THE GENETICS OF SCHIZOPHRENIA: AN fMRI INVESTIGATION OF ENDOPHENOTYPES IN UNAFFECTED SIBLINGS AND AN EXAMINATION OF THE NEUROPSYCHOLOGICAL CORRELATES OF COMT VAL108/158MET GENOTYPE IN PATIENTS

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To My Parents

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CHAPTER I

INTRODUCTION

A genetic basis for schizophrenia is well established as there is a clear relationship between risk for schizophrenia and genetic relatedness (Gottesman, 1991). The offspring of a parent with schizophrenia are at substantially greater risk for developing schizophrenia than subjects in the general population, and monozygotic twins demonstrate concordance rates of approximately 46% (Cardno & Gottesman, 2000; Kringlen, 2000; Shields & Gottesman, 1972). However, the lack of 100% concordance in monozygotic twins and the non-Mendellian pattern of disease distribution in affected families clearly implies that schizophrenia is a polygenetic disorder with a complicated etiology involving a dynamic interplay between multiple susceptibility genes and environmental factors (Gottesman & Shields, 1967; Gottesman & Gould, 2003; Shields et al., 1972; Caspi et al., 2005). Unfortunately, linkage studies have yielded few consistent findings (Tsuang & Faraone, 2000; Riley & McGuffin, 2000). Part of the difficulty in identifying susceptibility genes undoubtedly relates to the complex etiology of schizophrenia, the heterogeneous nature of the disorder, and the difficulty in parsing the phenotype(s) of the disorder (Tsuang & Faraone, 1995; Tsuang et al., 2000; Weinberger et al., 2001; Gottesman et al., 2003).

The identification of endophenotypes can greatly assist in the search for genes that confer susceptibility for psychiatric illnesses with complex inheritance patterns such as schizophrenia (Gottesman et al., 2003). Endophenotypes are essentially covert phenotypes not detectable to the “unaided eye” and include neurophysiological, biochemical, or neuropsychological markers of an illness that are frequently present in affected individuals. The basic rationale for using endophenotypes to identify susceptibility genes is based on the underlying assumption that the complexity between genotype and the emergent phenotype can be reduced by identifying more basic phenomena, as opposed to complex behavioral repertoires or overarching diagnostic

categorizations, related to the disease process under investigation (Gottesman et al., 2003). That is, fewer genes underlie the production and expression of more elementary phenotypes associated with the illness and are, therefore, more amenable to genetic analysis than complex behavioral phenomena associated with diagnostic categorizations.

Examination of unaffected family members of diseases with complex inheritance patterns such as schizophrenia can provide insight into the underlying pathological mechanisms of disorders that are related to genetic liability. Unaffected relatives of index cases share a considerable portion of their genes with an effected family member and in certain cases may be disease carriers (so called "obligate carriers") that transmit liability for schizophrenia to their offspring without ever expressing the illness themselves. Moreover, examination of first-degree relatives provides a unique opportunity to examine the genetic components of schizophrenia without the presence of confounding factors such as the effect of chronic medication, hospitalization, or aspects related to the expression of the illness itself (i.e. poor motivation, illness symptomatology, etc.). In fact, for putative endophenotypes to be useful in assisting in the search for susceptibility genes they must be present in unaffected family members of index cases at a greater rate than observed in the general population. Deficits in smooth pursuit eye movements, prepulse inhibition, and cognitive function, along with alterations in cerebral neurophysiological response during the performance of various cognitive tasks that are markedly similar to those observed in patients have been detected in unaffected relatives suggesting that they may be useful endophenotypes for guiding genetic research (Myles-Worsley & Park, 2002; Callicott et al., 2003a; Gottesman et al., 2003; Raemaekers, Ramsey, Vink, van den Heuval, & Kahn, 2005). Findings from functional imaging studies are especially intriguing as dysfunction of the frontal lobes and basal ganglia has been identified in both patients and their unaffected siblings, even in the absence of overt deficits in cognitive performance (Callicott et al., 2003a; Raemaekers et al., 2005). This indicates that abnormal cerebral physiological response to cognitive tasks may be more tightly coupled to the underlying neuropathology and genetic liability for schizophrenia than actual cognitive performance and, as such, may be a more sensitive endophenotype for genetic analysis.

Experiment One of this dissertation examined functional brain activity in normal controls and unaffected siblings of patients with schizophrenia during performance of a procedural learning task, the Serial Reaction Time task. This task is associated with robust activations in frontal, parietal, and striatal brain regions. Two studies have shown that patients with schizophrenia fail to recruit the same brain regions, frontal lobes and striatum in particular, during performance of this task (Kumari et al., 2002), despite the fact that patients demonstrated relatively normal performance in one of the studies . Thus, examination of unaffected siblings may further strengthen evidence that fronto-striatal dysfunction is a candidate endophenotype for schizophrenia and potentially may implicate additional brain regions that are relevant to the pathophysiology and genetic risk for schizophrenia.

Genes related to cognition, particularly processes that tap prefrontal cognitive functions such as working memory and executive functions, have attracted considerable attention as potential susceptibility genes for schizophrenia due to the fact that abnormalities in prefrontal cognitive functions, neurophysiology, and morphology are ubiquitous in schizophrenia (for review see Weinberger et al., 2001). Moreover, there is considerable evidence that the cognitive deficits and negative symptoms of schizophrenia are due, in part, to hypo-function of the mesocortical dopamine (DA) system and subsequent alterations in prefrontal cortical neurophysiology (Doran et al., 1987; Goldberg et al., 1988; Knable & Weinberger, 1997; Abi-Dargham et al., 2002; Akil et al., 1999; Davis, Kahn, Ko, & Davidson, 1991; Weinberger, Berman, & Illowsky, 1988). The endophenotype approach has already identified at least one potential susceptibility gene, the COMT val108/158met single nucleotide polymorphism (SNP), that is linked to working memory and prefrontal cortex (PFC) physiology by virtue of it's impact on the mesocortical DA system (Egan et al., 2001). The val allele of the COMT polymorphism results in the transcription of an enzyme with greater activity, and, as such, reduced availability of DA in the PFC (Weinshilboum & Raymond, 1977; Weinshilboum, Otterness, & Szumlanski, 1999; Matsumoto et al., 2003; Chen et al., 2004). Individuals homozygous for the val allele demonstrate relative impairments in working memory and

executive function and altered physiological activity of the dorsolateral PFC during performance of working memory tasks (Egan et al., 2001), presumably as a consequence of altered mesocortical DA function (Meyer-Lindenberg et al., 2005; Gogos et al., 1998). As such, the COMT val108/158met polymorphism may be a susceptibility gene given that working memory impairment is a cardinal feature of schizophrenia and is also present in unaffected relatives of schizophrenia patients (Park & Holzman, 1992; Park, Holzman, & Goldman-Rakic, 1995; Myles-Worsley et al., 2002). This indeed appears to be the case as family-based genetic studies have identified greater transmission of the val allele to schizophrenia probands (Egan et al., 2001), although the magnitude of the relationship is small (Fan et al., 2005). Moreover, two recent studies identified associations between COMT genotype and working memory improvement with atypical antipsychotics (Bertolino et al., 2004; Weickert et al., 2004), suggesting that the COMT val108/158met polymorphism also has important consequences for the treatment of schizophrenia. However, the small sample sizes included in these studies and circumscribed neuropsychological battery administered in both cases limit the conclusions that can be drawn and provide little insight into the specificity of the interactions.

Experiment Two of this dissertation begins with an overview of the anatomy and function of the mesocortical DA in cognition, and a brief description of the genetic regulation and role of COMT in the metabolism of DA in the PFC. Following that, evidence that COMT genotype is related to cognition is reviewed and the results from two experiments desgined to examine associations between COMT genotype, cognitive function, and cognitive change with the atypical antipsychotic clozapine in a sample of patients with schizophrenia are reported.

CHAPTER II. AN FMRI INVESTIGATION OF PROCEDURAL LEARNING IN UNAFFECTED SIBLINGS OF PATIENTS WITH SCHIZOPHRENIA

Introduction

Cognitive impairment is a hallmark of schizophrenia and is arguably the most debilitating aspect of the illness (Heinrichs & Zakzanis, 1998; Kolb & Whishaw, 1983; Green, 1996; Green, Kern, Braff, & Mintz, 2000). Patients perform well below normal on a broad array of neuropsychological tests; however, deficits in attention, learning and memory, and executive functions, including working memory, are especially robust (Heinrichs et al., 1998; Bilder et al., 2000; Park et al., 1992). The deficits are enduring, relatively stable features of the illness detectable at the first psychotic episode and not attributable to florid psychotic symptoms or medications (Hoff, Riordan, O'Donnell, Morris, & DeLisi, 1992; Hoff et al., 1999; Bilder et al., 2000; Heaton et al., 2001). Furthermore, retrospective studies of patients and longitudinal investigations of prodromal at-risk individuals indicate that cognitive impairments, particularly deficits in attention and fine motor coordination, are present prior to the onset of psychotic symptoms, are detectable as early as childhood, and are likely neurodevelopmental in origin (Cornblatt, Obuchowski, Roberts, Pollack, & Erlenmeyer-Kimling, 1999; Cornblatt, Obuchowski, Schnur, & O'Brien, 1998; Cornblatt et al., 2003; Erlenmeyer-Kimling et al., 2000; Erlenmeyer-Kimling & Cornblatt, 1992).

Just as important from an etiological perspective is the fact that some of the deficits observed in schizophrenia patients are also present in their unaffected relatives, indicating that cognitive dysfunction is related to genetic liability for schizophrenia and may be a useful endophenotype for genetic analysis. Relatives demonstrate moderate deficits on experimentally derived measure of attention, the Continuous Performance Test-Identical Pairs version (Keilp, Herrera, Stritzke, & Cornblatt, 1997), and spatial working memory (Cornblatt & Keilp, 1994; Erlenmeyer-Kimling et al., 1992; Myles-

Worsley et al., 2002; Park et al., 1995; Cornblatt & Malhotra, 2001). Relatives of patients with schizophrenia also demonstrate moderate impairments on the order of one half standard deviation below controls on clinical neuropsychological tests of verbal memory and visuomotor/set-shifting skills such as the California Verbal Learning Test (CVLT) and Trailmaking B test (TMB) (Sitskoorn, Aleman, Ebisch, Appels, & Kahn, 2004). Lesser, but still significant, impairments are also observed on tests of visuomotor speed, and non-verbal memory such as the Trailmaking A (TMA), and Wechsler Memory Scale Visual Reproduction Sub-test, respectively (Sitskoorn et al., 2004). The deficits observed in patients and unaffected first-degree relatives for selected neuropsychological measures are presented in Figure 1. It is noteworthy that the cognitive functions most impaired in schizophrenia, verbal memory and aspects of executive functions, are also

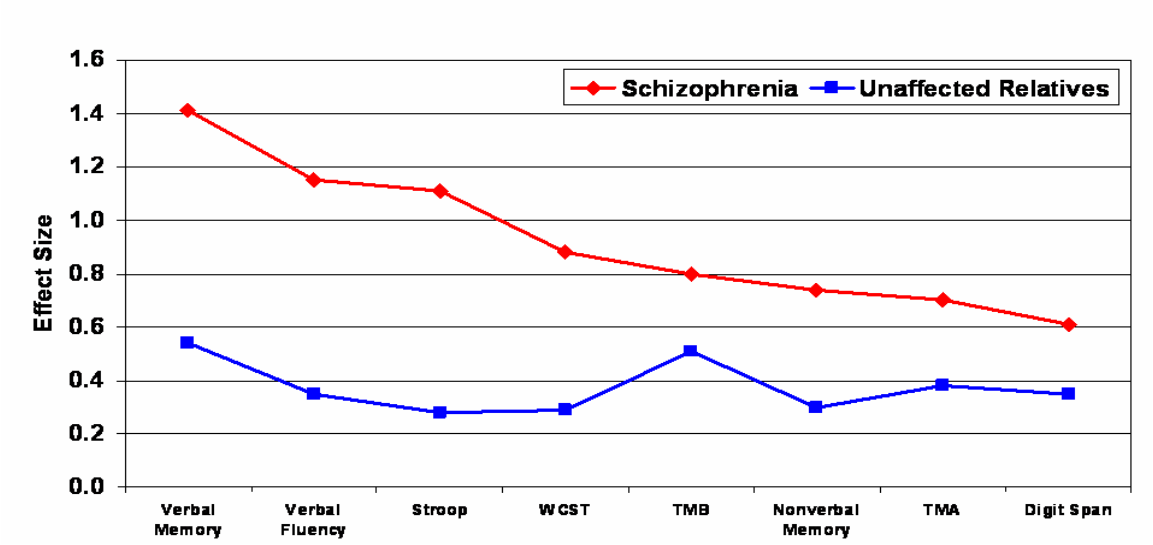

Figure 1. Neuropsychological impairment in patients with schizophrenia and their unaffected relatives. Derived from Sistkoorn et al., 2004 and Heinrichs and Zakzanis, 1998. Abbreviations: WCST: Wisconsin Card Sorting Test; TMA: Trailmaking A Test; TMB: Trailmaking B Test.

There are two important features regarding the magnitude and pattern of cognitive deficits observed in unaffected relatives of patients with schizophrenia. The first is that the magnitude of cognitive impairment detected in unaffected relatives is substantially less than that observed in patients. This is understandable given that unaffected relatives

share, at most, 50% of their genes with an ill family member. However, even unaffected monozygotic co-twins discordant for schizophrenia do not show the same degree of impairment as affected co-twins (Goldberg et al., 1993; Cannon et al., 2000). Thus, while there is evidence for genetic loading (i.e. greater impairment with increasing genetic liability) for some cognitive deficits, it is clear that environmental factors play a role in the etiology of cognitive impairment schizophrenia. The second major finding to come out of cognitive studies of unaffected relatives is that the patterns of impairments observed in patients and relatives do not always correspond with one another. That is, the cognitive functions most impaired in schizophrenia are not always the most impaired functions in their unaffected relatives. For example, schizophrenia patients perform well below controls on tests of attention, such as the Stroop Test, and language functioning, such as verbal fluency; however, unaffected family members perform only marginally below controls on these tests further indicating that although patients and relatives share many of the same cognitive deficits, there are some aspects of cognitive impairment that are not shared and are likely unrelated to genetic liability for the disorder.

A multitude of studies indicate that alterations in cerebral morphology and function underlie cognitive impairment in schizophrenia. With respect to cerebral morphology, reports of enlarged lateral ventricles and grey matter volume reductions within structures of the temporal lobe, superior temporal gyrus in particular and hippocampus to a lesser extent, are numerous (for review see Shenton, Dickey, Frumin, & McCarley, 2001). Reductions in PFC grey matter volume and cortical thickness are also consistently reported (Molina et al., 2005; Ananth et al., 2002; Hulshoff Pol et al., 2002; Crespo-Facorro, Kim, Andreasen, O'Leary, & Magnotta, 2000; Nopoulos et al., 1995; Narr et al., 2005). Volumetric reductions in additional cortical areas, regions of the parietal lobes in particular, and sub-cortical structures, thalamus and basal ganglia, is also be present, but the findings for the latter remain equivocal due to the difficulty in measuring the thalamus on MRI scans and the fact that antipsychotic treatments can alter dorsal striatum volumes (Shenton et al., 2001; Lang et al., 2004; Corson, Nopoulos, Miller, Arndt, & Andreasen, 1999; Chakos et al., 1994; Narr et al., 2005). Overall the findings from structural neuroimaging studies indicate that alterations in diverse cortical-

cortical and cortical-sub-cortical networks may be related to the pathophysiology of schizophrenia. Moreover, there is compelling evidence that many of the morphological alterations are related to cognitive impairment. Significant inverse associations between whole brain volume and premorbid intellect and between PFC volumes or temporal lobe volumes and executive, memory, and psychomotor functions have been detected (Antonova et al., 2005; for review see Antonova, Sharma, Morris, & Kumari, 2004).

In terms of cerebral function, early PET studies suggested that schizophrenia patients might be best described as "hypofrontal" at rest and when performing cognitive tasks (Gur & Gur, 1995; Weinberger & Berman, 1996). However, uncertainty as to exactly what "at rest" implied for brain imaging and the fact that patients often performed well below controls on cognitive tasks performed during scanning posed significant interpretational challenges to these studies (Weinberger et al., 1996). More recently, a picture of functional impairments in schizophrenia supporting and extending the structural findings implicating dysfunction in cortico-cortical and cortical-sub-cortical networks has emerged. Indeed, proponents of a network model for schizophrenia have speculated that not only cognitive impairment, but also many of the symptoms observed in the illness may relate to a core deficit in cortico-cerrebellar-thalamo-cortical circuits (CCTCC) and/or cortico-striatal circuits (e.g. Pantelis, Barnes, & Nelson, 1992; Andreasen et al., 1999; Buchsbaum, Hazlett, Haznedar, Spiegel-Cohen, & Wei, 1999). In a series of PET studies, Andreasen and colleagues consistently identified dysfunction in cortical, thalamic, and cerrebellar regions across an array of tasks that could not be explained by performance differences between patients and controls. These findings prompted them to forward the "cognitive dysmetria" model of schizophrenia which posits that the symptoms and cognitive deficits of schizophrenia relate to a core deficit in CCTCC (for review see Andreasen et al., 1999). Recent fMRI findings continue to support this model (Mendrek et al., 2005; Mendrek et al., 2004; Honey et al., 2005). Additional findings also suggested that some of the deficits in attention and working memory in particular may relate to abnormal function of fronto-parietal and fronto-striatal networks (Morey et al., 2005; O'Driscoll et al., 1999; Kumari et al., 2002; Manoach, 2003; Stevens, Goldman-Rakic, Gore, Fulbright, & Wexler, 1998; Callicott et

al., 2003b; Manoach et al., 2000; Braus, Weber-Fahr, Tost, Ruf, & Henn, 2002). It should be noted that although the findings strongly imply that dysfunction of neural networks is related to cognitive impairment in schizophrenia, it does not rule out the distinct possibility that some of the abnormalities detected may represent downstream effects of a core deficit within a specific brain region(s) (Lipska & Weinberger, 2002; Weinberger et al., 2001).

Given the associations between cognitive impairment, abnormalities in cerebral structure, and altered neurophysiology in schizophrenia, and the observation that many of the same cognitive deficits observed in patients are also detected in their unaffected relatives, it stands to reason that unaffected relatives of patients may also demonstrate abnormalities in cerebral structure and/or function and that these abnormalities are linked to genetic liability for schizophrenia. Results from a growing literature suggest that this is indeed the case, although, similar to the findings reported for cognitive function, some aspects of the alterations in cerebral morphology appear related to genetic liability whereas other deficits appear specific to the disease process itself (van Erp et al., 2004; Cannon et al., 2002; Narr et al., 2002; Steel et al., 2002). Grey matter volume reductions in structures of the temporal lobe, hippocampus and temporal pole in particular, and regions of the frontal lobe, dorsolateral, polar, and premotor cortex, that are qualitatively similar, although quantitatively less, than the volumetric reductions observed in patients are present in relatives (Cannon et al., 2002; Narr et al., 2002; McDonald et al., 2004; Tepest, Wang, Miller, Falkai, & Csernansky, 2003; Steel et al., 2002; Staal, Hulshoff Pol, Schnack, van der Schot, & Kahn, 1998). Volumetric reductions in sub-cortical structures, including striatum and thalamus, may also be present (McDonald et al., 2004; McIntosh et al., 2004). Imaging studies of monzygotic and dizygotic twins discordant for schizophrenia and healthy twins indicate that cortical grey matter volume reductions in the dorsolateral and polar PFC in particular are strongly correlated with genetic risk for schizophrenia (Cannon et al., 2002). On the other hand, reduced grey matter volumes in other sub-regions of the PFC, portions of the superior temporal gyrus, and parietal lobe appear unique to the disease process and unrelated to genetic susceptibility (Cannon et al., 2002).

There is also compelling evidence that some aspects of the abnormalities in cerebral activity detected in patients during performance of a variety of cognitive tasks are also observed in their unaffected relatives; although relatively few studies have been carried out and most focused on working memory. A preliminary investigation of spatial working memory using the ocular delayed response (ODR) task identified hypo-activation of the dorsolateral PFC, inferior parietal cortex, and middle frontal gyrus in a small sample of four offspring of schizophrenia patients; however, lack of behavioral data collection during the scanning session limit interpretation of the results (Keshavan et al., 2002). Abnormal frontal lobe activity during performance of verbal working memory tasks has been reported in two studies (Callicott et al., 2003a; Thermenos et al., 2004). Specifically, in a series of studies by Callicott and colleagues (Callicott et al., 2000; Callicott et al., 2003b; Callicott et al., 2003a) both patients and two independent samples of unaffected siblings evinced greater activity in the dorsolateral and inferior PFC during performance of a verbal n-back task despite performing relatively normally compared to controls. Unaffected siblings also demonstrated relatively increased activity in the inferior parietal lobule, and reduced activity in the medial frontal gyrus, posterior cingulate, thalamus, and cerebellum (Callicott et al., 2003a). Increased activation of the PFC in unaffected relatives was also observed in a more recent investigation of verbal working memory (Thermenos et al., 2004), although the magnitude of the differences in cerebral activity observed between the first degree relatives and controls were attenuated somewhat after controlling for task performance.

The observation that patients and unaffected siblings tend to demonstrate greater activity than expected during performance of working memory tasks is, at first glance, somewhat counter-intuitive as greater activity is generally interpreted as beneficial within the context of most neuroimaging studies. Imaging studies have revealed that individual differences in working memory performance relates directly to the degree of activity observed in the PFC (Mattay et al., 2000; Rypma et al., 2002). Specifically, individuals with greater working memory capacity exhibit less activation at any given working memory load level than individuals with lower working memory capacity; although

dorsolateral PFC activity plateaus at peak working memory loads and decreases once load exceeds capacity for all subjects (Callicott et al., 1999). Some investigators have interpreted these observation as evidence that subjects with better working memory performance have greater dorsolateral cortical "efficiency" (Callicott et al., 1999; Callicott et al., 2003b; Weinberger et al., 2001) than lower performing subjects, perhaps as a result of employing more efficient memory strategies (Rypma et al., 2002). That is, they demonstrate an activity-response curve that is shifted to right of those with lower working memory capacity. Thus, patients and siblings, who generally tend to demonstrate reduced working memory capacity compared to controls, can demonstrate hypo- or hyper-active activity in the PFC depending on the working memory load. Specifically, they can appear hyperactive if examined at a working memory load level below capacity, and hypo-active when examined at a load level beyond capacity, relative to control subjects.

Unaffected relatives also demonstrate abnormal neurophysiological response in the frontal lobes and basal ganglia during performance of other tasks such as eye tracking and antisaccade tasks, respectively (O'Driscoll et al., 1999; Raemaekers et al., 2005). Specifically, first-degree relatives with eye tracking deficits fail to activate the frontal eye fields to the same extent as first-degree relatives and controls without eye tracking deficits. In addition, failure to recruit the caudate when making antisaccades has been detected in both patients and their unaffected siblings and in the case of the siblings the abnormal cerebral response was detected in the absence of an overt deficit in performance (Raemaekers et al., 2005; Raemaekers et al., 2002). Combined, the studies suggest that unaffected relatives, especially siblings, demonstrate abnormal activation of frontal-sub-cortical circuits during performance of a variety of tasks and that the alterations in brain activity are not an artifact of impaired behavioral performance. The latter point is especially important as it indicates that altered cerebral physiological response during cognitive challenge may be a more useful endophenotype for genetic analysis than overt behavioral performance (Callicott & Weinberger, 2003c).

The goal of the current experiment was to identify the neural correlates of performance on the Serial Reaction Time (SRT) task (Nissen & Bullemer, 1987), a commonly used test of procedural learning, in a sample of unaffected siblings of patients with schizophrenia and an age matched group of controls with no family history of schizophrenia. Procedural learning refers to the ability to acquire a motor skill or cognitive routine in the absence of declarative knowledge (Cohen & Squire, 1980). Briefly, the SRT task requires subjects to react to the location of a target presented sequentially in one of several spatial locations as rapidly as possibly by depressing one of several keys, each of which corresponds with a specific target location (e.g. Destrebecqz & Cleeremans, 2001). Unbeknownst to subjects, during some blocks of trials the location of the target follows a predetermined sequence, typically 10-12 locations in length, whereas in other blocks the location of the targets is pseudorandom. Over time procedural learning occurs as subjects get faster at responding during blocks where the target follows a repeating sequence relative to blocks where targets are presented pseudorandomly and this advantage often occurs in the absence of explicit knowledge about the structure of the sequence (Destrebecqz et al., 2001; Willingham, Salidis, & Gabrieli, 2002). The task consistently dissociates fronto-sub-cortical implicit memory systems from medial temporal lobe systems related to explicit memory. Specifically, the SRT is sensitive to disease processes involving dysfunction of the dorsal striatum, such as Huntington's (HD) and Parkinson's Diseases (PD), basal ganglia infarct, and lesions of the PFC or cerebellum (Knopman & Nissen, 1991; Gomez, Grafman, Ruiz, I, Pascual-Leone, & Garcia-Monco, 2002; Stefanova, Kostic, Ziropadja, Markovic, & Ocic, 2000; Gomez, Grafman, Pascual-Leone, & Garcia-Monco, 1999; Sommer, Grafman, Clark, & Hallett, 1999; Gomez-Beldarrain, Garcia-Monco, Rubio, & Pascual-Leone, 1998; Vakil, Kahan, Huberman, & Osimani, 2000), but not primarily temporal lobe degenerative dementias such as Alzheimer's disease (Knopman & Nissen, 1987) or amnestic disorders (Nissen, Willingham, & Hartman, 1989). Imaging studies have confirmed the importance of sub-cortical structures to the procedural learning component of the SRT task and elaborated on the cortical contributions. Despite variability in sample characteristics, imaging methods, and experimental design, both PET and fMRI studies have consistently identify activations in regions of the caudate/putamen, globus pallidum,

superior and inferior frontal cortex, anterior cingulate, inferior parietal lobe, and cerebellum when sequenced blocks are contrasted with random blocks of the SRT task (Willingham et al., 2002; Martis, Wright, McMullin, Shin, & Rauch, 2004; Rauch et al., 1997; Rauch et al., 2001; Schendan, Searl, Melrose, & Stern, 2003; Kumari et al., 2002; Daselaar, Rombouts, Veltman, Raaijmakers, & Jonker, 2003; Thomas et al., 2004; Zedkova, Woodward, Harding, Tibbo, & Purdon, 2006).

