

**Functional Neurocircuitries of Working Memory in Chronic Schizophrenia**

by

Emanuela Tura  
B.Sc., University of Victoria, 2005

A Thesis Submitted in Partial Fulfillment of the  
Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

@ Emanuela Tura, 2008

All right(s) reserved. This thesis may not be reproduced in whole or in part, by photocopy or other means, without the permission of the author.

# **Functional Neurocircuitries of Working Memory in Chronic Schizophrenia**

by

Emanuela Tura  
B.Sc., University of Victoria, 2005

## **Supervisory Committee**

Dr. Nigel Livingston, Supervisor  
(Department of Biology)

Dr. Juan Ausió, Outside member  
(Department of Biochemistry)

Dr. Johan de Boer, Departmental member  
(Department of Biology)

Dr. Patrick MacLeod, Departmental member  
(Department of Biology)

**Supervisory Committee**

Dr. Nigel Livingston, Supervisor  
(Department of Biology)

Dr. Juan Ausió, Outside Member  
(Department of Biochemistry)

Dr. Johan de Boer, Departmental Member  
(Department of Biology)

Dr. Patrick MacLeod, Departmental Member  
(Department of Biology)

**Abstract**

Deficits in working memory are typical symptoms in schizophrenia. The gene for the Dopamine Receptor 1 (DRD1) is one of the candidate genes for schizophrenia, and it is critical for memory function. Magnetic Resonance Imaging (fMRI) was used to detect neurocircuitries engaged during a behavioral task of subjects with chronic schizophrenia and healthy people. Multivariate analysis in particular Partial Least Squares, was adopted to quantitatively capture diagnosis-specific patterns. The brain-behavior analyses identified diagnosis-specific circuitries that included many cortical areas. Furthermore, we compared two groups of schizophrenics with different DRD1 genotype. The imaging-genetics analysis showed that covariance patterns of different areas (including the dorsolateral prefrontal cortex and the inferior parietal lobule) were inversely related between the two genotypes. Therefore, it appears that the speed in subjects' response may be indicative of diagnostic-specific networks, and that DRD1 genotype may suggest differential use of neural networks.

## Table of Contents

Supervisory Committee.....	ii
Abstract.....	iii
Table of Contents .....	iv
List of Tables.....	v
List of Figures .....	vi
Acknowledgments .....	vii
Dedication.....	ix
Epigraph.....	x
Introduction .....	1
<b>Chapter 1 : Working Memory Network in Schizophrenia Modulate with Response Time.....</b>	<b>4</b>
1.1. INTRODUCTION.....	5
1.2. METHODS.....	7
1.2.1. Subjects.....	7
1.2.2. Scanning protocols.....	7
1.2.3. Cognitive protocol.....	8
1.2.4. Analysis methods.....	9
1.3. RESULTS.....	11
1.3.1. Behavioral analysis.....	11
1.3.2. Task-related PLS results.....	11
1.3.3. Behavioral PLS analysis.....	11
1.4 DISCUSSION.....	14
<b>Chapter 2 : Multivariate analyses suggest genetic impacts on neurocircuitry in schizophrenia.....</b>	<b>31</b>
2.1 INTRODUCTION.....	32
2.2. METHODS.....	34
2.2.1. Subjects.....	34
2.2.2. Genetic methods.....	34
2.2.3. MRI data collection.....	35
2.2.4. PLS – Image analysis.....	36
2.4. RESULTS.....	38
2.4.1. Genetic data and demographic variables.....	38
2.4.2. Behavioral analysis by genotype.....	38
2.5 DISCUSSION.....	40
<b>Chapter 3 : Working Memory Capacity and Effect of Working Memory Load in Functional circuitries.....</b>	<b>46</b>
3.1 WORKING MEMORY CAPACITY.....	46
3.2 WORKING MEMORY LOAD EFFECT.....	48
<b>Conclusion and Future Directions.....</b>	<b>54</b>
<b>References.....</b>	<b>57</b>

## List of Tables

TABLE 1.1: DEMOGRAPHIC SPECIFICS ON HEALTHY AND SCHIZOPHRENIC SUBJECTS. ....	24
TABLE 1.2: SIGNIFICANT CLUSTERS IDENTIFIED FOR LV1 AND LV3 IN THE RT ANALYSIS: POSITIVE AND NEGATIVE SALIENCES IN THE FRONTAL AREAS. ....	25
TABLE 1.3: SIGNIFICANT CLUSTERS IDENTIFIED FOR LV1 AND LV3 IN THE RT ANALYSIS: POSITIVE AND NEGATIVE SALIENCES IN THE CINGULATE AREAS. ....	26
TABLE 1.4.: SIGNIFICANT CLUSTERS IDENTIFIED FOR LV1 AND LV3 IN THE RT ANALYSIS: POSITIVE AND NEGATIVE SALIENCES IN THE TEMPORAL AREAS. ....	27
TABLE 1.5: SIGNIFICANT CLUSTERS IDENTIFIED FOR LV1 AND LV3 IN THE RT ANALYSIS: POSITIVE AND NEGATIVE SALIENCES IN THE PARIETAL AREAS. ....	28
TABLE 1.6: SIGNIFICANT CLUSTERS IDENTIFIED FOR LV1 AND LV3 IN THE RT ANALYSIS: POSITIVE AND NEGATIVE SALIENCES IN THE OCCIPITAL AREAS. ....	29
TABLE 1.7: SIGNIFICANT CLUSTERS IDENTIFIED FOR LV1 AND LV3 IN THE RT ANALYSIS: POSITIVE AND NEGATIVE SALIENCES IN ALL THE OTHER AREAS. ....	30
TABLE 2.1: POSITIVE AND NEGATIVE SYNDROME SCALE (PANSS) SCORES AND PREMORBID VERBAL IQ ESTIMATES (PVIE) SPECIFICS. ....	44

## List of Figures

FIGURE 1.1: SAMPLE RUN: IN THE SIX SECOND ENCODE CONDITION SUBJECTS MEMORIZED 5, 3 OR 1 TARGETS (DIGITS), AND IN THE THIRTY-EIGHT SECOND PROBE CONDITION 14 PROBES (DIGITS) WERE DISPLAYED. EACH CONDITION AND FIXATION BLOCKS WERE ALTERNATED. THE CONDITION ORDER AND THE FIXATION LENGTH WERE PSEUDORANDOM (AVERAGE OF 6 SECONDS WITH 4 AND 20 FOR MIN AND MAX VALUES).....	21
FIGURE 1.2: EFFECT OF MEMORY LOAD ON ACCURACY (A) AND REACTION TIME (B) IN BOTH HEALTHY AND SCHIZOPHRENIC SUBJECTS. ....	22
FIGURE 1.3: FIRST AND THIRD LATENT VARIABLES FOR THE REACTION TIME (RT) ANALYSIS. (A, C) THE SINGULAR IMAGE IDENTIFIES THE VOXELS COVARYING WITH RT VALUES DURING THE TASK (SHOWN IN RADIOLOGICAL CONVENTION, L = R). (B, D) THE CORRELATIONAL PROFILE DESCRIBES THE CORRELATION AMONG VOXELS, TASK AND BEHAVIOR. YELLOW AREAS REPRESENT REGIONS THAT ARE POSITIVELY CORRELATED WITH THE RT PROFILE. BLUE AREAS REPRESENT THE INVERSE CORRELATION. ....	23
FIGURE 2.1: THE VOXELS ARE THRESHOLDED AT 3.0 STANDARD DEVIATIONS FOR LV1. (A) YELLOW AREAS TEND TO BE MORE ENGAGED AS ACCURACY INCREASES IN GROUP AG, BUT NOT IN AA IN BOTH MEMORY CONDITIONS; BLUE AREAS REPRESENT THE INVERSE PATTERN. (B) THE CORRELATIONAL PROFILE DESCRIBES THE CORRELATION BETWEEN VOXELS AND ACCURACY VALUES. ....	45
FIGURE 3.1 : FIRST LATENT VARIABLE IN THE ONE-DIGIT ANALYSIS. (A ) THE SINGULAR IMAGE IDENTIFIES THE VOXELS COVARYING DURING THE ONE DIGIT TASK (SHOWN IN RADIOLOGICAL CONVENTION, L = R). (B) THE CORRELATIONAL PROFILE DESCRIBES THE CORRELATION AMONG VOXELS AND TASK. YELLOW AREAS REPRESENT REGIONS THAT ARE POSITIVELY CORRELATED WITH THE TASK. BLUE AREAS REPRESENT THE INVERSE CORRELATION. ....	51
FIGURE 3.2: FIRST LATENT VARIABLE IN HIGH AND LOW MEMORY LOAD ANALYSIS IN THE TWO GROUPS. (A) THE SINGULAR IMAGE IDENTIFIES VOXELS ACTIVATING IN HIGHER MEMORY LOAD RELATIVE TO LOWER MEMORY LOAD (SHOWN IN RADIOLOGICAL CONVENTION, L = R). (B) THE CORRELATIONAL PROFILE DESCRIBES THE CORRELATION AMONG VOXELS AND TASK IN THE TWO GROUPS. THE CORRELATIONAL PROFILE SHOWS A PATTERN THAT IS FOUND IN SCHIZOPHRENIC SUBJECTS ONLY. YELLOW AREAS REPRESENT REGIONS THAT ARE ACTIVE DURING THE HIGHER MEMORY LOAD COMPARED TO LOW MEMORY LOAD IN SCHIZOPHRENIC SUBJECTS ONLY. BLUE AREAS REPRESENT THE INVERSE CORRELATION. ....	52
FIGURE 3.3: FIRST LATENT VARIABLE IN HIGH AND LOW MEMORY LOAD ANALYSIS IN HEALTHY SUBJECTS ONLY. (A) THE SINGULAR IMAGE IDENTIFIES VOXELS ACTIVATING IN HIGHER MEMORY LOAD RELATIVE TO LOWER MEMORY LOAD (SHOWN IN RADIOLOGICAL CONVENTION, L = R). (B). YELLOW AREAS REPRESENT REGIONS THAT ARE ACTIVE DURING THE HIGHER MEMORY LOAD COMPARED TO LOW MEMORY LOAD IN HEALTHY SUBJECTS ONLY. BLUE AREAS REPRESENT THE INVERSE CORRELATION. ...	53

## Acknowledgments

I acknowledge and thank my supervisor, Dr. Nigel Livingston, for guiding me, trusting my pioneering work, and infusing his distinct enthusiasm for helping people with cognitive impairments.

I would also like to express my deep gratitude to my co-supervisors Dr. Johan de Boer and Dr. Juan Ausió for their invaluable assistance in this challenging endeavor, for all their thoughtful suggestions and helpful advice.

