

Treatment of Cognitive Deficits Associated with Schizophrenia

Potential Role of Catechol-*O*-Methyltransferase Inhibitors

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Abstract

In the last two decades, understanding of the dynamics of dopamine function in the prefrontal cortex and its role in prefrontal cortex physiology has opened up new avenues for therapeutic interventions in conditions in which prefrontal cortex function is compromised. Neuropsychological and imaging studies of prefrontal information processing have confirmed specific cognitive and neurophysiological abnormalities in individuals with schizophrenia. Because such findings are also observed in the healthy siblings of patients with schizophrenia, they may represent intermediate phenotypes related to schizophrenia susceptibility genes.

Catechol-*O*-methyltransferase (COMT) represents an important candidate as a susceptibility gene for cognitive dysfunction in schizophrenia because of the unique role this enzyme plays in regulating prefrontal dopaminergic function. A functional COMT polymorphism (Val158Met) predicts performance in tasks of prefrontal executive function and the neurophysiological response measured with electroencephalography and functional magnetic resonance imaging in tasks assessing working memory. In fact, individuals with the Val/Val genotype, which encodes for the high-activity enzyme resulting in lower dopamine concentrations in the prefrontal cortex, perform less well and are less efficient physiologically than Met/Met individuals.

These findings raise the possibility of new pharmacological interventions for the treatment of prefrontal cortex dysfunction and of predicting outcome based on COMT genotype. One strategy consists of the use of CNS-penetrant COMT inhibitors such as tolcapone. A second strategy is to increase extracellular dopamine concentrations in the frontal cortex by blocking the noradrenaline (norepinephrine) reuptake system, a secondary mechanism responsible for the disposal of dopamine from synaptic clefts in the prefrontal cortex. A third possibility involves the use of modafinil, a drug with an unclear mechanism of action but with positive effects on working memory in rodents.

The potential of these drugs to improve executive cognitive function by selectively increasing dopamine load in the frontal cortex but not in subcortical

territories, and the possibility that response to them may be modified by a COMT polymorphism, provides a novel genotype-based targeted pharmacological approach without abuse potential for the treatment of cognitive disorder in schizophrenia and in other conditions involving prefrontal cortex dysfunction.

During the last few decades, a growing body of evidence has pointed towards the dorsolateral prefrontal cortex (DLPFC) as an important anatomical area involved in the pathophysiology of schizophrenia. In fact, anatomical, neurochemical, neurophysiological, neuropsychological and imaging studies have provided extensive evidence on abnormal prefrontal neuronal circuits in individuals with schizophrenia.^[1-21] However, while the prefrontal cortex has been a centrepiece of systems neuroscience approaches to understanding schizophrenia, from a neuropharmacological perspective the focus has been on dopamine, particularly in the striatum.

Dopamine has long been observed to play a critical role in the pharmacological treatment of schizophrenia. In fact, functional dopamine receptor antagonists are endowed with a potent antipsychotic action^[22,23] and their clinical efficacy is correlated with their potency in displacing dopamine D₂ receptor ligands from brain membranes.^[24,25] More recently, it has been proposed that negative symptoms of schizophrenia such as social withdrawal, apathy, lack of motivation and poverty of speech are associated with DLPFC hypodopaminergia, whereas positive symptoms such as auditory hallucinations, paranoid or bizarre delusions and disorganisation are associated with an increased dopaminergic tone in subcortical areas.^[26,27] Dopaminergic dysregulation of prefrontal cortical circuits may also contribute to the cognitive difficulties typically associated with schizophrenia, such as problems with working memory, attention and executive functions.^[18,21]

Both typical and atypical antipsychotics have been the main pharmacotherapeutic avenue for the treatment of schizophrenia. These drugs, however,

even though effective in mitigating the positive symptoms of schizophrenia, are less effective in treating negative symptoms, particularly cognitive impairments, and they have considerable potential for adverse effects. The understanding of pathophysiological mechanisms in schizophrenia and the recent identification of a number of genetic risk factors for this disease has led to the search for new, more specific and focused strategies for the treatment of cognitive deficits associated with schizophrenia.

1. The Dorsolateral Prefrontal Cortex (DLPFC) and Schizophrenia

The availability of antibodies against tyrosine hydroxylase (TH), the enzyme involved in the synthesis of dopamine, and the development of refined histochemical techniques allowed the selective identification of dopaminergic terminals in the DLPFC, both in monkeys and human brain samples.^[1] Dopaminergic neurons in the mammalian neocortex are widely distributed in all cortical regions. The distribution of these monoaminergic neurons shows a specific pattern, with preferential innervation of motor over sensory regions, sensory associations over primary sensory regions, and auditory association cortex over visual association cortex.^[28] In densely innervated areas (e.g. area 9), the TH-containing fibres are evenly distributed across all cortical layers. In the dorsomedial convexity of the frontal lobe, fibre density increases in a rostral to caudal fashion from the frontal lobe to the premotor region. Additionally, the medial cortical surface displays a ventral to dorsal gradient of increasing fibre density. The laminar distribution of TH-containing neurons

shows that the relatively lightly innervated areas of the human prefrontal cortex tend to have fibres distributed in a bilaminar fashion, while the more densely innervated regions such as the dorsomedial region have a more uniform distribution.^[28]

The findings that the ventral tegmental area (VTA) is the main source for dopaminergic afferents to the limbic and DLPFC has been interpreted as having potential implications for understanding the role of dopamine in the pathophysiology of schizophrenia. In fact, dysregulation of the mesolimbic and mesocortical pathways may be at least partially responsible for the positive and negative symptoms described in schizophrenia, respectively.^[26,29,30] In this context, exaggerated mesolimbic transmission has been proposed as responsible for the hallucinations and delusional thoughts present in schizophrenia – the so-called positive symptoms.^[29,30] The proof for this assertion, however, is inconclusive since it derives from indirect evidence such as the psychotomimetic effect of chronically administered amfetamines, drugs that release dopamine in the brain, and the clinical efficacy of antipsychotics, drugs that block D₂ receptors in the brain.^[31,32] However, the question of how or even whether dopaminergic neurotransmission is disrupted in schizophrenia is still unanswered. Imaging studies in first-break patients with schizophrenia or in antipsychotic-naïve patients who have been chronically ill and are currently actively psychotic suggest that mesostriatal dopaminergic activity is increased, as assessed by exaggerated dopamine output in studies measuring D₂ receptor ligand displacement after amfetamine administration.^[32,33] Studies in stable patients with schizophrenia, i.e. those who are not actively psychotic, however, failed to confirm those results, suggesting that the increased mesostriatal activity is only patrimony of the acute stages of the disease.^[32] These findings indicate that increased dopaminergic neurotransmission in subcortical areas in schizophrenia is state dependent and

may be abnormal in response to the disruption of other main neuronal pathways.^[34,35]

The discovery of a direct correlation between the clinical efficacy of antipsychotics and their affinity for the D₂-type receptor raised the possibility that dopamine might be involved in the pathophysiology of schizophrenia.^[36] This evidence, together with the psychotomimetic effect of drugs that increase dopaminergic neurotransmission,^[29] fuelled the so-called ‘dopaminergic hypothesis of schizophrenia’.^[30] Even though this is an attractive hypothesis that unifies a pharmacological concept with a pathophysiological mechanism, the evidence for a primary pathogenic role of dopamine in schizophrenia is still uncertain and cannot fully explain the complex schizophrenia syndrome.