Procedural learning, as measured using a variety of measures is generally reported to be intact in schizophrenia (Altshuler et al., 2004; Purdon, Woodward, Lindborg, & Stip, 2003; Takano et al., 2002; Clare, McKenna, Mortimer, & Baddeley, 1993); although impairment has been reported in a minority of studies (Scherer, Stip, Paquet, & Bedard, 2003). In many cases it is difficult to ascribe the impairment to a pathophysiological disturbance related to schizophrenia due to the fact that patients were assessed while taking typical antipsychotic drugs (APDs) which are potent striatal D2 receptor antagonists and impair procedural learning in schizophrenia patients and controls (Kumari et al., 1997; Purdon, Woodward, Mintz, & LaBelle, 2002; Purdon et al., 2003; Bedard, Scherer, Delorimier, Stip, & Lalonde, 1996; Bedard et al., 2000; Peretti et al., 1997; Scherer et al., 2004; Danion et al., 1992; Schwartz, Rosse, Veazey, & Deutsch, 1996). Studies using the SRT task have been more equivocal with reports of intact procedural learning (Stevens et al., 2002; Zedkova et al., 2006), and slight (Green, Kern, Williams, McGurk, & Kee, 1997a) to significant impairment (Kumari et al., 2002; Exner, Weniger, Schmidt-Samoa, & Irle, 2006). As with other procedural learning measures, D2 receptor antagonism has been linked to impaired performance on the SRT task in patients with schizophrenia (Stevens et al., 2002). Interestingly, a longitudinal study of SRT task performance in patients found that patients were impaired during the acute phase of the illness, but not after a 20 month stabilization period (Exner, Boucsein, Degner, & Irle, 2006). Although no study has assessed procedural learning with the SRT task in unaffected relatives, impairment is unlikely based on findings of intact procedural learning on other measures in monozygotic twins discordant for schizophrenia (Goldberg et al., 1993) and the equivocal findings from the SRT task in patients. Methodological differences between studies likely account for some of the variable findings; however, the

failure to consistently find a deficit in procedural learning is incongruous with hypotheses that the pathophysiology of schizophrenia is related to dysfunction of CCTCC and/or fronto-striatal circuit dysfunction.

Two previous imaging studies of SRT performance in schizophrenia produced several important findings that may explain this discrepancy. The first study, by Kumari and colleagues, revealed a performance deficit in patients that was accompanied by an absence of activity in the frontal cortex, striatum, thalamus, and cerebellum relative to the control sample (Kumari et al., 2002); findings that are consistent with fronto-striatal and CCTCC dysfunction. Unfortunately, the results of this study are difficult to interpret due to the fact that patients were receiving typical APDs at the time of scanning, there were marked performance differences between patients and controls, and a failure to equate the rate of trial presentations resulted in controls being exposed to considerably more trials during sequenced blocks, relative to random blocks, than patients. A more recent fMRI study by Zedkova and colleagues (Zedkova et al., 2006) avoided many of these confounds by scanning subjects that were predominantly receiving atypical medications, drugs that have a more benign D2 binding profile and do not impair procedural learning (Purdon et al., 2002; Purdon et al., 2003; Stevens et al., 2002), and ensuring that patients and controls were exposed to the same number of trials during scanning. This study also revealed that patients fail to activate the PFC and striatum during performance of the SRT and identified an additional deficit within the parietal lobe. More importantly, the cerebral abnormalities were detected in the absence of a deficit in behavioral performance indicating that alterations in fronto-striatal and fronto-pareital networks are not related to performance differences, but are genuine alterations in neurophysiology, thereby supporting frontal-sub-cortical abnormalities in schizophrenia and buttressing the notion that neuroimaging is more sensitive at detecting schizophrenia phenotypes related to abnormal cerebral function than neuropsychological measures (Andreasen et al., 1999; Callicott et al., 2003c). Interestingly, patients demonstrated relative over-activations in the left globus pallidum, dorsal cingulate, and temporal lobe suggesting that they may compensate for a deficit in fronto-striatal circuit function by recruiting regions not typically involved in procedural learning on the SRT task. A similar compensation was

observed in saple of asymptomatic HD patients scanned while performing the SRT task (Kim et al., 2004).

Investigation of unaffected relatives of patients may help clarify the nature of the abnormal neurophysiological responses detected in patients when performing the SRT task. The findings reported by Kumari et al. (2002) suggest that SRT performance deficits in schizophrenia result from a failure to activate structures central to procedural learning and that this may reflect a core deficit in schizophrenia, but may also be a deleterious side effect of treatment with typical APDs. On the other hand, the findings reported by Zedkova et al. (2006) suggest that patients are capable of performing the SRT task normally, when receiving predominantly atypical APDs, and that patients may compensate for a failure to activate regions normally implicated in the SRT task by recruiting alternate regions, perhaps as a consequence of genetic liability and/or neurodevelopmental insult(s). If siblings also fail to activate the same regions compared to controls during SRT performance, in the absence of a performance deficit, then the alterations observed in patients may relate to genetic liability for schizophrenia. Conversely, if siblings demonstrate normal cerebral activity during SRT performance then the deficits observed in patients likely reflects disease specific alterations unrelated to genetic liability for schizophrenia or a deleterious effect of medications.

Methods

Subjects

Twelve unaffected siblings of patients with schizophrenia and fifteen age-matched controls between the ages of 18 and 55 were recruited for this study. All subjects were right handed. Subjects were recruited from the Edmonton, Alberta, Canada region and the study was approved by the Health Research Ethics Review Board of the University of Alberta. Subjects were provided a verbal and written description of the study prior to solicitation of written informed consent to participate. Exclusion criteria

included current or prior history of DSM-IV Axis I psychopathology, as determined using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID: First, Spitzer, Gibbon, & Williams, 1996), current use of any psychotropic medication, history of head injury or neurological disease, presence of systemic medical disease likely to affect central nervous system functions, current or previous alcohol/substance abuse, and the presence of ferromagnetic objects in the body. In addition, positive family history of schizophrenia was also an exclusion criterion for control subjects. Socioeconomic status was assessed according to the five-point scale presented in Appendix A. In addition, the Schizotypal Personality Questionnaire (SPQ: Raine, 1991) was mailed out to 11 of the siblings after they participated in the experiment (1 subject completed the SPQ during their scanning visit). SPQ data was available for 8 subjects and the mean score from these 8 subjects was 5.8, SD=2.9, which is well below the mean score of 26.9, SD=11.0, derived from the initial normative data gathered on the SPQ (Raine, 1991). Demographic data for the subjects is presented in Table 1.

Table 1: Experiment One Sample Characteristics*.

Variable	Controls	Siblings	**Test Statistic**
n	15	12	
Age	31.3 (11.2)	36.9 (13.3)	t(25)=1.19, p<.248
Education	17.2 (2.6)	15.0 (2.3)	t(25)=2.37, p<.027
Parental SES**	2.6 (0.5)	3.2 (0.8)	t(25)=2.18, p<.040
Sex (Men/Women)	10/5	5/7	x2=1.68, p<.195
SPQ Scores (n=8)	--	5.8 (2.9)	--

* Mean and (SD)

** Parental Socioeconomic Status (SES). Note: Lower scores equal higher status.

Behavioral Paradigm

The paradigm and procedures were identical to those used in our previous report on procedural learning in patients with schizophrenia (Zedkova et al., 2006) and is closely related to several previous fMRI investigations of procedural learning that used

the SRT task (Martis et al., 2004; Rauch et al., 1997; Rauch et al., 2001; Kim et al., 2004). The version of the SRT task used in the present investigation was created by the author using the E-Prime software package (Schneider, Eschman, & Zuccolotto, 2002). The task consisted of a circle that alternated location between four boxes arranged horizontally on a computer screen. Subjects were instructed to identify the location of the target as quickly and accurately as possible by pressing the response key that corresponded to the location of the target. The left two stimuli locations corresponded to the middle and index finger of the left hand, the right two stimuli to the index and middle finger of the right hand. On each trial the stimulus appeared for 800 ms prior to a 200 ms inter-trial interval. Sixty-trials comprised one block and were either sequenced (S) or random (R). Within S blocks the location of the circle followed a 12-element second order conditional (SOC) sequence corresponding to positions 3-4-2-3-1-2-1-4-3-2-4-1 repeated five times. In SOC sequences, two elements of temporal context are required to predict the location of the next stimulus (Reed & Johnson, 1994). The use of SOC sequences has been shown to protect against the formation of explicit knowledge during SRT tasks, even after extended practice (Destrebecqz et al., 2001; Reed et al., 1994). Each R block contained 60 trials with the location of the stimulus randomly assigned with the caveats that all 4 locations appear with equal frequency within a block, and no location repeated consecutively. Subjects completed two scanning runs, each consisting of 3 S and 3 R blocks alternating in a blocked AB manner, with each block separated by an 18 second fixation point resting period.

To facilitate procedural learning during the scanning session, subjects completed 5 consecutive blocks of 72 sequenced trials, each consisting of 6 repetitions of the SOC sequence, directly before entering the scanner. The pre-scanning and scanning procedure were similar in all other respects (i.e. 800 ms stimuli, 200 ms inter-stimulus interval, 18 second inter-block interval), except that subjects responded on a computer keyboard during the pre-scanning phase and button response boxes secured to each hand during the scanning phase. The pre-scanning training session was included to ensure that the imaging results were not biased towards reflecting early or late stages of procedural learning and promote stable performance during the scanning session. The length of the

pre-scanning session was based upon the minimum number of blocks needed to demonstrate procedural learning during the scanning session in pilot testing.

Statistical Analysis of Behavioral Data

The primary and secondary dependent variables for the analysis were median reaction times (RTs) for blocks of trials and accuracy, respectively. RTs for the pre-scanning and scanning sessions were examined separately, after excluding errors or timed out non-responses. Mixed effects repeated measures ANOVA were used to examine RTs for the pre-scan and scanning sessions. For the pre-scanning session, a repeated measures ANOVA, with block (1-5) entered as a within subjects variable and group (controls and siblings) as a between groups variable, was performed to examine mean changes in median RTs across blocks and between groups. In addition, a mean reaction time advantage was calculated for each subject by subtracting the median RT for block 1 from the median RT for block 5 and subjected to an independent groups t-test in order to further examine potential group differences on the critical procedural learning measure derived from the SRT. For the scanning session, a repeated measures ANOVA with block (1-6) and condition (S and R) entered as within subject variables and group (controls and siblings) entered as a between groups variable was performed. The multivariate method for repeated measures was employed in cases where sphericity was violated. Group differences in accuracy rates during the pre-scanning session were examined using independent groups t-test and accuracy rates during the scanning session were subjected to a repeated measures ANOVA with condition entered as a within subjects variable and group entered as a between subjects variable.

Functional MRI data Analysis

Image Acquisition

All structural and functional MRI images were acquired during a single session on a Siemens Sonata 1.5T scanner located at the University of Alberta In Vivo Imaging Center. 25 contiguous axial (approximate range Z=70 to Z=-30), 4 mm thick functional images acquired parallel to the AC-PC line using a single-shot, T2* EPI sequence (matrix=128x128; voxel size 1.72 x 1.72 x 4mm; TR=3000ms; TE=50ms) were collected. 159 volumes were acquired during each of the two runs but the first three volumes of each run were discarded. A high resolution, 144 slice, 1x1x1mm voxel size 3D structural image was also acquired using an MPRAGE sequence.

Functional Imaging Statistical Analysis

All processing of images and statistical analyses was carried out using Brainvoyager QX (Brain Innovation, Maastricht, The Netherlands) software except were noted. The raw fMRI images underwent motion correction, slice scan timing correction, spatial smoothing (8mm FWHM), and linear and non-linear drift removal prior to statistical analysis. The functional images for each subject were co-registered to their respective structural image using a two step semi-automated method that first utilized scanner positioning header data to initially align images and then finely aligned images using a multi-scale intensity alignment. The co-registration parameters were obtained for the motion-corrected fMRI images prior to spatial smoothing in order to maximize mutual information contained within the images. Following co-registration, each subjects structural brain image and corresponding functional images were warped into standard Talairach space (Talairach & Tournoux, 1988) and functional data were interpolated to a voxel size of 3x3x3 mm.

To reduce the number of voxels included in the statistical analysis and to examine activations in specific a-priori defined regions of the brain, two anatomical masks were used (Zedkova et al., 2006). The masks reduced the number of comparisons performed within the statistical parametric maps (SPMs) by limiting subsequent statistical analyses to only those voxels contained within the cortex and sub-cortical regions of interest (ROIs). The first mask, a cortex based mask, was created to examine areas of activation within the cortex. The mask was created using an automated procedure within the Brainvoyager software package (described in detail in Goebel, Khorram-Sefat, Muckli, Hacker, & Singer, 1998; Kriegeskorte & Goebel, 2001). Briefly, the procedure identifies the grey/white matter boundary of an anatomical data set that has been placed in Talairach space by first removing the cranium and cerebellum and filling in the ventricles and sub-cortical structures by application of a standard Talairach Brain mask. Following this step, a sigma filter is applied, which performs non-linear, edge preserving smoothing to reduce noise within grey and white matter, and grey and white matter intensity peaks are determined across all axial slices. The intensity peaks for grey and white matter establish the range within which a region growing process is initiated to segment the white matter. The region growing process is seeded automatically in the white matter of one hemisphere at a time beginning in a region of white matter above the corpus callosum. The growing process continues until the white/grey matter boundary is reached, all white matter is completely filled, and regions of cranium and cerebellum not previously removed are discarded. Since this process requires a uniform anatomical data set with consistent white/grey matter intensity separation across axial slices, 3D bias field correction is carried out using a Legendre multiplicative model polynomial inhomogeneity correction (Vaughan et al., 2001) prior to segmentation. The resulting segmentation was visually inspected by projecting the grey/white matter boundary onto the original structural image. Finally, the cortical mask is created by including all functional 3x3x3mm voxels that fall within or touch upon the borders of a -3 to 6 mm range from the vertex of the grey/white matter boundary. A wide range is used to ensure that the mask accounts for individual variation in gyral anatomy.

Since the majority of data available to date, including lesion, neurological, and functional imaging studies, implicates the caudate, putamen, thalamus, and globus pallidum in procedural learning, an ROI approach was undertaken to better identify activity in these relevant sub-cortical structures. The second structural, sub-cortical mask restricted the statistical analyses to only those functional voxels included in the thalamus, caudate, putamen, and globus pallidum. This mask was created from the 3D color coded Talairach Brain provided with the Brainvoyager QX software package (see above). The standardized 3D color coded Talairach Brain partitions the brain, including basal ganglia and thalamus, into different colors. Segmentation can be performed on the brain by removing unwanted color coded regions, such as cortex and ventricles, while keeping the color coded basal ganglia and thalamic regions of interest. The 3D ROI structure is then converted to a functional mask, as described above, and limits subsequent statistical analyses to only those voxels contained within the mask.

Statistical analyses proceeded by modeling the functional time course data at each voxel as a boxcar function, convolved with a gamma function to account for lag in the hemodynamic response, with S and R blocks entered as predictors in a fixed effects general linear model (GLM) analysis corrected for serial autocorrelations. SPMs comparing S to R blocks were created for each group in order to identify the pattern of activations unique to each sample. Since no cortical ROIs were specified a priori, the threshold for the cortex based statistical analysis was set to $p<0.005$ with a cluster threshold of 6 functional voxels (Forman et al., 1995). A statistical threshold of $p<0.01$ with no minimum cluster size threshold specified a priori was used to identify significant voxels for the sub-cortical ROI analysis. Significant differences in activations between groups were examined based on the results of the within groups analysis by entering all subjects into a voxelwise random effects GLM analysis that was restricted to only voxels that demonstrated a significant effect of condition in either the control group or sibling group. A p-value of 0.05, with a cluster size threshold of 6 functional voxels for the cortex based analysis, was applied to this analysis since the between groups contrast only included voxels that exceeded threshold in the within groups analyses. To further examine differences between groups in procedural learning related activity, BOLD signal

change (S minus R blocks) was extracted from each ROI identified in the between groups analysis for each subject. These values were plotted showing the distribution of BOLD signal change for each subject in order examine the frequency with which siblings demonstrate BOLD signal changes outside of the control range for each ROI. In addition, exploratory correlations between the magnitude of the RT advantage between S and R blocks during the scanning session and cerebral activity, S minus R blocks, were examined for both the cortical and sub-cortical ROI analyses. A liberal threshold of $p<.05$ was used due to the reduced power to detect correlations with these sample sizes and, for the cortex-based analysis, a minimum cluster size of 6 functional voxels was applied to the SPM generated for this analysis. The results presented in the subsequent section give the center Talairach coordinate for each cluster of activation, along with the spatial extent and maximum t, or r, statistic. The corresponding figures depicting the SPMs were interpolated to 1x1x1mm resolution for display purposes. Cortical brain surface representations of significant activations were created using MRIcro (MRIcro v1.39, Chris Rorden).

Results

Behavioral Data

Behavioral data from one control subject was lost due to experimenter error leaving complete behavioral data for 14 controls and 12 siblings. Mean of the median reaction times during the pre-scanning and scanning sessions are presented in Figure 2. Analysis of the SRT RTs for the pre-scanning session revealed a main effect of block ($F(4,21)=4.83$, $p<.007$), but no main effect of group ($F(1,24)=1.94$, $p<.177$) or block by group interaction ($F(4,21)=0.25$, $p<.908$). Subjects got progressively faster over blocks such that the median RT for the final block was approximately 40ms faster than it was for the first block of trials ($F(1,24)=16.39$, $p<.001$). The block 5 vs. block 1 time advantage did not differ between controls and siblings ($t(24)=0.36$, $p<.973$). Accuracy rates were

high in both controls (96%) and siblings (95%) and did not differ between groups (t(24)=0.81, p<.430).

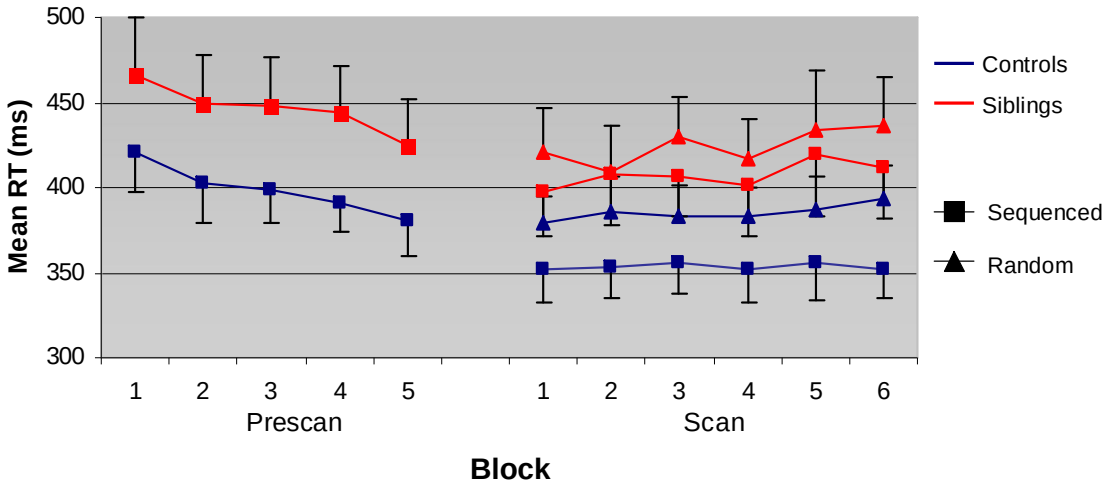

Figure 2. Mean reaction times on the SRT task in controls and unaffected siblings. Error bars represent standard errors.

A main effect of condition was observed during the scanning session (F(1,24)=29.78, p<.001) due to the fact that subjects responded approximately 24ms faster during S blocks compared to R blocks. The main effects of block (F(5,20)=1.92, p<.137) and group (F(1,24)=2.10, p<.161) were not significant nor were any of the interaction terms (all F-statistics<2.80, p<.108). Repeated measures analysis of the accuracy rates indicated that subjects performed equally well during S (97.1%) and R (96.7%) blocks (F(1,24)=1.60, p<.219); however there was trend for controls to be slightly more accurate overall than siblings (98.0% vs. 95.8%; F(1,24)=3.90, p<.061). There was no interaction between condition and group (F(1,24)=0.46, p<.504) with respect to accuracy rates.

Correlations between the RT advantage observed during scanning and demographic variables age, education, SES, and, in the unaffected siblings, SPQ scores, were performed to ensure that demographic differences between the two groups did not account for the behavioral results. Aside from a non-significant trend towards an inverse

relationship between age and procedural learning observed during scanning when both groups were combined ($r=-.38$, $p<.065$), none of the demographic variables was correlated with procedural learning in either the combined group, control group, or sibling group.

Imaging Results

The pattern of activations observed in controls and siblings is presented in Table 2 and Figure 3. In controls, significant activations during S, relative to R blocks, were observed in several cortical areas, primarily in the left hemisphere, including the rostral ventral anterior cingulate corresponding to Brodmann's Area (BA) 24/32, regions of the superior, middle, and inferior frontal gyri, and inferior parietal lobe, particularly those Brodmann's areas (BA) typically involved in motor function (BA 6), spatial attention (BA 39), and sub-regions of the PFC (BA 9 and 10). In addition, greater activity was also observed bilaterally in the middle temporal gyrus. Sub-cortical activations were observed primarily in the left caudate and anterior thalamic nucleus, and, to a lesser extent, right caudate and putamen. The reverse contrast revealed only three regions in the control group; right precuneus, right middle temporal gyrus, and left fusiform gyrus, that were more active during R blocks compared to S blocks.

Siblings also demonstrated greater activity when S blocks were contrasted with R blocks in several cortical regions including the rostral ventral anterior cingulate (BA 24/32), multiple regions of the PFC including premotor cortex (BA 6), middle frontal gyrus, and inferior frontal gyrus. The sibling group also demonstrated activity in the left middle temporal gyrus corresponding to BA 21 and fusiform gyrus corresponding to BA 21 and 37, respectively. Consistent with controls, the cortical activations were almost exclusively in the left hemisphere. With respect to the sub-cortical ROI analysis, the sibling group demonstrated greater activity bilaterally in the caudate. Several cortical and sub-cortical regions demonstrated greater activity during R blocks, relative to S

blocks, in the siblings. These included several foci bilaterally within the PFC, right caudate, left parahippocampal gyrus, and left globus pallidus.

Table 2. fMRI results in controls and unaffected siblings

		Talairach				Size
Group	**Brain Region**	**X**	**Y**	**Z**	**t Score**	**(mm3)**
Controls						
S>R Contrast: Cortex Based Analysis						
	L. Middle Frontal Gyrus (BA 6)	-30	6	52	4.22	459
	L. Superior Frontal Gyrus (BA 9)	-18	40	34	3.12	216
	L. Superior Frontal Gyrus (BA 10)	-22	58	12	3.22	351
	R. Superior Frontal Gyrus (BA 9)	14	52	19	3.99	378
	L. Angular Gyrus (BA 39)	-43	-67	32	3.77	1863
	R./L. Anterior Cingulate (BA 24/32)	-4	33	-5	3.87	1593
	L. Inferior Frontal Gyrus (BA 47)	-49	28	-8	3.66	351
	L. Inferior Frontal Gyrus (BA 10)	-48	43	-1	3.61	513
	L. Medial Frontal Gyrus (BA 10)	-10	53	7	3.46	513
	R. Middle Temporal Gyrus (BA 21)	60	-1	-9	3.78	837
		45	7	-24	3.49	324
	L. Middle Temporal Gyrus (BA 21)	-52	-9	-14	3.94	756
Sub-Cortical ROI Analysis						
	L. Caudate Body	-12	11	7	4.03	1944
	L. Anterior Thalamic Nucleus	-3	-2	7	2.94	135
	R. Caudate Body	15	17	4	2.72	27
	R. Caudate Body	11	2	4	2.83	54
	R. Putamen	30	-22	10	3.18	108
R>S Contrast: Cortex Based Analysis						
	R. Precuneus (BA 19)	25	-74	34	3.57	783
	R. Middle Temporal Gyrus (BA 37)	51	-57	0	3.36	378
	L. Fusiform Gyrus (BA 37)	-47	-45	-20	3.56	270
Siblings						
S>R Contrast: Cortex Based Analysis						
	L. Superior Frontal Gyrus (BA 6)	-15	14	49	3.97	918
	L. Middle Frontal Gyrus (BA 8)	-29	13	40	3.59	513
	L/R Anterior Cingulate (BA 24/32)	3	26	4	3.85	675
	L. Middle Temporal Gyrus (BA 21)	-52	-19	-17	3.14	216
	L. Inferior Frontal Gyrus (BA 47)	-22	17	-14	5.00	675
	L. Fusiform Gyrus (BA 37)	-41	-50	-18	3.59	189
S>R Contrast: Sub-Cortical ROI Analysis						
	L. Caudate	-18	14	7	2.55	27
	R. Caudate	18	14	6	2.82	54
R>S Contrast: Cortex Based Analysis						
	R. Precentral Gyrus (BA 6)	34	-7	58	3.80	270
	L. Middle Frontal Gyrus (BA 9)	-46	23	28	4.13	297
	R. Middle Frontal Gyrus (BA 10)	38	49	15	3.71	783
	L. Parahippocampal Gyrus	-17	-8	-11	3.41	270
R>S Contrast: Sub-Cortical ROI Analysis						
	L. Lateral Globus Pallidus	-27	-16	-5	2.68	27
	R. Globus Pallidus	18	-7	-7	4.62	2079

Abbreviations: L: left; R: right; BA: Brodmann's Area; ROI: Region of Interest

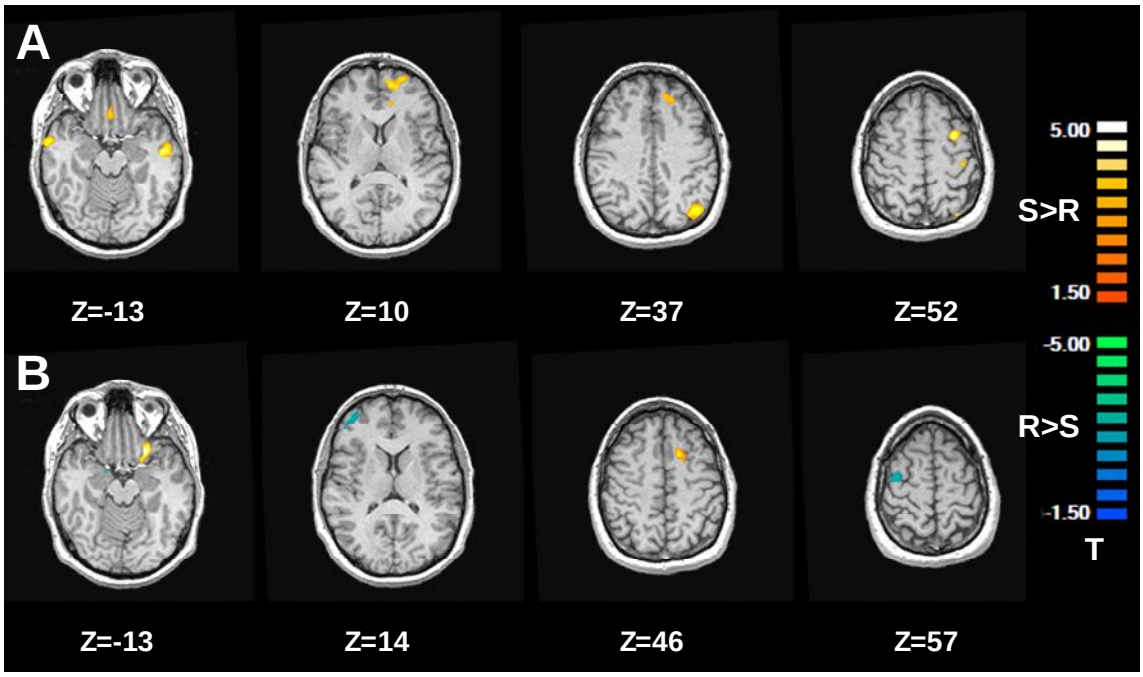

Figure 3. Cortical regions active during procedural learning in controls (panel A) and unaffected siblings (panel B). Regions more active in controls when S blocks were contrasted with R blocks (warm colors) included left superior and middle frontal gyri corresponding to Brodmann's Areas (BA) 6, 9, and 10, left angular gyrus (BA 39), and bilateral middle temporal gyrus (BA 21). Regions more active during S blocks in siblings included left premotor cortex (BA 6) and left inferior gyrus (BA 47). Siblings also demonstrated greater activity during R blocks (cool colors) in the right precentral gyrus (BA 6) and right middle frontal gyrus (BA 10). Images thresholded at $p<.005$ and minimum cluster size of 6 functional voxels. Note, left/right orientation reversed on axial slices. Abbreviations: R: random blocks; S=sequenced blocks.

