.2007.05.028

Männistö, P.T., and Kaakkola, S. (1999) Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacological reviews* 51: 593.

Männistö, P.T., Tuomainen, P., and Tuominen, R.K. (1992) Different in vivo properties of three new inhibitors of catechol O-methyltransferase in the rat. *Br J Pharmacol* 105: 569-574.

McDonald, C., and Murray, R.M. (2000) Early and late environmental risk factors for schizophrenia. *Brain Res Brain Res Rev* 31: 130-137.

McGrath, J., Saha, S., Welham, J., Saadi, O.E., MacCauley, C., and Chant, D. (2004) A systematic review of the incidence of schizophrenia: the distribution of rates and the influence of sex, urbanicity, migrant status and methodology. *BMC Medicine* 2: 13. doi:10.1186/1741-7015-2-13

McGrath, J., Welham, J., Scott, J., Varghese, D., Degenhardt, L., Hayatbakhsh, M.R., et al. (2010) Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults. *Arch Gen Psychiatry* 67: 440-447. doi:10.1001/archgenpsychiatry.2010.6

McLaughlin, P.J., Winston, K., Swezey, L., Wisniecki, A., Aberman, J., Tardif, D.J., et al. (2003) The cannabinoid CB1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. *Behav Pharmacol* 14: 583-588. doi:10.1097/01.fbp.0000104882.69384.aa

Mechoulam, R., and Parker, L.A. (2013) The endocannabinoid system and the brain. *Annu Rev Psychol* 64: 21-47. doi:10.1146/annurev-psych-113011-143739

Mehta, M.A., Manes, F.F., Magnolfi, G., Sahakian, B.J., and Robbins, T.W. (2004) Impaired set-shifting and dissociable effects on tests of spatial working memory following the dopamine D2 receptor antagonist sulpiride in human volunteers. *Psychopharmacology (Berl)* 176: 331-342. doi:10.1007/s00213-004-1899-2

Melis, M., Perra, S., Muntoni, A.L., Pillolla, G., Lutz, B., Marsicano, G., et al. (2004) Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. *J Neurosci* 24: 10707-10715. doi:10.1523/JNEUROSCI.3502-04.2004

De Melo, L.C.S., Cruz, A.P., Valentim, S.J.R., Marinho, A.R., Mendonça, J.B., and Nakamura-Palacios, E.M. (2005) Δ9-THC administered into the medial prefrontal cortex disrupts the spatial working memory. *Psychopharmacology* 183: 54-64.

Meloto, C.B., Segall, S.K., Smith, S., Parisien, M., Shabalina, S.A., Rizzatti-Barbosa, C.M., et al. (2015) COMT gene locus: new functional variants. *Pain* 156: 2072-2083. doi:10.1097/j.pain.0000000000000273

Mier, D., Kirsch, P., and Meyer-Lindenberg, A. (2010) Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Mol Psychiatry* 15: 918-927. doi:10.1038/mp.2009.36

Miller, B., Suvisaari, J., Miettunen, J., Järvelin, M.R., Haukka, J., Tanskanen, A., et al. (2011) Advanced paternal age and parental history of schizophrenia. *Schizophr Res* 133: 125-132. doi:10.1016/j.schres.2011.08.013

Miller, L.L., McFarland, D., Cornett, T.L., and Brightwell, D. (1977) Marijuana and memory impairment: effect on free recall and recognition memory. *Pharmacol Biochem Behav* 7: 99-103.

Moghaddam, B., and Javitt, D. (2012) From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* 37: 4-15. doi:10.1038/npp.2011.181

Monory, K., Blaudzun, H., Massa, F., Kaiser, N., Lemberger, T., Schütz, G., et al. (2007) Genetic dissection of behavioural and autonomic effects of Delta(9)-tetrahydrocannabinol in mice. *PLoS Biol* 5: e269. doi:10.1371/journal.pbio.0050269

Moore, T.H.M., Zammit, S., Lingford-Hughes, A., Barnes, T.R.E., Jones, P.B., Burke, M., and Lewis, G. (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *The Lancet* 370: 319-328. doi:10.1016/S0140-6736

Moreira, F.A., and Wotjak, C.T. (2009) *Behavioral neurobiology of anxiety and its treatment.* Springer.

Morgan, C.J., Freeman, T.P., Powell, J., and Curran, H.V. (2016) AKT1 genotype moderates the acute psychotomimetic effects of naturalistically smoked cannabis in young cannabis smokers. *Transl Psychiatry* 6: e738. doi:10.1038/tp.2015.219

Morón, J.A., Brockington, A., Wise, R.A., Rocha, B.A., and Hope, B.T. (2002) Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. *The Journal of neuroscience* 22: 389-395.

Morrison, P.D., and Stone, J.M. (2011) Synthetic delta-9-tetrahydrocannabinol elicits schizophrenia-like negative symptoms which are distinct from sedation. *Hum Psychopharmacol* 26: 77-80. doi:10.1002/hup.1166

Morrison, P.D., Zois, V., McKeown, D.A., Lee, T.D., Holt, D.W., Powell, J.F., et al. (2009) The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychol Med* 39: 1607-1616. doi:10.1017/S0033291709005522

Munafò, M.R., Bowes, L., Clark, T.G., and Flint, J. (2005) Lack of association of the COMT (Val158/108 Met) gene and schizophrenia: a meta-analysis of case-control studies. *Mol Psychiatry* 10: 765-770. doi:10.1038/sj.mp.4001664

Munro, S., Thomas, K.L., and Abu-Shaar, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365: 61-65. doi:10.1038/365061a0

Murphy, K.C., Jones, L.A., and Owen, M.J. (1999) High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch Gen Psychiatry* 56: 940-945.

Murray, G.K., Clark, L., Corlett, P.R., Blackwell, A.D., Cools, R., Jones, P.B., et al. (2008) Incentive motivation in first-episode psychosis: a behavioural study. *BMC Psychiatry* 8: 34. doi:10.1186/1471-244X-8-34

Murray, G.K., Corlett, P.R., Clark, L., Pessiglione, M., Blackwell, A.D., Honey, G., et al. (2008) Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Mol Psychiatry* 13: 239, 267-239, 276. doi:10.1038/sj.mp.4002058

Murray, R.M., and Lewis, S.W. (1987) Is schizophrenia a neurodevelopmental disorder? *Br Med J (Clin Res Ed)* 295: 681-682.