In contrast to the inconclusive data concerning dopamine in schizophrenia, more convincing evidence exists for a primary role of abnormal activity and connectivity in the DLPFC as the primary cause of negative symptoms and cognitive deficits present in this illness. In this context, the groundbreaking report of Ingvar and Franzen^[37] provided the first documented evidence of a reduced frontal lobe regional cerebral blood flow in schizophrenia. This hypoactivation or hypofrontality has been well documented in imaging studies performed during activation tasks that are associated with DLPFC activation, in particular working memory tasks.^[16,17,38–40] Moreover, animal studies indicate that prefrontal cortical function is critical for regulation of dopaminergic activity in the mesolimbic system,^[41] suggesting that abnormal DLPFC function might also relate to the dopaminergic component of psychosis.

The DLPFC regulates subcortical areas via direct descending facilitatory and indirect inhibitory pathways. In fact, chemical lesions aimed at disrupting DLPFC afferents to subcortical areas facilitate behaviours such as spontaneous motor activity and dopamine-dependent stereotyped behaviour,

and increase subcortical dopamine turnover, three indices associated with enhanced dopaminergic transmission in the nucleus accumbens septi and striatum.^[42-44] This relationship, i.e. abnormal prefrontal cortex biology predicting upregulated mesostriatal dopamine activity, has been confirmed in the nonhuman primate^[45] and in imaging studies in patients with schizophrenia.^[46,47] In addition, Akil and coworkers^[48] showed that expression of TH messenger RNA (mRNA) was increased in post-mortem human brain tissue of individuals with Val alleles of the catechol-*O*-methyltransferase (COMT; EC 2.1.1.6) gene, a genotype associated with putatively lower dopamine concentrations in the DLPFC, when compared to brain tissue from individuals with Met alleles, a genotype associated with relatively higher DLPFC dopamine concentrations.^[48] This genetic association has also been confirmed in a study of living subjects imaged with F-dopa position emission tomography.^[46]

The pathophysiology of prefrontal deficits in patients with schizophrenia is beginning to be revealed thanks to the development of sophisticated imaging and neuropsychological tools. In patients with schizophrenia, cognitive testing has reliably demonstrated impairments in working memory paradigms when compared with healthy volunteers or even with siblings of patients with schizophrenia, and these impairments are not correlated with psychotic symptoms or medication status.^[9,13,49-52] These findings have been consistently found when patients are studied during the execution of tasks that are normally connected with prefrontal engagement, such as when performing the n-back test (a reliable and specific test that engages the working memory system in maintaining and updating information over short delays) or the Wisconsin Card Sorting Test (a complex problem-solving test involving many cognitive components, including information maintenance, abstraction, set shifting and inhibition of previously rewarded responses).^[17,38-40,53]

While it is likely that the underlying mechanisms involved in these deficits are complex, during active cognitive load that results in impaired performance, patients with schizophrenia seem to disengage working-memory related circuits and become hypofrontal when studied with a variety of neuroimaging paradigms. However, even when patients who perform at normal levels on these tasks are studied, they show evidence of abnormal prefrontal processing of the cognitive information. Under these circumstances, however, the flavour of the physiological abnormality is relatively changed. When patients can keep up with the cognitive load they tend to show exaggerated activation, as if they needed to enlist a more complex neuronal circuitry to perform the task at a similar level of accuracy when compared with healthy volunteers.^[18,54,55] This finding supports the notion that, under certain circumstances, when patients are able to keep up with their cognitive demands, they do it less efficiently than healthy volunteers as they activate more of the DLPFC neuronal network to perform the task at the same level of performance. Overall, while performing cognitive tasks at different levels of load, patients with schizophrenia tend to display a reduced ability to stay on task, and while they are on task they are unable to process information with the same efficiency as healthy volunteers.

While the mechanism for the exaggerated functional magnetic resonance imaging (fMRI) response is not clearly understood, the decreased efficiency of the frontal cortex is most likely to be related to the emergence of hypofrontality when capacity is exceeded. This cortical functional profile, i.e. inefficient processing at lower loads and dissolution of the network profile at higher loads, has been linked to the phenomena in nonhuman primates of cortical tuning during working memory and the prominent role of cortical dopamine signalling in this process.^[56,57]

2. Dopamine Modulation of the DLPFC

Dopamine neurons in the mesencephalon projecting to the DLPFC, named the mesocortical dopaminergic system, regulate a number of cognitive processes, including executive functions, planning and attention.^[58,59] This neuronal system manifests specific characteristics that differentiate it from the other two main dopaminergic neuronal systems in the brain (the mesolimbic and the nigrostriatal dopaminergic systems), including a faster firing rate, more action potentials in bursts, much higher dopamine turnover^[60,61] and enhanced sensitivity to stress.^[62,63] In line with the concept of volume transmission in the cortex,^[64,65] the mesocortical dopaminergic system also exhibits greater extrasynaptic diffusion and slower metabolic clearance^[66,67] when compared with the mesolimbic and nigrostriatal systems. In this context, intracerebral dialysis studies showed that dopamine in the dialysate is more concentrated in the DLPFC than in the striatum and the nucleus accumbens, suggesting a higher percentage of extracellular dopamine relative to intrasynaptic sites.^[66,68] Studies on mice lacking dopamine transporter (DAT) gene expression indicate that in the striatum, factors affecting dopamine synthesis, autoreceptor density and metabolism^[69,70] have much less impact on extracellular dopamine accumulation than the DAT protein itself.^[71] In the prefrontal cortex, however, the role of the DAT is much less important^[72] and its expression is much lower.^[73] Thus, the lack or scantiness of DATs in the cortical synaptic cleft provides the basis to understand why the mesocortical system presents higher extracellular dopamine concentrations and a greater diffusion space at the level of the synaptic cleft when compared to the mesolimbic or nigrostriatal systems. Moreover, the lack of terminal recycling of released dopamine probably accounts for the greater metabolic turnover of cortical dopamine and for the stress sensitivity of cortical dopamine terminals to become dopamine depleted. The limited efficacy of

dopamine reuptake blocking drugs in the DLPFC when compared with other dopaminergic areas further supports these findings.^[74-76]

The mechanisms by which the DLPFC removes dopamine from the synaptic cleft have been explored from several directions. The main mechanisms involved are (i) the reuptake of dopamine by the noradrenaline (norepinephrine) transporter (NET); (ii) diffusion of dopamine into nonsynaptic areas; and (iii) enzymatic processing through COMT.^[68,76,77]