A special thanks to my mentor, Dr. Patrick MacLeod, who inspired me to think creatively and provided me with unique opportunities of exploring research with innovative and cutting-edge technology throughout my graduate program and most of my undergraduate preparation.

I am very grateful to Dr. Aaron Devor, Dean of graduate study, and his assistant, Ms. Anitra Winje for offering an immense support.

I also wish to thank Dr. Jessica Turner, Dr. James Fallon and Dr. Potkin, faculty members and fMRI specialists at the University of California, Irvine (UCI) for providing me with fMRI data, teaching me to analyze it and offering a pleasant working environment.

I thank Dr. Tugan Muftuler, Dr. Paul Rodriguez and Dr. Koroush Saberi, (UCI) for letting me participate in their classes and providing mentorship in learning fMRI basic concepts and programming.

I would like to thank Dr. Randy McIntosh, director of the Baycrest Institute at the University of Toronto for teaching me Partial Least Squares (PLS) and his warm hospitality in receiving me as a “visiting scientist”.

I am deeply grateful to the University of Victoria and the Biology department for their immense support and incredible opportunity they offered.

Last but not least, I would like thank Scuola Maya for donating \$5,000 towards my research.

**Dedication**

**Alla Famiglia**

**Epigraph**

**Γνώθι Σαυτόν**

*- Socrates*

## Introduction

Schizophrenia is a major public health concern. The world health organization rates schizophrenia among the five top disabilities in the world with a high suicide and unemployment rate, and a cost of \$40 billion per year in the US (WHO 2001). Over 230,000 people in Canada were estimated to be schizophrenic in 2004, resulting in a combined direct and indirect cost of approximately \$2 billion (Goeree et al 2005). Clinical symptoms usually emerge in late adolescence or early adulthood, followed, for some, by a steady clinical decline (Melle et al 2004). Schizophrenia is widely considered to be associated with gene dysfunction (Turner et al 2006).

Schizophrenia shows a wide spectrum of clinical symptoms. Negative symptoms of schizophrenia include: apathy, amotivation, poverty of speech, and social withdrawal. Positive symptoms include hallucinations and delusions. While these symptoms do not lead to a consistent classification in increasing understanding of the pathophysiology of schizophrenia, neuroimaging and genetic studies have shown many quantifiable aspects of the illness. The combination of clinical, imaging and genetic study are critical to reach a reliable quantifiable stratification of the disease.

Post-mortem examination and structural MRI brain studies have shown widespread aberrant volumetry, disconnectivity and cytoarchitecture in many brain areas of people with schizophrenia (Bonilha et al 2007; Cheung et al 2007; Iritani 2007; Lloyd in press). Schizophrenia has been thought to be a circuit-related pathology; hence main areas of the brains are expected to be functionally aberrant (Goldman-Rakic 1999). Furthermore, schizophrenia has been associated with the alterations in many genes (Carter 2006; Turner et al 2006).

Working memory is essential for temporary storage of information and processing. Many functional MRI studies have captured aspects of functional circuitry of working memory in schizophrenia. Taken all together, these studies showed aberrant functions in many areas of the frontal, temporal, cingulate, parietal, thalamic and striatal regions confirming the multifactorial nature of the disease as found by structural, molecular and genetic study and perhaps also suggesting that schizophrenia may be a circuit-related disorder. Furthermore, many studies have shown that schizophrenic subjects have a limited working memory capacity. Generally, in normal individuals activation in the prefrontal areas is low at light memory loads, and as the memory load increases this activation also increases until it reaches a plateau value. The activity in these prefrontal areas subsequently decreases. Schizophrenic subjects reach the highest level of activation in prefrontal areas earlier than healthy subjects.

Although the etiology of schizophrenia is unknown, there is a large amount of evidence that schizophrenia is associated with a dysfunction of the dopaminergic system. As antipsychotic drugs affect dopamine transmission and DRD1 regulation, DRD1 has been thought to be one of the candidate genes (Castner et al 2000). In schizophrenic patients, certain alleles of the Dopamine Receptor D1 (DRD1) gene are associated with clinical response, as well as a corresponding brain metabolic response to clozapine treatment. The AA allele of the DRD1 polymorphic site identified by the DDeI restriction enzyme upstream the initiation codon was found in individuals who were more responsive to the clozapine treatment and had a frequency of about 30 % in the healthy Caucasian population (Potkin et al 2003). The AG allele was found in individuals who were less responsive to clozapine treatment and had a frequency of about 60 %.

Only a few studies could find a snapshot view of the entire functional circuitry for working memory since standard univariate analyses allow only for a focal view of the brain. Reducing a complex cognitive task to a few areas may not capture different aspects of the pathology at once. On the other end, multivariate analysis methods applied to functional MRI data can identify coherence patterns in the changing BOLD (Blood-Oxygen-Level-Dependent) signal across the brain, possibly indicating brain regions with similar firing behaviors (McIntosh 2000).

For our research we chose a multivariate model known as Partial Least Squares (PLS) to analyze changes in brain activity patterns. As a data-driven approach, PLS identifies areas of the brain with the strongest signal coherence and the relationship of these brain patterns to the task conditions and performance (McIntosh et al 1996; McIntosh and Lobaugh 2004). However, *a priori*, we do not know if the majority of the covariance in the signal difference is due to groups' similarities or differences.

We investigated the differences in brain coherence patterns between healthy subjects and individuals affected by chronic schizophrenia. Furthermore, we investigated whether there are any differences in a cohort of chronic schizophrenics with similar symptomatology and behavioral performance on working memory tasks, but with different polymorphisms in the DRD1 gene.

## **Chapter 1 : Working Memory Network in Schizophrenia Modulate with Response Time**

Emanuela Tura, Jessica A. Turner, Liv C. Tronsden James H. Fallon, Steven G. Potkin.

Department of Biology, University of Victoria, Victoria, British Columbia, Canada, V8W 3N5  
Department of Psychiatry and Human Behavior, University of California, Irvine, Irvine, California, United States, 92617  
Department of Anatomy and Neurobiology, University of California, Irvine, Irvine, California, United States, 92697

### **Biology Psychiatry (to be submitted)**

#### **Abstract**

**Background.** Functional differences underlying working memory dysfunction in schizophrenia are likely expressed throughout the brain. We studied brain and behavior patterns in schizophrenia during a working memory task with a data-driven multivariate model. **Methods.** We conducted functional magnetic resonance imaging (fMRI) on 18 healthy subjects and 13 schizophrenic subjects in a 3T scanner while they performed a working memory task. The task required remembering one, three, and five digits--increasing memory load--and recognizing them among single random digits. We adopted the method of Partial Least Squares (PLS) to perform the multivariate analysis with and without mean response time (RT) as a covariate. **Results.** Only the analyses using mean RT by memory load distinguished subjects with schizophrenia from healthy subjects. Schizophrenic subjects' fast RT was associated with many prefrontal areas including the dorsolateral prefrontal cortex areas. When schizophrenic subjects were slower they relied on the thalamus and no longer on the prefrontal areas. Healthy subjects' fast RT correlated mostly with the cingulate, striatal and temporal areas as well as many prefrontal areas. When healthy subjects were slower, they still relied on temporal and cingulate areas even if to a smaller extent. **Conclusions.** Overall we found difference of RT correlation with a number of specific areas, in the frontal, occipital, temporal, parietal, and striatal regions. Hence, the speed in subjects' response may be indicative of a differential diagnosis-specific use of the brain regions engaged.

**Key Words:** schizophrenia, working memory, Partial Least Squares, multivariate analysis, reaction time, functional Magnetic Resonance Imaging

## 1.1. Introduction.

Deficits in working memory are core symptoms in schizophrenia (Callicott 2003; Callicott et al 2003). Although the exact functional system is still under investigation, many studies showed aberrant behavior in many areas including the dorsolateral prefrontal cortex (DLPFC), the anterior cingulate and the inferior parietal lobe in individuals with schizophrenia during a working memory task (Callicott 2003; Callicott et al 2000; Callicott et al 1999; Manoach 2003; Manoach et al 1999; Ragland et al 2007; Williams and Castner 2006). Slower processing speeds may contribute to memory deficits, but not many studies have investigated its correlation to neurocircuitry (Brebion et al 1998; Honey et al 2002) .

As the DLPFC seemed to be an important region for working memory, we have previously shown differential activation in the DLPFC between these groups of subjects with a region of interest investigation (Potkin et al 2008) confirming previous work (Callicott et al 1999; Callicott et al 2003; Johnson et al 2006; Manoach 2003; Van Snellenberg et al 2006; Williams and Castner 2006).

However, reducing neurophysiological differences between people with and without schizophrenia to one area may capture only certain aspects of the underlying pathology as post-mortem and structural MRI brain studies have shown widespread aberrant volumetry, disconnectivity and cytoarchitecture in many brain areas of people with schizophrenia (Bonilha et al 2007; Cheung et al 2007; Iritani 2007; Lloyd in press). Furthermore, schizophrenia has been associated with the dysfunction of many genes

(Carter 2006; Turner et al 2006). And perhaps most obviously, most brain regions are involved in multiple cognitive functions (Lloyd in press).

Multivariate analysis methods applied to functional MRI data can identify coherence patterns in the changing BOLD (Blood-Oxygen-Level-Dependent) signal across the brain, possibly indicating brain regions with similar firing behaviors (McIntosh 2000). We chose a multivariate model, Partial Least Squares (PLS), to analyze brain patterns of a cohort of people with and without schizophrenia while performing a working memory task, the Serial Item Recognition Paradigm (SIRP) (Manoach et al 1997; Manoach 2003; Manoach et al 2000; Manoach et al 1999); (Potkin et al 2008).

As a data-driven approach, PLS identifies areas of the brain with the strongest signal coherence and the relationship of these brain patterns to the task conditions and performance (McIntosh et al 1996; McIntosh and Lobaugh 2004). This allows a more global view of the brain rather than describing focal areas as per univariate analyses (McIntosh and Lobaugh 2004). However, *a priori*, we do not know if the pattern which accounts for the majority of the covariance in the signal is a difference between subject groups, or a change with memory load, or an interaction between the two. PLS identifies which of the possible patterns are the most robust in this dataset.

With this study, we investigated brain coherence patterns differing between healthy and schizophrenic subjects. Furthermore, we aimed to detect response-time-related circuitries for working memory in schizophrenia adopting a multivariate approach, as no other published material contributes to this notion.

## **1.2. Methods.**

### ***1.2.1. Subjects.***

The study was approved by the University of California, Irvine Institutional Research Board. Eighteen healthy comparison subjects and 13 schizophrenic or schizoaffective male and female adults were recruited for this study. Their demographics are summarized in Table 1.1.