Myöhänen, T.T., and Männistö, P.T. (2010) Distribution and functions of catechol-O-methyltransferase proteins: do recent findings change the picture? *Int Rev Neurobiol* 95: 29-47. doi:10.1016/B978-0-12-381326-8.00003-X

Myöhänen, T.T., Schendzielorz, N., and Männistö, P.T. (2010) Distribution of catechol-O-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. *J Neurochem* 113: 1632-1643. doi:10.1111/j.1471-4159.2010.06723.x

Nackley, A.G., Shabalina, S.A., Tchivileva, I.E., Satterfield, K., Korchynskyi, O., Makarov, S.S., et al. (2006) Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* 314: 1930-1933.

Nagai, H., Egashira, N., Sano, K., Ogata, A., Mizuki, A., Mishima, K., et al. (2006) Antipsychotics improve Delta9-tetrahydrocannabinol-induced impairment of the prepulse inhibition of the startle reflex in mice. *Pharmacol Biochem Behav* 84: 330-336. doi:10.1016/j.pbb.2006.05.018

Nakazawa, K., Zsiros, V., Jiang, Z., Nakao, K., Kolata, S., Zhang, S., and Belforte, J.E. (2012) GABAergic interneuron origin of schizophrenia pathophysiology. *Neuropharmacology* 62: 1574-1583. doi:10.1016/j.neuropharm.2011.01.022

Napolitano, A., Bellini, G., Borroni, E., Zürcher, G., and Bonuccelli, U. (2003) Effects of peripheral and central catechol-O-methyltransferase inhibition on striatal extracellular levels of dopamine: a microdialysis study in freely moving rats. *Parkinsonism Relat Disord* 9: 145-150.

Napolitano, A., Zürcher, G., and Da Prada, M. (1995) Effects of tolcapone, a novel catechol-O-methyltransferase inhibitor, on striatal metabolism of L-dopa and dopamine in rats. *Eur J Pharmacol* 273: 215-221.

Ng Cheong Ton, J.M., Gerhardt, G.A., Friedemann, M., Etgen, A.M., Rose, G.M., Sharpless, N.S., and Gardner, E.L. (1988) The effects of delta 9-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: an in vivo electrochemical and in vivo microdialysis study. *Brain Res* 451: 59-68.

Nicodemus, K.K., Kolachana, B.S., Vakkalanka, R., Straub, R.E., Giegling, I., Egan, M.F., et al. (2007) Evidence for statistical epistasis between catechol-O-methyltransferase (COMT) and polymorphisms in RGS4, G72 (DAOA), GRM3, and DISC1: influence on risk of schizophrenia. *Hum Genet* 120: 889-906. doi:10.1007/s00439-006-0257-3

Nicodemus, K.K., Marenco, S., Batten, A.J., Vakkalanka, R., Egan, M.F., Straub, R.E., and Weinberger, D.R. (2008) Serious obstetric complications interact with hypoxia-regulated/vascular-expression genes to influence schizophrenia risk. *Mol Psychiatry* 13: 873-877. doi:10.1038/sj.mp.4002153

Nikolova, Y.S., Ferrell, R.E., Manuck, S.B., and Hariri, A.R. (2011) Multilocus Genetic Profile for Dopamine Signaling Predicts Ventral Striatum Reactivity. *Neuropsychopharmacology* 36: 1940-1947. doi:10.1038/npp.2011.82

Nolan, K.A., Bilder, R.M., Lachman, H.M., and Volavka, J. (2004) Catechol O-methyltransferase Val158Met polymorphism in schizophrenia: differential effects of Val and Met alleles on cognitive stability and flexibility. *Am J Psychiatry* 161: 359-361.

Ohmori, O., Shinkai, T., Kojima, H., Terao, T., Suzuki, T., Mita, T., and Abe, K. (1998) Association study of a functional catechol-O-methyltransferase gene polymorphism in Japanese schizophrenics. *Neurosci Lett* 243: 109-112.

Okochi, T., Ikeda, M., Kishi, T., Kawashima, K., Kinoshita, Y., Kitajima, T., et al. (2009) Meta-analysis of association between genetic variants in COMT and schizophrenia: an update. *Schizophr Res* 110: 140-148. doi:10.1016/j.schres.2009.02.019

Okubo, Y., Suhara, T., Suzuki, K., Kobayashi, K., Inoue, O., Terasaki, O., et al. (1997) Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 385: 634-636. doi:10.1038/385634a0

Olarte-Sánchez, C.M., Valencia-Torres, L., Cassaday, H.J., Bradshaw, C.M., and Szabadi, E. (2015) Quantitative analysis of performance on a progressive-ratio schedule: effects of reinforcer type, food deprivation and acute treatment with Δ^9-tetrahydrocannabinol (THC). *Behav Processes* 113: 122-131. doi:10.1016/j.beproc.2015.01.014

Olson, E.N., Arnold, H.-H., Rigby, P.W.J., and Wold, B.J. (1996) Know your neighbors: three phenotypes in null mutants of the myogenic bHLH gene MRF4. *Cell* 85: 1-4.

O'Tuathaigh, C.M., Clarke, G., Walsh, J., Desbonnet, L., Petit, E., O'Leary, C., et al. (2011) Genetic vs. pharmacological inactivation of COMT influences cannabinoid-induced expression of schizophrenia-related phenotypes. *Int J Neuropsychopharmacol* : 1-12. doi:10.1017/S1461145711001581

O'Tuathaigh, C.M., Hryniewiecka, M., Behan, A., Tighe, O., Coughlan, C., Desbonnet, L., et al. (2010) Chronic Adolescent Exposure to Delta-9-Tetrahydrocannabinol in COMT Mutant Mice: Impact on Psychosis-Related and Other Phenotypes. *Neuropsychopharmacology* . doi:10.1038/npp.2010.100

Ovaska, M., and Yliniemelä, A. (1998) A semiempirical study on inhibition of catechol O-methyltransferase by substituted catechols. *J Comput Aided Mol Des* 12: 301-307.