Extracellular dopamine in the DLPFC is primarily cleared by NET mechanisms. In fact, in a cerebral dialysis model, the noradrenaline reuptake inhibitor desipramine elevates both dopamine and noradrenaline concentrations in the prefrontal cortex, consistent with it being an area with high NET concentration and scarce DATs.^[74,78] In line with these findings, studies using a knockout mice model for the NET further validated the contention that the uptake of dopamine in the frontal cortex depends primarily on the NET.^[72]

A second mechanism by which dopamine is cleared from the DLPFC is the volume transmission mechanism. Volume transmission refers to three-dimensional signal diffusion within the brain extracellular fluid that includes short- (~20nm) and long-distance diffusion of signals through the extracellular and cerebrospinal fluid. The inefficient DAT mechanism in the DLPFC enables diffusion over distances approaching 100µm.^[64,79] This diffusion increases the likelihood of extrasynaptic transmission, with subsequent catabolism of dopamine by an extrasynaptic DAT mechanism in an area far from where the neurotransmitter release took place. Extrasynaptic diffusion and volume transmission may be an important factor in D₁ receptor stimulation, as these receptors are primarily extrasynaptic,^[80] and in dopamine stimulation of GABAergic neurons, both of which are critical for tuning cortical circuitry during working memory.^[64]

A third mechanism by which synaptic dopamine is inactivated is metabolism by the enzyme COMT. This is a ubiquitous enzyme that catalyses the transfer of a methyl group from substrates carrying the catechol moiety^[81] and plays an important role in the metabolism of dopamine, adrenaline (epinephrine) and noradrenaline. In nervous tissue, COMT is located in postsynaptic processes surrounding synapses, capillary walls and postsynaptic dendritic spines; little or no COMT is found in presynaptic neurons.^[82,83] Early immunohistochemistry studies^[84] suggesting that COMT was expressed primarily in glia have been shown to be erroneous. COMT is expressed primarily in cortical neurons with little, if any, expression in glia.^[85] Moreover, the regional distribution of COMT follows an inverse pattern with expression of DAT; thus, it is highest in cortical areas, including neocortex and hippocampus, and lowest in striatum and brainstem.^[85] In the DLPFC, and probably in the hippocampus, the high levels of COMT and the lack or scarcity of DATs at synapses most likely contribute to the relative functional specificity for COMT to cortical dopamine metabolism.^[66,67]

Pharmacological studies have also confirmed that the catabolic flux of synaptic dopamine through the COMT pathway is much greater in the prefrontal cortex compared with other brain dopamine pathways.^[77] Recent studies in COMT knockout mice revealed an allele load-dependent increase of dopamine concentrations in the prefrontal cortex but not in the striatum when compared with the wild-type mice.^[86,87] No change in noradrenaline concentrations or turnover were found in these animals, consistent with the assumption that the abundance of NET in cortex minimises the role of COMT in cortical noradrenergic flux. Similarly, an *in vivo* microdialysis study in the rat confirmed that COMT inhibition, induced with the drug tolcapone, selectively enhanced drug-induced dopamine release in the prefrontal cortex but not in the striatum.^[88] As in

the COMT knockout mice, no change in noradrenaline concentrations was seen.

3. Catechol-O-Methyltransferase (COMT) Polymorphism and Genetic Predisposition to Psychosis

In the last 15 years, two different isoforms of COMT (soluble COMT [S-COMT] and membrane-bound COMT [MB-COMT]) have been characterised.^[89-91] Both rat and human S-COMTs contain 221 amino acids, and their molecular masses are 24.8 and 24.4 kDa, respectively. The peptide sequence of the human S-COMT form is approximately 81% identical to that of the rat enzyme.^[92,93] The respective molecular masses for rat and human MB-COMT are 29.6 and 30.0 kDa. One single gene localised in chromosome 22, band q11.2, encodes both the S-COMT and MB-COMT forms (figure 1).^[89,91,94,95]

The level of activity of COMT is genetically polymorphic in human red blood cells (RBCs) and liver. The enzyme has a trimodal distribution of low, intermediate and high levels of activity.^[96-99] This genetic variation, which is largely explained by a single nucleotide polymorphism (SNP) at position 158 in the membrane-bound form of the protein (this is at position 108 in the soluble form), results in substantial, 3- to 4-fold differences in COMT activity in RBCs and liver,^[96,98] which may explain individual variation in metabolism of catecholamines and monoaminergic drugs.^[100-102] In the postmortem DLPFC, however, determination of COMT activity revealed that, in human subjects, the COMT-Val allele activity is approximately 40% higher and shows higher thermostability at 37°C than the COMT-Met allele.^[103] Under analogous experimental conditions, similar results were confirmed in human lymphocytes, indicating that the findings in postmortem tissue are not artifacts of potential differences in postmortem protein stability based on genotype.^[103]

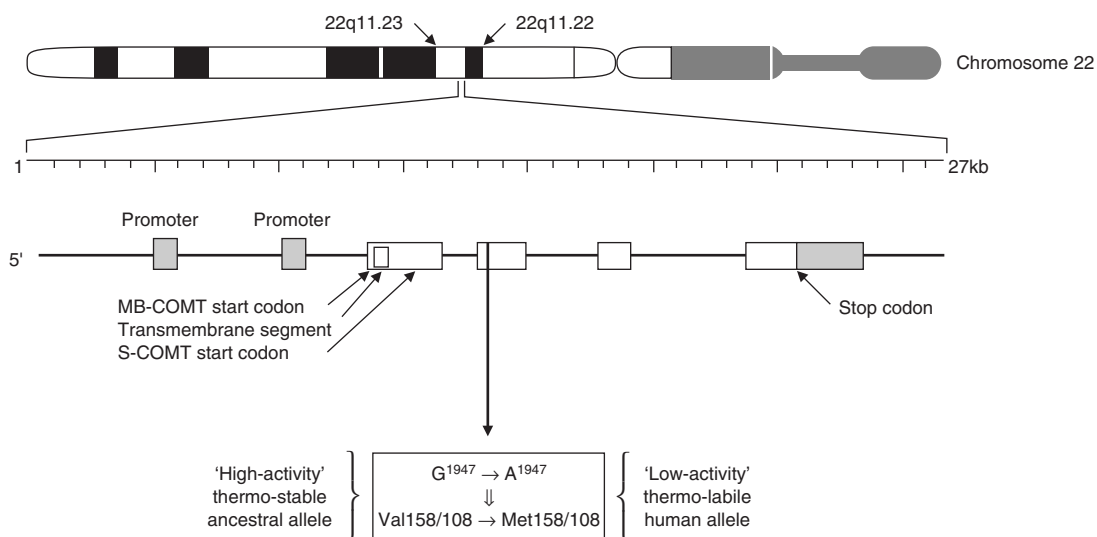


Fig. 1. The catechol-*O*-methyltransferase (COMT) Val158/108Met polymorphism determines the level of activity of the COMT enzyme. **MB-COMT** = membrane-bound COMT; **S-COMT** = soluble COMT.