Subjects were excluded if they had a current or past history of a major neurological, medical illness; previous head injury; substance or alcohol dependence; IQ less than 75 (as measured by the North American Adult Reading Test (Uttl 2002)); or if they used migraine treatments. Exclusion criteria for healthy volunteers were current or past major psychiatric diagnosis, or any first-degree family members. Subjects with schizophrenia or schizoaffective disorder meeting DSM-IV criteria were recruited for the study; schizophreniform subjects were excluded, as were subjects with significant extrapyramidal symptoms or tardive dyskinesia. Movement disorders were measured with the Abnormal Involuntary Movement Scale (AIMS). Subjects with schizophrenia were required to be clinically stable with no significant changes in their psychotropic medications in the previous two months.

### ***1.2.2. Scanning protocols***

All MRI scans were collected on a 1.5 Tesla Picker Eclipse scanner (Picker International, Cleveland, OH) at the University of California, Irvine Research Imaging Center. The scanning session consisted of a localizer scan; software shimming of the images to reduce ghosting in the echo planar imaging (EPI) acquisitions; a 3D T1-weighted scan, (FSPGR, 24 cm FOV, 1.5mm slice thickness, 160-170 slices as needed to cover the entire head,

sagittal orientation); and the functional imaging scans. The functional scans were T2\*-weighted gradient echo EPI sequences, with TR = 2s, TE = 30 ms, flip angle 90 deg, acquisition matrix 64x64, 22 cm FOV, 22 slices, 4 mm thick with 1 mm gap, AC-PC aligned.

Subjects who smoked refrained from smoking starting 40 minutes before lying down in the scanner.

The stimuli and responses were presented and collected using E-prime software, using an SRBox response device (Psychology Software Tools, Inc., <http://www.pstnet.com/products/e-prime/>), now available at [www.nbirn.net](http://www.nbirn.net). Visual stimuli were delivered using Resonance Technology goggles (<http://www.mrvideo.com/>).

### ***1.2.3. Cognitive protocol.***

The Serial Item Recognition Paradigm (SIRP) task is a choice response time task that requires working memory (WM); it has been reported to activate the dorsolateral prefrontal cortex in healthy subjects and people with schizophrenia (Johnson et al 2006; Manoach 2003). Subjects memorized a set of target digits that were shown in an MRI-compatible screen (the *encode* condition). The encode condition lasted six seconds. The SIRP task (illustrated in Figure 1.1) consisted of three working memory loads: 1, 3 or 5 target digits. After encoding, random digits (probes) were displayed one at the time on the screen (the *probe* condition). Fourteen probes were displayed in 38 seconds. Subjects were asked to recognize whether or not digits were part of the memory set (*targets* or *foils*, respectively). For each run or scan, each memory level was tested twice. The order of the three memory load conditions was pseudorandom. Between conditions, subjects

fixated on a flashing cross (baseline). There was a fifty percent chance to identify the correct answer. The entire block (encode and probe) lasted for a total of 46 seconds. There were six blocks in a run. Each run lasted six minutes. There were a total of three runs. Analyses were performed on the probe condition.

#### ***1.2.4. Analysis methods.***

The preprocessing steps for the functional imaging scans included motion detection and correction, co-registration and normalization to a Montreal Neurological Institute brain template (Montreal Neurological Institute, Montreal, Quebec, Canada) and smoothing with an 8 mm FWHM 3D Gaussian filter. The preprocessing steps were performed with the SPM2 software (<http://www.fil.ion.ucl.ac.uk/spm/software/spm2/>), using default settings where applicable (Friston et al 1995; Poline et al 1995). All runs for a subject were realigned using bilinear interpolation to the first image of the first run after discarding the first three unstable images. The motion-corrected, normalized, and smoothed images are the input to the PLS analysis.

The analyses used Partial Least Squares (PLS) version 5.0705021 (<http://www.rotman-baycrest.on.ca/>). We performed analyses with and without the mean response time as a covariate – “task PLS” and “response time PLS” analyses, respectively.

*Task PLS.* In order to identify areas of the brain presenting the same activations at the same time (covariance), PLS follows two steps (McIntosh et al 1996; McIntosh and Lobaugh 2004). First, it calculates the correlation between the design matrix and the data matrix (including information related to all subjects across all sessions). The resulting matrix is decomposed using singular value decomposition (SVD). This operation generates simultaneously a brain image showing voxels covarying with the task (*singular*

*image*), a *correlation profile* which is a plot expressing the relationship between the task and activation change in each condition for each group, and a *singular value* which is a scalar number reflecting the strength of the relationship between the correlation profile and voxel activity in the whole brain. The numerical weights within the singular image are termed *saliencies*; saliencies can be positive or negative. The singular image, singular values and correlational profile describe an overall pattern termed a latent variable (LV). Each SVD produces a number of LVs. The first LV accounts for the largest proportion of covariance, and thus is the primary pattern in the dataset; the second LV accounts for the next largest proportion, and so on.

The significance of the singular value is determined by permutation sampling. This involves randomly reassigning the subjects across groups and the conditions within subjects, and comparing the singular value of the original data with the distribution of singular values that arise with in this case 100 permutations. The bootstrap method was employed to reliably assess voxel intensity and derive estimates of standard errors of the LV saliencies for each voxel (McIntosh and Lobaugh, 2004).

*Reaction-Time PLS.* In this analysis, in the first step, the PLS process calculates a correlation between RT values by subject, condition, and voxel intensities. Thus the singular image shows the brain image with voxels whose activations covary with RT by condition, the correlation profile is the plot expressing the relationship between RT and activation change in each condition for each group, and the singular value is a scalar number giving the strength of the relationship between the task and all brain voxels intensity.

### **1.3. Results.**

#### ***1.3.1. Behavioral analysis***

Table 1.1 shows the behavioral results for both groups. Increasing memory load significantly decreased accuracy ( $F(2,58) = 3.9, p < .025$ ). Subjects with schizophrenia showed significantly lower accuracy than did healthy volunteers ( $F(1,29) = 4.5, p < .04$ ). The interaction of memory load and diagnosis was not significant. Increasing memory load significantly increased mean response time for both groups ( $F(2,58) = 39.7, p < .0001$ ). The effect of diagnosis was not significant as mean RT nor was the interaction of memory load and diagnosis. Figure 1.2 shows the relationship of behavior measures and diagnostic groups.

#### ***1.3.2. Task-related PLS results***

The first and second LVs accounted for significant amounts of covariance by the permutation test (53 % and 23% of the covariance for LV1 and LV2 respectively,  $p < .0001$ ). LVs did not identify any differences between the two groups, missing the purpose of this investigation looking for diagnosis-specific patterns.

#### ***1.3.3. Behavioral PLS analysis***

The response time PLS analysis also showed that the first and third LVs were significant by permutation testing ( $p < .05$ ). LVs are both illustrated in Figure 1.3 (thresholded to 3 standard errors) and regions of interest are detailed in Tables 1.2 to 1.7. Regions supporting these LVs are shown in Figure 1.3a and 1.3c. LV1 accounted for 28.5 % of the variance. The LV1 correlation profile describes a brain pattern that is unique to schizophrenics and is found in all memory loads (Figure 1.3b). In LV1 positive saliences expressed a positive correlation between decreased (faster) RT in all memory load

conditions; negative saliences showed a negative correlation. This LV did not correlate in the data from healthy volunteers. In the prefrontal lobe, positive saliences included many areas in the superior, middle, medial and inferior frontal gyri. A large cluster was found in BA11 (in the medial frontal gyrus), BA 9 (in the superior frontal gyrus), small and large clusters were found in BA 46 (in middle, medial, and inferior frontal gyri). There was a large cluster in the claustrum, medium-sized loci in the inferior and superior parietal lobules, small loci in the cerebellum and caudate body. Many small loci were also found in the middle occipital and lingual gyrus. The cingulate and temporal regions showed almost no correlation with RT.

Negatively-weighted areas expressed the correlation of slower RT with increased activation in schizophrenics. The frontal lobe showed little involvement in the circuitry. There was a large cluster in the superior frontal gyrus and a small cluster in the middle frontal gyrus. A large cluster in the thalamus was found. Numerous small to medium sized loci were present all over the temporal lobe. The parietal lobe showed many regions correlated with RT. There were small and large clusters in both the inferior and superior parietal lobules and post-central gyrus. Many small areas were found in the lingual and occipital gyrus. Again, the cingulate regions showed almost no correlation except of two small loci in the posterior cingulate gyrus.

The second LV in the RT-PLS results accounted for 19.2 % of the covariance, but it was not significant ( $p > 0.1$ ). The third LV in the RT-PLS results accounted for 18.5 % of the covariance and, in contrast to LV1, represented a pattern that was found in all memory conditions for healthy subjects and only in the lowest memory load for schizophrenics. The LV singular image is shown in Figure 1.3b. Areas with positive and negative

salience are listed in tables 1.3a and 1.3b respectively. Positively weighted areas expressed the correlation of faster RT with activation increases in all memory conditions for healthy volunteers and only in the lowest memory condition for schizophrenic subjects. In the frontal lobe there were a number of small to medium-sized clusters all over the brain. Only one large cluster was found in the inferior frontal gyrus (BA47). Many large and small clusters in the limbic areas strongly correlated with RT. There were two large clusters in the cingulate gyrus (BA31/32) and smaller loci in the cingulate and anterior cingulate gyrus (BA 23 and BA24). The posterior cingulate gyrus was barely represented. There were large clusters in the insula and putamen. The temporal lobe was largely involved. A large cluster in the transverse temporal gyrus and many areas in the middle temporal gyrus were found. In the parietal lobe, there only two small loci were noticed in inferior parietal lobule. Neither the superior parietal lobule nor the post-central gyrus were involved. The occipital lobe showed small sparse loci. Hippocampus and parahippocampal gyrus also showed some small loci.

Negatively-weighted areas expressed the opposite pattern describing areas correlated with slower RT. In the frontal lobe there were numerous clusters all over the brain except in the inferior frontal gyrus. Two large clusters were found the superior frontal gyrus (BA6/8). The limbic areas showed bilateral involvement of the BA 32 in the anterior cingulate. The posterior cingulate gyrus was not represented. The temporal lobe was barely represented. In the parietal lobe, there were was a large cluster in the post-central gyrus (BA7) and in many loci in the superior and inferior parietal lobule, in the precuneus and in other regions of the post-central gyrus. The occipital lobe showed small loci especially in the cuneus.