Direct comparison between groups revealed several cortical and sub-cortical regions that were more active in the controls than siblings when S blocks were contrasted with R blocks. Specifically, controls activated regions of the superior and middle frontal gyri corresponding to BA 9 and 10 bilaterally, left angular gyrus (BA 39), and left parahippocampal gyrus to a greater extent than siblings. With respect to sub-cortical regions, controls activated the left caudate, and right anterior thalamic nucleus, putamen, and medial globus pallidus to a greater extent than siblings. In contrast, siblings activated the left fusiform gyrus corresponding to BA 37 more than controls. Percent signal change was extracted from each cluster identified in the between groups comparison and an ANCOVA with each subjects procedural learning score, RT advantage, during the

scanning session entered as a covariate was performed to verify that the between groups differences identified in the above regions was not due to any potential differences in SRT performance. All clusters identified in the between groups analysis remained significant after co-varying for SRT performance with the exception of the left parahippocampal cluster which was significant at the trend level ($p<.075$). Group differences in procedural learning related activity are presented in Table 3 and Figure 4.

Table 3. Group differences in activations during procedural learning.

		Talairach			t Score	Size (mm3)
Contrast	**Brain Region**	**X**	**Y**	**Z**		
Controls > Siblings						
Cortex Based Analysis	L. Middle Frontal Gyrus (BA 9)	-46	24	29	3.22	162
	R. Middle Frontal Gyrus (BA 10)	35	51	15	2.36	270
	R. Superior Frontal Gyrus (BA 9)	14	53	20	2.71	243
	L. Angular Gyrus (BA 39)	-45	-66	32	2.79	999
	L. Parahippocampal Gyrus	-16	-8	-12	2.53	189
Sub-Cortical ROI Analysis	R. Anterior Thalamic Nucleus	18	-7	15	3.29	54
	L. Caudate Head	-15	23	6	2.25	54
	R. Putamen	27	-22	10	2.07	27
	R. Medial Globus Pallidus	18	-6	-7	4.03	1593
Siblings > Controls						
Cortex Based Analysis	L. Fusiform Gyrus (BA 37)	-44	-48	-19	3.05	243

Abbreviations: L: left; R: right; BA: Brodmann's Area; ROI: Region of Interest

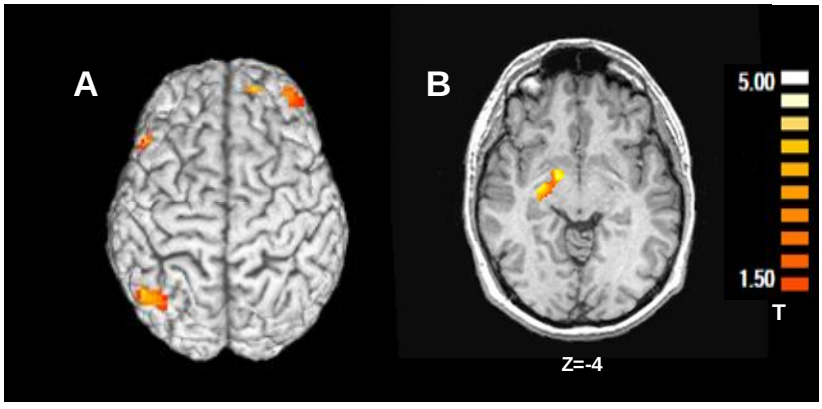

Figure 4. Differences between controls and unaffected siblings in procedural learning related activity on the SRT task. Controls demonstrated greater activity during procedural learning in (A) prefrontal regions corresponding to right superior and middle frontal gyri (BA 9 and 10), left middle frontal gyrus (BA 9), left angular gyrus (BA 39), and (B) right medial globus pallidus. Images thresholded at $p<.05$ and minimum cluster size of 6 functional voxels. Note, left/right orientation reversed on axial slice.

The distribution of BOLD signal change (S minus R blocks) for controls and siblings is presented in Figure 5. The frequency with which siblings demonstrated BOLD signal changes outside the range observed in controls varied across ROIs. As can be seen, signal changes outside the range observed in controls tended to be more prevelant in the left and right middle frontal gyrus, left angular gyrus, left fusiform gyrus, left caudate head, and right medial globus pallidus (25% or more siblings demonstrating activity outside the range observed in controls). On the other hand, fewer (<25 of siblings) demonstrated BOLD signal changes outside the range observed in controls in the right superior frontal gyrus, right anterior thalamus, and right putamen. Morevoer, the distribiution of BOLD signal changes in some regions appeared bimodal within the siblings. For example, a subset of five siblings demonstrated BOLD signal changes below the range observed in controls in the angular gyrus. Conseqeuntly, abnormal function in some regions may be more sensitive to genetic vulnerability for schizophrenia.

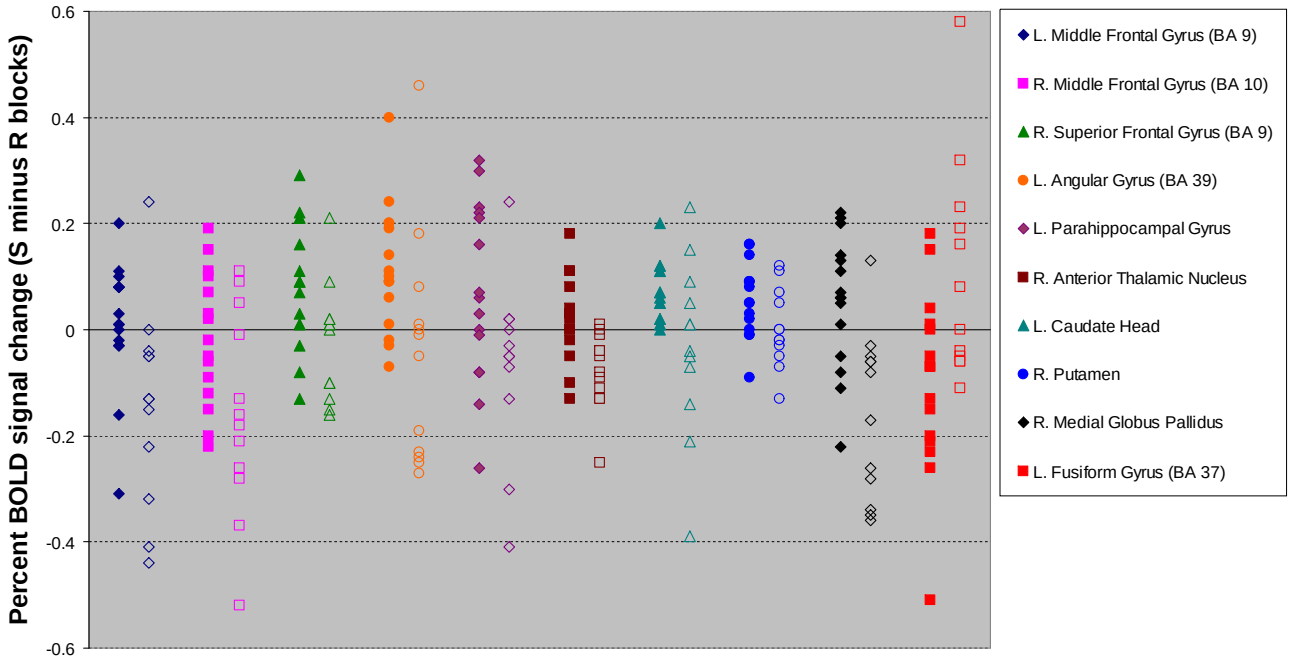

Figure 5. Percent BOLD signal change (S minus R blocks) for ROIs identified in the between groups comparison. Filled and open symbols represent control and siblings, respectively.

Exploratory correlations between RT advantage (R blocks minus S blocks) and cerebral activity when S blocks were contrasted with R blocks identified several regions in both controls and siblings that were correlated with procedural learning. The correlations are presented in Table 4. In controls, positive correlations were observed in the right superior frontal gyrus (BA 8), left precentral gyrus (BA 6), and bilateral middle occipital gyrus (BA 18). Conversely, activity in several regions of the PFC, including left middle and medial frontal gyri, and right superior and precentral gyrus was inversely correlated with RT advantage. Similarly, bilateral insula was also inversely correlated with RT advantage. No significant correlations were observed in sub-cortical regions. Compared to controls, siblings demonstrated fewer correlations within the PFC; however, they too demonstrated positive correlations, left medial and middle frontal gyri, and negative correlations, left precentral gyrus. Siblings also demonstrated several negative correlations in regions of the temporal lobe and lingual gyrus. In contrast to controls, siblings demonstrated numerous positive correlations between activity in sub-cortical regions and RT advantage. These included bilateral caudate, left lateral globus pallidus, right thalamus, and right brainstem. Caution is warranted when interpreting the correlations due to the reduced power for correlation analysis with small sample sizes and the liberal threshold applied to the correlation SPMs.

Table 4. Correlations between procedural learning and cerebral activity.

Group	Brain Region	Talairach X	Talairach Y	Talairach Z	r	Size (mm3)
Controls						
Cortex Based Analysis						
	Right Superior Frontal Gyrus (BA 8)	25	28	52	.70	294
	Left Precentral Gyrus (BA 6)	-42	-3	54	.67	162
	Right Paracentral Lobule (BA 31)	3	-11	44	-.74	634
	Left Middle Frontal Gyrus (BA 46)	-43	19	24	-.66	862
	Left Precentral Gyrus (BA 6)	-57	-2	28	-.72	420
	Left Insula (BA 13)	-37	-1	21	-.65	212
	Left Medial Frontal Gyrus (BA 9)	-3	48	20	-.64	233
	Right Superior Frontal Gyrus (BA 9)	13	55	19	-.66	574
	Right Precentral Gyrus (BA 4)	58	-2	18	-.76	614
	Right Insula (BA 13)	46	-12	12	-.73	783
	Left/Right Middle Occipital Gyrus (BA 18)	-20	-88	-8	.84	4886
	Left Middle Frontal Gyrus (BA 11)	-38	35	-9	-.70	368
	Right Fusiform Gyrus (BA 37)	47	-52	-16	.70	585
Siblings						
Cortex Based Analysis						
	Left Precentral Gyrus (BA 4)	-37	-14	58	-.71	172
	Right Inferior Parietal Lobule (BA 40)	50	-55	41	.75	191
	Left Medial Frontal Gyrus (BA 10)	-12	58	9	-.80	306
	Left Middle Temporal Gyrus (BA 19)	-36	-79	17	-.82	202
	Left Middle Frontal Gyrus (BA 47)	-43	33	-6	.82	238
	Right Parahippocampal Gyrus (BA 28)	22	-20	-18	.71	380
	Right Fusiform Gyrus (BA 37)	50	-45	-12	-.85	652
	Left Fusiform Gyrus (BA 19)	-26	-71	-20	-.72	1231
	Right Lingual Gyrus (BA 18)	16	-74	-19	-.76	189
Sub-Cortical ROI Analysis						
	Left Caudate Body	-18	-7	28	.72	149
		-11	13	21	.67	123
	Right Caudate Body	15	-6	27	.64	62
		21	14	19	.72	121
	Left Lateral Globus Pallidus	-19	-3	-2	.66	160
	Right Thalamus	1	-5	0	.68	401
	Right Brainstem Midbrain	3	-9	-12	.85	1154

Abbreviations: L: left; R: right; BA: Brodmann's Area; ROI: Region of Interest

Discussion

The present study examined behavioral performance and cerebral activity related to procedural learning, as quantified using the SRT task, in a sample of unaffected siblings of patients with schizophrenia and an aged matched control sample with no family history of schizophrenia. As anticipated, during scanning both siblings and controls demonstrated a significant reaction time advantage to blocks where the location of the target followed a repeating pattern relative to blocks in which the location of the target appeared pseudorandomly. Moreover, there was no difference between siblings and controls with respect to the magnitude of this advantage.

It has been hypothesized that cortico-striatal loops consisting of projections from the premotor, dorsolateral PFC, and parietal lobe to structures of the basal ganglia, caudate and putamen in particular, and cortico-cortical connections between the PFC and parietal lobe comprise neural circuits related to learning complex visuospatial motor sequences and that procedural motor learning relies on the integrity of these parallel networks (Alexander, DeLong, & Strick, 1986; Rauch et al., 1997; Hikosaka et al., 1999). Specifically, based on experiments conducted on non-human primates, and imaging and lesion data acquired in humans, Hikosaka and colleagues speculated that learning a sequential motor procedure activates two of these parallel networks; one consisting of connections between the dorsolateral PFC, parietal cortex, and caudate that is primarily involved in acquiring spatial knowledge about the procedure, and a second network comprised of the premotor cortex and putamen that is primarily concerned with learning the corresponding motor output sequence (Hikosaka et al., 1999). Moreover, they further speculated that the network broadly corresponding to PFC association areas and the caudate is preferentially involved in early stages of learning, whereas the premotor-putamen network is more involved in the later stages of learning. Although not specifically designed to test the validity of this hypothesis, the current results in the control sample are in broad agreement with this hypothesis and prior fMRI studies of SRT task performance. Specifically, greater activity was observed in multiple regions of

the PFC, including premotor and dorsolateral PFC, inferior parietal cortex, and structures of the basal ganglia, primarily the caudate. In addition, exploratory correlations indicated that activity in several PFC regions was inversely correlated with procedural learning RT advantage during scanning, with the exception of premotor cortex which was positively correlated with RT advantage. These findings are consistent with the idea that PFC association areas became less active as subjects get better at the task (i.e. later stage of learning), whereas premotor regions become more active. The current results are also consistent with the tendency for the cortical activations identified to be more prominent in the left hemisphere when bimanual versions of the task are used (Kumari et al., 2002; Rauch et al., 1997; Willingham et al., 2002). Additional activations were observed bilaterally in BA 21 of the temporal lobe in the control group. This observation is not unprecedented (Thomas et al., 2004; Daselaar et al., 2003), although fewer studies have identified procedural learning related activity in this region relative to the activations observed in the frontal lobe, inferior parietal cortex, and striatum (Kumari et al., 2002; Rauch et al., 1997; Willingham et al., 2002; Schendan et al., 2003; Daselaar et al., 2003).

These results are similar in some respects to the results from our previous application of the same methods to a sample of patients with chronic schizophrenia (Zedkova et al., 2006). Patients also demonstrated intact procedural learning on the SRT task, and they demonstrated less activity than controls in multiple regions of the PFC, left angular gyrus, and bilateral caudate. In the current study, siblings demonstrated considerably less activity in many of the same regions including multiple areas of the PFC, and left angular gyrus. However, in contrast to patients, the difference between siblings and controls in the degree of activity in the caudate was spatially circumscribed; limited to a very small region of the left caudate. This observation suggests that the abnormal cortical responses detected in both patients and siblings may relate to genetic vulnerability for schizophrenia, but that the abnormal striatal response observed primarily in patients may relate more so to disease specific components of the illness or the effects of treatment with APDs.

However, siblings did demonstrate some abnormalities in basal ganglia function. Specifically, siblings demonstrated an idiosyncratic pattern of greater activity in the globus pallidus during R blocks, relative to S blocks, and relatively less activity than controls in this region. Interestingly, the exact opposite pattern was observed in patients in our prior study. Specifically, patients demonstrated greater activity in the globus pallidus when S blocks were contrasted with R blocks and the increased activity was greater than that observed in controls (Zedkova et al., 2006). The globus pallidus is the main output structure of the basal ganglia and it can down regulate cortical activity via inhibitory inputs to excitatory thalamo-cortical projections (Alexander, Crutcher, & DeLong, 1990). As such, in siblings decreased activity in the globus pallidus during S blocks may be a compensatory mechanism to promote cortical activity. In patients enhanced activity in the globus pallidus may serve to down-regulate the PFC, perhaps in favor of promoting activity in another neural system to facilitate learning. Recall that patients demonstrated greater activity in the temporal lobe during procedural learning in our previous study. Combined, these findings may suggest a gradient of impairment in fronto-striatal circuits underlying procedural learning in siblings and patients. Specifically, in patients the fronto-striatal system may be impaired to the point that another circuit, possibly involving the temporal lobe, takes over to facilitate learning. In siblings, the degree of dysfunction may be modest enough to allow compensation within the system to take place. It is noteworthy that a sub-set of voxels in the same region of the globus pallidus identified in the between conditions contrast were positively correlated with RT advantage suggesting that in siblings the abnormal pattern of activity observed in the globus pallidus was inversely related to procedural learning. This hypothesis is parsimonious in that it explains the gradient of activity observed in the globus pallidus and frontal cortex across siblings and patients; increasing globus pallidus activity going from siblings to patients corresponds with decreasing frontal lobe activity during procedural learning.

The unique functions of specific cortical nodes within the network that underlies procedural learning on the SRT are poorly understood. Repetitive transcranial magnetic stimulation (rTMS) applied to the dorsolateral PFC, disrupts learning on the SRT task

when spatial location is used to cue the motor response, but not when color cues are used, suggesting that this region is necessary for acquiring spatial information about the sequence, but not motor sequence learning per se (Robertson, Tormos, Maeda, & Pascual-Leone, 2001). The role of the parietal cortex in SRT performance is less well known; however, it has been implicated in several other procedural learning tasks such as reverse mirror reading and artificial grammar learning (Callan, Callan, & Masaki, 2005; Xiang, Holowka, & Chuang, 2004; Rusconi, Walsh, & Butterworth, 2005; Skosnik et al., 2002). In addition, the left angular gyrus specifically has been implicated in anticipating visual target locations during smooth pursuit eye movements and, as such, may also be involved in coordinating eye movements during performance of the SRT task (Nagel et al., 2006). Indeed, subjects make greater anticipatory eye movements during procedural learning on the SRT, even when no explicit knowledge about the sequence occurs (Marcus, Karatekin, & Markiewicz, 2006). These findings suggest that the absence of activity in the PFC and parietal cortex may relate to a disruption in the neural circuitry underlying spatial learning and/or oculomotor learning in schizophrenia patients and their unaffected relatives.

The idiosyncratic finding of relatively greater activity during R compared to S blocks in the sibling group, especially in BA 10 of the right middle frontal gyrus, is also strikingly similar to the findings in our previous investigation of procedural learning in patients (Zedkova et al., 2006). In the prior investigation, patients also demonstrated significantly greater activity during R blocks compared to S blocks in BA 10 of the right middle frontal gyrus. However, patients also demonstrated relatively greater activity during R blocks in additional regions of the right PFC including superior, middle, and inferior frontal gyri corresponding to Brodmann's areas 9, 10, and 47, respectively. The fact that the regions demonstrating greater activity during R blocks relative to S blocks was more widespread in patients compared to unaffected siblings suggests that this aspect of altered cerebral activity may reflect both a genetic vulnerability for the disorder and disease specific processes.

The relative over-activation of the PFC during performance of the control task is somewhat reminiscent of the findings reported by Callicott and colleagues that identified regionally specific hypo and hyperactivations in the PFC in both patients and siblings, relative to controls, during performance of an N-back working memory task (Callicott et al., 2003b; Callicott et al., 2003a). Interestingly, verbal working memory has been linked to learning on the SRT task or variants of it, regardless of whether or not the sequence is learned implicitly or explicitly (Unsworth & Engle, 2005; Schwartz, Howard, Howard, Jr., Hovaguimian, & Deutsch, 2003). Moreover, executive deficits in patients with frontal lobe lesions covary with deficits in sequence learning on the SRT task (Barker, Andrade, Romanowski, Morton, & Wasti, 2006). As such, it is possible that the complex pattern of PFC dysfunction consisting of relative hyper- and hypo-activation may relate to disruption of a fundamental cognitive process tapped by both motor sequence learning and working memory, such as the allocation of visual attention, in schizophrenia (Bleckley, Durso, Crutchfield, Engle, & Khanna, 2003). This might also explain why patients failed to demonstrate procedural learning on a more spatially demanding version of the SRT than the one used here (Kumari et al., 2002), but relatively preserved performance on the SRT task used in the current study.

There are several caveats to the current study that may limit generalization of the results. The first relates to the lack of independent verification that declarative memory processes did not contribute to performance on the SRT task. This is typically accomplished by direct query of the subject regarding conscious awareness of the repeating sequence, but this would alert the unaware subject to the implicit sequence rendering them unsuitable for participation in our other ongoing fMRI studies of SRT test-retest reliability and stability through time. Without independent verification a contribution from declarative processes cannot be excluded with complete confidence, but this seems unlikely. Previous investigations with the SRT task have reported some instances of conscious awareness of the sequence (e.g. Shanks & Johnstone, 1999), but this appears specific to experimental designs with substantially more trials than the current design (1200-1400 vs. 720), when subjects are informed about the presence of a repeating pattern of the start of training, or when much shorter non-SOC sequences are

used (Perruchet & Amorim, 1992; Wilkinson & Shanks, 2004; Destrebecqz et al., 2001; Willingham et al., 2002). Also, it is unlikely that idiosyncratic activation of declarative processes in a few subjects would mitigate the current results because prior imaging studies of the SRT have reported similar regional activations, PFC and striatum in particular, under implicit and explicit learning (Willingham et al., 2002). Moreover, explicitly learning a sequence typically results in faster response times compared to implicit learning over the same amount of practice and greater activation of the PFC (Willingham et al., 2002; Unsworth et al., 2005). As such, it is unlikely that siblings differed from controls with respect to the amount of explicit knowledge acquired about the sequence given that controls and siblings performed the task equivalently and siblings actually demonstrated less PFC activation during procedural learning.

Second, although behavioral differences were not observed between groups, it is possible that controls and siblings were at different stages of learning during the scanning session and/or there were differences between groups with respect to the difficulty of the task (i.e the results may reflect greater general processing demands in siblings relative to controls rather than being specific to procedural learning). For example, it is possible that siblings had reached the later stages of learning during scanning, whereas controls might still have been in the early stages of learning and may have demonstrated greater procedural learning than siblings had more pre-scanning trails been included. Indeed, the relative absence of PFC activity in siblings could suggest that they had reached the later stages of learning, compared to controls. Moreover, the pattern of correlations between RT advantage and activity was markedly different between controls and siblings in several sub-cortical regions. In controls, no correlations were observed sub-cortically suggesting that they had reached a stage of proficiency on the SRT task whereby sub-cortical activity is an obligatory, but not fluctuating response, whereas in siblings it appeared as though sub-cortical activations were more closely related to actual performance. The significance of this is unclear, but again it might suggest that controls and siblings were at different stages of learning during the scanning session. Alternatively, it is possible that patients relied on a different set of neural structures during procedural learning, perhaps regardless of the stage, and that this network was not

as effective as controls. Unfortunately, such hypotheses are difficult to verify using the current analysis strategy. Imaging studies of SRT performance always include a pre-scanning learning phase then employ a block design whereby pseudorandom and sequenced blocks alternate during scanning. Such designs make it difficult to examine changes that occur in procedural learning across the experiment due to the fact that the relatively small number of blocks included in a run results in diminished power. Moreover, comparing activations between conditions that are obtained in different scanning runs can lead to spurious results due to potential baseline differences between runs related to subtle fluctuations in magnetic field homogeneity and scanner drift. Further analysis of this data set using functional connectivity or independent components analysis (ICA) may assist in identifying differential patterns of activity between controls and siblings. Additionally, parametric modulation of the task may assist in determining the relationship between cerebral activity and performance on the SRT task, as has been done for some working memory paradigms.

A final caveat pertains to the validation of the atypical SRT induced physiological activation as an endophenotype of schizophrenia. To meet criteria for an endophenotype, an anomaly must be detected in both patients and their unaffected family members. This appears to be true for the atypical SRT activation, though both observations require confirmation through replication. However, the heritability of an endophenotype must also be known (Gottesman et al., 2003), and at this time the heritability of SRT task performance, or the heritability of cerebral activation patterns from any cognitive task, have not been demonstrated. Although heritability is often assumed when similar deficits observed in patients are detected in their unaffected family members, definitive heritability estimates are required before atypical cerebral physiology can be accepted as a valid endophenotype with value to the delineation of susceptibility genes for psychiatric disorders (Callicott et al., 2003c). It has been proposed that abnormal cerebral physiological activity be used to define the phenotype of schizophrenia, either in addition to or in place of other phenotypes (e.g. Andreasen et al., 1999). Clearly the greater sensitivity of functional imaging and relative proximity to gene products, compared to actual behaviors, are advantages of this approach. The present results suggest that

physiological anomalies can be identified in patients and generalized to unaffected family members, but the true value of the anomaly will rest upon replication of the present results and a clear demonstration that functional brain activity is heritable. Moreover, the specificity of the deficits is also unknown and additional investigation is required to determine if the imaging endophenotypes identified herein, and in other studies, are specific to schizophrenia or represent a neuropathological substrate common to other mental illnesses.