Owen, M.J., O'Donovan, M.C., Thapar, A., and Craddock, N. (2011) Neurodevelopmental hypothesis of schizophrenia. *Br J Psychiatry* 198: 173-175. doi:10.1192/bjp.bp.110.084384

Palmatier, M.A., Kang, A.M., and Kidd, K.K. (1999) Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles. *Biol Psychiatry* 46: 557-567.

Papaleo, F., Crawley, J.N., Song, J., Lipska, B.K., Pickel, J., Weinberger, D.R., and Chen, J. (2008) Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice. *J Neurosci* 28: 8709-8723. doi:10.1523/JNEUROSCI.2077-08.2008

Papaleo, F., Erickson, L., Liu, G., Chen, J., and Weinberger, D.R. (2012) Effects of sex and COMT genotype on environmentally modulated cognitive control in mice. *Proc Natl Acad Sci U S A* 109: 20160-20165. doi:10.1073/pnas.1214397109

Paterlini, M., Zakharenko, S.S., Lai, W.S., Qin, J., Zhang, H., Mukai, J., et al. (2005) Transcriptional and behavioral interaction between 22q11.2 orthologs modulates schizophrenia-related phenotypes in mice. *Nat Neurosci* 8: 1586-1594. doi:10.1038/nn1562

Paxinos, G., and Franklin, K.B.J. (2004) *The mouse brain in stereotaxic coordinates.* Amsterdam ; Boston: Elsevier Academic Press.

Peciña, S., Cagniard, B., Berridge, K.C., Aldridge, J.W., and Zhuang, X. (2003) Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. *J Neurosci* 23: 9395-9402.

Pelayo-Terán, J.M., Pérez-Iglesias, R., Mata, I., Carrasco-Marín, E., Vázquez-Barquero, J.L., and Crespo-Facorro, B. (2010) Catechol-O-Methyltransferase (COMT) Val158Met variations and cannabis use in first-episode non-affective psychosis: clinical-onset implications. *Psychiatry Res* 179: 291-296. doi:10.1016/j.psychres.2009.08.022

Perrin, M.C., Brown, A.S., and Malaspina, D. (2007) Aberrant Epigenetic Regulation Could Explain the Relationship of Paternal Age to Schizophrenia. *Schizophrenia Bulletin* 33: 1270-1273. doi:10.1093/schbul/sbm093

Pertwee, R.G. (2006) The pharmacology of cannabinoid receptors and their ligands: an overview. *Int J Obes (Lond)* 30 Suppl 1: S13-S18. doi:10.1038/sj.ijo.0803272

Pertwee, R.G. (2010) Receptors and channels targeted by synthetic cannabinoid receptor agonists and antagonists. *Curr Med Chem* 17: 1360-1381.

Pistis, M., Ferraro, L., Pira, L., Flore, G., Tanganelli, S., Gessa, G.L., and Devoto, P. (2002) Delta(9)-tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study. *Brain Res* 948: 155-158.

Powell, S.B., Zhou, X., and Geyer, M.A. (2009) Prepulse inhibition and genetic mouse models of schizophrenia. *Behav Brain Res* 204: 282-294. doi:10.1016/j.bbr.2009.04.021

Pycock, .J., Kerwin, .W., and Carter, .J. (1980) Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. *Nature* 286: 74-77. doi:10.1038/286074a0

Qin, S., Cousijn, H., Rijpkema, M., Luo, J., Franke, B., Hermans, E.J., and Fernández, G. (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. *Front Integr Neurosci* 6: 16. doi:10.3389/fnint.2012.00016

Quednow, B.B., Schmechtig, A., Ettinger, U., Petrovsky, N., Collier, D.A., Vollenweider, F.X., et al. (2009) Sensorimotor gating depends on polymorphisms of the serotonin-2A receptor and catechol-O-methyltransferase, but not on neuregulin-1 Arg38Gln genotype: a replication study. *Biol Psychiatry* 66: 614-620. doi:10.1016/j.biopsych.2009.05.007

Quinn, H.R., Matsumoto, I., Callaghan, P.D., Long, L.E., Arnold, J.C., Gunasekaran, N., et al. (2008) Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology* 33: 1113-1126. doi:10.1038/sj.npp.1301475

Radhakrishnan, R., Wilkinson, S.T., and D'Souza, D.C. (2014) Gone to Pot - A Review of the Association between Cannabis and Psychosis. *Front Psychiatry* 5: 54. doi:10.3389/fpsyt.2014.00054

Randall, P.A., Lee, C.A., Nunes, E.J., Yohn, S.E., Nowak, V., Khan, B., et al. (2014) The VMAT-2 inhibitor tetrabenazine affects effort-related decision making in a progressive ratio/chow feeding choice task: reversal with antidepressant drugs. *PLoS One* 9: e99320. doi:10.1371/journal.pone.0099320

Ranganathan, M., and D'Souza, D.C. (2006) The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl)* 188: 425-444. doi:10.1007/s00213-006-0508-y

Raxworthy, M.J., Gulliver, P.A., and Hughes, P.J. (1982) The cellular location of catechol-O-methyltransferase in rat liver. *Naunyn Schmiedebergs Arch Pharmacol* 320: 182-188.

Realini, N., Vigano', D., Guidali, C., Zamberletti, E., Rubino, T., and Parolaro, D. (2011) Chronic URB597 treatment at adulthood reverted most depressive-like symptoms induced by adolescent exposure to THC in female rats. *Neuropharmacology* 60: 235-243. doi:10.1016/j.neuropharm.2010.09.003

Reenilä, I., Tuomainen, P., Soinila, S., and Männistö, P.T. (1997) Increase of catechol-O-methyltransferase activity in rat brain microglia after intrastriatal infusion of fluorocitrate, a glial toxin. *Neurosci Lett* 230: 155-158.

Richardson, N.R., and Roberts, D.C. (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Methods* 66: 1-11.

Riegel, A.C., and Lupica, C.R. (2004) Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. *J Neurosci* 24: 11070-11078. doi:10.1523/JNEUROSCI.3695-04.2004

Risbrough, V., Ji, B., Hauger, R., and Zhou, X. (2014) Generation and characterization of humanized mice carrying COMT158 Met/Val alleles. *Neuropsychopharmacology* 39: 1823-1832. doi:10.1038/npp.2014.29

Rivett, A.J., Francis, A., and Roth, J.A. (1983) Distinct cellular localization of membrane-bound and soluble forms of catechol-O-methyltransferase in brain. *J Neurochem* 40: 215-219.