#### **1.4 Discussion.**

This study shows the complexity of cognitive processes in pathological and non-pathological working memory. PLS was used to identify covariance patterns in both healthy and schizophrenic subjects. The RT analysis but not the task analysis identified a diagnosis effect. Accuracy levels decreased as the task became more challenging in both groups; however, the accuracy scores were significantly lower overall for schizophrenics, as expected (Callicott et al 2000; Carter et al 1996; Fleming et al 1997; Goldberg and Weinberger 1995). At the same time, as the task became harder, both groups were slower at retrieving information. While SZ subjects tended to have slower response times than controls, there was not a significant difference in response times between the two groups. This argues against any RT-specific responses being due merely to the fact that the SZ subjects are slower.

Significant differences were found in RT-related networks between the two groups. Overall we found diagnostic patterns of RT correlation with a number of areas with a number of specific areas found in 1) frontal, 2) cingulate, 3) insular, 4) basal ganglia, 5) occipital, 6) parietal, 7) thalamic and 8) temporal regions, corroborating the theory that schizophrenia may be a dysfunction affecting cortical connections (Goldman-Rakic 1995; Goldman-Rakic 1999) and that multivariate analysis is an appropriate tool as it allows to detect distributed network (Calhoun et al 2006; Kim et al in preparation; Lloyd in press; McIntosh et al 1996; McIntosh and Lobaugh 2004).

RT-analysis shows some unique aspects of the neurocircuitry of the schizophrenic subjects as well as some regions shared by the schizophrenic and healthy individuals.

Specifically, some capacity issues are noticed; there are areas that are used by healthy subjects in all conditions and by the schizophrenics only in the lowest memory load, suggesting that a different mechanism may be in place for harder tasks in subjects with schizophrenia. Some of the most distinctive patterns show that healthy subjects in all three conditions and schizophrenics in only the easiest condition relied heavily on cingulate-striatal circuitries to give a fast response. However, schizophrenics heavily relied on the prefrontal areas, including the dorsolateral-prefrontal cortex in the higher as well lower memory loads. This suggests that healthy subjects rely on error checking system for fast responses and that the error-checking system of schizophrenic subjects may be dysfunctional.

There were frontal regions that correlated with faster performance, were found in all three conditions, but were group-specific. Schizophrenic subjects relied on BA 9/46 and BA 11 and numerous loci in the superior, middle, medial and inferior gyri. Healthy subject also had loci in many frontal areas; however, they relied more on the inferior frontal gyrus and did not use the dorsolateral prefrontal cortex for a faster performance. This pattern was also found in schizophrenics, but only in the lower memory load, again suggesting capacity differences. When subjects were slower, different patterns were observed. Healthy subjects still used the frontal regions with a noticeable shift to superior frontal areas (from inferior frontal areas correlating to fast responses). This was again found in all three memory conditions for healthy subjects and only in the lowest memory condition for schizophrenic subjects. Schizophrenic subjects were unable to keep up with the frontal regions when they were slower. Adopting independent component analysis (ICA), another type of multivariate analysis, our collaborators found again a differential

use of the prefrontal areas during the same memory task (Calhoun et al 2006; Kim et al in preparation). Our findings may also indicate capacity limits reflecting previous investigations showing differential activations between schizophrenic and healthy subjects (Calhoun et al 2006; Callicott et al 2000; Callicott et al 2003; Manoach 2003).

For fast performance, we observed a differential use of the limbic lobe in the two groups. Healthy subjects relied heavily on the anterior cingulate and cingulate gyri (BA32, 31 and 24) in all three memory loads. Schizophrenic subjects were unable to use those areas for the highest memory load. Koch et al also have found that the increased BOLD signal in these areas was associated with faster response behavior only in healthy individuals (Koch et al 2008). The impact of the limbic lobe was not as strong for slower responses, but it was still present in healthy subjects, but not in schizophrenic subjects (except for the lowest memory load). Dysfunctions in the anterior cingulate gyrus in schizophrenics has been reported consistently in the literature (Callicott et al 1999; Callicott et al 2003; Kim et al in preparation; Ragland et al 2007; Snitz et al 2005) possibly reflecting error checking and self-monitoring deficits (Brown and Braver 2005).

The insula was strongly associated with RT in probe for healthy subjects and in the lowest memory load for schizophrenic subjects. Insula hyperactivation has also been consistently reported in the literature (Johnson et al 2006; Koch et al 2008). Our analysis may be detecting the neuroanatomical network of insular projections in the anterior cingulate (BA24) (Martin 2003). Schizophrenic subjects did not show any correlation of insular activation with fast response in higher memory loads. However, an equivalent cluster in the claustrum correlated with fast response in higher memory loads for schizophrenics. This may indicate a disruption along the insulo-cingulate projections.

Further differences were found in the basal ganglia areas. Both the putamen and basal ganglia correlated with fast response during probe for healthy subjects, but only for the lowest working memory condition in schizophrenics corroborating previous notion that these areas are functionally and structurally aberrant in schizophrenics (Agid et al 2007; Glenthøj et al 2007; Murray et al 2008).

The occipital patterns for LV 1 and LV3 were also different and may reflect eye-related abnormalities (Maccabe et al 2005; Spengler et al 2006). Calhoun's investigations have also found differential use of occipital regions using multivariate analyses (Calhoun et al 2006; Kim et al in preparation).

In schizophrenics, fast response time seems to correlate more to the superior and not the inferior parietal lobule (IPL) as in healthy subjects reflecting previous RT-activation correlation studies (Bullmore et al 1996). Functional studies have also found dysfunction of the IPL (Assaf et al 2006; Calhoun et al 2006; Callicott et al 2000; Thermenos et al 2005). Differential use of the thalamus was also noticed. When both group were slower, schizophrenics used the thalamus much more than healthy subjects. Thus, IPL-thalamic dysfunction together with aberrant activation in occipital regions may indicate an abnormality to integrate visual and cognitive information (Pierrot-Deseilligny et al 1995). Differential correlation patterns found in the IPL, thalamus, cuneus, precuneus, supplementary eye fields and lateral frontal eye fields provides further support to previous findings showing basal ganglia-thalamocortical circuitry dysfunction (Camchong et al 2006).

Distinct differences in the temporal wiring were also evident reflecting the consolidated notion of dysfunction and abnormal cortical structure in the temporal lobe (Bonilha et al

2007; Calhoun et al 2006; Iritani 2007; Kim et al in preparation; Ragland et al 2007). Many temporal regions were strongly associated with RT in probe for healthy subjects and in the lowest memory load for schizophrenic subjects. Schizophrenic subjects barely relied on temporal areas for fast performance in higher memory loads. However, this pattern was the opposite when subjects were slower. Healthy individuals barely used the temporal regions throughout probe, while schizophrenics showed a number of small loci in many temporal areas.

Our findings may detect RT-related circuitries reflecting a slightly different level of effort or fundamentally different brain structures between the two groups. It is also possible that schizophrenic subjects reached maximum capacity earlier, and it is also possible that they cannot efficiently use many temporal areas. Multivariate analyses on activations when memory load exceeds capacity for both healthy and schizophrenic subjects will give deeper insight of network capacity and restraints.

Whether or not these differences and similarities in circuitries were due to medications, we are not able to assess it in this group. Chronic schizophrenics take a number of different medications such as typical and atypical antipsychotics, mood stabilizers and antidepressants. Because of the nature of the clinical approach, it is not possible to identify how each medication affects the brain, specifically. However, specific brain features are found in first-episode as well as in chronic schizophrenics. Structural studies have shown decreased brain volume in the DLPFC and fronto-temporal areas in unmedicated schizophrenics or subjects in the first episode of schizophrenia (Bonilha et al 2007; Nakamura et al 2007). There are not many on-set functional studies; the few studies also indicate DLPFC dysfunction (Barch et al 2001).

Multivariate analyses are opening new ways of capturing neurons' firing behaviors in a way to detect entire functional networks. However, we do not have any information pertaining to any temporal aspect. In fact, in an elegant analogy, McIntosh observes that as notes and timing create a symphony, the neural system shows temporal dependency of cognitive functions (McIntosh 2000). In a preliminary, Lloyd's suggested that a generalized temporal discontinuity characterizes schizophrenic cognition (Lloyd in press).

Task analysis identified a load effect across the two groups. It is possible that common circuitry relates to load manipulation and has a high BOLD signal. Thus, with multivariate analyses, it may be important to develop strategies to uncover the different brain activities depending on smaller BOLD signals such as incorporating the use of covariates.

### **Conclusion**

In conclusion, our analyses capture several aspects of working memory relative to diagnosis. Our analysis found diagnostic patterns of RT correlation with a number of areas in the frontal, cingulate, insular, basal ganglia, parietal, thalamic and temporal regions reflecting the notion that schizophrenia may be a dysfunction affecting cortical connections. Thus, the speed in subjects' response may be indicative of a differential diagnosis-specific use of the brain regions engaged.

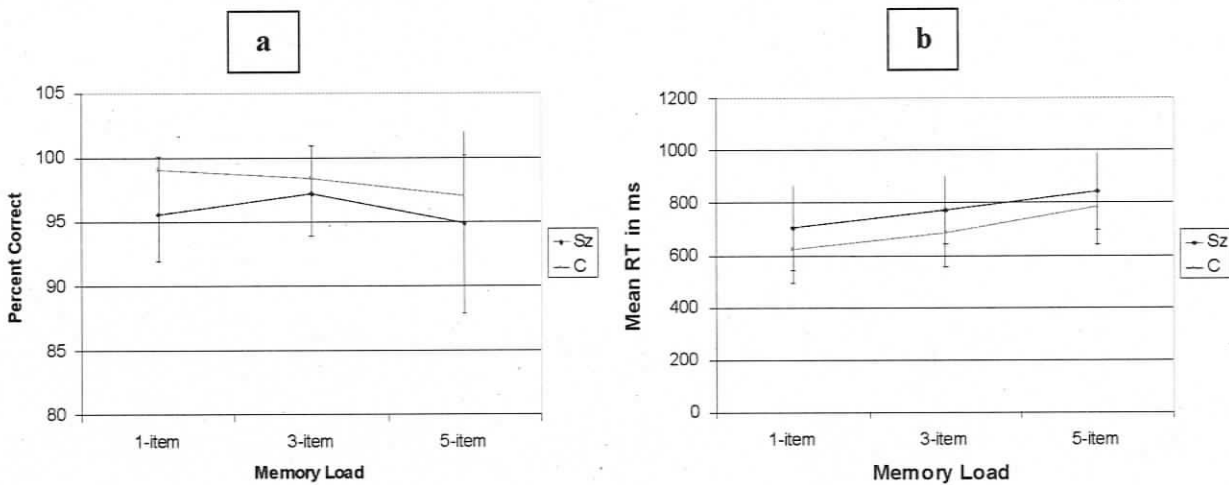
**Acknowledgments.**

This research was supported by U24 RR021992 to the FBIRN and by P20 RR020837-01 to the Transdisciplinary Imaging Genetics Center, from the National Center for Research Resources/National Institutes of Health. We acknowledge and thank Randy McIntosh, Ph.D., from the University of Toronto for his helpful PLS workshops, Paul Rodriguez, Ph.D., from the University of California, Irvine, for his input on these discussions. We also thank Johan De Boer, Ph.D, Juan Ausio, Ph.D. and the University of Victoria for their invaluable assistance.