CHAPTER TWO. ASSOCIATIONS BETWEEN COMT VAL108/158MET GENOTYPE, COGNITIVE FUNCTION, AND COGNITIVE IMPROVEMENT WITH CLOZAPINE IN SCHIZOPHRENIA

Introduction

Catechol-O-methyltransferase (COMT) is a key enzyme involved in the catabolism of catecholamine neurotransmitters, especially dopamine (DA), in the brain (Axelrod & Tomchick , 1958; Axelrod , SENOH, & WITKOP, 1958). Several lines of evidence suggest that COMT plays an especially prominent role in inactivating DA in the prefrontal cortex (PFC) (Gogos et al., 1998; Karoum, Chrapusta, & Egan, 1994). Findings in rodents, non-human primates, and, to a lesser extent, humans, indicate that DA alters PFC physiology and cognitive functions such as working memory (Goldman-Rakic, 1990; Sawaguchi, Matsumura, & Kubota, 1988; Aalto, Bruck, Laine, Nagren, & Rinne, 2005). It has been known for some time that the activity of COMT is under genetic regulation, at least in other organs such as the kidney and in lymphocytes; however, until the relatively recent development of modern gene sequencing techniques it was not possible to identify the specific gene(s) that govern COMT activity in humans (Weinshilboum et al., 1999). It is now clear that a functional single nucleotide polymorphism (SNP) consisting of a valine (val) to methionine (met) substitution at codon 108/158 of the COMT gene (COMT val108/158met) results in the transcription of a protein with significantly less enzyme activity (Lotta et al., 1995). As such, alterations in COMT activity and subsequently DA resulting from this SNP may alter PFC physiology and cognition.

The COMT val108/158met polymorphism (alternatively referred to as COMT genotype) may also have important implications for the etiology of schizophrenia. There is a growing consensus that the cognitive deficits and negative symptoms of schizophrenia are due, in part, to hypo-function of the mesocortical DA system (Doran et al., 1987; Goldberg et al., 1988; Knable et al., 1997; Weinberger et al., 1988; Abi-

Dargham et al., 2002; Akil et al., 1999; Davis et al., 1991). Thus, the val allele of the COMT gene is a candidate susceptibility gene given that this allele is associated with greater COMT activity, and, presumably, reduced cortical DA, and possibly altered cognitive function. This indeed appears to be the case as family-based studies support an association between the val allele and schizophrenia (Egan et al., 2001; Li et al., 2000). This association has been confirmed by meta-analysis, although the magnitude of the association is modest (Munafo, Bowes, Clark, & Flint, 2005; Glatt, Faraone, & Tsuang, 2003).

In the following sections, the anatomy and impact of the mesocortical DA system on PFC cognitive functions is reviewed as is the genetic regulation and role that COMT plays in metabolizing PFC DA. Following that, the results from two experiments designed to examine associations between the COMT val108/158met genotype, cognitive function, and cognitive changes with clozapine treatment in schizophrenia are described.

Anatomy of the Mesocortical Dopamine System

Three DA pathways, the nigrostriatal, mesolimbic (mesoaccumbal), and mesocortical, emerge from a heterogenous group of midbrain nuclei, the ventral tegmental area (VTA: cell group A10), retrorubral area (RRA: A8), and substantia nigra (SN: A9), and send axons primarily to the cortex, nucleus accumbens (NAS) and paralimbic regions, and dorsal striatum respectively (Cooper, Bloom, & Roth, 2003). Historically, these three pathways were viewed as distinct systems with each arising from specific nuclei and having a unique set of anatomical targets, however, it is apparent that the organization and projections of midbrain DA nuclei is considerably more complicated than originally thought (Berger, Gaspar, & Verney, 1991; Williams & Goldman-Rakic, 1993; Williams & Goldman-Rakic, 1998; Tzschentke, 2001). Brainstem projections comprising the mesocortical system arise primarily from the nucleus parabrachialis pigmentosus of the VTA and RRA but also include projections from the dorsomedial

substantia nigra compacta (SNc, area 9) (Thierry, Blanc, Sobel, Stinus, & Golwinski, 1973; Verney, 1999; Tzschentke, 2001; Williams et al., 1993; Williams et al., 1998).

Much of the anatomy of the mesocortical system is based on the rodent and while many of these findings generalize to human and non-human primates, there are marked differences between species, particularly with respect to the extent of DA innervation observed in the cortex and the laminar distribution of midbrain DA afferents (Berger, 1992; Berger, Trottier, Verney, Gaspar, & Alvarez, 1988; Gaspar, Berger, Febvret, Vigny, & Henry, 1989a; Berger et al., 1991; Williams et al., 1993; Verney, 1999). In the rodent, DA axons terminate almost exclusively in the medial prefrontal, entorhinal, and piriform cortices (Thierry et al., 1973; Bannon & Roth, 1983; Felten & Sladek, Jr., 1983). In contrast, DA innervation of the cortex is much denser in primates and extends to all cortical regions, although medial frontal regions remain the most richly innervated (Williams et al., 1993). In general, agranular regions, including anterior cingulate area 24 and motor areas 4-6, are more heavily innervated than granular cortical regions, such as prefrontal areas 9-12 and 46, parietal regions 1-3, 5, and 7, and temporal areas 21 and 22 (Berger et al., 1988). In the frontal cortex, two gradients in the density of DA innervations exist; 1) a prominent medial to lateral gradient of decreasing density of innervation centered on medial areas 6 and 24, and 2) a less prominent caudal to rostral gradient, again centered on medial areas 6 and 24 (Gaspar, Berger, Febvret, Vigny, & Henry, 1989b; Williams et al., 1993). It is also apparent that the source of projections to areas within the prefrontal cortex are arranged in an oblique topographical manner in primates (Williams et al., 1998). In general, lateral prefrontal cortical regions receive inputs from the lateral aspects of midbrain DA nuclei, whereas medial prefrontal regions receive greater inputs from ventromedial areas of DA brainstem nuclei (Williams et al., 1998).

Five dopamine receptors, denoted D1 through D5, are expressed in the CNS but they are generally grouped into two main categories, D1-like and D2-like, that include the D1, D5 and D2, D3, D4 subtypes respectively (Cooper et al., 2003). D1-like and D2-like receptors are differentiated from one another based on their g-protein coupling and

subsequent effects on adenylate cyclase activity; D1-like receptors activate adenylate cyclase whereas D2-like g-coupled receptors inhibit adenylate cyclase activity. In general, D1, D4, and to a lesser degree D5, receptors are more prevalent in the cortex than D2 and D3 receptors (Meador-Woodruff et al., 1996; Lidow, Wang, Cao, & Goldman-Rakic, 1998). In non-human primates, D1 and D2 receptors are expressed throughout the cortex, however, prefrontal and cingulate regions are particularly enriched (Goldman-Rakic, Lidow, & Gallager, 1990; Lidow, Goldman-Rakic, Gallager, & Rakic, 1991; De Keyser et al., 1988). D1 receptors are expressed to a much greater extent in non-human primates compared to rodents and are up to 20 times more prevalent than D2 receptors (Goldman-Rakic, Muly, III, & Williams, 2000; Goldman-Rakic et al., 1990; Lidow et al., 1991; Richfield, Young, & Penney, 1989). Direct comparison of dopamine receptor mRNA and radioligand binding in post mortem human brain tissue has confirmed the prominent expression of D1 sites within prefrontal cortex and, consistent with data from non-human primates, indicates that D1 receptors are more common than D2 receptors (Meador-Woodruff et al., 1996; De Keyser et al., 1988). Interestingly, immunoreactivity studies indicate that many dopamine D1 receptors are located at extra-synaptic sites suggesting that they are activated predominantly by diffusion of dopamine beyond the synapse (Smiley, Levey, Ciliax, & Goldman-Rakic, 1994). The D4 receptor is also densely expressed in the cortex, in fact the D4 receptor is more prevalent in the cortex than striatum, a feature shared with D5 receptors (Lidow et al., 1998; Boy et al., 1998; Meador-Woodruff et al., 1996). D4 mRNA expression in post-mortem human brain tissue is highest in the middle frontal gyrus and cingulate gyrus with slightly lower levels detected in superior frontal gyrus (Mulcrone & Kerwin, 1997). Both in situ hybridization and immunocytochemistry studies in non-human primates have confirmed the presence of significant levels of D5 receptors in the prefrontal cortex, relative to striatum where D5 receptors levels appear negligible (Lidow et al., 1998; Choi, Machida, & Ronnekleiv, 1995; Ciliax et al., 2000). The same pattern of distribution is observed in humans, however, the expression of D5 mRNA indicates that medial prefrontal regions and occipital area 17 may be particularly enriched in D5 receptors (Khan et al., 2000; Meador-Woodruff et al., 1996). D3 receptors are expressed at relatively low levels throughout much of the cortex, however, they are present at moderately high levels in the

anterior cingulate (Meador-Woodruff et al., 1996; Suzuki, Hurd, Sokoloff, Schwartz, & Sedvall, 1998).

Dopamine Modulation of Prefrontal Cortex Cognitive Functions in Non-Human Primates

Dopamine Modulation of Working Memory

Working memory refers to the ability to maintain and/or manipulate relevant goal directed information over relatively short periods of time (i.e. seconds) and delayed-response tasks such as delayed matching-to-sample (DMS), delayed alternation, and occulomotor delayed response (ODR) (Fuster, 1997; Funahashi, Bruce, & Goldman-Rakic, 1989; Funahashi, Bruce, & Goldman-Rakic, 1990) are frequently used to investigate the neural basis of working memory in animals. Although there are differences between these commonly used probes of PFC function, the internal representation of a prior stimulus location or direction of a motor response over the delay period is critical for successful performance of each of them.

Prefrontal ablations profoundly impair performance on delayed-response tasks (Jacobsen, 1935; Jacobsen, 1936) especially at longer delay intervals (Fuster, 1997). Electrophysiological investigations indicate that sustained neuronal activity in the dorsolateral aspect of the PFC during the delay period is essential for successful performance of delayed-response tasks and that disruptions of delay related activity impairs performance (Fuster & Alexander, 1970; Fuster, 1973). The delay-related activity is highly specific to the features of the stimuli to be maintained in working memory buffers (Goldman-Rakic, 1999; Rao, Williams, & Goldman-Rakic, 1999b). For example, individual pyramidal cells and adjacent interneurons preferentially respond to stimuli in discrete spatial locations or directions, as mapped in egocentric space (Funahashi, Bruce, & Goldman-Rakic, 1993; Goldman-Rakic, 1999), and are thus said to have “memory fields” or “directional tuning”, respectively.

The considerable degree of dopaminergic innervation of the cortex suggested to researchers early on that DA may be involved in working memory. There is now considerable evidence that this is the case. PFC DA levels increase during performance of a delayed alternation task (Watanabe, Kodama, & Hikosaka, 1997) and depletion of PFC DA results in an impairment in working memory (Collins, Roberts, Dias, Everitt, & Robbins, 1998; Roberts et al., 1994) that can be partly reversed by treatment with L-DOPA (Brozoski, Brown, Rosvold, & Goldman, 1979). Similarly, age related decreases in PFC DA and associated impairments in working memory can be reversed with DA agonists (Arnsten, Cai, Murphy, & Goldman-Rakic, 1994; Arnsten, 1993; Cai & Arnsten, 1997). Conversely, there is also evidence that too much DA in the PFC may adversely affect working memory. For example, loud noise or pharmacologically induced stress increases PFC DA activity and disrupts ODR performance (Murphy, Arnsten, Jentsch, & Roth, 1996; Murphy, Arnsten, Goldman-Rakic, & Roth, 1996; Murphy, Roth, & Arnsten, 1997; Arnsten & Goldman-Rakic, 1998).

It is clear that DA modulates cell firing during the delay period and that this underlies its effects on working memory. Iontophoretic application of DA directly on to PFC neurons enhances delay-related activity and directional tuning (Sawaguchi, Matsumura, & Kubota, 1990a; Sawaguchi, 2001; Sawaguchi et al., 1988). Further work has revealed that activity at D1 sites in particular underlies most of DA effects on delay-related neuronal activity and working memory (Sawaguchi & Goldman-Rakic, 1991). Local injections of selective D1 antagonists or non-selective DA antagonists into monkey PFC impairs working memory in a dose dependent manner, especially at longer delays (Sawaguchi & Goldman-Rakic, 1994; Sawaguchi et al., 1991; Arnsten et al., 1994; Cai et al., 1997), and decreases directional delay-related neuronal activity (Wang, Vijayraghavan, & Goldman-Rakic, 2004; Sawaguchi, 2001). However, low doses of D1 antagonists enhance delay-related neuronal firing in the PFC suggesting that optimal DA D1 stimulation may be required for normal working memory (Williams & Goldman-Rakic, 1995). Observations that moderate doses of D1 agonists improve working memory in young and aged monkeys, whereas high doses impair performance (Arnsten et al., 1994; Cai et al., 1997), and that D1 agonists enhance working memory in monkeys

with down-regulated D1 receptor levels (Castner, Williams, & Goldman-Rakic, 2000) are consistent with this interpretation. In addition, stress induced impairments can be prevented by pretreatment with D1 antagonists such as clozapine (Murphy et al., 1996; Murphy et al., 1997). In contrast, D2 antagonists appear to have either no effect or only a slight deleterious effect on delay related activity and working memory performance (Sawaguchi, 2001; Williams et al., 1995; Sawaguchi et al., 1994; Sawaguchi, Matsumura, & Kubota, 1990b; Sawaguchi et al., 1988).

Dopamine Modulation of Set-Shifting

Many of the delay-related tasks that are sensitive to PFC lesions and manipulation of DA also require other cognitive processes in addition to the active maintenance of a stimulus in working memory. For example, in delayed alternation subjects must not only actively maintain the direction of the upcoming response in memory but also inhibit or suppress the tendency to respond to the previously rewarded location. Furthermore, all delayed-response tasks require the subject to ignore irrelevant stimuli during both the initial cue presentation and subsequent delay period. Attention as a whole encompasses a remarkably wide array of processes and involves diverse cortical networks broadly consisting of connections between posterior and anterior brain regions (Fan & Posner, 2004; Posner & Petersen, 1990). However, this section will focus on a specific aspect of attention called set shifting that has been linked to the dorsolateral PFC and DA (Dias, Robbins, & Roberts, 1996; Crofts et al., 2001; Roberts et al., 1994).

Attentional set shifting refers to a subject's ability to shift their "attentional set", the bias to respond to a specific feature of a stimulus following training, from one perceptual dimension of a compound stimulus to another. Visual discrimination tests of set shifting are modeled after the well-known clinical neuropsychology tool, the Wisconsin Card Sorting Test (WCST) (Milner, 1982), but allow for a finer decomposition of the cognitive processes involved in such a complicated task. For example, tests of set shifting delineate between reversal learning, intra-dimensional set

shifting (IDS), and extra-dimensional set shifting (EDS) (Crofts et al., 2001; Dias et al., 1996; Roberts et al., 1994; Dias, Robbins, & Roberts, 1997).

In a typical lesion study of set shifting, the animal is first trained on a visual discrimination task in which it must learn to respond to one aspect of a pair of compound stimuli (e.g. shape of a polygon that has lines superimposed on it) prior to ablating the PFC. Following surgery, retention of the learned discrimination is tested and the ability to learn new discriminations in which the previously trained dimension (e.g. shape of polygon) is still rewarded is examined (i.e. IDS) using a novel pair of stimuli. The animal's ability to shift attention to the previously irrelevant feature of the compound stimulus (e.g. superimposed lines) is then tested with a series of novel compound stimuli (i.e. EDS). Control animals typically demonstrate some degree of perseverate responding during learning of the new stimulus-reward pairing and this is taken as proof of the establishment of an attentional set to the previously rewarded stimulus dimension. Finally, reversal learning is then tested by seeing if the animal can alter its responding when previously learned stimulus-reward contingencies are reversed within the same pair of exemplars. Lesions to different sub-regions of the PFC differentially affect EDS and reversal learning. Specifically, EDS, but not reversal learning, is impaired following lesions of the dorsolateral PFC, whereas the reverse is true following orbital frontal lesions (Dias et al., 1996). Interestingly, both dorsolateral and orbital frontal lesioned animals are capable of EDS and reversal learning at subsequent testing sessions when they are required to shift back to a previously learned attentional set indicating that the deficits are especially apparent when the task is novel to the animal (Dias et al., 1997).

Remarkably, Roberts et al. (1994) found that 6-OHDA lesion of the dorsolateral PFC following the establishment of attentional set actually *enhances* post-surgery EDS without impairing IDS or reversal learning in marmosets (Roberts et al., 1994). Moreover, the same animals demonstrated the expected impairment in spatial working memory following PFC DA depletion. However, the implications of this finding were ambiguous since the lesions were carried out after an attentional set had been formed (Arnsten & Robbins, 2002). A subsequent study that examined both the establishment of

an attentional set and set shifting after depleting PFC DA revealed a complex pattern of deficits that were not entirely consistent with the earlier report by Roberts et al. (1994) (Crofts et al., 2001). Specifically, animals with PFC DA lesions had more difficulty forming an attentional set, where more easily distracted by novel exemplars from the irrelevant stimulus dimension after formation of an attentional set, and made more errors during EDS (Crofts et al., 2001).

Summary: the Inverted U-Curve Hypothesis of Dopamine Function

There is strong evidence that DA modulates PFC cognitive functions and physiology in monkeys. Greater DA activity in the PFC is associated with enhanced working memory performance and greater delay related activity in PFC neurons. However, too much stimulation, at D1 sites in particular, leads to a decrease in working memory performance and a reduction in directional tuning of PFC neurons. These observations have led some investigators to speculate that working memory and DA transmission in the PFC adheres to an inverted U curve whereby increases or decreases from an optimal level leads to a degradation in performance and a reduction in delay related activity.

There is compelling evidence that DA modulates another PFC function, attentional set shifting, although the neuronal basis underlying this effect has not been as clearly elucidated as it has for working memory. Also, it remains to be determined if attentional processes follow a similar inverted U-curve as working memory appears to. Work in rodents using the five choice reaction time task suggests that this may be case (Granon et al., 2000). Granon and colleagues trained rats to perform a five choice reaction time task that required the animals to react quickly to receive reward when a cue indicating the presence of a reward appeared in one of five locations. Rats were trained until their performance stabilized and then divided into two groups; one with accuracy above 75% and the other with accuracy below 75% percent, that putatively related to differences in endogenous PFC DA release. Infusions of DA D1 agonists and antagonists

directly into medial PFC differentially affected performance. Specifically, infusion of the D1 agonist SKF 38393 improved accuracy in the low baseline group but decreased accuracy in the high baseline group (Granon et al., 2000). Thus, although the evidence is limited, attentional processes mediated by PFC DA stimulation may also adhere to an inverted U function. In addition, not all cognition functions relying on the PFC are affected by DA transmission in this region. For example, self-ordered sequencing, a task impaired by PFC lesions, is not sensitive to PFC DA depletion (Collins et al., 1998).

Dopamine Modulation of Pre-Frontal Cortex Cognitive Functions in Humans

There is overwhelming evidence that many of the cognitive deficits observed after PFC ablations in non-human primates are also seen in humans with similar lesions and the relatively recent development of functional imaging has further clarified the role of the PFC in human cognition (Fuster, 1997; Cabeza & Nyberg, 2000; Stuss et al., 2002). Not surprisingly, the cognitive functions of the PFC in humans differ quantitatively from non-human primates and this is likely a direct consequence of greater phylogenetic development. However, aside from the obvious development of language, they are qualitatively similar across species.

The cognitive functions of the PFC in humans have become synonymous with the term "executive functions"; a general descriptor that often subsumes a number of conceptually complicated processes such as planning, volition, purposive action, and effective performance (Lezak, 1995). While these terms may be useful within the framework of clinical neuropsychology, they are generally not useful from a cognitive neuroscience point of view since they are broad and difficult to operationally define. Unfortunately, attempts to fractionate executive functions into more circumscribed constituent parts has proven especially difficult in humans (Baddeley, 2002; Miyake et al., 2000; Miyake, Emerson, & Friedman, 2000; Stuss, Shallice, Alexander, & Picton, 1995; Stuss et al., 2002). Part of the problem in dissociating processes undoubtedly has to do with the way executive functions are usually assessed in both clinical and

experimental settings. A variety of experimentally and clinically derived tools are used to assay executive functions. In some cases, the paradigms and tests tap a relatively specific function. However, more often than not, the tasks used are more complicated and tap multiple cognitive processes of the PFC. Even seemingly disparate tests can tap similar executive subcomponents. Some success has been made in identifying specific cognitive sub-components that collectively make up executive functions (Royall et al., 2002; Miyake et al., 2000; Stuss et al., 2002; Collette & Van der Linden, 2002). Generally, several inter-correlated but dissociable factors identified as working memory (updating and monitoring in particular), inhibition and resistance to distraction, and mental set shifting are thought to exist (Miyake et al., 2000; Dias et al., 1996; Baddeley & Hitch, 1974).

Linking the specific sub-components to their respective anatomies has also proven difficult (Stuss et al., 2002; Duncan & Owen, 2000). Lesion studies in non-human primates have identified a broad dissociation between dorsolateral cortex, which is involved primarily in tasks that require integration of events and responses across time and selective attention (i.e. delayed response tasks and set shifting), the ventral cortex, which appears to play a role in inhibition, especially when affect is involved (i.e. reversal learning), and the anterior cingulate, which is largely involved in monitoring conflict between competing responses and outcome (Fuster, 1997; Dias et al., 1996; Dias et al., 1997; Ito, Stuphorn, Brown, & Schall, 2003; Gemba, Sasaki, & Brooks, 1986; Meunier, Bachevalier, & Mishkin, 1997). This broad compartmentalization of functions is largely present in humans also, although there is considerable overlap of function (Stuss et al., 2002; Jonides, Badre, Curtis, Thompson-Schill, & Smith, 2002; Bechara, Tranel, & Damasio, 2000; Bechara, 2004; Tranel, Bechara, & Denburg, 2002; Botvinick, Braver, Barch, Carter, & Cohen, 2001).

Surprisingly, imaging studies have not been as beneficial as hoped at delineating the anatomical components unique to each sub-process (Duncan et al., 2000; Collette et al., 2002; Cabeza et al., 2000). Indeed, imaging studies indicate that diverse executive tasks often recruit a relatively homogenous set of regions in the PFC including the

dorsolateral and ventrolateral PFC, and anterior cingulate (Duncan et al., 2000; Collette et al., 2002); although there are differences in the degree to which each brain region is recruited by executive sub-processes. For example, tasks that require a high degree of conflict monitoring strongly activate the dorsal anterior cingulate relative to tasks that place lesser demands on this function (Collette et al., 2002; Carter, Botvinick, & Cohen, 1999; Carter et al., 1998; Jonides et al., 2002). Based on lesion and imaging data it is tempting to speculate that executive functions may be anatomically situated within the PFC; however, it is important to keep in mind that many of the operations described as executive functions rely on distributed networks that include not only PFC regions but posterior cortical areas and sub-cortical structures as well. Furthermore, lesions to the frontal lobe do not always result in impairments in executive functions and damage outside of the PFC can also impair executive functions (Lezak, 1995; Stuss et al., 2002).

Insights into DAs effects on PFC cognitive functions in humans have been hampered by a number of methodological limitations. The first stems from the fact discussed above that many of the most commonly used tests of executive functions tap multiple cognitive processes and, thus, make it difficult to isolate and detect neurotransmitter specific effects on cognition. A second major limitation relates to the way one major function of the PFC, working memory, is assessed in monkeys and humans. There is a wealth of data indicating that the PFC is also critical for working memory in humans (Fuster, 1997; Kolb & Whishaw, 2003; Passingham & Sakai, 2004). However, there are important differences between humans and non-human primates regarding what "working memory" tasks are in fact assessing. In non-human primates, working memory often refers exclusively to the short term maintenance of information for future use (Fuster, 1997; Goldman-Rakic, 1990). While this definition has been applied to paradigms used in human research, the broader concept of working memory, as defined by Baddeley and Hitch (1974), posits that information is not only transiently stored in limited capacity systems but also manipulated, presumably by one or several executive sub-components (Baddeley, 2003; Baddeley et al., 1974). In working memory, executive functions come into play when various operations are to be performed on information contained within storage systems. Thus, typical working memory paradigms

used in non-human primates place little, if any, demands on other executive functions while working memory tasks used in humans often place variable demands on them.

This distinction between non-human and human primates with respect to working memory has important implications for the neuroanatomical substrates of memory related functions in the PFC. As discussed in the previous section, working memory tasks in non-human primates relies primarily on the integrity of the dorsolateral PFC. However, in humans the transient maintenance of information appears to rely primarily on the ventrolateral PFC (Baddeley, 2003; Owen, 2000; D'Esposito, Postle, Ballard, & Lease, 1999; D'Esposito et al., 1998) and the dorsolateral PFC typically doesn't come into play unless delay periods are relatively long, the information is actively rehearsed in memory, the to be remembered stimulus is complex (i.e. faces), or some manipulation or updating of information is required (Owen, 2000; Baddeley, 2003; Passingham et al., 2004; McCarthy et al., 1994; Courtney, Ungerleider, Keil, & Haxby, 1997b; Manoach et al., 1997; Stern et al., 2000; Leung, Gore, & Goldman-Rakic, 2002; Passingham & Rowe, 2002). This does not imply that the dorsolateral PFC does not display sustained delay related activity too, it does (Courtney et al., 1997), but indicates that it is more sensitive to the executive demands of the task (Curtis, Rao, & D'Esposito, 2004; Callicott et al., 2003b; Manoach et al., 1997; Owen, 2000). Moreover, individual differences in working memory performance relates directly to the degree of activity observed in the PFC (Rypma, Berger, & D'Esposito, 2002; Mattay et al., 2000). Individuals with greater working memory performance exhibit less activation at any given working memory load level than individuals with poorer performance; although dorsolateral PFC activity plateaus at high working memory loads and decreases once accuracy begins to significantly decrease for all subjects (Callicott et al., 1999). Some investigators have interpreted these observations as evidence that subjects with better working memory performance have greater dorsolateral cortical "efficiency" (Callicott et al., 2003b; Callicott et al., 1999; Weinberger et al., 2001) than lower performing subjects, perhaps as a result of employing more efficient memory strategies (Rypma et al., 2002). One implication of such a hypothesis is that the dorsolateral PFC plays a more general role in PFC cognitive functions such as attentional selection (Passinghan & Rowe, 1995;

D'Esposito, Ballard, Aguirre, & Zarahn, 1998) and may explain why this region is crucial for many tasks.