Roberts, A.C., De Salvia, M.A., Wilkinson, L.S., Collins, P., Muir, J.L., Everitt, B.J., and Robbins, T.W. (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. *J Neurosci* 14: 2531-2544.

Robinson, T.E., and Berridge, K.C. (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* 18: 247-291.

Robledo, P., Trigo, J.M., Panayi, F., de la Torre, R., and Maldonado, R. (2007) Behavioural and neurochemical effects of combined MDMA and THC administration in mice. *Psychopharmacology (Berl)* 195: 255-264. doi:10.1007/s00213-007-0879-8

Roesch, M.R., and Olson, C.R. (2007) Neuronal activity related to anticipated reward in frontal cortex. *Annals of the New York Academy of Sciences* 1121: 431-446.

Roiser, J.P., Stephan, K.E., den Ouden, H.E., Barnes, T.R., Friston, K.J., and Joyce, E.M. (2009) Do patients with schizophrenia exhibit aberrant salience? *Psychol Med* 39: 199-209. doi:10.1017/S0033291708003863

Rosa, A., Peralta, V., Cuesta, M.J., Zarzuela, A., Serrano, F., Martínez-Larrea, A., and Fañanás, L. (2004) New evidence of association between COMT gene and prefrontal neurocognitive function in healthy individuals from sibling pairs discordant for psychosis. *Am J Psychiatry* 161: 1110-1112.

Rosa, E.C., Dickinson, D., Apud, J., Weinberger, D.R., and Elvevåg, B. (2010) COMT Val158Met polymorphism, cognitive stability and cognitive flexibility: an experimental examination. *Behav Brain Funct* 6: 53. doi:10.1186/1744-9081-6-53

Roth, J.A. (1992) Membrane-bound catechol-O-methyltransferase: a reevaluation of its role in the O-methylation of the catecholamine neurotransmitters. *Rev Physiol Biochem Pharmacol* 120: 1-29.

Roussos, P., Giakoumaki, S.G., and Bitsios, P. (2009) Tolcapone effects on gating, working memory, and mood interact with the synonymous catechol-O-methyltransferase rs4818c/g polymorphism. *Biol Psychiatry* 66: 997-1004. doi:10.1016/j.biopsych.2009.07.008

Roussos, P., Giakoumaki, S.G., Rogdaki, M., Pavlakis, S., Frangou, S., Bitsios, P., et al. (2008) Prepulse inhibition of the startle reflex depends on the catechol *O*-methyltransferase Val158Met gene polymorphism. *Psychological Medicine* 38: 1651-1658. doi:10.1017/S0033291708002912

Rubino, T., Realini, N., Braida, D., Guidi, S., Capurro, V., Viganò, D., et al. (2009) Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus* 19: 763-772. doi:10.1002/hipo.20554

Saddoris, M.P., Cacciapaglia, F., Wightman, R.M., and Carelli, R.M. (2015) Differential Dopamine Release Dynamics in the Nucleus Accumbens Core and Shell Reveal Complementary Signals for Error Prediction and Incentive Motivation. *J Neurosci* 35: 11572-11582. doi:10.1523/JNEUROSCI.2344-15.2015

Salamone, J.D., Correa, M., Farrar, A., and Mingote, S.M. (2007) Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology (Berl)* 191: 461-482. doi:10.1007/s00213-006-0668-9

Salamone, J.D., Wisniecki, A., Carlson, B.B., and Correa, M. (2001) Nucleus accumbens dopamine depletions make animals highly sensitive to high fixed ratio requirements but do not impair primary food reinforcement. *Neuroscience* 105: 863-870.

Sanchis-Segura, C., Cline, B.H., Marsicano, G., Lutz, B., and Spanagel, R. (2004) Reduced sensitivity to reward in CB1 knockout mice. *Psychopharmacology (Berl)* 176: 223-232. doi:10.1007/s00213-004-1877-8

Sañudo-Peña, M.C., Romero, J., Seale, G.E., Fernandez-Ruiz, J.J., and Walker, J.M. (2000) Activational role of cannabinoids on movement. *Eur J Pharmacol* 391: 269-274.

Satoh, T., Nakai, S., Sato, T., and Kimura, M. (2003) Correlated coding of motivation and outcome of decision by dopamine neurons. *Journal of neuroscience* 23: 9913-9923.

Sawaguchi, T. (2000) The role of D1-dopamine receptors in working memory-guided movements mediated by frontal cortical areas. *Parkinsonism Relat Disord* 7: 9-19.

Sawaguchi, T., and Goldman-Rakic, P.S. (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* 251: 947-950.

Sazci, A., Ergul, E., Kucukali, I., Kilic, G., Kaya, G., and Kara, I. (2004) Catechol-O-methyltransferase gene Val108/158Met polymorphism, and susceptibility to schizophrenia: association is more significant in women. *Brain Res Mol Brain Res* 132: 51-56. doi:10.1016/j.molbrainres.2004.09.005

Scheggia, D., Bebensee, A., Weinberger, D.R., and Papaleo, F. (2013) The Ultimate Intra-/Extra-dimensional Attentional Set-Shifting Task for Mice. *Biological Psychiatry* : -. doi:10.1016/j.biopsych.2013.05.021

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511: 421-427. doi:10.1038/nature13595

Schmack, K., Schlagenhauf, F., Sterzer, P., Wrase, J., Beck, A., Dembler, T., et al. (2008) Catechol-O-methyltransferase val158met genotype influences neural processing of reward anticipation. *Neuroimage* 42: 1631-1638. doi:10.1016/j.neuroimage.2008.06.019

Schmidt-Kastner, R., van Os, J., W M Steinbusch, H., and Schmitz, C. (2006) Gene regulation by hypoxia and the neurodevelopmental origin of schizophrenia. *Schizophr Res* 84: 253-271. doi:10.1016/j.schres.2006.02.022

Schneider, M., and Koch, M. (2002) The cannabinoid agonist WIN 55,212-2 reduces sensorimotor gating and recognition memory in rats. *Behavioural pharmacology* 13: 29-37.