Figure 1.1: Sample Run: in the six second encode condition subjects memorized 5, 3 or 1 targets (digits), and in the thirty-eight second probe condition 14 probes (digits) were displayed. Each condition and fixation blocks were alternated. The condition order and the fixation length were pseudorandom (average of 6 seconds with 4 and 20 for min and max values)

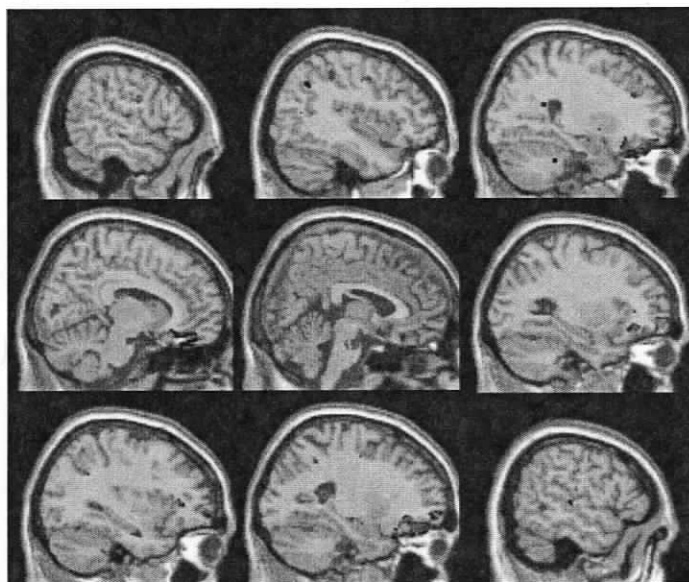
	1.5" + .5"	6"	38"	= 46" block
	Prompt	Encode	14 probes	
5t	Learn	0 2 1 3 8	9 7 2 4 ...	
3t	Learn	7 * 5 * 4		
1t	Learn	* * 9 * *		

Figure 1.2: Effect of memory load on accuracy (a) and reaction time (b) in both healthy and schizophrenic subjects.

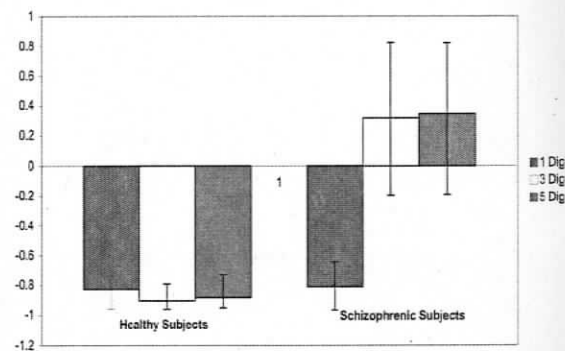
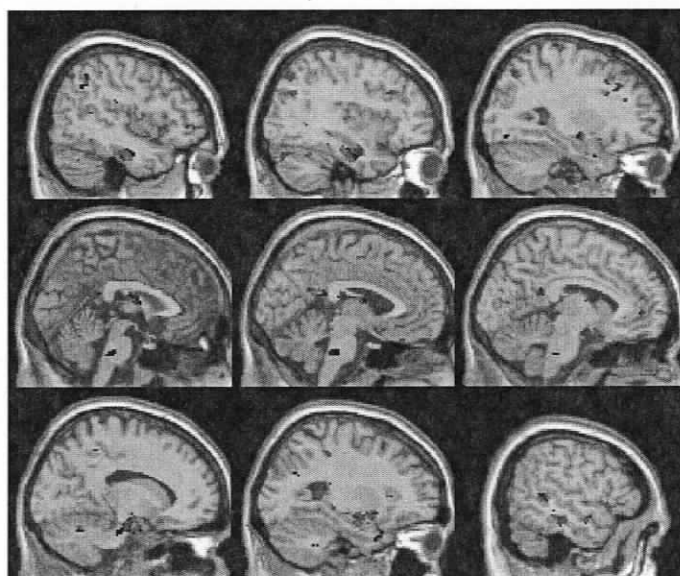
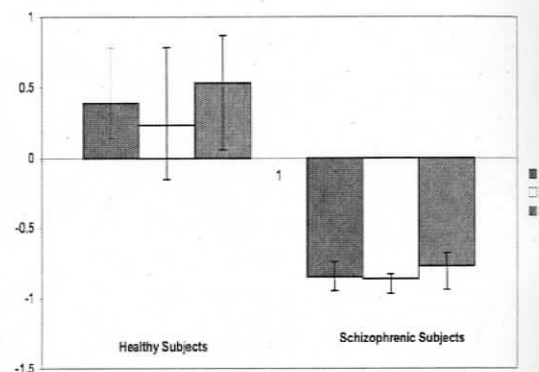


**Figure 1.3: First and third latent variables for the Reaction Time (RT) analysis. (a, c) The singular image identifies the voxels covarying with RT values during the task (shown in radiological convention, L = R). (b, d) The correlational profile describes the correlation among voxels, task and behavior. Yellow areas represent regions that are positively correlated with the RT profile. Blue areas represent the inverse correlation.**

(a)



(b)



(d)

(c)

**Table 1.1: Demographic specifics on healthy and schizophrenic subjects.**

	Subjects	
	Schizophrenic	Healthy
Total number of participants	13	18
Men; women	10; 3	13; 5
Mean Age in Years	40.2	40.9
Right Handed	11	15
Mean total scores for Schedule for Assessment for Negative Syndrome (SANS)	44.9	NA
Mean total scores for Schedule for Assessment for Positive Syndrome (SAPS)	29.9	NA
Mean proportion of accurate answers		
One-Digit-Load	.95 ± 3.64	.99 ± 1.05
Three-Digit-Load	.97 ± 3.30	.98 ± 2.55
Five-Digit-Load	.94 ± 7.03	.97 ± 3.14
Mean response time (RT)		
One-Digit-Load	703 ± 160.35	697 ± 129.11
Three-Digit-Load	772 ± 127.76	734 ± 129.54
Five-Digit-Load	843 ± 144.00	783 ± 146.74

Table 1.2: Significant Clusters identified for LV1 and LV3 in the RT analysis: positive and negative saliences in the frontal areas

Anatomical Region	RT -LV1		RT -LV3	
	Positive XYZ (cluster) [BSR]	Negat. XYZ (cluster)	Positive XYZ (cluster)	Negat. XYZ (cluster)
Superior Frontal Gyrus				
BA 6				-6 34 55 (124) [-5.6]
BA 8				8 30 50 (204) [-3.9]
BA 9	24 50 32 (106) [4.9]			
BA 10		-18 66 6 (160) [-6.4]	-18 61 17 (14) [3.7]	14 56 -4 (8) [-4.0]
Middle Frontal Gyrus				
BA 6		28 -9 59 (34) [-3.8]	32 -8 43 (8) [3.2]	
BA 8				-32 31 46 (65) [-4.2]
BA 9	42 42 40 (34) [3.8]			42 13 36 (21) [-3.7] -38 43 35 (6) -3.0
BA 10			-32 46 22 (51) [3.5]	
BA 46	57 38 15 (96) [4.8] -44 28 24 (42) [3.4]			
Medial Frontal Gyrus				
BA 6	-18 -15 47 (22) [3.6]			
BA 8	12 29 43 (44) [4.2]		-16 33 37 (17) [3.6]	-4 49 40 (77) [-4.3]
BA 11	-10 29 -12 (3482) [9.4]			
Inferior frontal				
BA 9				
BA 45	-61 26 19 (6) [3.2]		-57 16 3 (76) [3.8]	
BA 46	-61 32 9 (7) [3.8]			
BA 47	-34 15 -6 (63) [3.7]		-34 27 -8 (113) [4.3] 48 21 -9 (38) [3.7]	
Precentral Gyrus				
BA 4		-28 -23 53 (50) [-5.1]		-28 -26 68 (12) [3.5]
BA 6		44 -8 43 (30) [-3.6] -34 -8 63 (15) [-3.0]	63 2 7 (49) [4.1]	30 -18 67 (15) [-3.9]

**Table 1.3: Significant Clusters identified for LV1 and LV3 in the RT analysis: positive and negative saliences in the cingulate areas.**

Anatomical Region	RT-LV1		RT-LV3	
	<i>Positive XYZ (cluster) [BSR]</i>	<i>Negat. XYZ (cluster) [BSR]</i>	<i>Positive XYZ (cluster) [BSR]</i>	<i>Negat. XYZ (cluster) [BSR]</i>
Cingulate Gyrus				
BA 23			2 -16 27 (7) [3.3]	
BA 24	0 4 31 (24) [3.8]		12 9 35 (56) [5.2]	
BA 31			-14 -29 38 (180) [5.7] 12 -43 43 (14) [3.3]	26 -42 24 (39) [-3.5]
BA 32			-14 9 31(396) [5.7]	
Anterior Cingulate Gyrus				
BA 24			-10 30 13 (27) [4.0]	
BA 32				-12 44 -6 (55) [-3.6] 6 23 -8 (18) [-3.3]
Posterior Cingulate Gyrus				
BA 23		2 -34 20 (27) [-3.3]		
BA 29	-16 -48 10 (22) [4.8]	4 -56 12 (20) [-3.7]	20 -60 14 (11) [3.2]	

**Table 1.4.: Significant Clusters identified for LV1 and LV3 in the RT analysis: positive and negative saliences in the temporal areas.**

Anatomical Region	RT-LV1		RT-LV3	
	<i>Positive X Y Z</i> <i>(cluster)</i> <i>[BSR]</i>	<i>Negat. X Y Z</i> <i>(cluster)</i> <i>[BSR]</i>	<i>Positive X Y Z</i> <i>(cluster)</i> <i>[BSR]</i>	<i>Negat. X Y Z</i> <i>(cluster)</i> <i>[BSR]</i>
Superior Temporal Gyr.				
BA 22	73 -38 11 (33) [4.7] -69 -38 17 (8) [3.5]		-53 -44 11 (41) [3.5]	
BA 38		55 6 -5 (49) [-4.1]		
BA 39		-61 -55 25 (72) [-4.5]		
BA 42				-71 -25 5 (11) [-4.3]
Middle Temporal Gyr.				
BA 20		53 -36 -12 (6) [-3.3]		
BA 21	67 -56 5 (32) [4.9]		-61 -33 -7 (97) [4.6] 67 -54 6 (8) [3.4]	59 -37 -12 (36) [-4.7] -46 -18 -13 (41) [-4.3]
BA 22			-48 -37 4 (7) [3.1]	
BA 39		-57 -71 13 (6) [-3.4]	55 -67 11 (33) [3.6]	
Inferior Temporal Gyr.				
BA 37		63 -60 -5 (8) [-3.2]		
Transverse Temporal Gyr.				
BA 41			-48 -25 12 (6) [3.0]	
BA42			-59 -19 10 (197) [4.4]	