With these limitations in mind the available literature on DA effects on PFC cognitive functions in humans are reviewed briefly in the section below.

Dopamine modulation of working memory and executive functions

Executive functions and working memory in Parkinson's disease

Parkinson's disease (PD) results from a substantial loss of DA at the striatum due to degeneration of substantia nigra cells (Forno & Alvord, Jr., 1971; Alvord, Jr., 1971) and lesser reductions in cortical DA due to degeneration of cells in the VTA (Scatton, Javoy-Agid, Rouquier, Dubois, & Agid, 1983). As such, PD provides a crude model for examining alterations in PFC function and physiology that result from a loss of DA inputs to the cortex. Not surprisingly, deficits in PFC cognitive functions are also observed in addition to the classic extrapyramidal motor symptoms that define the illness (Owen, 2004). Patients with PD are often prescribed L-dopa and studies of patients in the off and on medication states have proved fruitful for investigating dopaminergic modulation of PFC cognitive functions. L-dopa treatment has been shown to improve working memory (Lewis, Slabosz, Robbins, Barker, & Owen, 2005), task switching (Cools, Barker, Sahakian, & Robbins, 2003), and set shifting (Lange et al., 1992).

Pharmacological studies in healthy individuals

The effects of non-specific and specific DA receptor agonists have been investigated in healthy individuals in order to further elucidate the effects of DA on cognitive functioning. Amphetamine improves performance on a selective attention task, the Eriksen Flanker paradigm, and increases the amplitude of the error-related negativity

response thought to originate in the anterior cingulate (Servan-Schreiber, Carter, Bruno, & Cohen, 1998; de Bruijn, Hulstijn, Verkes, Ruigt, & Sabbe, 2004). Improvements in spatial working memory with bromocriptine, a D2 agonist, or pergolide, a partial D1/D2 agonist, have been reported in some (Kimberg, D'Esposito, & Farah, 1997; Luciana, Collins, & Depue, 1998; Muller, von Cramon, & Pollmann, 1998; Mehta, Swainson, Ogilvie, Sahakian, & Robbins, 2001) but not other studies (Kimberg, Aguirre, Lease, & D'Esposito, 2001; Bartholomeusz, Box, Van Rooy, & Nathan, 2003; McDowell, Whyte, & D'Esposito, 1998; Roesch-Ely et al., 2005). Improvements have tended to be found in studies that included delay periods of 8 seconds or longer (Luciana et al., 1998; Muller et al., 1998).

The effects of these medications on other executive functions have not been studied as extensively; however, the results have also been inconsistent (Kimberg et al., 2001; Kimberg et al., 1997; McDowell et al., 1998). A number of studies have also divided subjects into high and low performance groups that putatively differ in their degree of endogenous DA activity and, hence, their positions on the inverted U-curve, in order to examine interactions between baseline performance and change following treatment. Results have been inconsistent in this regard also. In general no interaction between baseline performance and response to D2 agonists has been observed for working memory (Kimberg et al., 2001; Kimberg et al., 1997; Roesch-Ely et al., 2005). McDowell et al. (1998) found that bromocriptine improved performance on a composite measure of executive function that included the Stroop, verbal fluency, and Trailmaking B tests in subjects with low baseline performance whereas it decreased performance in the high baseline group. Similarly, Kimberg et al. (1997) reported improvement in WCST with bromocriptine in low performers and impairment in the high baseline group. However, a subsequent study by the same group found the exact opposite (Kimberg et al., 2001).

In contrast to studies using DA agonists, investigations on the effects of DA antagonists have tended to be more consistent. In general, non-specific and D2 specific antagonists impair a wide range of executive functions in healthy controls including

working memory, task switching, and extra-dimensional set shifting (Mehta, Manes, Magnolfi, Sahakian, & Robbins, 2004; Mehta, Sahakian, McKenna, & Robbins, 1999; Beuzen, Taylor, Wesnes, & Wood, 1999; Ramaekers et al., 1999; Vitiello et al., 1997). Interestingly, the impairment observed in extra-dimensional set-shifting with sulpride, a selective D2 antagonist, was only observed during the first testing session in two double-blind, crossover studies, again suggesting that DA is critical when this task is novel to the subject (Mehta et al., 2004; Mehta et al., 1999). Examination of the effects of DA agonists or antagonists on executive function is limited though by the lack of specific D1 agonists or antagonists. This is unfortunate as the bulk of the non-human primate literature suggests that DA exerts many of its effects on PFC cognitive functions through its actions at the D1 receptor.

Neuroimaging Studies of Dopamine

Studies of the effects of DA manipulations on executive functions in PD and healthy individuals are informative; however, the inferences that can be drawn from such investigations are limited by a number of factors. In the case of PD, it is unclear to what extent deficits in executive functions reflect pathology of the mesocortical system or disruption of striatal outflow to the PFC (Monchi et al., 2004) since DA is depleted throughout the cerebrum. This is also a problem for pharmacological investigations in healthy controls since all of them utilized partial or selective D2 agonists/antagonists that likely exerted most of their effects at the striatum since the density of D2 receptors is much greater in this region than cortex.

Although the data is limited to a single recent PET study; there is indirect evidence that, consistent with findings in rodents and non-human primates, endogenous DA release in the lateral PFC and ventral anterior cingulate is greater during performance of a verbal working memory task than during a vigilance control task (Aalto et al., 2005). Moreover, the amount of DA released in ventrolateral PFC and ventral anterior cingulate has been shown to correlate with reaction times and errors on a verbal working memory

task suggesting that greater DA release is related to better performance (Aalto et al., 2005).

Alterations in cognitive function and PFC physiology following exogenous modulation of DA in controls and PD have also been examined with functional neuroimaging. PD patients demonstrate greater activation of dorsolateral PFC and anterior cingulate during performance of a working memory or planning task, respectively, when off L-dopa relative to when they are receiving treatment (Cools, Stefanova, Barker, Robbins, & Owen, 2002; Mattay et al., 2002). Over-activation of the dorsolateral PFC, relative to controls, was also observed during performance of a set-shifting task in patients withdrawn from L-dopa for at least 12 hours (Monchi et al., 2004). Moreover, increased activation in the dorsolateral cortex when off L-dopa has been shown to correlate with the number of errors subjects made on an n-back working memory task or the Tower of London planning task. These studies are reminiscent of the L-dopa induced restoration of spatial working memory in DA lesioned monkeys and suggest that L-dopa therapy may in essence normalize PFC physiology by promoting more focal activity and improving the efficiency of PFC function (Mattay et al., 2002; Goldman-Rakic, 1999; Sawaguchi, 2001; Cools et al., 2002). A similar normalization of PFC over-activity during working memory has also been observed following l-dopa treatment in patients with Tourettes' syndrome (Hershey, Lillie, Sadler, & White, 2004).

Imaging studies in healthy individuals have also tended to report decreases in task related PFC activity following administration of non-selective DA agonists. Amphetamine decreases fMRI blood-oxygen-level-dependent (BOLD) response in left or right dorsolateral PFC during performance of a verbal working memory or n-back task, respectively (Willson, Wilman, Bell, Asghar, & Silverstone, 2004; Mattay et al., 2000). Similarly, decreased working memory related regional cerebral blood flow (rCBF) was observed in the left prefrontal cortex following administration of apomorphine (Friston et al., 1992; Grasby et al., 1992). However, amphetamine has also been shown to increase PET regional cerebral blood flow in the left inferior frontal cortex during performance of the WCST (Mattay et al., 1996).

The discrepant findings may relate to individual differences in PFC cognitive function and physiology. Mattay et al. (2002) found that amphetamine related BOLD increase in the dorsolateral PFC was greater in subjects who demonstrated high 3-back working memory at baseline compared to subjects with low baseline performance. Moreover, amphetamine worsened working memory performance in the high baseline group and increased performance in the low baseline group (Mattay et al., 2000). A recent fMRI study did not identify a similar relationship between baseline performance and PFC activity on a different and less demanding version of the 2-back working memory task with bromocriptine (Kimberg et al., 2001) suggesting that associations between baseline performance and response to DA agonists may not be mediated by activity at D2 receptors and/or are not as apparent on tasks that place less demands on executive functions.

Pre-Frontal Dopamine Dysfunction and Cognitive Impairment in Schizophrenia

There is growing consensus that many of the cognitive deficits observed in schizophrenia, especially those related to executive functions and working memory, arise to some extent from abnormal DA transmission in the PFC (Doran et al., 1987; Abi-Dargham, 2004; Knable et al., 1997; Akil et al., 1999; Weinberger et al., 2001; Davis et al., 1991; Weinberger et al., 1988). Evidence in support of altered mesocortical DA function has come from at least two lines of research. First, imaging studies suggest that DA is involved in some of the deficits. In vivo binding of the specific D1 receptor radiotracer [11C]NNC 112 is higher in the PFC of patients, compared to controls, and positively correlated with the degree of working memory impairment in patients (Abi-Dargham et al., 2002). The up-regulation of D1 receptors is attributed to reduced DA innervation of the PFC, which is consistent with post-mortem data (Akil et al., 1999). However, the failure of earlier studies to consistently identify a similar increase in binding using the D1 radiotracer [11C]SCH 23390 (Okubo et al., 1997; Karlsson, Farde, Halldin, & Sedvall, 2002) along with observations that cortical D2 binding potentials are reduced in PD patients questions this assumption (Kaasinen et al., 2003; Kaasinen et al.,

2000). The reasons for this discrepancy are unclear; however, it may relate to the fact that the D1 radiotracer [11C]SCH 23390 appears insensitive to chronic cortical DA depletion and D1 receptor up-regulation, whereas [11C]NNC 112 does, at least in rodents (Guo et al., 2003). Nonetheless, additional imaging studies suggest that mesocortical DA dysfunction is related to cognitive impairment in schizophrenia. rCBF in the dorsolateral cortex during performance of the WCST is positively correlated with CSF concentrations of the DA metabolite HVA indicating that greater DA activity may be related to improved PFC function (Weinberger et al., 1988). Observations that apomorphine or amphetamine improve rCBF in the dorsolateral PFC and anterior cingulate during performance of the WCST or a verbal fluency task, respectively (Daniel, Berman, & Weinberger, 1989; Daniel et al., 1991; Dolan et al., 1995), are consistent with this interpretation. Furthermore, dopamine agonists have proven beneficial for the treatment of PFC cognitive dysfunction (Goldberg, Bigelow, Weinberger, Daniel, & Kleinman, 1991).

A second line of evidence supporting the hypothesis that PFC DA transmission is abnormal in schizophrenia has come from studies that examined cognitive change to various atypical, or second generation antipsychotic drugs (APDs). Atypical APDs are distinguished from earlier typical or first generations APDs based on the fact that they produce fewer extrapyramidal side effects (Meltzer, 2004). Atypical APDs also increase the release of DA in the PFC in rodents, a feature not observed with typical APDs (Ichikawa, Li, Dai, & Meltzer, 2002; Meltzer, 2002; Ichikawa & Meltzer, 1999). Accordingly, atypical APDs improve some PFC cognitive functions in schizophrenia such as selective attention and verbal fluency (Meltzer & Sumiyoshi, 2003; Woodward, Purdon, Meltzer, & Zald, 2005), and increase task-related fMRI responses during performance of working memory, verbal fluency, or paced motor tasks (Honey et al., 1999; Stephan et al., 2001; Jones et al., 2004).

Summary of Dopaminergic Modulation of Prefrontal Cortex Cognitive Functions in Humans

Overall the evidence that DA modulates PFC cognition and physiology in humans is consistent with findings from non-human primates; although there are areas that require further investigation. There is compelling evidence that normal DA transmission in the PFC is critical for working memory and other executive functions in humans. Evidence in support of this has come from a variety of sources including pharmacological and neuroimaging investigations of healthy individuals and patient populations with established or suspected mesocortical DA system pathology. Imaging studies of healthy individuals indicate that greater DA release or activity in the PFC is associated with better working memory performance and selective attention. Similarly, investigations in PD and schizophrenia have generally found that non-specific DA agonists normalize PFC physiology and improve PFC cognitive functions. Evidence that specific DA agonists improve PFC physiology and cognitive function in healthy individuals is equivocal at this time; however, investigations have been hampered by the lack of a specific D1 agonist. On the other hand, there is considerable evidence that DA antagonists have a deleterious effect on PFC functions.

Evidence that PFC cognitive functions and physiology follow and inverted U-curve in humans whereby to much or too little DA impairs performance and function is limited. While there is clearly evidence from patient groups and normal controls that too little DA impairs certain cognitive functions, the effects of too much DA stimulation on PFC cognitive functions is not clearly understood. Consistent with findings in non-human primates, stress impairs selective attention in humans, but it remains to be confirmed that this is a direct result of over stimulation of DA receptors in the PFC (Hartley & Adams, 1974). There is some evidence that individual differences in response to amphetamine or bromocriptine is related to baseline performance; however, the findings have not always been in the anticipated direction (i.e. those with worse performance at baseline demonstrate greater improvement after treatment and vice versa).

Imaging studies of individual differences have produced some evidence that DA over-stimulation promotes greater activity and reduced cortical "efficiency", but the sample sizes yielded after stratification have been very small.

Genetic Regulation and Role of COMT in the Metabolism of Dopamine in the Prefrontal Cortex

COMT exists in both soluble (S-COMT) and membrane-bound (MB-COMT) forms but MB-COMT is more highly expressed in the brain (Tenhunen et al., 1994; Rivett, Francis, & Roth, 1983b). The two forms are also expressed in different cell populations within the CNS; S-COMT is localized to glial cells and MB-COMT is localized to neurons, at post-synaptic dendritic process in particular (Matsumoto et al., 2003; Mannisto & Kaakkola, 1999; Rivett, Francis, & Roth, 1983a).

In the CNS, DA is inactivated by active reuptake, via the dopamine (DAT) and norepinephrine (NET) transporters, or metabolized to 3-methoxytyramine (3-MT) and homovanillic acid (HVA) by COMT and monamine oxidase (Harvey, Napolitano, Mao, & Gharabawi, 2003) respectively (Cooper et al., 2003; Moron, Brockington, Wise, Rocha, & Hope, 2002; Weinshilboum et al., 1999). In the striatum, the vast majority of DA is quickly removed from the synapse by DAT, which is present in high concentrations on pre-synaptic DA terminals, and only moderate levels of COMT are present (Grace, 1995; Giros, Jaber, Jones, Wightman, & Caron, 1996; Hong, Shu-Leong, Tao, & Lap-Ping, 1998). The situation is markedly different in the cortex. DAT levels are variable throughout the cortex with motor cortex and dorsal anterior cingulate demonstrating relatively high levels, parietal and ventral cingulate regions demonstrating moderate levels, and dorsolateral areas demonstrating little or no DAT reactivity (Lewis et al., 2001; Ciliax et al., 1999; Ciliax et al., 1995; Sesack, Hawrylak, Matus, Guido, & Levey, 1998). In contrast, COMT levels are high throughout much of the cortex, relative to striatum (Hong et al., 1998). This distribution pattern along with evidence that; 1) 3-

MT formation accounts for up to 60% of DA turnover in the PFC (Karoum et al., 1994); 2) DAT antagonists such as cocaine inhibit uptake by 95% in the striatum but only 40-70% in medial prefrontal cortex (Wayment, Schenk, & Sorg, 2001; Tzschentke, 2001; Garris, Collins, Jones, & Wightman, 1993; Hadfield & Nugent, 1983; Izenwasser, Werling, & Cox, 1990; Wheeler, Edwards, & Ondo, 1993) and increase extracellular DA levels by 5000% in striatum but only 250% in PFC (Saunders, Kolachana, & Weinberger, 1994); and, 3) DA clearance is slower and diffusion greater in prefrontal cortex than striatum (Garris et al., 1993; Tzschentke, 2001) suggest that COMT plays a greater role and DAT a reduced role, relative to striatum, in regulating prefrontal DA flux.

Gene knockout and pharmacological studies have shed some light on COMT's role in regulating prefrontal DA flux. An initial study of genetic knockout mice identified an increase in prefrontal DA levels, but not other catecholamines, in male, but not female, knockout mice suggesting that a direct inverse relationship between COMT activity and basal DA levels may exist (Gogos et al., 1998). Moreover, male knockout mice demonstrated increased levels of aggression when caged with other male mice, and female knockout mice demonstrated decreased locomotion in the dark/light exploratory model of anxiety-like behavior, relative to their wild-type counterparts (Gogos et al., 1998). However, a second gene knockout study failed to identify a similar increase in basal DA levels in male and female mice lacking the COMT gene (Huotari et al., 2002). The authors attributed this discrepancy to several methodological differences between studies including the difference in DA sampling techniques and the extensive behavioral testing of animals in the initial study. The latter may be particularly relevant in light of evidence that DA release induced by the DA precursor L-DOPA or the depolarization agent potassium chloride is greater in knockout mice or rats pretreated with the COMT inhibitor tolcapone, respectively (Huotari et al., 2002; Tunbridge, Bannerman, Sharp, & Harrison, 2004). Interestingly, tolcapone also potentiated clozapine induced DA release in rats. Thus, the available evidence suggests that COMT has little or no effect on basal DA levels under normal conditions, but may potentiate DA accumulation in the PFC under pharmacologically and, speculatively, stimulus induced DA release.

A single gene located on chromosome 22q11 encodes both S-COMT and MB-COMT, although S-COMT is assembled from a shorter mRNA sequence (Lundstrom, Salminen, Jalanko, Savolainen, & Ulmanen, 1991; Salminen et al., 1990; Tenhunen et al., 1994; Mannisto et al., 1999; Grossman, Emanuel, & Budarf, 1992). A common single nucleotide polymorphism (Li et al., 2000) consisting of a valine (val) to methionine (met) substitution at codon 108 of S-COMT and 158 of MB-COMT exists that alters COMT activity in humans (Lachman et al., 1996; Lotta et al., 1995). The substitution of met for val produces a thermally unstable variant of COMT such that individuals homozygous for the met allele exhibit considerably less enzyme activity than val homozygous subjects (Lotta et al., 1995; Lachman et al., 1996). Since neither allele is dominant, heterozygous individuals demonstrate intermediate COMT activity.

The exact difference in enzyme activity between genotype groups is unclear. Differences in activity in peripheral organs expressing COMT have ranged from 3 to 4 fold (Weinshilboum et al., 1999; for review see Mannisto et al., 1999); however, the methods used to measure enzyme activity in these studies may have over-estimated the actual differences that exist between genotype groups (Chen et al., 2004). A recent study that examined enzyme activity in both post-mortem PFC brain tissue and lymphocytes under simulated normal physiological temperatures (i.e. 37 degrees C) found that the met/met haplotype was associated with approximately 40% less enzyme activity than the val/val haplotype (Chen et al., 2004). Samples obtained from women had less enzyme activity at all genotype groups than samples from men, but variation by genotype was consistent in Caucasians and African Americans.

Experiment 2A: The Neuropsychological Correlates of COMT val(108/158)met Genotype in Schizophrenia

Introduction

It is clear based on the review in the preceeding section that greater endogenous DA activity in the PFC is associated with better cognition (Aalto et al., 2005; Watanabe et al., 1997; Phillips, Ahn, & Floresco, 2004). Moreover, evidence, primarily from rodents and non-human primates suggests that DAs effects on cognitive functions follow an inverted U-curve whereby either increases or decreases from an optimal level leads to cognitive impairment (Goldman-Rakic et al., 2000; Goldman-Rakic, Castner, Svensson, Siever, & Williams, 2004; Tipper et al., 2005). DA enhances the physiological response of the PFC during performance of working memory and executive function tasks by promoting more focal neuronal activity; an effect that has been described as increasing physiological "signal-to-noise" or cortical "efficiency" (Sawaguchi et al., 1988; Sawaguchi, 2001; Mattay et al., 2000; Mattay et al., 2002; Servan-Schreiber, Printz, & Cohen, 1990; Servan-Schreiber et al., 1998; Weinberger et al., 2001; Tipper et al., 2005).

Preliminary findings from rodents or humans treated with COMT inhibitors indicate that reducing COMT activity improves aspects of PFC mediated cognitive functions, presumably by increasing the availability of DA. Rats treated with tolcapone, a COMT inhibitor, demonstrate better spatial working memory and extra-dimensional set shifting than control animals (Tunbridge et al., 2004; Liljequist, Haapalinna, Ahlander, Li, & Mannisto, 1997). Similarly, tolcapone improves performance on several tests sensitive to PFC cognitive functions in patients with PD (Gasparini, Fabrizio, Bonifati, & Meco, 1997). These findings suggest that COMT genotype may impact performance on tests of working memory and attention, such as set shifting, but not other cognitive tasks that are not sensitive to DA transmission in the PFC. More specifically, individuals with the met/met genotype should outperform val/val subjects given that the met allele is associated with reduced enzymatic activity and, speculatively, greater prefrontal DA

activity in vivo in humans. Heterozygous individuals should perform somewhere in between homozygous subjects.

Most studies have examined COMT genotype effects on the WCST, a widely used neuropsychological test that is sensitive to PFC damage and, speculatively, DA transmission in the PFC. As can be seen in Table 5, with relatively few exceptions, most studies found that, as anticipated, individuals homozygous for the met allele demonstrated fewer perseverate errors than subjects homozygous for the val allele. In some cases, the failure to identify a significant genotype effect on the WCST likely related to small sample sizes within genotype groups, COMT activity variation by ethnicity (Lee et al., 2005), and, in the case of schizophrenia patients, possible interactions between medications and genotype.

Table 5. Associations between COMT val108/158met genotype and WCST.

Study	Subject Population	N				Findings
		Total	m/m	m/v	v/v	
Egan et al., (2001)	Ctrl, Scz, Sib	452	77	229	146	m/m > v/v; m/v > v/v (Scz & Ctrl only)
Bilder et al., (2002)	Scz	58	7	31	20	NS
Joober et al., (2002)	Ctrl, Scz	125	31	59	35	m/m > v/v; m/m > m/v
Malhotra et al., (2002)	Ctrl	73	13	31	29	m/m > v/v
Tsai et al., (2003)	Ctrl (women)	120	6	43	71	NS
Rosa et al., (2004)	Scz, Sib pairs	178	NR	NR	NR	m/m > v/v (Sib only)
Bruder et al., (2005)	Ctrl	246	43	116	87	m/m > v/v
Ho et al., (2005)	Ctrl, Scz	243	56	144	43	NS
Galderisi et al., (2005)	Scz	106	28	42	36	m/m > v/v on combined CPT & WCST score
Minzenberg et al., (2006)	Ctrl/SPD	98	26	50	22	met carriers > val/val
Rybakowski et al., (2006)	Scz	79	24	41	14	val/val > met carriers in females
Szoke et al., (2006)	Ctrl, Scz, Bpd, Sib	303	68	165	70	NS

Abbreviations: Bpd: Bipolar Disorder; CPT: Continous Performance Test; Ctrl: Control; m: met; NR: Not Reported; NS: Not Significant; Sib: Sibling; Scz: Schizophrenia; v: val; WCST: Wisconsin Card Sorting Test.

The effect of genotype on tests that are, putatively, more specific to working memory than the WCST are somewhat less consistent, most likely because of variability in the instruments used to assess working memory. A significant effect of genotype was observed on 1 and 2-back accuracy in a large sample of patients, siblings, and controls (Goldberg et al., 2003), however, this finding was not replicated in two large samples of healthy subjects (Stefanis et al., 2004; Bruder et al., 2005). The discrepancy may relate to subtle differences between the versions of the n-back test used in the studies. The study reporting the positive finding (Goldberg et al., 2003) employed a demanding free

recall version that has been shown on several occasions to be sensitive to DA modulation of PFC activity (Mattay et al., 2002; Mattay et al., 2000), whereas the version employed in the two negative studies (Bruder et al., 2005; Stefanis et al., 2004) taps recognition memory and has not been consistently linked with DA transmission (Kimberg et al., 2001; Bartholomeusz et al., 2003). Bruder et al. (2005) examined the specificity of COMT genotype effects on working memory by examining performance differences across a battery of working memory tests that included the Letter-Number Sequencing (LNS) subtest from the Wechsler Adult Intelligence Scales-Revised (WAIS-R), Spatial Delayed Response Task (SDR), Word Serial Position Test (WSPT), and N-back task. A significant effect of COMT genotype was observed only on the LNS. A significant genotype effect was observed on digit span subtest of the Korean-Wechsler Adult Intelligence Scale in a sample of first-episode Korean schizophrenia patients (Han et al., 2006); however, a similar finding was not observed on the WAIS-R digit span backwards in a Caucasian sample (Ho, Wassink, O'Leary, Sheffield, & Andreasen, 2005). Minzenberg et al. (2006) identified a significant genotype effect on the Paced Auditory Serial Addition Test (PASAT), a demanding test of verbal working memory, but not on the DOT visuospatial working memory test. Combined, these findings suggest that verbal working memory may be related to COMT genotype, particularly on demanding paradigms such as the free recall N-back test, LNS, and PASAT that tap executive processes similar to the WCST such as manipulation and/or temporal updating of information, but the evidence is not conclusive in this regard.

The reported effects of genotype have been inconsistent for tests of executive functions that do not tap working memory per se but require a high degree of cognitive control. The met allele has been linked to better performance on a non-verbal Stroop-like task in a sample of children (Diamond, Briand, Fossella, & Gehlbach, 2004) but not an antisaccade task or flanker task in adults (Stefanis et al., 2004; Fossella et al., 2002). Interestingly, a main effect of genotype was identified on a flanker-like task in which the degree of perceptual conflict varied parametrically (Blasi et al., 2005). Specifically, Blasi et al. (2005), found that val homozygous subjects made significantly more errors than met homozygous subjects when the degree of stimulus-response conflict was greatest, but

not at lower levels of conflict. This is reminiscent of findings from studies that used the n-back task which have tended to find the greatest associations between dopamine function and performance on the more demanding 3-back version of the task (Mattay et al., 2000; Goldberg et al., 2003). Combined, the findings lend further support to the hypotheses that associations between COMT and performance may be most apparent when executive functions are highly taxed.