If dopamine innervation of the DLPFC is an important factor in the regulation of prefrontal information processing, and COMT impacts on cortical dopamine signalling, it is logical to assume that genetic variation in COMT is a key mechanism involved in prefrontal dopamine metabolism and will have a significant impact in prefrontal physiology and prefrontally mediated cognitive function. As noted above, the COMT gene contains a highly functional common nucleotide polymorphism at position 427 (guanine to adenine substitution) that results in a Val-Met change in peptide sequence that affects the thermo-lability and activity of the enzyme. In line with these various considerations, it is predictable that COMT Val/Met genotype should have effects on prefrontal cortical function, and that even in healthy young individuals, the Val allele, because of its higher capacity to metabolise dopamine in the frontal cortex and the presumed reduction in synaptic dopamine, would be associated with a relatively compromised DLPFC function. An increasing number of recent studies have confirmed this assumption, including studies of executive cognition tasks, such as the Wisconsin Card

Sorting Test and the n-back task, and studies of prefrontal cortical physiology during performance of such tasks.^[46,52,104-106]

Changes in performance on tests that measure executive cognitive function have been shown to predict changes in prefrontal neuronal physiological activity.^[39,107,108] This being the case, COMT genotype-mediated changes in dorsolateral prefrontal efficiency may also predict the physiological response of the DLPFC, as assessed by blood oxygen level dependent (BOLD) fMRI, during the performance of tests that measure prefrontal executive cognition. Initial studies in three different cohorts, which included a group of patients with schizophrenia and two different cohorts of unaffected siblings of patients with schizophrenia, revealed a reduced efficiency in information processing during the n-back working memory task in patients and their unaffected siblings (figure 2).^[104] The fMRI response was analysed by COMT genotype during the two-back version of the n-back task in the two groups of siblings that did not differ in mean age, gender, education, handedness or performance accuracy. Under these conditions, areas in dorsolateral pre-

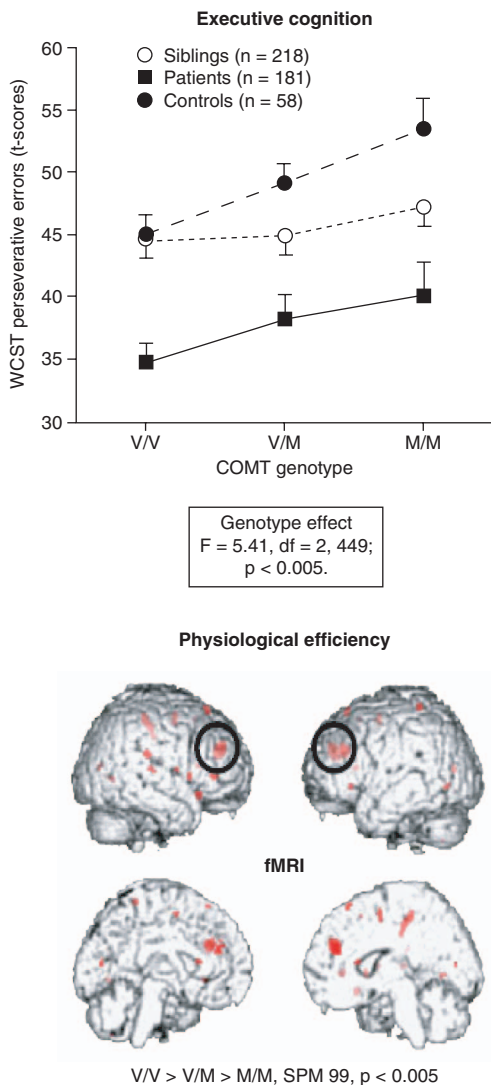


Fig. 2. Catechol-O-methyltransferase (COMT) Val158/108Met genotype modulates executive function and physiological efficiency in the dorsolateral prefrontal cortex.^[104] **fMRI** = functional magnetic resonance imaging; **M/M** = Met/Met; **V/M** = Val/Met; **V/V** = Val/Val; **WCST** = Wisconsin Card Sorting Test.

frontal and cingulate cortices show the predicted genotype effects, with Val/Val individuals having the greatest activation (i.e. being least efficient), followed by Val/Vet and then Met/Met individuals (figure 2).^[104] This set of results confirmed the hypothesis that COMT genotype would predict the

efficiency of the cortical response and that the Val allele, because of its association with a more effective catabolic process leading to greater dopamine inactivation in the frontal cortex, would be linked to a decreased prefrontal physiological efficiency.

In light of the evidence that abnormal prefrontal function is a central feature of schizophrenia and is related to an increased genetic risk for schizophrenia,^[49,109,110] it would not be surprising if COMT were a candidate susceptibility gene for schizophrenia. Early case-control studies comparing allele frequencies between patients with schizophrenia and healthy volunteers revealed either a positive association^[111] or no association.^[112] These studies, however, are prone to a number of artifacts, including underpowered cohorts and problems with population admixture or stratification, which is especially problematic because COMT allele frequencies vary across certain ethnic groups.^[112]

A different approach to study genetic association in schizophrenia is represented by the Transmission Disequilibrium Test (TDT), in which the proportion of alleles transmitted from heterozygous parents to their sick progeny can be reliably assessed.^[97] One of the advantages of this approach is that the method is not susceptible to population stratification effects because the frequencies of transmitted alleles are determined always within families. Early TDT studies of COMT alleles in schizophrenia concluded that the Val allele was more frequently transmitted from parents to their ill offspring than the Met allele.^[113-115] In line with these studies, Egan and co-workers^[104] found that the Val allele was transmitted more frequently to the schizophrenic progeny than would be predicted by random assortment, while transmission to the healthy sibling was not significantly different between alleles. However, studies that have followed these earlier reports have been decidedly negative,^[116-119] and a meta-analysis^[120] argued that in cases of European ancestry, the

COMT Val allele is of borderline significance as having a role in risk for schizophrenia.

There are a number of possible explanations for these apparent inconsistencies. One possibility is that other functional sites in COMT may balance the Val/Met allele.^[121] Indeed, Shifman and co-workers,^[122] studying an Ashkenazi Jewish founder population, reported a highly significant association between schizophrenia and a COMT haplotype that includes two noncoding SNPs at either end of the COMT gene. The 3' flanking region SNP, which is associated with lower expression of COMT mRNA, is transcribed in human brain and exhibits significant differences in allelic expression.^[123] Their finding that the Val158Met allele primarily affected women in their sample, while the 3' flanking region SNP affected both genders, raises the possibility that the susceptibility for schizophrenia may be affected by more than one functional COMT polymorphism.^[122]

The possibility also exists that the effect of COMT genotype will vary based on environmental modifiers. Caspi and co-workers^[124] recently reported that the effect of Val alleles on predicting psychosis risk is dramatically enhanced in the context of adolescence marijuana use. In fact, in this study, early adolescent marijuana use in individuals with Val/Val genotypes increased the risk of adult schizophrenia 10-fold compared with the general population.