**Table 1.5: Significant Clusters identified for LV1 and LV3 in the RT analysis: positive and negative saliences in the parietal areas.**

Anatomical Region	RT-LV1		RT-LV3	
	<i>Positive XYZ (cluster) [BSR]</i>	<i>Negat. XYZ (cluster) [BSR]</i>	<i>Positive XYZ (cluster) [BSR]</i>	<i>Negat. XYZ (cluster) [BSR]</i>
Superior Parietal Lobule				
BA7	32 -59 58 (56) [3.9]	-30 -53 63 (123) [-3.7]		42 -59 58 (15) [-3.4]
Inferior Parietal Lobule				
BA 40	-59 -39 42 (83) [4.6]	-51 -43 37 (34) [-3.7]	50 -43 43 (31) [4.5] -65 -30 25 (53) [4.4]	63 -34 50 (19) [-3.8]
Precuneus				
BA 7	12 -52 39 (15) [3.0]			26 -52 50 (28) [-3.6]
Post-Central Gyrus				
BA 1		-65 -23 36 (7) [-3.1]	32 -34 64 (62) [4.4]	
BA 2		34 -38 65 (168) [-7.7]		38 -31 31 (16) [-3.0]
BA 3		59 -21 38 (85) [-4.7]		
BA 7		10 -47 63 (19) [-3.9]		18 -51 67 (185) [-5.6]

**Table 1.6: Significant Clusters identified for LV1 and LV3 in the RT analysis: positive and negative saliences in the occipital areas.**

Anatomical Region	RT-LV1		RT-LV3	
	Positive X Y Z (cluster) [BSR]	Negat. X Y Z (cluster) [BSR]	Positive X Y Z (cluster) [BSR]	Negat. X Y Z (cluster) [BSR]
Middle Occipital Gyrus		50 -83 2 (46) [-3.6]		
BA 18	-28 -100 12 (18) (3.3) 22 -99 10 (16) [3.2]			
BA 19	-40 -66 7 (93) [4.0]			-28 -80 22(6) [-3.3]
Inferior Occipital Gyrus				
BA 18				30 -91 -4 (24) [-3.8]
Lingual Gyrus				
BA 17	20 -84 -1 (7) [3.0]	8 -88 -2 (13) [-4.0]		
BA 18	24 -74 -5 (19) [4.2] -12 -76 -1 (9) [3.4]		8 -70 -2 (13) [3.6]	
Cuneus		-10 -99 3 (18) [-3.2]		
BA17			4 -81 10 (45) [4.7]	-20 -87 6 (36) [-3.3]
BA 18		-12 -82 24 (26) [-4.3] 10 -105 4 (9) [-3.6]		-14 -99 7(6) [-3.5] 8 -98 12 (6) [-3.2]
BA 19		0 -84 34 (51) [-4.1]		
Fusiform Gyurs				
BA 19			26 -66 -5 (88) [5.2]	

**Table 1.7: Significant Clusters identified for LV1 and LV3 in the RT analysis: positive and negative saliences in all the other areas.**

Anatomical Region	RT-LV1		RT-LV3	
	<i>Positive XYZ (cluster) [BSR]</i>	<i>Negat. XYZ (cluster) [BSR]</i>	<i>Positive XYZ (cluster) [BSR]</i>	<i>Negat. XYZ (cluster) [BSR]</i>
Other Regions				
Hippocampus			24 -39 -3 (14) [3.7]	
Parahippocampal Gyrus				
BA 19			-28 -43 0(25) [3.5]	
Insula		42 -25 14 (32) [-4.0]	42 6 -2 (412) [5.4] -40 -17 17 (18) [3.6]	
Thalamus	-18 -36 -11 (14) [3.9]	-12 -25 -2 (563) [-5.6]		
Pulvinar				16 -33 2 (15) [-3.4]
Clastrum	-22 20 10 (457) [4.7]			
Cerebellum				
Culmen	10 -47 -6 (62) [5.5]			
Striatum				
Caudate Body	10 -3 15 (17) [3.4]			
Caudate Tail		38 -18 -8 (36) [-3.6]		
Putamen			-30 4 2 (547) [6.5] 20 4 11 (61) [4.3]	
Substantia Nigra			-12 -18 -13 (47) [4.0]	

## **Chapter 2: Multivariate analyses suggest genetic impacts on neurocircuitry in schizophrenia**

Emanuela Tura, Jessica A. Turner, James H. Fallon, James L. Kennedy, Steven G. Potkin.

Department of Biology, University of Victoria, Victoria, British Columbia, Canada, V8W 3N5  
Department of Psychiatry and Human Behavior, University of California, Irvine, Irvine, California, United States, 92617  
Department of Anatomy and Neurobiology, University of California, Irvine, Irvine, California, United States, 92697

**Neuroreport** (in press)

### **Abstract.**

We investigated the relationship of functional neurocircuitries and dopamine receptor D1 (DRD1) polymorphisms in schizophrenics during a working memory task. Subjects performed the Serial Item Recognition Paradigm memory task during functional magnetic resonance imaging acquisition. We performed a data-driven multivariate analysis (Partial Least Squares) to characterize brain network (covariance) patterns. Genetic testing identified two main genotypes. Accuracy did not differ between the groups. Covariance patterns of different areas (including the dorsolateral prefrontal cortex and the inferior parietal lobule) were inversely related between the two genotypes.

Two groups of schizophrenic subjects with similar symptomatology and performance on a working memory task, but with distinct dopamine receptor genotypes, may use distinct neural systems to retrieve information.

**Keywords: schizophrenia, fMRI, genes, Partial Least Squares (PLS)**

## 2.1 Introduction

A hallmark of schizophrenia is cognitive impairment, including working memory dysfunction. Functional Magnetic Resonance Imaging (fMRI) studies of working memory have shown differential activations in specific areas including in the dorsolateral prefrontal cortex, the anterior cingulate, inferior parietal lobule, basal ganglia and superior frontal gyrus (Callicott et al 2003; Johnson et al 2006; Karlsgodt et al 2007; Manoach et al 1999; Schneider et al 2007). It has been suggested that genetic differences may explain the phenomenon (Williams and Castner 2006). The Dopamine receptor D1 (DRD1) is one of the candidate genes for schizophrenia and was also found to be essential for working memory (Hirvonen et al 2006; Williams and Castner 2006).

In our previous study we found that the DRD1 polymorphic site located one kilobase upstream from the initiation codon (DDeI) was predictive of clinical response to clozapine in schizophrenia (Potkin et al 2003); thus, we investigated whether this polymorphic site was also associated with specific functional neurocircuitries in the same population.

In a cohort of people with schizophrenia, we used partial least squares (PLS) as a data-driven tool to see whether patients with different dopaminergic genotypes recruited the same circuitry to perform a working memory task. PLS methods in neuroimaging identify areas of the brain with the strongest signal coherence (McIntosh et al 1996; McIntosh and Lobaugh 2004). Rather than hypothesizing a specific relationship between fMRI signal changes and performance in the experimental conditions, a PLS analysis

determines what relationship there is between the signal changes and the conditions and performance covariates, and where in the brain that relationship is in evidence.

## **2.2. Methods.**

### **2.2.1. Subjects.**

Informed consent was obtained from all participants. UCI institutional review board approved the study protocol. Twenty-one medically stable chronic schizophrenic patients were recruited for the study. Nineteen of these subjects were included in the final analysis (two were homozygous for GG allele comprising too small a sample to analyze), and they were all right handed. There were 6 females and 13 males. The subjects were on average 43 years of age (standard deviation,  $\pm 10.5$  years) and had been ill 13.9 years ( $\pm 9.1$  years). All except for two subjects had been ill for at least 5 years. The Positive and Negative Syndrome Scale (PANSS) and the Premorbid Verbal IQ Estimates (PVIE) (Grober and Sliwinski 1991) are detailed in Table 2.1. All subjects were on stable doses of atypical antipsychotic drugs, except for two on conventional antipsychotic agents. Six subjects were also on mood stabilizers, four on antidepressants, and two on antiparkinson agents, all with doses consistent with the Food and Drug Administration approved package inserts. The PANSS scores and medications are typical for patients with chronic schizophrenia.

### **2.2.2. Genetic methods**

DNA was extracted from blood samples using the high-salt method (Lahiri and Nurnberger 1991) and polymerase chain reaction (PCR) was used to amplify the upstream DRD1 polymorphic site. The DRD1 polymorphism that was used is recognized by the restriction enzyme DdeI and is located about one kilobase upstream of the initiation codon. PCR amplification was done according to Cichon et al (Cichon et al 1994) and DdeI restriction digest was performed according to the enzyme manufacturer's

instructions (Fermentas Inc, Hamilton, Ontario, Canada). The DdeI restriction fragments were visualized using 2% agarose gel electrophoresis. No studies have yet been carried out to determine whether this site alters promoter function. Subjects were divided into groups based on DRD1 DdeI polymorphism, which could be AA, AG, or GG.

### ***2.2.3. MRI data collection***

All imaging data were collected on a 1.5T Philips scanner (Marconi/Picker Eclipse model). The fMRI scans consisted of a T2\*-weighted gradient echo planar imaging sequence (24 cm FOV, 28 slices, 5 mm thick with no gap, axially oriented; TR = 3s, TE = 40 ms, 90 deg flip angle, 80 frames per scan) tuned to Blood Oxygenation Level Dependent (BOLD) signal. During the fMRI scans, subjects performed a Serial Item Recognition Paradigm, a working memory task based on Manoach et al. (Manoach et al 1999). The Serial Item Recognition Paradigm has been repeatedly reported to activate the dorsolateral prefrontal cortex in healthy subjects and people with schizophrenia (Johnson et al 2006; Manoach 2003). Three runs (240 s each) of the working memory task were collected within the same scanning session.

The task included three conditions in a blocked design: a baseline condition, a low memory load condition, and a high memory load condition. In the baseline condition blocks, subjects were presented a series of arrows and asked to indicate the direction in which the arrow pointed (left or right). In both memory load conditions blocks, subjects were presented with a set of numbers (presented simultaneously for 5 s), then presented with a series of ten probe trials each consisting of a single number presented for 2 s. Subjects indicated whether the probe was in the memory set of numbers, or not. In the low memory load condition, there were only two numbers in the memory set; in the high

memory load condition, there were five. The memory sets were different in every block and every run. Each run consisted of nine blocks, beginning and ending with a baseline condition block.