The specificity of COMT val108/158met polymorphism effects on cognition is poorly understood. The most comprehensive examination was carried out by Bilder and colleagues (2002b) who examined gene effects on a battery of 15 neuropsychological tests grouped into five domains in a small sample of patients with schizophrenia. They identified a significant relationship in the expected direction (i.e. met/met > val/val) on the Processing Speed and Attention domain that included the Trailmaking tests and WAIS-R Digit Symbol test, but not on the Executive and Perceptual Organization domain that included the WCST, or Verbal Learning and Memory, and Simple Motor domains in a sample of chronic patients with schizophrenia (Bilder et al., 2002b). However, the conclusions reached by this study are tenuous at best given that five or fewer met homozygous subjects completed many of the tests. Nonetheless, Somewhat similar findings were reported on an executive domain that included Trailmaking B (TMB) in a small sample of patients with Velo-Cardio-Facial Syndrome tested on a circumscribed battery composed of four measures (Bearden et al., 2004). De Frias et al. (2005) reported a significant effect of genotype on a composite measure of executive function that included a verbal fluency test, the Tower of Hanoi, and an experimentally derived measure of working memory. However, Ho et al. (2005) failed to find a significant effect of genotype on TMB or WCST perseverate errors in a sample of 243 schizophrenia patients and controls tested on a limited battery composed of the WCST, TMB, and digit span tests (Ho et al., 2005). Similarly, no effect of genotype was observed on the WCST and TMB in a mixed sample of 318 controls, bipolar patients, schizophrenia patients, and their unaffected relatives (Szoke et al., 2006). Mixed results have also been reported for specific tests of verbal learning and episodic memory (de Frias et al., 2004; Minzenberg et al., 2006). Thus, while considerable evidence indicates

that COMT is associated with the WCST and working memory, there is a paucity of data on the associations between COMT genotype and other aspects of cognitive function. This is unfortunate given existing data in schizophrenia patients indicating that enhanced DA transmission may be associated with broad neuropsychological improvement.

The goal of the experiment described below was to 1) replicate and extend previous findings of an association between COMT genotype and executive functions, including working memory, and 2) examine the specificity of COMT effects on cognitive functions using a battery of validated neuropsychological tests in a relatively large sample of mostly unmedicated patients with schizophrenia.

Methods

Subjects

93 schizophrenia patients with preserved blood samples available for genetic analysis were included in this study and the prospective component of the study discussed in the following experiment. All subjects underwent at least one neuropsychological evaluation at baseline and after 6-weeks and 6-months of treatment with clozapine. The subjects included in this study represent the subset of patients with available DNA for genotyping from a group of approximately 280 schizophrenia patients obtained from Case Western Reserve University. The majority of patients (78%) were unmedicated at baseline. The rest were receiving typical APDs. The methods surrounding the recruitment and screening of subjects are described in detail in several prior reports (Kenny & Meltzer, 1991; Lee et al., 1999; Hwang et al., 2005). Briefly, diagnoses were based on a structured interview from which DSM-III-R or DSM-IV criteria were extracted and reviewed by a research psychiatrist (H.Y. Meltzer). Exclusion criteria included history of learning disabilities, drug/alcohol abuse, head trauma, stroke, or neurological illness. The demographics for the subjects included in the baseline cross-sectional study and prospective investigation reported in the following section are

presented in Table 6. The only variable the genotype groups differed on was ethnicity ($\chi^2(2)=12,48$, $p<.003$). A significantly greater proportion of the val homozygous group was composed of African Americans than the met homozygous ($\chi^2(1)=10.89$, $p<.002$) and val/met heterozygous groups ($\chi^2(1)=4.79$, $p<.030$).

Table 6: Experiment Two Sample Demographics*.

	Genotype			Total	
Variable	met/met	val/met	val/val	**Sample**	**Test Statistic**
n	22	37	34	93	$x^2=3.39$, $p<.436$
Age	29.0 (7.9)	31.0 (7.9)	31.5 (8.5)	30.7 (8.1)	$F(2,90)=0.67$, $p<.514$
Education[a]	11.6 (1.6)	12.2 (2.1)	11.6 (1.6)	11.8 (1.8)	$F(2,79)=1.07$, $p<.348$
Sex (Men/Women)	13/9	27/10	28/6	68/25	$x^2=3.68$, $p<.160$
Ethnicity (White/AA)	20/1	28/8	18/16	66/25	$x^2=12.48$, $p<.003$
Age at Onset[b]	19.4 (7.1)	20.9 (5.2)	21.3 (5.6)	20.7 (5.8)	$F(2,89)=0.72$, $p<.489$
Illness Duration[b]	9.6 (5.7)	10.1 (7.0)	10.3 (8.3)	10.0 (7.1)	$F(2.89)=0.70$, $p<.933$
Previous Hospitalizations[b]	6.0 (7.4)	5.3 (4.5)	6.0 (6.1)	5.7 (5.8)	$F(2,89)=0.17$, $p<.847$
Medicated at Baseline (No/Yes)[c]	17/5	26/9	26/6	72/17	$x^2=0.28$, $p<.872$

* Mean and (SD)

[a] Unavailable for 11 subjects.

[b] Unavailable for 1 subject.

[c] Unavailable for 2 subjects.

Neuropsychological Testing

Patients were administered a neuropsychological battery to determine neurocognitive functioning at baseline and after six weeks and six months of treatment with clozapine. The battery consisted of neuropsychological tests sensitive to cognitive impairment in schizophrenia (Kenny and Meltzer 1991) and included tests of working memory (Auditory Consonant Trigram Test (ACTT): Peterson & Peterson, 1959), executive function (WCST: Heaton, 1980; Wechsler Intelligence Scale for Children-Revised (WISC-R) Mazes Subtest: Wechsler, 1974), verbal learning and memory (Buschke Selective Reminding Test (BSRT); Buschke & Fuld, 1974), verbal fluency (Category Instance Generation Test (CIGT): Newcombe, 1969; Controlled Oral Word Association Test (COWAT): Benton, 1968), and processing speed (Digit Symbol Subtest (DSST) from the WAIS-R: Wechsler, 1981)). A brief description of each test and the dependent variables derived from them is given in Appendix B. All tests were

administered manually according to published guidelines by trained psychology assistants.

Genotyping

Subjects were genotyped by the Vanderbilt University Human Genetics Core Laboratory for the COMT val108/158met SNP using previously described methods (Bilder et al., 2002b). Genomic DNA was extracted from whole blood following standard protocols and polymerase chain reaction (PCR) was used to amplify the fragments of genomic DNA containing the valine 158 methionine polymorphism of COMT. The primers 5_-TCA CCA TCG AGA TCA ACC CC-3_ and 5_-GAA CGT GGT TGT AAC ACC TG-3_ were used (Kunugi et al., 1997). PCR was carried out in a 25 mL volume containing 150 ng genomic DNA, 10 mmol/L Tris-HCl, 50 mmol/L KCl, 1.0 mmol/L MgCl2, 0.001% gelatin, 5% DMSO, 0.2 mmol/L of each dNTP, 1 ng/mL of each primer and 1 unit of AmpliTaq DNA polymerase (Perkin-Elmer, Toronto, Canada). The initial denaturation was at 95°C for 3 min followed by 35 temperature cycles consisting of 30 sec at 94°C, 30 sec at 62°C, and 30 sec at 72°C. A final extension at 72°C for 7 min finished the reactions. Polymerase chain reaction products were digested by restriction enzymes Nla III and then subjected to electrophoresis on a 3.5% high resolution agarose (Bioshop Canada, Burlington, Ontario) gel, which was stained with ethidium bromide. The bands were visualized under ultraviolet light; DNA bands were assigned allele numbers based on their size: allele 1 (val158) _ 82bp_54bp_48bp and allele 2 (met158) _ 63bp_54bp_48bp_19bp.

Statistical Analysis

The set of nine neuropsychological variables derived from the seven tests was reduced to three domain scores based on a principal components analysis carried out on the larger sample of 280 patients and a sample of 26 healthy controls that completed the

same battery of tests in order to reduce the number of dependent variables included in the statistical analyses. Based on the observed scree plot and Eigen values, along with the proportion of variance explained by the model, a reliable three factor model was selected (Jayathilake et al., in prep.). The three factors explained 69% of the total variance and the minimum loading of any test on its respective factor was .58. The three domain scores, and individual tests within each, were: 1) Memory Function (BSRT immediate and delayed recall, ACTT, WISC-R Mazes subtest); 2) Attention and Verbal Fluency (COWAT, CIGT, DSST); and 3) Executive Function (WCST categories and percent perseverative errors scores). The factor scores are reported as Z-scores which were created by standardizing each neurocognitive variable to the control sample and averaging the standardized scores included in each factor. In addition, a global cognitive score was created by averaging the mean Z-scores of the nine neurocognitive variables.

The global cognitive summary and domain scores were the primary variables of interest and, with the exception of the WCST and ACTT, group differences on individual neuropsychological tests were not examined unless they were included in a domain score that demonstrated a significant main effect of genotype. Group differences were examined using one-way ANOVAs or the equivalent non-parametric test (Welch's test) if the assumption of variance homogeneity was violated, as indicated by Levene's test of homogeneity of variance. Several planned contrasts were performed regardless of the one-way ANOVA or Welch test results for selected measures based on theoretical considerations and previous findings. Specifically, based on previous findings of a significant association between COMT genotype, WCST and working memory, it was anticipated that met/met homozygous subjects would outperform val/val homozygous subjects on the WCST percent perseverative errors score and ACTT. As such, one tailed contrasts between the met/met and val/val groups on the WCST percent perseverative errors score and the ACTT were performed.

Fisher's Least Significance Difference (LSD) method was used for pairwise contrasts on the remaining measures provided that the overall ANOVA reached significance. An alpha level of .05 was used for all ANOVA and pairwise contrasts. No

correction to the critical alpha was required for the remaining tests since the LSD method sufficiently corrects for family-wise error rates by limiting pairwise contrasts to only those omnibus ANOVA tests reaching significance. Significant Welch's tests were followed up with pair-wise contrasts using the Games-Howell procedure, which is based on Welch's degrees of freedom correction, and is appropriate to use when variances and sample sizes are unequal (Maxwell & Delaney, 2004).

Results

Group means at baseline for the global cognitive measure, three domain scores, and each neuropsychological test are presented in Table 7. No genotype effects were observed on the global cognitive score or any of the domain variables at baseline (all F statistics<1.27, p-values<.820). The results remained unchanged when the analysis was limited to unmedicated patients.

Table 7: Neuropsychological Test Scores and COMT val108/158met Genotype at Baseline*.

	Genotype			Total	
Variable	met/met	val/met	val/val	**Sample**	**Test Statistic**
Cognitive Domain Z-scores					
Global Cognitive Score	-1.28 (0.78)	-1.52 (1.03)	-1.71 (0.85)	-1.52 (0.92)	F(2,78)=1.30, p<.279
Memory	-1.33 (0.97)	-1.50 (1.31)	-1.94 (1.15)	-1.60 (1.19)	F(2,79)=1.84, p<.166
Attention & Verbal Fluency	-1.40 (0.76)	-1.51 (0.90)	-1.51 (0.79)	-1.48 (0.82)	F(2,80)=0.13, p<.876
Executive Function	-1.13 (1.48)	-1.60 (1.57)	-1.67 (1.30)	-1.51 (1.46)	F(2,83)=0.95, p<.391
Neuropsychological Test Raw Scores					
ACTT	30.0 (6.9)	30.0 (9.1)	23.6 (8.7)	27.7 (8.9)	F(2,82)=5.61, p<.006
BSRT Immediate Recall	7.6 (1.7)	6.8 (2.9)	6.7 (2.7)	7.0 (2.6)	F(2,82)=0.87, p<.426
BSRT Delayed Recall	6.7 (2.5)	5.8 (3.5)	5.7 (3.3)	6.0 (3.2)	F(2,82)=0.68, p<.509
COWAT	29.8 (11.6)	30.5 (14.3)	29.9 (13.9)	30.1 (13.4)	F(2,81)=0.22, p<.979
CIGT	41.0 (11.5)	37.9 (16.6)	41.6 (11.9)	39.9 (14.0)	F(2,81)=0.62, p<.544
WAIS-R DSST	6.4 (2.2)	6.3 (2.2)	5.6 (2.2)	6.1 (2.2)	F(2,82)=1.10, p<.339
WCST Categories	3.1 (2.5)	2.5 (2.3)	2.5 (2.2)	2.7 (2.3)	F(2,83)=0.47, p<.626
WCST Perseverative Errors**	16.9 (9.2)	20.3 (10.1)	24.1 (9.0)	20.9 (9.8)	F(2,75)=3.30, p<.043
WISC-R Mazes Subtest	8.4 (5.7)	8.8 (4.0)	7.5 (5.2)	8.3 (4.8)	F(2,80)=0.54, p<.585

* Mean and (SD)

** Excluding Outliers

A main effect of genotype was observed on the ACTT at baseline ($F(2,82)=5.61$, $p<.006$). The pre-planned contrast between met and val homozygous patients indicated that met homozygous patients performed better on the ACTT than val homozygous patients ($t(82)=2.67$, one-tailed $p<.005$). In addition, val/met heterozygous patients also performed better on the ACTT than val homozygous patients ($t(82)=3.04$, two-tailed $p<.004$). The results remained unchanged when global cognitive score was entered as a covariate. The main effect of genotype observed on the ACTT remained significant when the analysis was restricted to patients who were unmedicated at baseline ($F(2,63)=3.30$, $p<.044$; as did the pre-planned contrast between met and val homozygous groups ($t(63)=1.76$, one-tailed $p<.045$) and the post-hoc contrast between val/met and val homozygous groups ($t(63)=2.48$, two-tailed $p<.017$). As can been seen in Figure 5a, the means for medicated and unmedicated patients were similar within each genotype group. ACTT scores did not differ between Caucasians and African Americans within the val/val genotype group ($t(28)=0.04$, $p<.966$) indicating that the different ethnic composition of the val homozygous group did not account for the results.

There was no main effect of genotype on the WCST percent perseverative errors score at baseline ($F(2,83)=1.17$, $p<.315$). However, inspection of the distribution of WCST percent perseverative errors scores revealed that the distribution was highly skewed (skewness=1.24; SE=0.26) due to the presence of eight outlier scores. The distribution of scores improved substantially after removing these subjects from the analysis (skewness=0.24; SE=0.27). Of the eight patients excluded from the WCST analysis at baseline, two, four, and two were from the met/met, val/met, and val/val groups, respectively. After exluding these subjects, a main effect of genotype was observed for WCST percent perseverative errors scores at baseline ($F(2,75)=3.30$, $p<.043$) due to the fact that met homozygous subjects made fewer perseverative errors than val homozygous subjects ($t(75)=2.54$, one-tailed $p<.007$). No difference was observed between the val/met and val/val groups ($t(75)=1.52$, two-tailed $p<.133$). The met/met vs. val/val contrast remained significant after covarying for global cognitive scores. These results remained significant when the analysis was restricted to unmedicated patients ($F(2,57)=5.29$, $p<.009$; met/met vs. val/val contrast $t(57)=3.24$,

one-tailed p<.001). The means for WCST percent perseverative errors scores for medicated and unmedicated patients within each genotype group are presented in Figure 6. There was no difference in WCST perseverative error scores between African Americans and Caucasians within the val homozygous group (t(26)=0.81, two-tailed p<.427) indicating that the different ethnic composition of the val homozygous group did not account for the results.

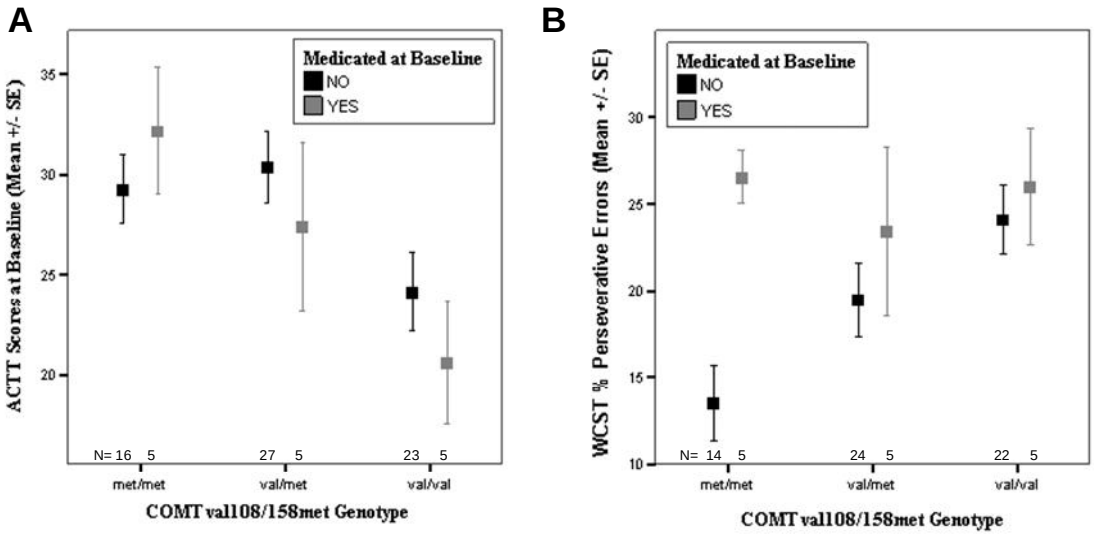

Figure 6. Association between COMT val108/158met genotype and (A) working memory and (B) perseverative errors on the WCST in medicated and un-mediated patients with schizophrenia. Error bars represent standard error of the mean.

Discussion

The current results provide further evidence of an association between COMT val108/158met genotype and cognitive function. As anticipated, at baseline patients homozygous for the met allele made fewer perseverative errors on the WCST and demonstrated superior performance on the ACTT compared to val allele homozygous patients. The differences observed at baseline on the WCST replicates previous studies that have, for the most part, also identified an association between COMT genotype and performance on the WCST in patients with schizophrenia and healthy controls

(Minzenberg et al., 2006; Egan et al., 2001; Joober et al., 2002; Rosa et al., 2004; Malhotra et al., 2002; Bruder et al., 2005). Moreover, COMT genotype accounted for approximately 8% of the variance in WCST perseverative errors scores which is consistent with other studies that have generally reported R Sqaured values of 4-10% (Szoke et al., 2006). In contrast to previous investigations in schizophrenia, the current results at baseline were obtained in a largely unmedicated sample of patients. In fact, the main effect of genotype on the WCST percent perseverative errors score was substantially more robust in unmedicated patients. Unfortunately, the small number of medicated patients at baseline precluded a formal comparison between medicated and un-medicated patients. Nonetheless, this observation suggests that the association between genotype and WCST might be slightly attenuated when subjects receiving typical APDs are included in a study and could explain why some investigations in schizophrenia did not find a significant effect of genotype on the WCST (Ho et al., 2005; Szoke et al., 2006).

The relationship between working memory and COMT genotype has not been examined before with the ACTT, although the current results are in agreement with several prior studies that found met homozygous subjects performed better on working memory tasks such as the N-back, LNS, and the PASAT than val homozygous subjects (Goldberg et al., 2003; Bruder et al., 2005; Minzenberg et al., 2006). The version of the ACTT used in the current study required subjects to recall three consonants read to them following a 15 second interference delay period during which subjects counted backwards aloud (Peterson et al., 1959). The inclusion of an interference component is unique to the ACTT compared to other tests of working memory such as digit span and letter-number sequencing. However, the introduction of a self-paced interference component raises the possibility that individual differences in mathematical ability and/or processing speed, rather than working memory and resistance to distraction, may better account for individual differences in performance. It is noteworthy in this regard that although mathematical ability is related to the number of mathematical errors made on the interference task, it does not affect recall of the consonants following the delay (i.e. the dependent variable) (Tsiakas, Gagnon, Awad, & Messier, 2004). Moreover, factor

analytic studies have found that the ACTT tends to load on factors related to working memory and complex attention and is relatively unrelated to processing speed, as measured using the digit symbol coding task of the WAIS-R (Boone, Ponton, Gorsuch, Gonzalez, & Miller, 1998; Mertens, Gagnon, Coulombe, & Messier, 2006). Thus, the available data indicate that the ACTT measures a relatively unique cognitive function, the ability to maintain items in working memory in the face of distraction, and is relatively unaffected by individual differences in the ability to perform the interference task. The fact that lesion studies suggest that the ACTT is sensitive to the integrity of the PFC are in accordance with the psychometric data indicating that the ACTT measures a relatively unique executive cognitive function (Stuss et al., 1982). Furthermore, imaging studies strongly suggest that activity in dorsolateral PFC specifically is related to resistance to distraction, and executive processes carried out on information transiently maintained in working memory buffers such as manipulation and updating (Owen, 2000; Baddeley, 2003; Passingham et al., 2004; McCarthy et al., 1994; Courtney, Ungerleider, Keil, & Haxby, 1997a; Manoach et al., 1997; Stern et al., 2000; Leung et al., 2002; Passingham et al., 2002; Sakai, Rowe, & Passingham, 2002).

It is noteworthy that studies identifying an association between COMT genotype and working memory, including the present study, all included free recall, as opposed to recognition, working memory tasks that significantly tapped executive sub-processes such as manipulation and updating of information, and resistance to distraction (Goldberg et al., 2003; Bruder et al., 2005). Conversely, studies that included recognition working memory and/or tasks that do not tax executive processes have not found an association between COMT and working memory (Bruder et al., 2005; Stefanis et al., 2004). The pharmacological studies reviwed earlier suggest that recall working memory tasks that tap executive processes are especially sensitive to PFC DA activity (Mattay et al., 2000; Mattay et al., 2002; Mattay et al., 2003), whereas recognition working memory tasks that do not tax executive sub-components are not (Kimberg et al., 2001; Bartholomeusz et al., 2003). As such, it is plausible that the effect of COMT genotype observed in the current and previous studies relates more to the executive components of the task than simply the on-line maintenance of information.

The current results also add to the specificity of the association between COMT genotype and cognition. Consistent with a previous study (Bilder et al., 2002b), no genotype effect was observed on the global cognitive summary score, suggesting that COMT genotype has a relatively circumscribed effect on cognition. Similarly, consistent with the majority of other studies, no significant effects were detected on tests of verbal learning and memory (Bilder et al., 2002b; Minzenberg et al., 2006; Diamond et al., 2004). However, in contrast to one other study, that included only 7 met homozygous subjects (Bilder et al., 2002b), a significant effect was not observed on the DSST. Although a significant effect was observed on a composite measure that included verbal fluency in a prior study (de Frias et al., 2005), the results here are inline with other studies that failed to identify a significant genotype on more specific measures of verbal fluency (Bilder et al., 2002b; Bearden et al., 2004). Thus, overall the results derived from the current experiment are consistent with the majority of other studies, and support the hypothesis that COMT genotype effects on cognition are specific to executive functions, the WCST perseverative errors scores in particular, and working memory.

There are several caveats to the current results. It is possible that significant genotype effects might have been detected on the global cognitive summary score and specific domains had the sample size been larger. For example, the power to detect a small (f=.10) and medium (f=.25) effect size for a one-way ANOVA with 90 subjects is approximately 12% and 54%, respectively. The effect sizes here for the difference between met and val homozygous subjects on the ACTT and WCST were .82 and .79, respectively. Inspection of the means for the remaining individual tests suggested that if a genotype effect exists it is quite small. It is also possible that demographic differences between groups might account for the gene effects. A pre-morbid test of intelligence was not included, thus, it's possible that met/met individuals performed better because they had greater overall intellectual abilities despite the fact that genotype effects observed on the ACTT and WCST remained significant after accounting for global cognitive function based on the mean of all neuropsychological tests administerred. However, the absence of a significant difference between groups in education suggests that this is unlikely and

it is also likely that such an effect would have been observed on the global cognitive score. Finally, it is possible that differences in ethnicity between groups accounted for the results. The val homozygous group included significantly more African American subjects than both the met homozygous and val/met heterozygous groups. However, the ACTT and WCST scores for Caucasians and African Americans were virtually identical within the val homozygous group suggesting that the lower scores associated with val allele were not related to ethnicity. Moreover, the magnitude of the effect of COMT genotype and COMT activity is the same in Caucasians and African Americans (Chen et al., 2004).

Experiment 2B: Interactions Between Neuropsychological Change with Clozapine Treatment and COMT val(108/158)met Genotype in Schizophrenia

Introduction

As discussed throughout this manuscript, schizophrenia is characterized by prominent deficits in neuropsychological functioning (Heinrichs et al., 1998). Patients demonstrate deficits in a broad array of functions; however, impairments in executive skills, including working memory, verbal skills, and learning and memory are especially severe (Bilder et al., 2000; Hoff et al., 1992; Heinrichs et al., 1998). Moreover, cognitive impairment, along with negative symptoms, is a critical determinant of functional outcome in schizophrenia (Green, 1996; Green et al., 2000). Specifically, cognitive functioning is related to dimensions of functional outcome such as vocational/occupational and psychosocial functioning (Green, 1996). Not surprisingly cognitive impairment has become an important therapeutic target based upon the rationale that improvements in cognitive function will enhance functional outcome.

Early studies by Meltzer (1992) following the re-introduction of clozapine in 1988 suggested that cognitive impairment may be ameliorated to some extent by clozapine, the original atypical antipsychotic drug (APD). Subsequent double-blind, random assignment trials confirmed and extended the cognitive advantages identified with clozapine (Bilder et al., 2002a; Lee, Jayathilake, & Meltzer, 1999a; Potkin, Fleming, Jin, & Gulasekaram, 2001) to a host of newer atypical APDs including risperidone (Green et al., 1997b; Harvey, Green, McGurk, & Meltzer, 2003a; Green et al., 2002; Purdon et al., 2000), olanzapine (Purdon et al., 2000; Harvey, Siu, & Romano, 2003b; Harvey et al., 2003a; Keefe et al., 2004), quetiapine (Purdon, Malla, LaBelle, & Lit, 2001; Velligan et al., 2002), and, to a lesser extent, ziprasidone (Harvey et al., 2003b) and amisulpride (Wagner et al., 2005). Improvements occur in several cognitive domains and vary to some extent by treatment; however, gains in learning and processing speed are especially robust (Woodward et al., 2005). The advantages to cognition associated with

atypical APDs are often attributed to the novel pharmacological actions of these agents (Meltzer & McGurk, 1999) which include, but are not limited to, their ability to enhance dopamine and acetylcholine release in the prefrontal cortex (Rao, Williams, & Goldman-Rakic, 1999a; Ichikawa, Dai, O'Laughlin, Fowler, & Meltzer, 2002b; Meltzer, 2004). The latter likely has important consequences for cognitive functions of the PFC given that hypo-function of the mesocortical DA system is thought to underlie aspects of cognitive impairment in schizophrenia.