Another interesting association of COMT and psychosis is in patients with Alzheimer's disease. Val alleles have been associated with increased risk of psychosis in two recent, relatively large case-control studies.^[125,126]

4. COMT Inhibitors and Cognitive Function

A number of compounds that inhibit the activity of COMT were synthesised after the discovery of the enzyme.^[81,127] Among these, gallates, tropolone,

U-0521 and 3',4'-dihydroxy-2-methyl-propionophenone represented the first-generation of COMT inhibitors, which were used mostly for experimental purposes (figure 3).^[128] However, these products were rather toxic, nonselective, short acting and scarcely potent in *in vitro* systems, and the limited clinical experience with them was rather unsatisfactory.^[129-131]

Interest in COMT inhibitors as therapeutic tools for Parkinson's disease led to the development of a second generation of more selective, potent and clinically useful compounds with inhibitory activity against peripheral and/or central COMT (figure 3).^[132-135] The 'classic' second-generation COMT inhibitors, such as tolcapone, entacapone and nitecapone, possess a nitrocatechol structure, and their primary therapeutic use has been as adjuncts to levodopa for the treatment of the 'on-off phenomenon' in Parkinson's disease.^[136-138] Levodopa is a catechol that is peripherally *O*-methylated by COMT to 3-*O*-methyldopa. The action of COMT is mainly exerted at the level of liver, intestine and kidney, where high levels of the enzyme (as the S-COMT isoform) are present.^[134,139] Thus, COMT inhibition increases the peripheral availability of levodopa and, thus, the amount that can be transported into the brain. The more hydrophobic COMT inhibitors have the ability to cross the blood-brain barrier (BBB) and thus further increase the synaptic pool of dopamine in areas lacking specific DATs, such as the prefrontal cortex and hippocampus.

Nitrocatechol compounds bind tightly but reversibly to COMT^[132,140] and the speed of reaction of COMT in the presence of various concentrations of the inhibitor increases progressively with escalating enzyme concentrations. Also, the action of the 'classic' nitrocatechol inhibitors can be effectively and fully reversed by dialysis.^[132]

Among the 'classic' compounds, tolcapone^[141,142] proved to be the most potent *in vitro* and can penetrate the BBB, reaching a brain level

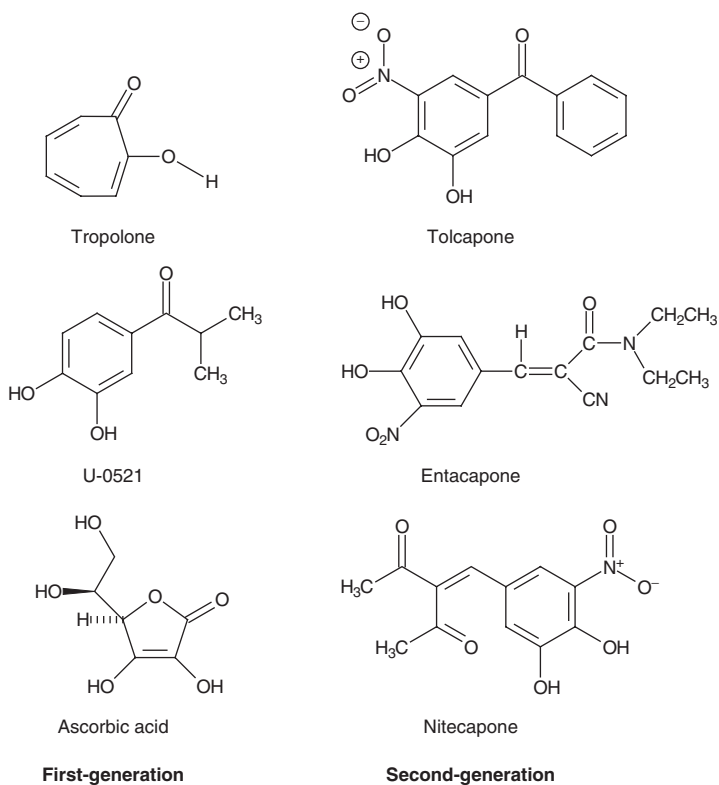


Fig. 3. Chemical structure of some first- and second-generation catechol-*O*-methyltransferase inhibitors.

equivalent to 1% of the plasma concentration.^[143] Tolcapone inhibits brain COMT activity *in vivo*, with a dose that inhibits activity by 50% (ID₅₀) of 26–28 mg/kg.^[144,145] Tolcapone was shown to be particularly potent in inhibiting MB-COMT from brain *in vivo*, although it does not discriminate between MB- and S-COMT under *in vitro* experimental conditions when using the same amount of enzyme in the assay.^[146] The ability of tolcapone to decrease 3-*O*-methyl-dopa, 3-methoxytyramine and homovanillic acid concentrations in the brain provides further evidence of the BBB-penetrating properties of the drug.^[141,142,147] In fact, the formation of 3-methoxytyramine was suppressed by 90% 1 hour after drug administration, while homovanillic acid formation was suppressed by 80% 4 hours after administration.^[145]

Microdialysis studies investigating the effect of tolcapone in the DLPFC revealed that the drug did not affect baseline extracellular catecholamine concentrations, but significantly increased the concentration of extracellular dopamine, although not that of noradrenaline, after potentiation with a depolarising agent such as potassium chloride or following administration of the atypical antipsychotic clozapine.^[88] Voltametric evidence revealed that after stimulation of ascending dopamine fibres at a 20Hz frequency, tolcapone administration produced a 50% increase of dopamine efflux in the caudate putamen area and basolateral amygdaloid nucleus.^[67] Neurochemical studies indicate that tolcapone seems to be a quite specific inhibitor of COMT since it does not affect the reuptake of tritiated catecholamines, or the activities of monoamine oxidase, hydroxyindole-*O*-methyltransferase or phenyl-etha-

nolamine-*N*-methyltransferase. Similarly, it does not interact with monoaminergic, serotonergic or cholinergic receptors.^[141,142,145]

In cognitive function studies, such as the single-trial passive avoidance retention test, intraperitoneal administration of tolcapone following pre-training in rats pre-treated with scopolamine (a drug that impairs working memory performance) counteracted the effect of scopolamine and facilitated spatial working memory in intact rats. Similarly, tolcapone also prolonged the time lag before entering the dark compartment when administered during memory extinction to rats bearing nucleus basalis magnocellularis (NBM) lesions.^[148,149] Further evidence for a positive effect of tolcapone on cognition was provided by studies in which the COMT inhibitor improved the performance of senescent poor-performing rats in a spatial memory task.^[150] However, in the senescent rat model task, tolcapone was unable to counteract memory performance deficits in animals treated with scopolamine or with lesions of the NBM.^[150]

The use of more specific experimental models, such as the rat model of attentional set shifting, to test the performance and efficiency of the DLPFC, provided more cogent evidence on the usefulness of COMT inhibitors in the treatment of cognitive deficits associated with mesocortical dysfunction. In this context, it was observed that the increase in extracellular cortical dopamine concentration (but not that of noradrenaline) observed after tolcapone administration in rats pre-treated with a depolarising agent or with clozapine was significantly and specifically correlated with an improvement in the intradimensional set-shifting task.^[88]

These studies underline the role of dopamine and, mainly, COMT in the modulation of the activity of the DLPFC, and suggest potential benefits of COMT inhibitors for the treatment of cognitive disorders associated with impaired DLPFC function.