The preprocessing steps included motion detection and correction, co-registration and normalization to a Montreal Neurological Institute brain template (Montreal Neurological Institute, Montreal, Quebec, Canada), and smoothing with an 8 mm FWHM 3D Gaussian filter. The preprocessing steps were performed with the SPM99 software (<http://www.fil.ion.ucl.ac.uk/spm/software/spm99/>), using default settings where applicable (Friston et al 1995; Poline et al 1995). The motion-corrected, normalized, and smoothed images were the input to the PLS analysis.

#### ***2.2.4. PLS – Image analysis***

The PLS analyses used PLS version 5.0701151 (<http://www.rotman-baycrest.on.ca/>). To quantitatively determine differences between circuitry of the two genotypes, a combined analysis was performed analyzing the two groups together. Separate analyses by genotype group were also performed to confirm results.

A blocked analysis of the fMRI data was used. Averages of the last six frames (18 s) of the baseline blocks were used as a baseline. Average images from the last six frames of each block during the low and high memory load conditions were included so that the hemodynamic response function could best reflect the subject's efforts at recall, rather than at the stimuli presentation. The preceding baseline block was subtracted from each memory condition block to provide a measure of BOLD signal change.

The main goal of PLS is to identify areas of the brain presenting the same activations at the same time (covariance) (McIntosh et al 1996; McIntosh et al 2004).

The strongest covariance within each block is expected to describe the brain pattern related to that specific task. Singular value decomposition (SVD) is performed on correlations between accuracy values averaged within each block and BOLD values at each voxel. This operation generates simultaneously a *singular image* which is the brain image with the covarying voxels correlated with accuracy, a *correlation profile* which is a plot expressing the relationship between accuracy and activation change in each memory condition for each group, and a *singular value* which is a scalar number accounting for the covariance. The numerical weights within the singular image are termed saliencies; saliencies can be positive or negative. Singular image, singular value and correlational profile describe an overall pattern termed a latent variable (LV). Each SVD produces a number of LVs. The first LV accounts for the largest proportion of covariance, and thus is the primary pattern in the dataset; the second LV accounts for the next largest proportion, etc.

The significance of the singular value (i.e. whether the LV accounts for an amount of covariance that is unlikely to have arisen by chance—in essence, the strength of the LV signal relative to random noise) is determined by permutation sampling. This involves randomly reassigning the subjects across groups (McIntosh et al 2004). The reliability of the saliencies in the singular image (i.e., which voxels' saliencies in the singular image are significantly different from zero) are determined by the bootstrap method (McIntosh et al 2004). The bootstrap procedure involves sampling the dataset with replacement in order to derive estimates of standard errors of the LV saliencies for each voxel.

## 2.4. Results.

### 2.4.1. Genetic data and demographic variables

Nine and ten subjects carried the AA and AG genotypes respectively in the DRD1. Two subjects carried the GG genotype and were excluded from analysis due to the inability to perform significance tests on such a small sample size. The distribution of males and females within the different groups was not significant (Fisher's exact test,  $p > 0.05$ ). The mean age, symptom severity as measured by the PANSS subscores, and duration of illness did not significantly differ across genotypes ( $p > 0.05$ ).

### 2.4.2. Behavioral analysis by genotype

The mean proportion  $\pm$  standard deviation of correct answers was  $0.90 \pm 0.18$  and  $0.93 \pm 0.07$  for the baseline (arrows) condition,  $0.80 \pm 0.23$  and  $0.90 \pm 0.09$  for the low memory condition and  $0.81 \pm 0.21$  and  $0.81 \pm 0.11$  for the high memory load in the two genotypes respectively. The main effect of condition was significant ( $F(2,34) = 5.98$ ,  $p < .006$ ), confirming the overall decrease in accuracy with increasing load. The effect of genotype group on performance was not significant ( $F(1,17) < 1$ ); accuracy in both groups was equivalent and there was no interaction by genotype with memory load. This was also true for the response time measures: response times slowed with increasing load ( $F(2,34) = 97$ ,  $p < .00001$ ), while the effect of genotype group was not significant ( $F(1,17) = 1.5$ ,  $p < 0.25$ ).

*PLS results:* This analysis was performed to contrast the brain behavior between the two groups. Of the four latent variables (LVs) identified by the analysis, only the first one was significant ( $p < 0.02$  by permutation testing) and stable, and it accounted for 37% of the cross-block covariance between behavior and fMRI data.

The significant LV indicated the two groups had very different patterns of BOLD signal relationship with accuracy. The network (singular image thresholded for significant voxels) and the correlation profile are plotted in Figure 2.1(a) and 2.1(b) respectively. The LV shows a different profile by genotype grouping. The voxels shown in yellow/red are positively weighted on the underlying profile, while the voxels shown in blue are negatively weighted on the underlying profile for the LV.

In Figure 1a, yellow areas (positive weights) represent areas where increasing and covarying activation levels correlated with increased accuracy values within the AG group compared to the AA group; these included the temporal pole, the dorsolateral prefrontal cortex (Brodmann Area 46), the inferior parietal lobule (Brodmann Area 40) and the Brodmann Areas 6, 21, 22 and 37. Blue areas (negative weights), in contrast, represent brain patterns that correlated with increased accuracy within the AA group relative to the AG group; these included the tectum, retrosplenial, vermis, medial amygdala, posterior inferior temporal lobe, hippocampal area, anterior insula (Brodmann Area 15), Brodmann Area 7, 8, 18, and 20.

The correlations specific to the dorsolateral prefrontal cortex, temporal pole, tectum and vermis are inverted for the two genotypes. The same areas show the opposite relationships with accuracy and memory between the two genotypic groups: What is positively correlated for the AA group is negatively correlated for the AG group, and vice versa. Each group analyzed individually also showed the pattern it showed in the combined group analysis.

## 2.5 Discussion

The subjects with schizophrenia presented a similar symptomatology and working memory performance, but fell into two genotypic groups. The data driven analysis showed that the strongest patterns of BOLD coherence identified primarily differences between the two genotypes. It appears that patients used distinct neural systems to retrieve information based on their DRD1 genotype. The AG genotype may engage a more conscious visual and verbal attentional neocortical system compared to the AA genotype in order to achieve an increasing level of accuracy. On the other hand, the AA genotype seems to adopt a more tecto-pulvinar pathway, involving an unconscious visual pathway part of the posterior attentional system compared to the other.

Our study corroborated previous findings showing differential activations during working memory tasks in the dorsolateral prefrontal cortex, the anterior cingulate area, the inferior parietal lobule, and the basal ganglia (Callicott et al 2003; Johnson et al 2006; Karlsgodt et al 2007; Manoach et al 1999; Schneider et al 2007). It is possible that the pathology cannot be simplified to a reduced or increased activation during working memory, but to specific underlying neuronal pathways determined by specific genetic mechanisms (Williams and Castner 2006).

In our study, the higher the accuracy, the more the dorsolateral prefrontal cortex seemed to be engaged in the AG and not in the AA genotype, although their performance was equivalent. This finding may contribute to aspects of the inverted U model for dopaminergic regulation perhaps based on genotype. According to the model, the dorsolateral prefrontal cortex is increasingly engaged as the working memory task

becomes more difficult until it reaches a maximum capacity (Callicott et al 1999). At the highest memory efforts, dorsolateral prefrontal cortex activation declines. While both healthy subjects and patients follow this model, the curve for people with schizophrenia peaks and falls off with lower memory load reflecting lower working memory capacity (Callicott et al 2003).

These analyses were performed on subjects with schizophrenia only. As such, the results relate to working memory issues within the context of the disease. They should not be interpreted as indicating causal relationships with the clinical diagnosis *per se*. However, finding a genetic influence on the circuitry underlying memory performance within the patient sample indicates the feasibility of using this integrated imaging-genetics approach to tease apart potentially different subtypes of the diagnosis.

A data-driven analysis was necessary to answer the question of whether or not there was a significant difference in the neurocircuitry of patients with a specific genotype. This was a rich dataset, with many possible outcomes: One group may have accounted for the most coherent pattern, while the other group was more variable and had no significant coherent pattern, or shown a coherent pattern in an entirely different group of brain regions. Or, the two groups may have shown the same pattern, either increasing or decreasing with memory load condition and performance. A data-driven approach extracts the most important patterns, and when followed by permutation testing and bootstrapping, indicates the reliability of those findings.

Permutation testing has been found to be particularly suitable for neuroimaging datasets with small sample sizes where parametric assumptions may not be met (Nichols and Holmes 2002). Our findings reflect brain-behavior patterns in a small group;

however, our sample size is similar to the majority of neuroimaging studies that use multivariate analysis combined with permutation testing (Caplan et al 2007; Della-Maggiore et al 2000; McIntosh et al 2004; McIntosh and Lobaugh 2004). Studies with a larger sample can investigate imaging-genetics patterns including a larger number of polymorphisms and genes in order to be able fully characterize common features of schizophrenia.

This preliminary study indicates that the DRD1 DdeI marker, which was previously identified as a potential pharmacogenetic target, may also be a useful imaging-genetics biomarker. The effect of the GG genotype is currently undetermined: It may be more like the AG group, or it may show a different pattern altogether.

### **Conclusion**

The DRD1 (DdeI) genotype is associated with different brain patterns in schizophrenic subjects. The AG genotype uses a specific network that includes the dorsolateral prefrontal cortex positively correlated with accuracy. The AA genotype, in contrast, has a negative correlation in the dorsolateral prefrontal cortex with accuracy, and has positive correlations in a different network. Therefore, even though the two groups achieve the same performance, the circuitry is possibly associated with DRD1 genotype. This study may provide imaging-genetics insights in order to develop targeted therapies reflecting an individual genetic and physiological variations.