Although the benefits of atypical APDs to cognitive functions have been confirmed by meta-analysis, there is evidence that the degree of improvement observed on some neuropsychological instruments is quite variable, not only across, but also within studies. For example, mean group improvements in Trailmaking A (TMA) and TMB with atypical APDs ranges from approximately 4 to 15, and 10 to 30 seconds, respectively. Moreover, the average within study variation in improvement for verbal fluency and TMB ranges from 2 to 10 words and 40 to 70 seconds, respectively. These observations are striking when compared to normal controls who typically demonstrate mean group improvements on the order of 4 and 5 seconds on TMA and TMB, respectively, when tested over comparable intervals and average within group variation in change scores ranging from 4 to 7 words and 15 to 25 seconds for verbal fluency and TMB, respectively (Woodward, Purdon, Meltzer, & Zald, 2007).

Undoubtedly some of the variability relates to the nature of the tests, patient characteristics, and study design (Woodward et al., 2005). However, preliminary evidence in both animals and humans suggests that variation in COMT activity may influence the degree of cognitive improvement and cerebral physiological changes observed with atypical APDs, including clozapine (Tunbridge et al., 2004; Bertolino et al., 2004; Weickert et al., 2004). For example, rodents treated with clozapine and tolcapone, a COMT inhibitor, demonstrate greater DA release in the PFC compared to control animals treated with tolcapone alone (Tunbridge et al., 2004). The investigation of variation in treatment response resulting from genetic differences between individuals is termed pharmacogenetics and there is growing evidence that the COMT

val108/158met polymorphism may account for some of the variation in cognitive improvement and alterations in neurophysiology with atypical APDs. The results from two independent studies indicate that met homozygous schizophrenia patients demonstrate greater improvement in working memory, as measured using the n-back task, than val homozygous subjects after several weeks of treatment with a variety of atypical APDs (Weickert et al., 2004) or just olanzapine (Bertolino et al., 2004). Remarkably, the improvement also corresponds with enhanced function of the dorsolateral PFC during working memory performance (Bertolino et al., 2004). The improved physiological response observed in the PFC is consistent with data from normal control subjects that demonstrate improved performance on the same N-back task following amphetamine treatment and implies that met homozygous patients may benefit more from enhanced cortical DA transmission as a result of treatment with atypical APDs than val homozygous subjects (Mattay et al., 2003).

Unfortunately the conclusions reached regarding potential interactions between atypical APDs and COMT genotype must be considered preliminary until studies with larger sample sizes and more comprehensive neuropsychological batteries are carried out. The two prior investigations of interactions between COMT genotype and atypical APD related cognitive improvement included 20 patients, including 5 or less met homozygous subjects in each case (Bertolino et al., 2004; Weickert et al., 2004). Moreover, both studies restricted their analyses largely to one test of working memory, the N-back test, and included few additional measures. As mentioned above, atypical APDs improve a broad array of functions in schizophrenia and it is plausible that interactions between COMT genotype and cognitive improvement are not limited to the N-back task. This experiment was undertaken to address the sample size limitations of previous longitudinal studies by prospectively examining a larger sample of patients with schizophrenia before and after 6 weeks and 6 months of treatment with clozapine. Moreover, the specificity of the interactions between cognitive improvement with clozapine and COMT genotype was addressed by employing a larger neuropsychological test battery than previous studies.

Methods

The patient sample, neuropsychological testing, and genotyping are the same as that reported for Experiment 2A.

Statistical Analysis

Longitudinal changes in cognitive function and interactions between COMT genotype and cognitive change with clozapine were examined using linear mixed model analyses due to the fact that not all subject completed every assessment. Linear mixed models provide a more powerful alternative to traditional repeated measures models in datasets where some subjects have incomplete data (Maxwell and Delaney 2004). Specifically, linear mixed models use the maximum likelihood-based method for estimating means rather than the method of moments which requires subjects to have complete data. The covariance matrix was modeled as compound symmetric in the mixed models analyses and baseline score was entered as a covariate. As with the cross-sectional analysis of baseline data, the analyses were restricted largely to the global cognitive and domain scores, with the exception of the WCST percent perseverative and ACTT measures, which were examined separately due to previous reports suggesting that COMT genotype may interact with working memory changes related to antipsychotic treatment.

Results

The global cognitive score, domain Z-scores, and raw neuropsychological test scores following 6-weeks and 6-months of clozapine treatment are listed in Table 8. A main effect of time was observed on the global cognitive score ($F(2,95)=3.43$, $p<.037$) and the Attention and Verbal Fluency domain ($F(2,96)=26.44$, $p<.001$); both in the

direction of improved performance over time, and a significant interaction between COMT genotype and improvement was detected on the Attention and Verbal Fluency domain score ($F(4,96)=2.78$, $p<.032$). Follow-up contrasts revealed that the global cognitive score improved significantly from baseline to 6 months ($t(95)=2.62$, $p<.011$). Follow-up contrasts for the Attention and Verbal Fluency domain indicated that both the met/met and val/met groups demonstrated superior performance at the 6 month assessment compared to the val/val group ($t(96)=2.48$, $p<.016$; and $t(96)=3.84$, $p<.001$, respectively), after controlling for baseline performance. As depicted in Figure 7, the differences observed between groups at 6 months were due to the fact that met homozygous and val/met heterozygous groups, but not the val homozygous group, demonstrated significant improvement from baseline to 6 months. The interaction observed on the Attention and Verbal Fluency domain remained significant when the analysis was restricted to patients that were unmedicated at baseline ($F(4,71)=4.06$, $p<.006$). Again, the interaction was due to the fact that both met homozygous and val/met heterozygous patients demonstrated superior performance at 6 months compared to val homozygous patients ($t(71)=2.21$, $p<.031$; and $t(71)=4.55$, $p<.001$).

Table 8: COMT val108/158met Genotype and Cognitive Change with Clozapine.

	Genotype											
	met/met				**val/met**				**val/val**			
	6-week		6-month		6-week		6-month		6-week		6-month	
Cognitive Domain	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Global Cognitive Score	12	-1.25 (0.92)	14	-1.14 (1.01)	26	-1.45 (0.92)	26	-1.27 (0.96)	18	-1.64 (0.89)	17	-1.81 (0.81)
Memory	13	-1.64 (1.09)	15	-1.56 (1.39)	26	-1.62 (1.06)	26	-1.33 (1.29)	19	-2.04 (1.10)	17	-2.04 (1.18)
Attentional & Verbal Fluency	13	-1.02 (0.95)	14	-0.80 (0.83)	26	-1.17 (0.84)	26	-0.96 (0.75)	18	-1.19 (1.06)	17	-1.35 (0.73)
Executive Function	14	-0.92 (1.27)	15	-0.96 (1.19)	26	-1.52 (1.69)	26	-1.63 (1.41)	19	-1.79 (1.51)	17	-2.03 (1.39)
Neuropsychological Test Scores												
ACTT	14	22.6 (8.2)	15	22.3 (10.3)	26	25.8 (6.3)	26	26.0 (8.9)	20	21.1 (7.1)	17	20.8 (8.4)
BSRT Immediate Recall	14	7.9 (1.9)	15	8.3 (2.3)	26	7.5 (2.5)	26	8.2 (2.5)	20	7.0 (2.5)	17	7.5 (2.8)
BSRT Delayed Recall	14	7.6 (2.7)	15	7.13 (3.2)	26	6.4 (3.1)	26	7.2 (2.7)	19	5.8 (3.0)	17	6.5 (3.4)
COWAT	14	37.6 (14.2)	15	38.5 (13.4)	26	34.4 (12.2)	26	37.5 (13.8)	19	31.6 (15.2)	17	33.9 (11.5)
CIGT	14	45.6 (14.9)	15	48.8 (14.2	26	42.9 (13.8)	26	45.3 (11.6)	19	41.5 (16.6)	17	39.5 (11.0)
WAIS-R DSST	13	6.7 (1.8)	14	7.3 (2.3)	26	7.1 (2.7)	26	7.6 (2.7)	18	7.3 (3.0)	17	6.5 (2.0)
WCST Categories	14	3.5 (2.2)	15	2.9 (2.5)	26	2.8 (2.5)	26	2.4 (2.2)	19	2.3 (2.1)	17	1.9 (2.1)
WCST Perseverative Errors	14	19.0 (14.2)	15	16.3 (9.2)	26	25.4 (17.8)	26	25.3 (15.2)	19	27.3 (17.2)	17	29.6 (15.3)
WISC-R Mazes Subtest	13	7.8 (3.0)	15	9.0 (4.2)	26	8.5 (3.3)	26	9.5 (4.3)	19	7.4 (4.3)	17	6.1 (3.1)

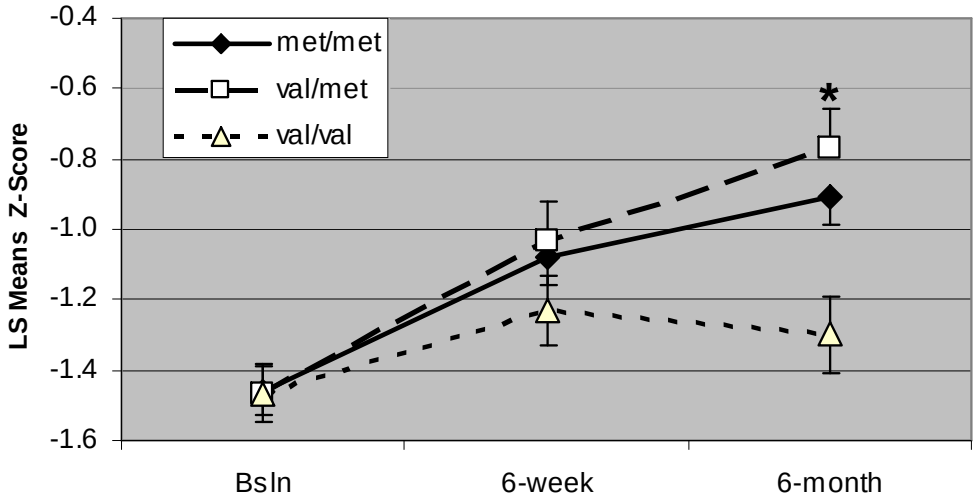

Figure 7. Change in Attention and Verbal Fluency domain score with clozapine in COMT val108/158met homozygous and heterozygous patients with schizophrenia.
* met/met and val/met groups significantly greater than val/val group; ANCOVA genotype by time interaction ($p<.05$) with baseline entered as a covariate.

Each neuropsychological measure within the Attention and Verbal Fluency domain (COWAT, CIGT, DSST) was subjected to a linear mixed model analysis in order to determine if the changes over time and interaction was observed on all or specific tests within this domain. A main effect of time was observed on all three measures included within this domain (all F statistics>9.41, $p<.001$); however, the interaction term only reached significance for the COWAT ($F(4,99)=4.40$, $p<.003$). Post-hoc contrasts indicated that met/met and val/met groups had higher COWAT scores, compared to the val homozygous group, at both the 6 week ($t(99)=3.45$, $p<.001$; and $t(99)=2.45$, $p<.017$, respectively) and 6 month evaluations ($t(99)=3.36$, $p<.002$; and $t(99)=4.24$, $p<.001$, respectively).

With respect to the ACTT and WCST percent perseverative errors scores, only a main effect of time was observed on the ACTT ($F(2,102)=7.05$, $p<.002$) due to the fact that ACTT scores decreased over time. No main effects or interactions were observed on the WCST percent perseverative errors scores (all F statistics<0.57, p-values<.573).

Post-hoc contrasts indicated that, compared to baseline, patients with the met/met genotype demonstrated lower ACTT scores at 6 weeks ($t(102)=2.13$, $p<.036$) and 6 months ($t(102)=2.66$, $p<.001$). Patients with the val/met genotype also demonstrated a reduction in ACTT scores at 6 weeks ($t(102)=2.32$, $p<.023$). Conversely, patients with the val/val genotype did not demonstrate any changes in ACTT scores at either 6 weeks ($t(102)=1.39$, $p<.167$) or 6 months ($t(102)=0.71$, $p<.482$), relative to baseline.

Discussion

Consistent with two previous smaller studies, a significant interaction between COMT genotype and cognitive improvement with atypical APDs was observed. Specifically, met carriers improved to a greater degree on the Attention and Verbal Fluency domain than val homozygous patients following treatment with clozapine. Moreover, the current study confirmed that the interaction was present in patients initially tested while unmedicated, thereby ruling out the possibility that the interaction was due to differential effects of withdrawal from prior APD treatments between groups. The Attention and Verbal Fluency domain consisted of the DSST, COWAT, and CIGT. Inspection of the individual tests within this domain indicated that the interaction between genotype and cognitive improvement with clozapine was most apparent on the COWAT test, although met carriers also improved substantially more than val homozygous patients on the CIGT as well. The magnitude of the difference in change between met carriers and val homozygous patients was notable as the improvement detected in the met homozygous and heterozygous patients was approximately 9 and 8 words on the COWAT and CIGT, respectively, compared to a 4 word improvement and no change on the COWAT and CIGT, respectively, in val homozygous patients.

With few exceptions (Buchanan, Holstein, & Breier, 1994; Bilder et al., 2002a), improvement in verbal fluency with clozapine has been consistently found in both double-blind, random assignment (Lee, Jayathilake, & Meltzer, 1999b; Potkin et al., 2001), and naturalistic open label trials (Hagger et al., 1993; Zahn, Pickar, & Haier, 1994; Purdon, LaBelle, & Boulay, 2001). The interaction between improvement with clozapine and COMT genotype suggests that the failure of a minority of studies to identify significant improvement in verbal fluency with clozapine might relate, in part, to the genotype of the subjects included in the studies. It is noteworthy in this regard that one study that failed to identify an improvement in verbal fluency with clozapine may have included up to 3 times more val homozygous than met homozygous patients (Bilder et al., 2002b; Bilder et al., 2002a). Future clinical trials on the cognitive efficacy of atypical

APDs may benefit by stratifying subjects on the basis of genotype. Moreover, the specific links between cognition and functional outcome suggest combined with evidence that cognitive improvement varies by COMT genotype suggests that the functional outcome may also vary by genotype. This hypothesis is discussed in greater detail in the conclusions section at the end of this dissertation.

The finding that the COMT val108/158met genotype is associated with improvement in the Attention and Verbal Fluency domain but not the Memory Function domain, which included the most specific test of working memory, the ACTT, and the Executive Function domain, which includes the WCST categories and percent perseverative error scores, indicates that the relationship between COMT genotype and response to treatment goes beyond working memory (Weickert et al., 2004; Bertolino et al., 2004; Mattay et al., 2003). The failure to find an association between improvement in working memory and COMT genotype might relate to the difference in medication and subject population, compared to previous studies. The potential to improve any domain of cognition would be expected to be, in part, a function of baseline DAergic activity and the extent of enhancement by further release. Clozapine produces much larger increases in cortical DA efflux in rat cortex than olanzapine (Kuroki, Meltzer, & Ichikawa, 1999), which was used in a previous study (Bertolino et al., 2004). Moreover, although the current results conflict with a report in patients that found olanzapine improves working memory in met homozygous patients, they are entirely consistent with evidence that amphetamine significantly reduces N-back working memory accuracy in healthy subjects homozygous for the met allele and has a slight, non-significant negative effect in val homozygous subjects (Mattay et al., 2003).

The observation that on the one hand clozapine improves verbal fluency, but on the other reduces working memory performance in met homozygous patients has several implications. First, it indicates that as in healthy subjects, and in accordance with the inverted U-curve hypotheses of DA function, too much PFC DA activity impairs working memory in schizophrenia patients. At baseline when most patients were unmedicated, met homozygous patients performed better than val homozygous patients on the ACTT

and, therefore, might have been operating at, or near the peak of the DA U-curve. The addition of clozapine, which evokes robust DA release in the PFC, could have increased cortical DAergic activity in these subjects beyond the optimal point for working memory. This hypothesis has important implications for pharmacological treatment of working memory impairment in schizophrenia. As discussed at the beginning of this chapter, cognitive impairment in schizophrenia presumably relates, at least in part, to hypo-function of the mesocortical DA system (Davis et al., 1991). However, the observation that clozapine actually significantly impairs working memory clearly implies that pharmacological treatments that promote significant DA release in the PFC are likely to have only a modest impact at best, and deleterious effect at worse, in some patients on working memory in schizophrenia (e.g. McGurk et al., 2005). As such, met/met genotype status may mark vulnerability for clozapine induced working memory impairment in schizophrenia. More broadly speaking, the current results also implie that correcting mesocortical DA hypofunction may not be as simple as increasing PFC DA levels. Indeed, evidence of DA receptor up-regulation in the PFC of patients (Abi-Dargham et al., 2002) suggests that some compensation for reduced PFC activity occurs and it is possible that the up-regulation of DA receptors is associated with increased receptor sensitivity.

The results also imply that the optimal level of DA function required for one task is not necessarily the same as that required for another task. Such a hypothesis is not unexpected given evidence presented earlier that: 1) mesocortical DA dysfunction impairs some cognitive functions, such as working memory, but can actually enhance some aspects of attention in rodents and primates (Granon et al. 2000; Roberts et al. 1994); and 2) findings from factor analytic studies in humans, including the current study, indicating that neuropsychological tests of "frontal" lobe function, such as the COWAT, WCST, and the ACTT, do not load onto a single unitary factor, but appear to rely on unique cognitive process (Miyake et al. 2000; Boone et al. 1998). As such, it is not unreasonable to expect that different cognitive tasks might be differentially sensitive to DA and require different levels of DA activity for optimal performance. Evidence that cognitive stability is better in met homozygous subjects, whereas cognitive flexibility is

better in val homozygous would appear provide support for this view. It may be the case that verbal fluency is a task that is more closely related to cognitive flexibility, while working memory is more closely related to cognitive stability. Moreover, an [18F] Fallypride PET study found that sub-cortical binding potential was inversely correlated with performance, whereas the reverse was true for cortical binding indicating that the relationship between DA function and verbal fluency is complex (Zald et al., 2005). If it can be confirmed that clozapine's effects on cognition vary by genotype and that different PFC cognitive functions require different levels of DA for optimal performance, then individualized treatment plans for patients will likely have to consider the genotype and baseline cognitive functioning of the individual. It is perhaps too early to speculate, but in combination with the earlier study of olanzapine, the current results suggest that olanzapine might be a better choice for improving working memory dysfunction in met homozygous patients.

A limitation of this experiment is the relatively small sample size. Despite the fact that this experiment included substantially more patients than the two previous prospective studies of interactions between genotype and cognitive improvement, it is still relatively small compared to recent clinical trials of atypical APDs that included well over 100 subjects (Harvey et al., 2003a; Keefe et al., 2005). Moreover, the same limitations regarding differential ethnicity across the genotype groups identified in Experiment 2A may also apply to the current results.

CHAPTER IV

CONCLUSIONS

The current results contribute to the growing body of evidence indicating that 1) some aspects of the abnormal neurophysiological responses observed in schizophrenia during performance of cognitive tasks may be related to genetic vulnerability for schizophrenia; 2) the COMT val108/158met SNP is related to cognitive impairment in schizophrenia; and 3) COMT genotype is associated with cognitive improvement with clozapine in schizophrenia. A broad overview of the results and implications of Experiments One and Two are presented in the remainder of this dissertation. Specific attention is drawn to the limitations of Experiment One, with respect to fulfilling endophenotype criteria, genes that might be related to PFC and parietal cortex function, factors that deserve consideration when searching for associations between specific genes and cognition, and the implication of genetics for cognitive improvement in schizophrenia including potential effects of genes on functional outcome.

Overview of Experiment One

Experiment One is one of just a handful of studies to employ fMRI to examine cerebral neurophysiology during performance of a cognitive task in unaffected siblings of patients with schizophrenia. Previous studies produced remarkably similar results in that all found abnormal neurophysiological responses in unaffected siblings that were similar in many respects to the deficits observed in patients, although there is a tendency for the deficits to be milder in unaffected relatives. For example, consistent with patients, unaffected relatives evinced reduced activity in the caudate and greater activity in the dorsolateral PFC during performance of an antisaccade task and working memory task, respectively, compared to controls (Raemaekers et al., 2005; Callicott et al., 2003a). Moreover, in both cases unaffected siblings performed the tasks as well as controls.

When considered in combination with our previous fMRI investigation of SRT task performance in patients, the current results are consistent with the prior imaging studies of unaffected siblings. Specifically, the abnormal responses detected in the PFC, parietal cortex, and, to a lesser extent, caudate, in patients were also observed in the current investigation of unaffected relatives. However, the magnitude of the abnormal responses observed in unaffected siblings tended to be quantitatively less than that observed in patients. For example, the effect size for the difference in activity observed in the caudate between patients and controls approached 0.75, whereas the effect size for the difference between controls and unaffected siblings was approximately 0.45. Similarly, the spatial extent of the difference was also larger in patients in most regions. These observations add to the growing body of evidence indicating that abnormalities in cerebral neurophysiology are related to genetic vulnerability for schizophrenia; however, it is also clear that the abnormalities reflect both genetic and environmental influences. Detailed parsing of genetic and non-genetic components of abnormal cerebral function in schizophrenia requires imaging monozygotic twins discordant for the illness and control subjects.

It is also noteworthy that the abnormal responses observed in unaffected siblings in Experiment One were not associated with an overt deficit in performance indicating that the differences in cerebral activity observed between groups was not likely a consequence of differential performance, as confirmed by ANCOVA. However, this does not confirm that unaffected siblings do not demonstrate behavioral deficits on the SRT task. Proving the null hypothesis is more difficult than disproving it and it is possible that group differences might have emerged had the sample sizes been larger. An alternative approach might have been to use a task in which difficulty could be modulated parametrically. Such an approach allows one to 1) examine group differences in imaging results at varying levels of difficulty, 2) learn more about the relationship between cerebral activations and task performance, and 3) identify the points at which group differences emerge, with respect to both behavior and neurophysiology. For example, it may be the case that group differences in cerebral function emerge before overt deficits in performance are detected. Unfortunately, it is not readily apparent how this approach

could have been implemented in the current study. Procedural learning is considered an automatic process that occurs outside of conscious awareness, thus, it is unclear how parametric manipulation of task difficulty, such as increasing working memory load in working memory paradigms, could have been implemented in the current task. Nonetheless, the current findings are in agreement with imaging studies in both patients and unaffected siblings that have also found abnormalities in cerebral function even when overt deficits in performance are not observed.

Evaluation of Endophenotype Criteria for Study One

Gottesman and Gould (2003) identified several criteria that neurophysiological and/or neuropsychological deficits detected in an illness must meet to be considered endophenotypes. These criteria were touched upon in Study One. They are discussed in more detail in the following sections and whether or not the neurophysiological results derived from Study One, particularly evidence of abnormal PFC function, meet these criteria is evaluated. For comparison purposed, the criteria are also applied to the behavioral and imaging results derived from several investigations of working memory that used the N-back task as implemented by Callicott et al. (2000).

Criteria #1: The Endophenotype is Associated with Illness in the Population

With respect to behavioral performance of the SRT task, there is no clear consensus on whether or not patients with schizophrenia demonstrate impairment. There are reports of both intact (Stevens et al., 2002) and impaired (Green et al., 1997a; Kumari et al., 2002; Exner et al., 2006) procedural learning on the SRT task. As with other procedural learning measures, variability in medication status of patients across studies may account for the discrepant findings (Stevens et al., 2002). For example, two studies reporting impairment included patients that were receiving typical APDs exclusively (Green et al., 1997a; Kumari et al., 2002), whereas patients included in the two studies

that did not find impairment were receiving predominantly or exclusively atypical APDs (Stevens et al., 2002). The picture is further complicated by a report that procedural learning on the SRT task is impaired during the acute, but not stabilized phase of the illness (Exner et al., 2006). In contrast, neuroimaging studies of SRT task performance have yielded consistent results, although only two studies have been carried out to date. Specifically, reduced activity in the PFC and striatum was observed in both studies (Kumari et al., 2002). An additional deficit in the parietal cortex was also observed in one study . Moreover, the deficits do not appear to result from differential performance between groups as patients in one study performed the task as well as controls, yet still demonstrated abnormal cerebral activity. Combined, these findings suggest that at the behavioral level, the SRT task may not consistently demarcate patients from controls, but the neuroimaging findings strongly suggest that patients do not demonstrate the same pattern of activity as controls during performance of the SRT task and dysfunction of the PFC, striatum, and perhaps parietal lobe, may be an endophenotype of schizophrenia.

There is ample evidence that patients with schizophrenia demonstrate deficits on the N-back working memory test (e.g. Goldberg et al., 2003). Indeed, working memory dysfunction, regardless of the type of task used, has been replicated in a large number of studies and appears to be a cardinal feature of cognitive impairment in schizophrenia (Park et al., 1992). With respect to neuroimaging findings, there is also growing consensus that patients demonstrate abnormal cerebral activity during performance of the N-back test, especially in the dorsolateral PFC (Callicott et al., 2000; Callicott et al., 2003b). However, the nature of the abnormal activity observed in the PFC appears complex. Specifically, patients that perform much worse than controls on the N-back task demonstrate less activity in the dorsolateral PFC, whereas patients with relatively normal performance on the task demonstrate regionally specific areas of hypo- and hyper-activation in the PFC (Callicott et al., 2003b; Callicott et al., 2000).

Thus, at the behavioral level there is mixed evidence for impairment on the SRT task, but strong evidence for impairment on the N-back working memory test. In terms of neuroimaging findings, there is compelling evidence for PFC dysfunction during performance of both tasks and strong evidence for striatal dysfunction on the SRT task,

although the results for the latter might be confounded by the potentially deleterious effects of APDs on the function of this region.