Studies on human volunteers revealed that the pharmacokinetics of tolcapone are linear over the dose range of 50–400mg. The elimination half-life of the drug is 2–3 hours and there is no significant accumulation after repeated administration. Tolcapone is absorbed, with a time to reach peak plasma concentration of approximately 1.5–2.0 hours, while the absolute bioavailability following oral administration is approximately 60–65%. The drug binds mostly to albumin and the plasma protein binding is >99.99%.^[83] Tolcapone is almost completely metabolised. It is excreted in urine and faeces, with only 0.5% excreted unchanged. Glucuronidation is the most important route of metabolism, and oxidative reactions through cytochrome P450 3A4 and 2A6 isoenzymes are of small significance.^[151] After administration of tolcapone at a dosage of 200mg three times daily for 7 days, COMT inhibition is rapid (time to maximum effect [tEmax] <2 hours), substantial (~80% inhibition) and reversible (return to baseline levels 15–21 hours following drug administration). At that same dosage, a 45% COMT inhibition is still evident in erythrocytes 6 hours following drug administration.^[152,153]

Clinical studies showed that tolcapone decreases COMT activity and increases levodopa availability when both compounds are administered in combination.^[154,155] This pharmacological property has led to the development of new pharmacotherapeutic strategies for the treatment of Parkinson's disease and a number of extensive reviews have been devoted to this issue.^[83,138,153,156] The only study where behavioural cognitive measures were assessed showed that, in patients with advanced Parkinson's disease who were evaluated with neuropsychological measures at baseline and after 6 months of treatment, the addition of tolcapone to levodopa improved function in memory and attentional tasks, including verbal short-term memory, cued recall, visuo-spatial recall, the trails B test and constructional praxia, as

well as motor symptoms.^[157] While it is unclear from this study whether these cognitive benefits are related directly to tolcapone or indirectly to an increased levodopa availability, the former interpretation has credibility because such changes are generally not seen after small increases in levodopa availability.

In line with a previous experimental study in which tolcapone displayed antidepressant-like properties in a rat model of depression,^[158] tolcapone by itself was effective for the treatment of depression in an outpatient sample of patients with a DSM-IV^[159] diagnosis of major depressive disorder. This indicates that tolcapone itself is able to exert a therapeutic action without the addition of levodopa.^[160]

5. COMT Polymorphisms and the Effect of COMT Inhibitors on Cognitive Function

Recent studies have evaluated the effects of tolcapone on measures of cognitive function and prefrontal cortical information processing in healthy volunteers. Consistent with extensive evidence in animals and humans of the role of dopamine in DLPFC information processing as noted in section 2, studies were designed based on the prediction that COMT inhibition would enhance cognition, as measured on specific cognitive tests that assess prefrontal performance, and the efficiency of prefrontal function analysed with fMRI. It was also predicted that because COMT Val108/158Met genotype influences performance on cognitive tests of DLPFC function, such as working memory and executive function,^[52,104] as well as prefrontal efficiency as assessed by fMRI^[104,106,161] and on episodic memory related to hippocampal function,^[162,163] there would be a genotype \times tolcapone interaction. This interaction was predicted to result in individuals with Val/Val alleles showing relatively greater benefit from the drug because of their higher en-

zyme activity and allegedly lower dopamine concentrations in the DLPFC.

We recently reported the results of a study of the effects of tolcapone in 47 healthy volunteers, who were matched for gender, age, education and IQ between COMT genotypes. Consistent with an earlier report on the effect of Val108/158Met genotype on RBC COMT activity,^[164] determination of whole blood baseline COMT activity in these volunteers revealed that Val/Val individuals had higher enzyme activity when compared with Val/Met and Met/Met individuals. In line with a previous study on the effect of tolcapone on human RBC COMT activity,^[165] repeated measure ANOVA of COMT activity determined 4 hours after treatment (placebo vs tolcapone) revealed a highly significant drug effect and a significant genotype \times drug interaction.^[166,167] Administration of tolcapone 600 mg/day resulted in an approximately 40% reduction in blood COMT activity.

Analysis of the effects of tolcapone and COMT Val108/158Met genotype on measures of cognitive function and DLPFC physiological efficiency assessed with fMRI provided the first evidence of a pharmacological enhancement of these functions in healthy controls by a drug without psychostimulant properties. Tolcapone significantly enhanced performance in healthy volunteers on tasks that demand speed of response in the context of working memory, namely Trails B (in which the sequencing of letters and numbers proceeds in an alternating fashion) and the n-back working memory task at high cognitive loads (in which updating of the working memory buffer is near continuous) [figure 4a].^[166,167] For intra-dimensional shifting from the CANTAB (Cambridge Neuropsychological Test Automated Battery) test battery (a measure of executive cognition) [figure 4c] and episodic memory tasks (figure 4b), the response to tolcapone was modulated by COMT genotype. In the intra-dimensional shifting test, a task that requires rule general-

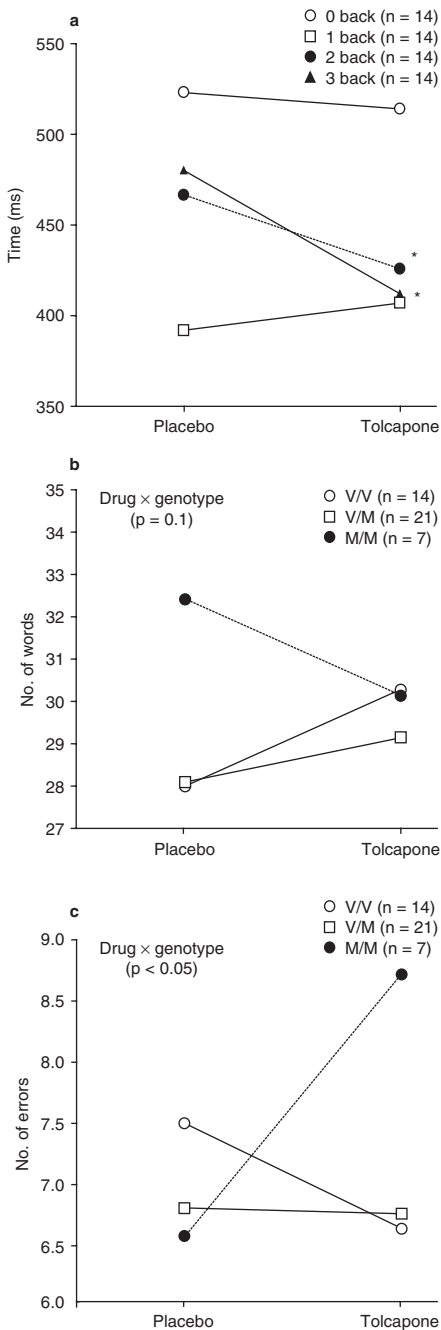


Fig. 4. Effect of placebo or tolcapone on (a) the n-back test in healthy volunteers; (b) verbal memory trials 1–3 in healthy volunteers; and (c) CANTAB (Cambridge Neuropsychological Test Automated Battery) intra-dimensional shift trials in healthy volunteers. M/M = Met/Met; V/M = Val/Met; V/V = Val/Val; * $p < 0.05$.^[166]