Schneider, M., and Koch, M. (2003) Chronic Pubertal, but not Adult Chronic Cannabinoid Treatment Impairs Sensorimotor Gating, Recognition Memory, and the Performance in a Progressive Ratio Task in Adult Rats. *Neuropsychopharmacology* . doi:10.1038/sj.npp.1300225

Schoeler, T., Petros, N., Di Forti, M., Klamerus, E., Foglia, E., Ajnakina, O., et al. (2016) Effects of continuation, frequency, and type of cannabis use on relapse in the first 2 years after onset of psychosis: an observational study. *Lancet Psychiatry* 3: 947-953. doi:10.1016/S2215-0366(16)30188-2

Schoeler, T., Petros, N., Di Forti, M., Pingault, J.B., Klamerus, E., Foglia, E., et al. (2016) Association Between Continued Cannabis Use and Risk of Relapse in First-Episode Psychosis: A Quasi-Experimental Investigation Within an Observational Study. *JAMA Psychiatry* 73: 1173-1179. doi:10.1001/jamapsychiatry.2016.2427

Schultz, W. (1997) A Neural Substrate of Prediction and Reward. *Science* 275: 1593-1599. doi:10.1126/science.275.5306.1593

Schultz, W. (2013) Updating dopamine reward signals. *Curr Opin Neurobiol* 23: 229-238. doi:10.1016/j.conb.2012.11.012

Schürhoff, F., Szöke, A., Chevalier, F., Roy, I., Méary, A., Bellivier, F., et al. (2007) Schizotypal dimensions: an intermediate phenotype associated with the COMT high activity allele. *Am J Med Genet B Neuropsychiatr Genet* 144B: 64-68. doi:10.1002/ajmg.b.30395

Seamans, J.K., and Yang, C.R. (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol* 74: 1-58. doi:10.1016/j.pneurobio.2004.05.006

Segovia, K.N., Correa, M., and Salamone, J.D. (2011) Slow phasic changes in nucleus accumbens dopamine release during fixed ratio acquisition: a microdialysis study. *Neuroscience* 196: 178-188. doi:10.1016/j.neuroscience.2011.07.078

Semple, D.M. (2005) Book Review: Cannabis as a risk factor for psychosis: systematic review. *Journal of Psychopharmacology* 19: 187-194. doi:10.1177/0269881105049040

Serrano, A., and Parsons, L.H. (2011) Endocannabinoid influence in drug reinforcement, dependence and addiction-related behaviors. *Pharmacol Ther* 132: 215-241. doi:10.1016/j.pharmthera.2011.06.005

Sesack, S.R., Hawrylak, V.A., Matus, C., Guido, M.A., and Levey, A.I. (1998) Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci* 18: 2697-2708.

Shafiei, N., Gray, M., Viau, V., and Floresco, S.B. (2012) Acute stress induces selective alterations in cost/benefit decision-making. *Neuropsychopharmacology* 37: 2194-2209. doi:10.1038/npp.2012.69

Sharp, T., and Zetterstrom, T. (1992) *Monitoring neuronal activity: a practical approach.* Oxford University Press Oxford.

Shifman, S., Bronstein, M., Sternfeld, M., Pisanté-Shalom, A., Lev-Lehman, E., Weizman, A., et al. (2002) A highly significant association between a COMT haplotype and schizophrenia. *Am J Hum Genet* 71: 1296-1302. doi:10.1086/344514

Simpson, E.H., Kellendonk, C., and Kandel, E. (2010) A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. *Neuron* 65: 585-596. doi:10.1016/j.neuron.2010.02.014

Simpson, E.H., Morud, J., Winiger, V., Biezonski, D., Zhu, J.P., Bach, M.E., et al. (2014) Genetic variation in COMT activity impacts learning and dopamine release capacity in the striatum. *Learn Mem* 21: 205-214. doi:10.1101/lm.032094.113

Slifstein, M., Kolachana, B., Simpson, E.H., Tabares, P., Cheng, B., Duvall, M., et al. (2008) COMT genotype predicts cortical-limbic D1 receptor availability measured with [11C]NNC112 and PET. *Mol Psychiatry* 13: 821-827. doi:10.1038/mp.2008.19

Slifstein, M., van de Giessen, E., Van Snellenberg, J., Thompson, J.L., Narendran, R., Gil, R., et al. (2015) Deficits in prefrontal cortical and extrastriatal dopamine release in schizophrenia: a positron emission tomographic functional magnetic resonance imaging study. *JAMA Psychiatry* 72: 316-324. doi:10.1001/jamapsychiatry.2014.2414

Smith, K.S., Berridge, K.C., and Aldridge, J.W. (2011) Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proc Natl Acad Sci U S A* 108: E255-E264. doi:10.1073/pnas.1101920108

Solinas, M., and Goldberg, S.R. (2005) Motivational effects of cannabinoids and opioids on food reinforcement depend on simultaneous activation of cannabinoid and opioid systems. *Neuropsychopharmacology* 30: 2035-2045. doi:10.1038/sj.npp.1300720

Solowij, N., and Battisti, R. (2008) The chronic effects of cannabis on memory in humans: a review. *Curr Drug Abuse Rev* 1: 81-98.

Solowji, N., Fernandez, F., Murray, R., and Yucel, M. (2012) G enetic modulation of the long-term effects of cannabis on brain structure, . *Shizophrenia Research* 136 (Suppl 1), S134.

Spauwen, J., Krabbendam, L., Lieb, R., Wittchen, H.U., and van Os, J. (2006) Evidence that the outcome of developmental expression of psychosis is worse for adolescents growing up in an urban environment. *Psychol Med* 36: 407-415. doi:10.1017/S0033291705006902

Stanton, H.C., Cornejeo, R.A., Mersmann, H.J., Brown, L.J., and Mueller, R.L. (1975) Ontogenesis of monoamine oxidase and catechol-O-methyl transferase in various tissues of domestic swine. *Archives internationales de pharmacodynamie et de therapie* 213: 128-144.