Criteria #2: The Endophenotype is Heritable

No studies have examined the heritability of procedural learning using the SRT task or working memory using the N-back task. As such, heritability estimates for these two tasks do not exist. Although simple reaction time does not appear to be under strong genetic influence, the heritability for choice reaction time tasks in which subjects must detect a target that can appear in one of several spatial locations is approximately 64% (Wright et al., 2001). This suggests that reaction times from the SRT are likely heritable to a certain degree; however, it does not confirm that procedural learning per se on the SRT is heritable. Although no studies have examined the heritability of N-back working memory performance, there is evidence that working memory, as a general cognitive process, is heritable with heritability estimates ranging from 30 to 50% (Wright et al., 2001; Tuulio-Henriksson et al., 2002; Ando, Ono, & Wright, 2001). Similar studies examining the heritability of functional cerebral networks that underlie specific cognitive functions have not been carried out yet, although it is generally assumed that these networks are at least as heritable as actual behavioral performance. Indeed, this may account for why both structural and functional imaging is more sensitive to genetic vulnerability for schizophrenia.

Criteria #3: The Endophenotype is Primarily State-Independent

The results from Study One were acquired from a larger, on-going project examining SRT performance and imaging findings in both chronic and first episode patients with schizophrenia, their unaffected relatives, and normal controls. Currently, data is being collected on approximately a dozen first episode patients and a second cohort of appropriately age-matched controls. In addition, a subset of the first episode

patients will be scanned again after starting APD treatment in order to identify the effects of APDs on SRT task performance and cerebral activity. It is anticipated that the results garnered from this project will assist in determining whether or not behavioral performance on the SRT and the associated abnormalities in cerebral function detected in patients are trait or state-dependent, to what extent they are affected by APDs, and if they are related to genetic vulnerability. The one previous longitudinal study of SRT task performance that initially assessed patients during the acute phase and then again after symptom stabilization found that an impairment detected first testing resolved at retesting 20 months later following partial remission of symptoms (Exner et al., 2006). This would suggest that SRT task deficits may fluctuate with the severity of symptoms. However, preliminary findings from the current ongoing study suggest that, consistent with chronic patients, unmedicated first episode patients also fail to activate regions of the PFC and striatum during performance of the SRT task. As such, preliminary findings at least suggest that the imaging endophenotypes identified using the SRT task are not state-dependent.

Similar data for the N-back test are lacking since the studies carried out to date have not separated patients into first-episode or chronic sub-sets. Although given the ubiquity of the behavioral deficits and abnormal cerebral function observed on other measures of working memory it seems likely that the deficits are not state-dependent.

Criteria #4: The Endophenotype Observed in Patients is Found in Unaffected Family Members at a Higher Rate Than in the General Population

With respect to behavioral performance on the SRT task, the results from Study One suggest that impairment in procedural learning is not observed in unaffected siblings of patients with schizophrenia. However, as mentioned earlier, it's possible that larger sample sizes might be required to identify any differences between controls and unaffected siblings. As such, the current findings must be considered preliminary until replicated. On the other hand, there is evidence that unaffected siblings demonstrate

impairment on the N-back test of working memory. Specifically, in study of 250 subjects, including 108 unaffected siblings, Goldberg et al. (2003) identified a trend towards impaired performance at the 1 and 2-back working memory loads of the N-back task. Obviously the imaging findings derived from Study One and prior studies of the N-back test reviewed earlier indicate that some of the functional cerebral abnormalities observed in patients are also observed in unaffected siblings; however, replication of these findings is essential. Preliminary results reported here indicate that the frequency with which unaffected siblings demonstrate cerebral activity outside the range observed in controls ranges from less than 25% to over 50% for selected ROIs.

Criteria #5: Within Families, Endophenotype and Illness Co-segregate

This criteria indicates that within families, the endophenotype is observed to a greater extent in index cases and, in conjunction with criteria #4, implies a gradient of impairment with index cases demonstrated the most impairment, relative to unrelated controls from the general population, and unaffected family members demonstrating intermediate performance relative to controls and affected family members (Waldman, 2005). It is unlikely that this criteria is met with respect to behavioral performance on the SRT given that no deficits were observed unaffected siblings and inconclusive results from patients. However, there is evidence that this may be the case on the N-back test (Goldberg et al., 2003). Specifically, Goldberg et al. (2003) found that patients performed significantly worse than their unaffected siblings and controls drawn from the general population, and unaffected siblings demonstrated poorer performance compared to controls at the trend level for significance. No imaging studies of the SRT or N-back tests have included both affected and unaffected members from the same family along with matched controls from the general population. Thus, it is not known if the imaging endophenotypes identified on the SRT and N-back tests co-segregate within families. Further imaging studies are required to confirm this criterion.

In summary, there is compelling evidence to suggest that the abnormal physiological responses observed in the PFC, and perhaps parietal lobe during performance of the SRT and the N-back working memory tasks is a potential endophenotype for schizophrenia. However, several criteria remain to be fulfilled before the findings can be confirmed. Until then, it remains possible that the deficits observed across patients and unaffected siblings reflect the effect of some deleterious environmental factor common to both patients and their unaffected siblings.

The specificity of a putative endophenotype is also important an important consideration; however, specificity is not necessarily a requirement. Indeed, genetic studies have identified several genes that confer risk for both schizophrenia and bipolar disorder (Cannon & Keller, 2006). Unfortunately, there has been relatively little research into the specificity of putative schizophrenia endophenotypes. Cognitive impairment related to psychiatric disorders is not unique to schizophrenia. Indeed, several disorders, including depression, post-traumatic stress disorder, and, perhaps most importantly, bipolar disorder, are associated with some degree of cognitive impairment. However, the relative breadth and severity of the impairments observed in schizophrenia differentiates it from other disorders. For example, the impairments in attention, learning and memory, and possibly executive functions are greater in schizophrenia than bipolar disorder, despite the fact that bipolar patients also tend to demonstrate impairment relative to healthy control subjects (Czobor, Jaeger, Berns, Gonzalez, & Loftus, 2007; Schretlen et al., 2007). Fewer studies of unaffected relatives of patients with bipolar disorder have been carried out; however, evidence of impairment is mixed with some studies reporting impairment and others not (Antila et al., 2007; McIntosh, Harrison, Forrester, Lawrie, & Johnstone, 2005). Far less effort has been directed towards examining the specificity of structural and functional brain abnormalities to schizophrenia. Limited evidence suggests that structural volume losses in the PFC and lateral ventricles may be specific to patients with schizophrenia and their unaffected relatives (McDonald et al., 2006; McIntosh et al., 2004). With respect to neurophysiology, studies directly comparing cerebral function in unaffected relatives of schizophrenia or bipolar disorder have not been carried out.

Candidate Genes Related to Prefrontal and Parietal Cortex Function

If the abnormal cerebral function observed in the PFC and parietal cortex in patients and siblings is assumed to be an endophenotype for schizophrenia, then the next question is: what specific genes contribute to the abnormal neurophysiological responses and are these genes related to susceptibility for schizophrenia? Few imaging studies examining the associations between specific genes and neurophysiology have been carried out. The COMT val08/158met SNP has been linked to PFC, anterior cingulate, and parietal cortex function in several studies (Egan et al., 2001; Ho et al., 2005; Blasi et al., 2005; Smolka et al., 2005; Schott et al., 2006). With respect to neuroimaging findings, two studies, one fMRI and one PET, found that val homozygous subjects demonstrated greater activity in the dorsolateral PFC than met homozygous subjects during performance of an N-back working memory task (Egan et al., 2001; Ho et al., 2005), a finding the authors interpreted as reflecting "inefficient" information processing in the PFC (Weinberger et al., 2001). However, in both cases the number of subjects within each genotype group was small, less than eight, questioning the degree to which theses results can be generalized. COMT gene effects do not appear to be limited to the PFC as val homozygous individuals also demonstrated greater activity in anterior cingulate compared to met homozygous subjects during performance of a flanker-like task (Blasi et al., 2005). More recently, COMT gene effects were also observed in multiple regions of the PFC during performance of an episodic memory task, again with val homozygous demonstrate inefficient processing, suggesting that COMT gene effects on physiology are not limited to working memory (Schott et al., 2006). Indeed, the COMT gene has also been linked to activity in the PFC, left inferior parietal cortex, amygdala, thalamus, and hippocampus during passing viewing of unpleasant stimuli suggesting that COMT genotype exerts widespread effects on cerebral activity during performance of a variety of tasks (Smolka et al., 2005). Interestingly, met homozygous individuals' demonstrated greater activity in these regions during passive viewing of negative images, the opposite of what is generally observed during performance of

cognitive tasks, suggesting that met homozygous subjects may be more sensitive to unpleasant stimuli.

Another gene that has been linked to abnormal PFC physiology and schizophrenia is the metabotropic glutamate receptor 3 (GRM3) SNP4 A allele (Egan et al., 2004). The A allele of this SNP is overtransmitted to schizophrenia probands and is associated with "inefficient" PFC physiology during performance of the N-back working memory task (Egan et al., 2004). Further investigation of the SNP found that the A allele is associated with a reduced expression of N-acetylasparate (NAA), a molecular marker of general neuronal integrity, in the dorsolateral PFC (Marenco et al., 2006). Combined, the COMT val108/158met and GRM3 SNPs are attractive candidates as they link abnormal PFC physiology, altered dopamine and glutamate function, and cognitive impairment to schizophrenia, all of which have been implicated in the illness (Davis et al., 1991; Weinberger et al., 2001; Heinrichs et al., 1998; Laruelle, Kegeles, & Abi-Dargham, 2003). Although relatively few studies have been carried out, early results suggest that the COMT gene might have a widespread effect on neuronal activity. Similarly, the Ubiquitous role glutamate plays in cerebral function suggests that the results obtained for the GRM3 SNP4 likely generalize to a wide array of neuronal processes.

With the exception of the COMT result discussed above, few genes have been linked to parietal lobe function. The apolipoprotein E epsilon4 (APOE) allele, the main susceptibility gene for Alzheimer's Disease (AD), has been linked to reduced parietal lobe function during performance of a semantic categorization task in cognitively intact subjects (Lind et al., 2006). However, the relevance of this finding to schizophrenia is unclear given that this gene has not been linked to schizophrenia. The variable number of tandem repeats (VNTR) SNP of the monoamine oxidase-A (MAO-A) gene has been linked to reduced parietal cortex activation during performance of a Go/No-Go task, but again the relevance of this finding is unclear given that the MAO-A VNTR SNP has not been linked to schizophrenia. One gene that preliminary evidence suggests is related to schizophrenia and parietal lobe function, at least at the behavioral level, is the nicotinic acetylcholine receptor alpha 4 (CHRNA4) gene (Parasuraman, Greenwood, Kumar, &

Fossella, 2005; De, V, Voineskos, Wong, & Kennedy, 2006). The parietal lobe is innervated by the cholinergic system and nicotinic receptors in the parietal cortex modulate cortical function in this region (Everitt & Robbins, 1997). Furthermore, nicotinic receptors have been implicated in attention (Sacco, Bannon, & George, 2004). The CHRNA4 C1545T SNP has been linked to visuospatial attention, putatively, via effects on parietal lobe function (Parasuraman et al., 2005). Specifically, the T allele is associated with slower RTs to spatial locations to valid spatial cues and greater RT costs to invalid spatial cues. A haplotype involving three CHRNA4 SNPs and a CHRNB2 SNP was significantly associated with schizophrenia a family study (De, V et al., 2006). However, this study did not genotype subjects for the CHRNA4 C1545T SNP, thus, it's unclear if this specific SNP is related to susceptibility for schizophrenia. Nonetheless, preliminary results are encouraging. Determining the functional relevance of this SNP to cerebral function would appear to be an important next step.

Overview of Experiment Two

The results of Experiment 2A contribute to the rapidly accumulating evidence linking COMT val108/158met genotype to cognitive function in humans, and schizophrenia more specifically. Indeed, in the past year alone over a half dozen studies examining associations between cognition and COMT genotype have been released. However, all these studies share the same caveat in that they are associative in nature. That is, in no case can it be said with absolute confidence that the observed effects are due to the COMT val108/158met SNP. It is entirely possibly that another functional polymorphism in linkage disequilibrium with COMT val108/158met exists and underlies the observations. Moreover, the impact of COMT genotype on PFC DA regulation in vivo remains to be fully articulated. Recent findings are consistent with expectations; however, more studies are needed. PET imaging investigations in particular may help clarify the effect of COMT genotype on DA function in vivo. Radioligands sensitive enough to image extra-striatal DA D2 and D3 receptors have recently been synthesized, and at least two of them, [F18] Fallypride and [11C] FLB457, are sensitive to

endogenous DA release and may be used to examine COMT genotype effects on cognitive task related or amphetamine induced DA release (Aalto et al., 2005; Riccardi et al. in press). By imaging changes in receptor occupancy during task performance it may be possible to directly identify differences between genotype groups in DA function.

Experiment 2B is the largest prospective investigations of COMT genotype interactions with APD related cognitive improvement in schizophrenia. As discussed earlier, two previous studies identified variation in working memory improvement with COMT genotype; however, these studies were hampered by their relatively small sample sizes and limited number of tests administered. Again though, confirmation of the underlying neurobiological interactions between genotype and APD treatment is required to confirm the results. Only one imaging study examining the nature of the interaction between genotype and APD treatment has been carried out to date. Clearly more investigation is needed. And, as with any preliminary findings, replication is essential.

In addition, a comprehensive understanding of COMT genotype effects on cognition and interaction with DAergic drugs requires a complete understanding of the role that DA plays in cognition and neurophysiology. Typically, our interpretations of findings concerning COMT effects on cognition and DAergic modulation of cognition in general, are made within the context of the inverted U-curve hypotheses (Goldman-Rakic et al., 2000), which is based primarily on findings from rodents and non-human primates. Although there is no reason to expect these findings don't generalize to humans, evidence that PFC cognitive functions and physiology follow and inverted U-curve in humans whereby to much or too little DA impairs performance and function is limited. There is compelling evidence from patient groups and normal controls that too little DA impairs certain cognitive functions; however, the effect of too much DA stimulation on PFC cognitive functions is not entirely understood. Consistent with findings in non-human primates, stress impairs selective attention in humans, but it remains to be confirmed that this is a direct result of over stimulation of DA receptors in the PFC (Hartley et al., 1974). There is some evidence that individual differences in response to amphetamine is related to baseline performance; however, the findings have not always

been in the anticipated direction (i.e. those with worse performance at baseline demonstrate greater improvement after treatment and vice versa).

It should also be kept in mind that COMT is not only involved in the regulation of dopamine, but norepinephrine (NE) too (Cooper et al., 2003). NE plays a prominent role in cognition (Aston-Jones & Cohen, 2005) and it is possible that some of the effects ascribed to DAergic variation by genotype are actually due to variation in NE activity. Again, a comprehensive understanding of the role that DA plays in cognition would assist in the interpretation of genetic associations. Similarly, with respect to the findings reported in Experiment 2B, it should also be kept in mind that clozapine has a complex receptor binding profile (Meltzer, 2002). Clozapine acts on muscarnic M1 and multiple serotonin receptors and enhances acetylcholine and, indirectly, glutamate activity in multiple brain regions including PFC and hippocampus, in addition to enhancing PFC dopamine transmission in animal models (Ichikawa et al., 2001; Ichikawa, Dai, O'Laughlin, Fowler, & Meltzer, 2002a; Meltzer, 2002; Meltzer, 2004). Perhaps more relevant is the fact that clozapine also evokes norepinephrine release in the PFC (Devoto et al., 2003). As such, it is possible that the current results reflect an interaction between clozapine evoked release of norepinephrine and genotype, although this is unlikely given the relatively selective effects COMT gene knockout has on PFC dopamine levels in rodents (Gogos et al., 1998). Moreover, dopamine also enhances dopamine release in the parietal cortex raising the possibility that the interaction between COMT genotype and improved cognition relates to dopaminergic activity beyond the PFC (Valentini, Frau, & Di Chiara, 2004).

With these caveats in mind, additional factors that are likely important for gene-cognition association studies and further implications of the findings reported from Experiment 2 are discussed below.

Factors to consider when examining genotype-cognition associations

Based on a consideration of studies examining COMT associations with cognition, including the current experiments, several factors that are likely important and explanations for some of the conflicting results regarding studies of COMT and cognition are offered below.

Psychometric Properties of the test and Subject Population Characteristics

Identification of SNP effects on normal variation in cognition will require detailed parsing of the cognitive processes under examination and the selection and/or development of tests and paradigms sensitive to the process (Goldberg & Weinberger, 2004). It is important that the psychometric properties of the test lend themselves to detecting gene effects on cognition that are likely subtle and process specific. Obviously, it is important that the test selected provide a good assay of the cognitive construct that is believed to be associated with the effects of the gene under investigation (i.e. the test should validly measure the cognitive process of interest). However, in most cases, neuropsychological tests are not process pure and tap a number of functions; this is especially true for "executive" or "frontal lobe" tasks. This does not necessarily mean that such tests can not be used though. In some cases, the set of cognitive processes underlying performance on complex tasks can be separated into unique, dissociable components and individual measures of these processes have been developed. A good example of this is the WCST. The WCST is often used as a holistic measure of executive cognitive functions; however, individual measures of relatively circumscribed cognitive processes (i.e. perseverative responding) exist and have been reliably dissociated from one another (Lezak, 1995; Spreen & Strauss, 1998). In addition, the test should not be susceptible to floor or ceiling effects. For example, it is extremely unlikely that gene effects would be detected on a task that subjects are capable of performing at or near perfect.

Floor or ceiling effects should be considered in conjunction with the abilities of the population under investigation in mind as the ranges of scores are likely to vary across populations in accordance with ability levels. For example, a working memory task that yields a good range of scores in a normal population may not produce the same spread of scores in a sample of patients with schizophrenia. Indeed, the relative difficulty of the task across subject populations may account for some of the discrepant findings observed for COMT. For example, in one study a significant effect was observed on the WAIS-R block design subtest in middle-aged, but not older adults (de Frias et al., 2005). Although the pattern of results is curious, the fact that there was variation in the COMT effect across ages is not entirely unexpected from a neurobiological perspective given evidence that the neural networks involved in the performance of some tasks very with age (Zysset, Schroeter, Neumann, & Yves, 2006; Prvulovic, Van, V, Sack, Maurer, & Linden, 2005). The frontal lobe is one of the last regions to reach maturation and is not the principle neural substrate involved in working memory in humans until after puberty (Lewis, 1997). Conversely, imaging studies of working memory suggest that older individuals appear to have "inefficient" working memory activity in the dorsolateral PFC compared to younger adults (Mattay et al., 2006). As such, if the effects of COMT are specific to executive sub-process that are often tapped in working memory paradigms, as speculated in Experiment 2A, and are regionally specific in the sense that COMT effects are most apparent on tasks that involve the dorsolateral PFC, then variation in the effects of COMT across age would be expected. In addition, a similar effect may underlie variable findings across subject populations. For example, a working memory task may be more sensitive to COMT effects in schizophrenia because patients are cortically "inefficient" in that they display greater activity in the dorsolateral PFC during working memory, yet the same task, at the same difficulty level, may not be sensitive to COMT genotype in healthy individuals if the dorsolateral PFC is not activated.

Heritability

More than just parsing and isolating the cognitive process of interest is required to examine genetic influences on task performance. A major requirement of the cognitive process under examination that is often overlooked is heritability. If a cognitive process is not at least partially heritable, then it is not influenced by genetics. In general, approximately 50% of the variance in overall cognitive ability (i.e. IQ) across individuals can be explained by genetic differences (Plomin & Spinath, 2004). Considerably less is known about the heritability of circumscribed cognitive process such as the executive components of attention and working memory (Winterer & Goldman, 2003). For example, reports on the heritability estimates for various indices of the WCST in monzygotic twins range from 0% to approximately 40% (Anokhin, Heath, & Ralano, 2003; Campana, Macciardi, Gambini, & Scarone, 1996). The heritability estimates for other executive measures such as Trailmaking B and the digit symbol substitution sub-test from the Wechsler Adult Intelligence Scale (WAIS) range from 40-60% (Swan & Carmelli, 2002). The heritability of working memory ranges from 30-50% depending on the task used (Ando et al., 2001; Wright et al., 2001). Clearly more investigation of the heritability of specific cognitive processes is needed. Ideally, one would select the test with highest heritability estimates for the cognitive process under investigation when examining the associations between cognition and genes. Knowing the hereditability of a specific cognitive process also places an upper limit on the amount of variability in performance any one SNP can account for and helps place a genotype effect in context. For example, the observation that COMT genotype accounted for 47% of the variance in task performance in a study conducted by Nolan et al. (2004) appears unrealistically high, assuming that the heritability of the task likely does not exceed 50-60%, and suggests that other factors (e.g. small sample size) likely accounted for the finding.

COMT Genotype and Schizophrenia: Beyond Cognition

Cognitive impairment is significantly related to outcome in schizophrenia (Green, 1996; Green et al., 2000). Moreover, specific associations between circumscribed cognitive domains and dimensions of outcome have been identified. For example, executive functions are linked to daily living and community skills, whereas attention may be more closely linked to job tenure (Green et al., 2000; Gold, Goldberg, McNary, Dixon, & Lehman, 2002). In light of these associations it is possible that the effects of COMT genotype might extend to functional outcome by virtue of its impact on cognition. Specifically, better functional outcome in met homozygous individuals might be anticipated given evidence of an association between better executive functions and working memory. However, enthusiasm for putative associations between genotype and functional outcome must be tempered by knowledge that genotype accounts for a relatively small proportion of the variance in cognition (typically less than 10%), which, in turn, accounts for only a proportion of the variance in outcome (up to approximately 40%).

It is possible that COMT genotype-environment interactions that contribute to functional outcome might be more pronounced. For example, stress increases PFC dopamine levels and, in schizophrenia, is associated with symptom exacerbation (Deutch & Roth, 1990; Lysaker, Davis, Lightfoot, Hunter, & Stasburger, 2005; Corcoran et al., 2003). Individuals with the met allele may be more sensitive to the deleterious effects of stress-induced PFC dopamine release given that they are, putatively, at or near the peak of the inverted U-curve of dopamine function already. As such, one might expect met homozygous individuals to demonstrate impaired cognition and exacerbation of symptoms when environmental stressors are present and subsequent alterations in functional outcome. One example of a significant gene-environment interaction influencing outcome is the relationship between MAO-A, childhood maltreatment, and the development of anti-social behavior (Kim-Cohen et al., 2006). However, in the current context outcome does not refer to development of psychopathology per se, but

specific dimensions of illness such as daily living skills, psychosocial skills, or symptomatology. It is noteworthy that the met allele has been linked to increased violence and aggression in schizophrenia in most (Han et al., 2006; Strous et al., 2003; Lachman, Nolan, Mohr, Saito, & Volavka, 1998; Strous, Bark, Parsia, Volavka, & Lachman, 1997), but not all investigations of this association (Zammit et al., 2004). Whether or not this association is mediated by genotype-environment interactions involving COMT and stress, and subsequent alterations in cognition is not known; however, this may be an interesting avenue to pursue given speculation that executive cognitive functions are associated with aggression and violence (Blair, 2001; McKay & Halperin, 2001).

Concluding Remarks

In essence, Experiments One and Two of this dissertation can be thought of as representing the early and later stages of the endophenotype approach, respectively. Specifically, Experiment One contributes to a small, but growing literature suggesting that abnormal cerebral physiology, especially in the PFC, is related to genetic vulnerability, while Experiment Two identified associations between discrete cognitive functions and a specific gene that transmission studies suggest is over-represented in patients with schizophrenia. The intermediate steps could include linkage analysis studies that link broad chromosomal regions to disease markers and/or the illness (e.g. Gasperoni et al., 2003), while the latter steps involve examining the effects of specific genes. Each step increases genetic specificity in the sense that the earliest stages look across the entire genome (i.e. do certain abnormalities, or the disease itself, run in families?), intermediate steps involve linking specific disease markers to chromosomal markers (i.e. are certain abnormalities, or the disease itself, linked to chromosomal markers?), until finally specific genes are implicated (i.e. are specific genes linked to a disease marker and is this gene preferentially transmitted to schizophrenia probands?). The advantage of the endophenotype approach over traditional linkage analysis studies that use the schizophrenia diagnosis alone to define the phenotype is the presumably

greater precision quantified endophenotypes offer compared to complex, subjective diagnostic classifications (Gottesman et al., 2003).

Appendix A: Parental Socioeconomic Scale

1= Families of wealth, education, top-rank social prestige

2= Families with adults having post-secondary education; in professional or high-rank positions

3= Small businessmen, white-collar and skilled workers; high school graduates

4= Semi-skilled workers, laborers; education below High School or equivalent

5= Unskilled and semiskilled workers; Formal education limited to elementary grades

6= Unknown

Appendix B: Description of cognitive battery.

Test	Brief Description	Dependent Variable
Working Memory		
Auditory Consonant Trigrams	Subjects are given three consonants aloud and asked to repeat them after a delay of 15 seconds. During the delay subjects are required to count backward from a variable starting number.	Total number of correct letters recalled, irrespective of order, over 14 trials.
Processing Speed		
WAIS-R Digit Symbol	Subjects are presented with sheet of paper consisting of 133 boxes that contain a number (1-9) in the top portion of each box. Subjects must draw the symbol that is paired with each number in the lower portion of each box. A key indicating the unique symbol that is paired with each number is presented at the top of the sheet.	Number of correctly matched symbols drawn in 2 minutes
Executive Function		
Wisconsin Card Sorting Test	See reference listed in text for detailed description	Number of categories achieved Percent Perseverate Errors
WISC-R Mazes (with no time limits)	Subjects are required to draw the correct path to the exits of a series of nine mazes of increasing complexity. Number of errors is deducted from the maximum attainable score of 30 and converted to an age-scaled score.	Age-scaled score (average=10)
Verbal Fluency		
Category Instance Generation Test	Subjects are required to generate as many category examplars as possible in 1 min. 4 trials are given- objects found outside, animals, fruits, and vegetables.	Total number of words generated
Controlled Oral Word Association Test	Subjects are required to generate as many words as possible that start with the letters F, A, S. Three trials lasting 1 min each are given for each letter.	Total number of words generated
Verbal Learning and Memory		
Buschke Selective Reminding Test	Subjects are read aloud a list of 12 words until they can accurately recall the list without missing any word over 2 consecutiv trials. Only words not recalled by subjects on two consecutive trials are repeated during the encoding stage. Subjects are then asked to repeat the list, without reminders, after attainment of list and again after a 45-min delay.	Number of words recalled after attainment (immediate recall) Number of words recalled after 45-min delay (delayed recall)

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