isation in the face of a salient distractor, a significant drug \times genotype interaction was present (figure 4c). In this test, healthy volunteers with Val/Val genotypes, who had relatively poorer working memory capacity at baseline, markedly improved while receiving tolcapone compared with those receiving placebo, while individuals with Met/Met genotypes, who had relatively higher working memory capacity at baseline, worsened. A similar, although not statistically significant, genotype effect was also observed on tests of verbal episodic memory, in which individuals with Val/Val genotypes noticeably improved on tolcapone, whereas individuals with Met/Met genotypes worsened (figure 4b).

In work from our group, COMT Val/Met genotype accounts for approximately 3–4% of the variance on tests of executive function and target detection.^[104] These results are in line with previous research on cognitive tasks in which dexamfetamine (dextroamphetamine), a drug that releases dopamine in the synaptic cleft, improved performance only in Val/Val subjects. In the Met/Met subjects, DLPFC performance deteriorated on dexamfetamine.^[168]

If treatment with tolcapone induces significant improvements in tasks that measure DLPFC cognitive performance, presumably because of its effect on dopamine availability in the frontal cortex, it may be expected that this drug may also be associated with an increased efficiency in the neurophysiological response during fMRI tasks. Analysis of the n-back-related activation responses as a main effect indicated that, when compared with placebo, tolcapone improves the information processing efficiency during the task. In fact, there is a more focused activation in brain regions related to working memory, particularly in the DLPFC bilaterally, without significant difference in accuracy or reaction time across the two drug conditions (figure 5). In this study, no overall significant genotype effects were observed on fMRI-based efficiency measures, most likely because of the limited power of the

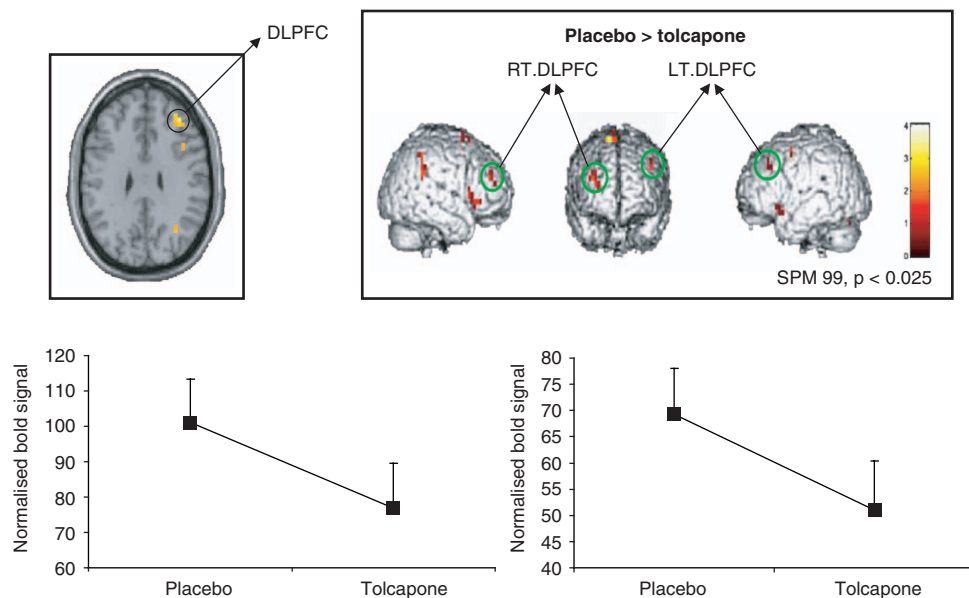


Fig. 5. Tolcapone improves the information processing efficiency in the dorsolateral prefrontal cortex (DLPFC) during the n-back task, as assessed by functional magnetic resonance imaging. **LT** = left; **RT** = right.^[166]

smaller sample available in the imaging component of the study.^[166,167]

6. Use of COMT Inhibitors and Other Dopaminergic Agents for the Treatment of Cognitive Deficits in Schizophrenia

Impairment of DLPFC function and prominent cognitive difficulties observed in patients with schizophrenia have been proposed as the main factors responsible for the poor function of these patients on activities of daily living, self-care and overall role performance.^[13,49,51,169,170] These cognitive difficulties, which are also associated with negative symptoms including blunted affect, lack of motivation, poverty of speech, avolition, etc., were proposed to be related, at least in part, to a decreased connectivity and reduced functional activity of the mesocortical afferents innervating the DLPFC.^[53,171]

The decrease in DLPFC efficiency observed in patients with schizophrenia during working memory tasks is most likely related, in part, to impaired activity of the dopamine mesocortical system, as

revealed by a number of reports.^[17,18,172-174] An increase in D₁ receptor number strongly predicts poor performance in the n-back task, a reliable test of working memory.^[175]

A number of pharmacological agents have been described as potential tools for the improvement of cognitive deficits in patients with schizophrenia. In this context, studies have shown that atypical antipsychotic agents are effective not only for the treatment of both positive and negative symptoms but also for some cognitive difficulties in those with schizophrenia.^[176-178] The mechanisms underlying this action are not yet clearly understood, although an effect was postulated through an interaction with either (a) serotonin 5-HT_{2A} receptors; or (b) a specific effect on dopamine release in the prefrontal cortex.^[179-181] Indeed, it has recently been proposed that these drugs work through a combination of postsynaptic D₂ receptor blockade and presynaptic dopamine release and D₁ receptor stimulation in the prefrontal cortex.^[57]

In line with this last hypothesis and the role of COMT in prefrontal cortical signalling, recent studies suggest that the COMT Val108/158Met polymorphism might be an important factor in the cognitive response to antipsychotic medication in patients with schizophrenia.^[182,183] In fact, patients with the low-activity COMT Met/Met allele displayed significant improvement on the n-back working memory task after administration of antipsychotics in both of these recent studies. In contrast, patients with the high-activity COMT Val/Val allele not only failed to improve but were significantly impaired on the working memory task after treatment with antipsychotics when compared with the COMT Met/Met patients. These results have implications for the understanding of antipsychotic response, and might partially explain the different responses observed in previous studies concerning cognitive improvement in schizophrenic patients treated with atypical antipsychotics.^[184]