Stefanis, N.C., Henquet, C., Avramopoulos, D., Smyrnis, N., Evdokimidis, I., Myin-Germeys, I., et al. (2007) COMT Val158Met moderation of stress-induced psychosis. *Psychol Med* 37: 1651-1656. doi:10.1017/S0033291707001080

Stefanis, N.C., Van Os, J., Avramopoulos, D., Smyrnis, N., Evdokimidis, I., Hantoumi, I., and Stefanis, C.N. (2004) Variation in catechol-o-methyltransferase val158 met genotype associated with schizotypy but not cognition: a population study in 543 young men. *Biol Psychiatry* 56: 510-515. doi:10.1016/j.biopsych.2004.06.038

Stokes, P.R., Egerton, A., Watson, B., Reid, A., Breen, G., Lingford-Hughes, A., et al. (2010) Significant decreases in frontal and temporal [11C]-raclopride binding after THC challenge. *Neuroimage* 52: 1521-1527. doi:10.1016/j.neuroimage.2010.04.274

Stokes, P.R., Mehta, M.A., Curran, H.V., Breen, G., and Grasby, P.M. (2009) Can recreational doses of THC produce significant dopamine release in the human striatum? *Neuroimage* 48: 186-190. doi:10.1016/j.neuroimage.2009.06.029

Sullivan, P.F., Kendler, K.S., and Neale, M.C. (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 60: 1187-1192. doi:10.1001/archpsyc.60.12.1187

Susser, E., Hoek, H.W., and Brown, A. (1998) Neurodevelopmental disorders after prenatal famine: The story of the Dutch Famine Study. *Am J Epidemiol* 147: 213-216.

Swerdlow, N., Geyer, M., and Braff, D. (2001) Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology* 156: 194-215. doi:10.1007/s002130100799

Swerdlow, N.R., Weber, M., Qu, Y., Light, G.A., and Braff, D.L. (2008) Realistic expectations of prepulse inhibition in translational models for schizophrenia research. *Psychopharmacology (Berl)* 199: 331-388. doi:10.1007/s00213-008-1072-4

Tammimaki, A., Aonurm-Helm, A., Zhang, F.P., Poutanen, M., Duran-Torres, G., Garcia-Horsman, A., and Mannisto, P.T. (2016) Generation of membrane-bound catechol-O-methyl transferase deficient mice with disctinct sex dependent behavioral phenotype. *J Physiol Pharmacol* 67: 827-842.

Tammimäki, A., Forsberg, M.M., Karayiorgou, M., Gogos, J.A., and Männistö, P.T. (2008) Increase in free choice oral ethanol self-administration in catechol-o-methyltransferase gene-disrupted male mice. *Basic Clin Pharmacol Toxicol* 103: 297-304. doi:10.1111/j.1742-7843.2008.00267.x

Tammimäki, A., Käenmäki, M., Kambur, O., Kulesskaya, N., Keisala, T., Karvonen, E., et al. (2010) Effect of S-COMT deficiency on behavior and extracellular brain dopamine concentrations in mice. *Psychopharmacology (Berl)* 211: 389-401. doi:10.1007/s00213-010-1944-2

Tanda, G. (1997) Cannabinoid and Heroin Activation of Mesolimbic Dopamine Transmission by a Common μ1 Opioid Receptor Mechanism. *Science* 276: 2048-2050. doi:10.1126/science.276.5321.2048

Taylor, S. (2013) Molecular genetics of obsessive-compulsive disorder: a comprehensive meta-analysis of genetic association studies. *Mol Psychiatry* 18: 799-805. doi:10.1038/mp.2012.76

Tenhunen, J., Salminen, M., Lundström, K., Kiviluoto, T., Savolainen, R., and Ulmanen, I. (1994) Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters. *Eur J Biochem* 223: 1049-1059.

Tobler, P.N., Fiorillo, C.D., and Schultz, W. (2005) Adaptive coding of reward value by dopamine neurons. *Science* 307: 1642-1645. doi:10.1126/science.1105370

Torrey, E.F., Buka, S., Cannon, T.D., Goldstein, J.M., Seidman, L.J., Liu, T., et al. (2009) Paternal age as a risk factor for schizophrenia: how important is it? *Schizophr Res* 114: 1-5. doi:10.1016/j.schres.2009.06.017

Torrey, E.F., Miller, J., Rawlings, R., and Yolken, R.H. (1997) Seasonality of births in schizophrenia and bipolar disorder: a review of the literature. *Schizophr Res* 28: 1-38.

Törnwall, M., Tuomainen, P., and Männistö, P.T. (1993) Modulation of rat brain endogenous dopamine metabolism by new inhibitors of catechol O-methyltransferase. *Eur J Pharmacol* 239: 39-45.

Traiger, G.J., and Calvert, D.N. (1969) O-Methylation of 3H-norepinephrine by epididymal adipose tissue. *Biochem Pharmacol* 18: 109-117.

Trujillo-Pisanty, I., Hernandez, G., Moreau-Debord, I., Cossette, M.P., Conover, K., Cheer, J.F., and Shizgal, P. (2011) Cannabinoid receptor blockade reduces the opportunity cost at which rats maintain operant performance for rewarding brain stimulation. *J Neurosci* 31: 5426-5435. doi:10.1523/JNEUROSCI.0079-11.2011

Tsou, K., Brown, S., Sañudo-Peña, M.C., Mackie, K., and Walker, J.M. (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 83: 393-411.

Tsuang, M. (2000) Schizophrenia: genes and environment. *Biol Psychiatry* 47: 210-220.

Tunbridge, E.M., and Harrison, P.J. (2011) Importance of the COMT gene for sex differences in brain function and predisposition to psychiatric disorders. *Curr Top Behav Neurosci* 8: 119-140. doi:10.1007/7854_2010_97

Tunbridge, E.M., Bannerman, D.M., Sharp, T., and Harrison, P.J. (2004) Catechol-o-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. *J Neurosci* 24: 5331-5335. doi:10.1523/JNEUROSCI.1124-04.2004

Tunbridge, E.M., Dunn, G., Murray, R.M., Evans, N., Lister, R., Stumpenhorst, K., et al. (2015) Genetic moderation of the effects of cannabis: catechol-O-methyltransferase (COMT) affects the impact of Δ9-tetrahydrocannabinol (THC) on working memory performance but not on the occurrence of psychotic experiences. *J Psychopharmacol* 29: 1146-1151. doi:10.1177/0269881115609073

Tunbridge, E.M., Harrison, P.J., and Weinberger, D.R. (2006) Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol Psychiatry* 60: 141-151. doi:10.1016/j.biopsych.2005.10.024

Tunbridge, E.M., Huber, A., Farrell, S.M., Stumpenhorst, K., Harrison, P.J., and Walton, M.E. (2012) The role of catechol-O-methyltransferase in reward processing and addiction. *CNS Neurol Disord Drug Targets* 11: 306-323.

Tunbridge, E.M., Lane, T.A., and Harrison, P.J. (2007) Expression of multiple catechol-o-methyltransferase (COMT) mRNA variants in human brain. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 144B: 834-839. doi:10.1002/ajmg.b.30539

Tunbridge, E.M., Weickert, C.S., Kleinman, J.E., Herman, M.M., Chen, J., Kolachana, B.S., et al. (2007) Catechol-o-methyltransferase enzyme activity and protein expression in human prefrontal cortex across the postnatal lifespan. *Cereb Cortex* 17: 1206-1212. doi:10.1093/cercor/bhl032

Tuomainen, P., Törnwall, M., and Männistö, P.T. (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: 392-396.

Twitchell, W., Brown, S., and Mackie, K. (1997) Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *J Neurophysiol* 78: 43-50.

Ulmanen, I., Peränen, J., Tenhunen, J., Tilgmann, C., Karhunen, T., Panula, P., et al. (1997) Expression and intracellular localization of catechol O-methyltransferase in transfected mammalian cells. *Eur J Biochem* 243: 452-459.

Ursini, G., Bollati, V., Fazio, L., Porcelli, A., Iacovelli, L., Catalani, A., et al. (2011) Stress-related methylation of the catechol-O-methyltransferase Val 158 allele predicts human prefrontal cognition and activity. *J Neurosci* 31: 6692-6698. doi:10.1523/JNEUROSCI.6631-10.2011

van Os, J., Hanssen, M., Bak, M., Bijl, R.V., and Vollebergh, W. (2003) Do urbanicity and familial liability coparticipate in causing psychosis? *Am J Psychiatry* 160: 477-482.

van Os, J., Kenis, G., and Rutten, B.P. (2010) The environment and schizophrenia. *Nature* 468: 203-212. doi:10.1038/nature09563

van Os, J., Rutten, B.P., and Poulton, R. (2008) Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. *Schizophr Bull* 34: 1066-1082. doi:10.1093/schbul/sbn117

van Winkel, R. (2010) Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. *Archives of general psychiatry* : archgenpsychiatry-archgenpsychi2010.

van Winkel, R., and Kuepper, R. (2014) Epidemiological, neurobiological, and genetic clues to the mechanisms linking cannabis use to risk for nonaffective psychosis. *Annu Rev Clin Psychol* 10: 767-791. doi:10.1146/annurev-clinpsy-032813-153631

van Winkel, R., van Beveren, N.J., Simons, C., and Genetic Risk and Outcome of Psychosis (GROUP) Investigators (2011) AKT1 moderation of cannabis-induced cognitive alterations in psychotic disorder. *Neuropsychopharmacology* 36: 2529-2537. doi:10.1038/npp.2011.141

Varese, F., Smeets, F., Drukker, M., Lieverse, R., Lataster, T., Viechtbauer, W., et al. (2012) Childhood Adversities Increase the Risk of Psychosis: A Meta-analysis of Patient-Control, Prospective- and Cross-sectional Cohort Studies. *Schizophrenia Bulletin* 38: 661-671. doi:10.1093/schbul/sbs050

Varvel, S.A., Bridgen, D.T., Tao, Q., Thomas, B.F., Martin, B.R., and Lichtman, A.H. (2005) Delta9-tetrahydrocannbinol accounts for the antinociceptive, hypothermic, and cataleptic effects of marijuana in mice. *J Pharmacol Exp Ther* 314: 329-337. doi:10.1124/jpet.104.080739

Verdejo-García, A., Fagundo, A.B., Cuenca, A., Rodriguez, J., Cuyás, E., Langohr, K., et al. (2013) COMT val158met and 5-HTTLPR genetic polymorphisms moderate executive control in cannabis users. *Neuropsychopharmacology* 38: 1598-1606. doi:10.1038/npp.2013.59

Verrico, C.D., Gu, H., Peterson, M.L., Sampson, A.R., and Lewis, D.A. (2014) Repeated Δ9-tetrahydrocannabinol exposure in adolescent monkeys: persistent effects selective for spatial working memory. *Am J Psychiatry* 171: 416-425. doi:10.1176/appi.ajp.2013.13030335

Verrico, C.D., Jentsch, J.D., and Roth, R.H. (2003) Persistent and anatomically selective reduction in prefrontal cortical dopamine metabolism after repeated, intermittent cannabinoid administration to rats. *Synapse* 49: 61-66. doi:10.1002/syn.10215

Vidgren, J., Svensson, L.A., and Liljas, A. (1994) Crystal structure of catechol O-methyltransferase. *Nature* 368: 354-358. doi:10.1038/368354a0

Vinkers, C.H., Van Gastel, W.A., Schubart, C.D., Van Eijk, K.R., Luykx, J.J., Van Winkel, R., et al. (2013) The effect of childhood maltreatment and cannabis use on adult psychotic symptoms is modified by the COMT Val^{158}Met polymorphism. *Schizophr Res* 150: 303-311. doi:10.1016/j.schres.2013.07.020

Vlachou, S., and Panagis, G. (2014) Regulation of Brain Reward by the Endocannabinoid System: A Critical Review of Behavioral Studies in Animals. *CPD* 20: 2072-2088. doi:10.2174/13816128113199990433

Volkow, N.D. (2009) Substance use disorders in schizophrenia--clinical implications of comorbidity. *Schizophr Bull* 35: 469-472. doi:10.1093/schbul/sbp016

Voruganti, L.N., Slomka, P., Zabel, P., Mattar, A., and Awad, A.G. (2001) Cannabis induced dopamine release: an in-vivo SPECT study. *Psychiatry Res* 107: 173-177.