Other pharmacological interventions aimed at boosting dopamine concentrations in the prefrontal synaptic cleft might represent important approaches to target dopamine prefrontal mechanisms and improve working memory performance and other cognitive deficits present in patients with schizophrenia. For example, dexamfetamine and other psychostimulants increase CNS alertness, modulate attention, and enhance mood and cognitive performance by potentiating monoaminergic neurotransmission. In patients with schizophrenia, amfetamine has been shown to improve performance on a measure of concept formation on the Wisconsin Card Sorting Test and enhanced psychomotor speed, and to have a positive effect in terms of affect, cooperation and engagement with the environment while improving the focusing of prefrontal regional blood flow. The treatment, however, did not result in changes in performance on other clusters of the Wisconsin Card Sorting Test or attention.^[107,185] These studies utilised amfetamine combined with a D₂ receptor

antagonist drug, presumably to block the potential psychotogenic effects of D₂ receptor activation. The possibility that the effect of amfetamine may also display a genotype effect on the prefrontal cortex of patients with schizophrenia has not yet been studied; however, in healthy volunteers, amfetamine enhanced performance on a working memory task at all levels of difficulty, as assayed with fMRI in subjects with the COMT Val/Val genotype. In contrast, in volunteers with the low-activity Met/Met genotype who display higher cognitive efficiency, amfetamine failed to affect cortical efficiency at low-to-moderate working memory load, and caused deterioration at high working memory load.^[106]

Even though dopaminomimetic drugs (e.g. stimulants) have the potential to be effective in improving cognition in patients with schizophrenia, they may also produce a number of adverse effects, such as worsening of psychosis or triggering of abnormal involuntary movements due to their effect on mesolimbic and nigrostriatal systems other than on the mesocortical pathway. From a mechanistic perspective, a more rational approach to the treatment of cognitive dysfunction in schizophrenia would be to use medications that specifically target the mesocortical system and, more selectively, the prefrontal cortex and hippocampal cortex, without acting on the other dopaminergic pathways.

As noted above, diverse evidence suggests that selective COMT inhibitors with the ability to cross the BBB specifically target the main catabolic mechanism involved in dopamine regulation in the frontal cortex and hippocampus. Thus, blockade of COMT with a COMT inhibitor such as tolcapone may result in a very circumscribed and selective increase of dopamine in the DLPFC and hippocampus without affecting subcortical regions.^[186] This strategy may prove very useful, not only to improve cognitive deficits in individuals with schizophrenia but also may significantly reduce the potential for unwanted effects related to impact on other

dopaminergic pathways. The studies showing increased cognitive performance and prefrontal cortex efficiency after tolcapone in healthy volunteers (see section 5) open up new avenues for the pharmacological treatment of cognitive disorders.

Strategies that selectively increase dopamine in the frontal cortex through mechanisms other than COMT blockade may also prove useful for the treatment of cognitive deficits associated with reduced DLPFC function. In this context, modafinil, an eugeroic drug developed as a wakefulness-promoting agent for the treatment of narcolepsy, is also able to increase extracellular dopamine in the prefrontal cortex^[187] and to improve short-term working memory in rodents.^[188] This agent may also lead to novel strategies for the treatment of frontal lobe dysfunction in patients with schizophrenia. A previous study in healthy subjects showed that modafinil at a dosage of 100–200 mg/day significantly improved cognitive ability in tests of digit span, visual pattern recognition memory, spatial planning and stop-signal reaction, and slowed latency on the delayed matching to sample and spatial planning tasks.^[189] While the pharmacological mechanism of these effects is not known, from a pharmacotherapeutic perspective, modafinil presents limited adverse effects and no major clinically relevant drug interactions with other psychotropic drugs.

Recent evidence further suggests that the response to modafinil in individuals with narcolepsy seems to be closely associated with the COMT Val/Met genotype. In fact, subjects who bear the low activity COMT Met/Met genotype have a more effective response to modafinil in terms of the reduction of recurrent daytime sleepiness episodes when compared with Val/Val individuals.^[190] Whether this effect indicates an action of modafinil at the level of cortical dopamine signalling or is an indirect reflection of other actions of this drug is not known.

Another potential target for the regulation of dopamine flux in the prefrontal cortex is represented

by the NET.^[72,74,78] As noted in section 2, NETs take up dopamine in the cortex, and blocking NETs leads to an increase in cortical dopamine concentrations without changes in subcortical dopamine concentrations. This effect has been demonstrated for the specific noradrenaline reuptake inhibitor, atomoxetine,^[191,192] a drug that does not have a psychotomimetic effect or abuse potential. Because of its unique mechanism of action that results in a selective increase of dopamine in the frontal cortex, atomoxetine represents a potentially important candidate as a tool for the treatment of conditions that involve working memory difficulties such as schizophrenia and other neuropsychiatric problems with associated frontal lobe dysfunction. This is yet to be systematically explored. Also, whether the response to atomoxetine is associated with COMT genotype is not yet known. Its low addictive potential, its good adverse effect profile and the limited potential for pharmacokinetic drug interactions make atomoxetine an interesting candidate for the treatment of cognitive dysfunction.

7. Conclusions

The identification of core mechanisms involved in the pathophysiology of cognitive impairments associated with schizophrenia coupled with the discovery of genetic polymorphisms that represent risk factors responsible for these impairments has opened up new avenues for the understanding and treatment of this complex and multidimensional disease. The COMT Val108/158Met polymorphism, which affects how prefrontal information is managed and processed, is associated with one of the core neurobiological abnormalities present in schizophrenia. The fact that subjects homozygous for the COMT Val polymorphism perform worst in tests of executive cognition, episodic memory and attentional control than those homozygous for the Met allele, even healthy individuals, underscores the importance of this gene in information processing

under physiological conditions. Because of their cognitive deterioration, patients with schizophrenia start at a cognitive capacity far below their unaffected siblings and healthy subjects; thus, the negative impact of the COMT Val polymorphism on their information processing abilities is more prominent.

The availability of COMT inhibitors that readily cross the BBB, such as tolcapone, provides a valuable tool to improve prefrontal executive cognitive function, putatively by selectively increasing dopamine turnover in the prefrontal and possibly temporal cortices without affecting other neuronal circuits that use dopamine as a neurotransmitter. The unique mechanism of action of tolcapone provides a distinct example of 'targeted' pharmacology for the treatment of cognitive disorders in patients with schizophrenia, and opens the field for the pharmacotherapy of nonschizophrenia-related cognitive disorders associated with dysfunction of the frontal cortex. Initial proof-of-concept studies involving healthy volunteers indicated that there may be an interaction of tolcapone with COMT genotype that may modify the effect of the drug on prefrontal efficiency. This clearly illustrates the possibility of genotype-targeted pharmacology for the treatment of cognitive dysfunction associated with schizophrenia. Even though tolcapone has proved useful in this regard, its hepatotoxicity proscribes its widespread use. The development of newer COMT inhibitors that can permeate the BBB effectively and are devoid of serious adverse effects will allow expansion of the search for more specific, selective and effective therapies for the treatment of cognitive disorders. Furthermore, the availability of newer drugs that affect dopamine concentrations in the frontal cortex through pharmacological mechanisms of action other than COMT inhibition, but whose therapeutic response is modified by COMT genotype, clearly represents another example of genotype-targeted pharmacology.

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