Völter, C., Riedel, M., Wöstmann, N., Aichert, D.S., Lobo, S., Costa, A., et al. (2012) Sensorimotor gating and D2 receptor signalling: evidence from a molecular genetic approach. *Int J Neuropsychopharmacol* 15: 1427-1440. doi:10.1017/S1461145711001787

Wagner, E.J. (2016) Sex differences in cannabinoid-regulated biology: A focus on energy homeostasis. *Front Neuroendocrinol* 40: 101-109. doi:10.1016/j.yfrne.2016.01.003

Ward, S.J., and Dykstra, L.A. (2005) The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940). *Behav Pharmacol* 16: 381-388.

Wardle, M.C., de Wit, H., Penton-Voak, I., Lewis, G., and Munafò, M.R. (2013) Lack of Association Between COMT and Working Memory in a Population-Based Cohort of Healthy Young Adults. *Neuropsychopharmacology* . doi:10.1038/npp.2013.24

Weickert, T.W., Goldberg, T.E., Gold, J.M., Bigelow, L.B., Egan, M.F., and Weinberger, D.R. (2000) Cognitive impairments in patients with schizophrenia displaying preserved and compromised intellect. *Arch Gen Psychiatry* 57: 907-913.

Weinberger, D.R. (1986) The pathogenesis of schizophrenia ; A neurodevelopmental theory. *The neurology of schizophrenia* : 397-406.

Weinberger, D.R. (1987) Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry* 44: 660-669.

Weinberger, D.R., Berman, K.F., Suddath, R., and Torrey, E.F. (1992) Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: a magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. *Am J Psychiatry* 149: 890-897. doi:10.1176/ajp.149.7.890

Weinstein, A.M., and Gorelick, D.A. (2011) Pharmacological treatment of cannabis dependence. *Current pharmaceutical design* 17: 1351.

Weisz, J., Fritz-Wolz, G., Gestl, S., Clawson, G.A., Creveling, C.R., Liehr, J.G., and Dabbs, D. (2000) Nuclear localization of catechol-O-methyltransferase in neoplastic and nonneoplastic mammary epithelial cells. *Am J Pathol* 156: 1841-1848. doi:10.1016/S0002-9440(10)65057-2

Williams, C.M., Rogers, P.J., and Kirkham, T.C. (1998) Hyperphagia in pre-fed rats following oral delta9-THC. *Physiol Behav* 65: 343-346.

Williams, N.M., O'Donovan, M.C., and Owen, M.J. (2006) Chromosome 22 Deletion Syndrome And Schizophrenia. Ronald J. Bradley, R. Adron Harris, and Jenner, P. (eds). Academic Press, pp. 1 - 27.

Winterer, G., and Weinberger, D.R. (2004) Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. *Trends Neurosci* 27: 683-690. doi:10.1016/j.tins.2004.08.002

Winton-Brown, T.T., Fusar-Poli, P., Ungless, M.A., and Howes, O.D. (2014) Dopaminergic basis of salience dysregulation in psychosis. *Trends Neurosci* 37: 85-94. doi:10.1016/j.tins.2013.11.003

Wise, R.A., Spindler, J., deWit, H., and Gerberg, G.J. (1978) Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. *Science* 201: 262-264.

Witte, A.V., and Flöel, A. (2012) Effects of COMT polymorphisms on brain function and behavior in health and disease. *Brain Res Bull* 88: 418-428. doi:10.1016/j.brainresbull.2011.11.012

Wright, M.J., Vandewater, S.A., Parsons, L.H., and Taffe, M.A. (2013) Δ(9)Tetrahydrocannabinol impairs reversal learning but not extra-dimensional shifts in rhesus macaques. *Neuroscience* 235: 51-58. doi:10.1016/j.neuroscience.2013.01.018

Wyvell, C.L., and Berridge, K.C. (2000) Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward wanting without enhanced liking or response reinforcement. *The Journal of Neuroscience* 20: 8122-8130.

Yacubian, J., Sommer, T., Schroeder, K., Gläscher, J., Kalisch, R., Leuenberger, B., et al. (2007) Gene-gene interaction associated with neural reward sensitivity. *Proc Natl Acad Sci U S A* 104: 8125-8130. doi:10.1073/pnas.0702029104

Yavich, L., Forsberg, M.M., Karayiorgou, M., Gogos, J.A., and Männistö, P.T. (2007) Site-specific role of catechol-O-methyltransferase in dopamine overflow within prefrontal cortex and dorsal striatum. *J Neurosci* 27: 10196-10209. doi:10.1523/JNEUROSCI.0665-07.2007

Yin, H.H., and Knowlton, B.J. (2006) The role of the basal ganglia in habit formation. *Nat Rev Neurosci* 7: 464-476. doi:10.1038/nrn1919

Yin, H.H., Knowlton, B.J., and Balleine, B.W. (2004) Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *European journal of neuroscience* 19: 181-189.

Zahrt, J., Taylor, J.R., Mathew, R.G., and Arnsten, A.F. (1997) Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J Neurosci* 17: 8528-8535.

Zammit, S., Allebeck, P., Dalman, C., Lundberg, I., Hemmingson, T., Owen, M.J., and Lewis, G. (2003) Paternal age and risk for schizophrenia. *The British journal of psychiatry : the journal of mental science* 183: 405-408. doi:10.1192/bjp.183.5.405

Zammit, S., Moore, T.H., Lingford-Hughes, A., Barnes, T.R., Jones, P.B., Burke, M., and Lewis, G. (2008) Effects of cannabis use on outcomes of psychotic disorders: systematic review. *Br J Psychiatry* 193: 357-363. doi:10.1192/bjp.bp.107.046375

Zammit, S., Owen, M.J., Evans, J., Heron, J., and Lewis, G. (2011) Cannabis, COMT and psychotic experiences. *Br J Psychiatry* 199: 380-385. doi:10.1192/bjp.bp.111.091421

Zammit, S., Spurlock, G., Williams, H., Norton, N., Williams, N., O'Donovan, M.C., and Owen, M.J. (2007) Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. *Br J Psychiatry* 191: 402-407. doi:10.1192/bjp.bp.107.036129

Zürcher, G., Keller, H.H., Kettler, R., Borgulya, J., Bonetti, E.P., Eigenmann, R., and Da Prada, M. (1990) Ro 40-7592, a novel, very potent, and orally active inhibitor of catechol-O-methyltransferase: a pharmacological study in rats. *Adv Neurol* 53: 497-503.