### **Acknowledgments**

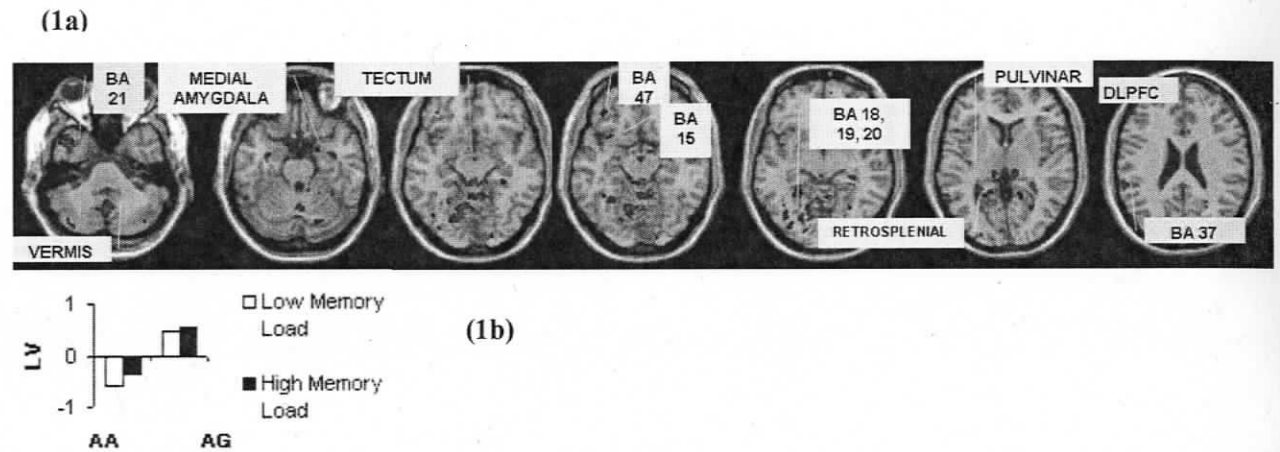
This research was supported by U24 RR021992 to the FBIRN and by P20 RR020837-01 to the Transdisciplinary Imaging Genetics Center, from the National Center for Research Resources/National Institutes of Health. We acknowledge and thank

Randy McIntosh, Ph.D., from the University of Toronto for his advice on PLS analysis, Hal Stern, Ph.D., from the University of California, Irvine, for his input on these discussions. We also thank Patrick MacLeod, M.D., Nigel Livingston, Ph.D. and the University of Victoria for their invaluable assistance.

**Table 2.1: Positive and Negative Syndrome Scale (PANSS) scores and Premorbid Verbal IQ Estimates (PVIE) specifics.**

	PANSS total	PANSS positive	PANSS negative	PANSS General	PVIE
Mean	75.3	16.2	20.1	39.0	108.8
Range	56 to 104	10 to 28	12 to 26	31 to 50	87.11 to 119.04

Figure 2.1: The voxels are thresholded at 3.0 standard deviations for LV1. (a) Yellow areas tend to be more engaged as accuracy increases in group AG, but not in AA in both memory conditions; blue areas represent the inverse pattern. (b) The correlational profile describes the correlation between voxels and accuracy values.



### **Chapter 3 : Working Memory Capacity and Effect of Working Memory Load in Functional circuitries**

Task analysis in Chapter 1 did not find a diagnostic effect in the most prominent BOLD covariance pattern. Therefore, we decided to break the analysis into smaller steps and analyze the working memory capacity and the effect of load in schizophrenia.

#### **3.1 Working Memory Capacity.**

Each working memory condition was analyzed in schizophrenic and healthy subjects. Specifically, the BOLD signal related to the lowest working memory condition was compared in schizophrenic and healthy subjects. In a separate analysis, the three digits memory condition was compared in schizophrenic and healthy subjects. In a further analysis the highest working memory condition was compared in schizophrenic and healthy subjects. Only the first analysis showed a significant pattern ( $p < 0.0001$ ). The other two analyses for higher memory loads were not significant ( $p > 0.1$ ).

The brain regions related to the low memory condition analysis are illustrated in Figure 3.1a with the correlational profile shown in Figure 3.1b. Positive saliences expressed a positive correlation between increased BOLD signal and the lowest (one digit) memory condition in healthy subjects compared to schizophrenic subjects. Negative saliences showed the inverse correlation; hence they expressed the correlation of increased BOLD signal in schizophrenic subjects relative to the control subjects.

Schizophrenics already engaged many areas in the frontal areas during the lowest memory condition. There were many large, medium-sized and small cluster in the superior, middle, medial and inferior frontal gyri. The dorsolateral prefrontal cortex was already heavily engaged. This reflects the current theory of the inverted U curve.

Schizophrenic subjects are hyperfrontal compared to healthy subjects in the lowest memory load, and as the memory effort increases, healthy subjects become hyperfrontal, suggesting that schizophrenic subjects have a limited working memory capacity (Calhoun et al 2006; Callicott et al 2000; Callicott et al 2003; Manoach 2003).

Differential activation was also found in the limbic lobe. Healthy subjects showed much activation in the cingulate and posterior cingulate gyrus. Schizophrenic subjects showed a very small locus in the anterior cingulate gyrus. This may indicate that for the lowest memory load, healthy subjects rely more on error checking (Callicott et al 1999; Callicott et al 2003; Kim et al in preparation; Ragland et al 2007; Snitz et al 2005).

Differential activations were also found in the temporal, parietal and occipital lobes. In the lowest memory load, healthy subjects relied on temporal lobe (superior and middle temporal gyrus), while schizophrenics engaged the inferior or superior parietal lobule and the occipital areas (cuneus, middle occipital and lingual gyri). IPL dysfunction together with aberrant activation in occipital regions may indicate an abnormality to integrate visual and cognitive information (Pierrot-Deseilligny et al 1995).

There were small loci in the insula and striatum in healthy but not in schizophrenic subjects as reported in previous studies (Agid et al 2007; Glenthøj et al 2007; Johnson et al 2006; Koch et al 2008; Murray et al 2008).

### 3.2 Working Memory Load Effect

The highest and lowest memory loads were compared in an analysis which included schizophrenic and healthy subjects. This analysis yielded to one significant pattern ( $p < 0.02$ ). The pattern decoupled lower and higher memory loads in schizophrenic subjects; however it did not show any correlation with healthy subjects. Therefore, a second analysis was performed comparing high and low memory loads in healthy subjects alone. This analysis yielded to one significant pattern ( $p < 0.004$ ). This pattern also decoupled lower and higher memory loads in schizophrenic subjects. The brain pattern and the correlational profile describing functional circuitries in schizophrenics are depicted in Figure 3.2 . The brain pattern and the correlational profile describing functional circuitries in healthy subjects are depicted in Figure 3.3. In both analyses correlation profiles showed that positive saliences expressed a positive correlation between increased BOLD signal and the highest memory condition compared to the lowest memory condition. Hence positive areas showed areas of activation of high memory load compared to low memory load. Negative saliences showed the inverse correlation; hence they showed areas of activation of low memory load compared to high memory load. These patterns were found in both schizophrenic and healthy subjects performing separate analyses.

Although in this way, we cannot directly compare the circuitry of high memory load relative to low memory load in schizophrenic and healthy subjects at the same time, we can have an idea on the changes in brain regions from low to high memory load and vice versa in the two groups separately.

Noticeable activation areas in high memory load (compared to low memory load) in healthy subjects were found in frontal areas. The same patterns were also found in schizophrenics. There were many large, small and medium sized clusters in the superior, middle, and medial frontal areas during the highest memory effort possibly reflecting the trend described by the inverted U curve theory. As the memory load increases, activation in frontal areas increases until reaching a maximum peak. After that, as memory load increases, frontal areas activation starts to decrease. According to this theory, schizophrenic subjects reach maximum capacity earlier and thus the curve is shifted to the left. In our experiment, it is possible that both schizophrenic and healthy subjects have not reached or have just passed the maximum capacity, and at this point they show hyperfrontality compared to the lowest memory load. However, the analysis does not tell us whether schizophrenics reached maximum capacity before healthy subjects.

The activation areas in higher memory loads (compared to lower memory load) in healthy subjects were also very noticeable in the inferior parietal lobule and in many occipital areas. These areas did not activate in schizophrenic subjects reflecting previous IPL dysfunction findings (Assaf et al 2006; Calhoun et al 2006; Callicott et al 2000; Thermenos et al 2005). Thus, IPL dysfunction suggests an abnormality of visual and cognitive information integration (Pierrot-Deseilligny et al 1995).

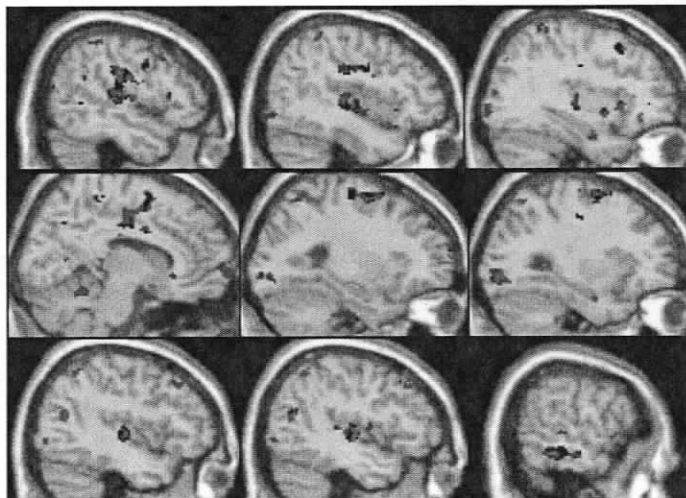
A large thalamic activation was found in higher memory loads (compared to lower memory load) only in schizophrenic subjects corroborating findings of thalamic-cortical dysfunction (Camchong et al 2006).

In healthy subjects, the limbic lobe was much more active in lower than higher memory loads. In schizophrenic subjects, there was only a small locus in the anterior cingulate

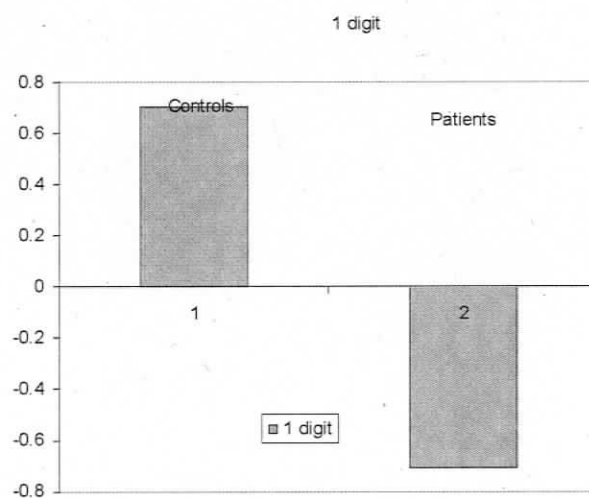
gyrus. It is possible again that healthy subjects more than schizophrenics used an error-checking system during the lowest memory load.

These analyses suggested functional dysfunction in schizophrenia relative to frontal, cingulate, occipital, parietal, thalamic and temporal regions. Healthy more than schizophrenic subjects appeared to rely on error checking system during the easiest memory load. It seemed that schizophrenic subjects heavily engaged the prefrontal areas and the dorsolateral prefrontal cortex since the lowest memory load, and as the difficulty increased the engagement of prefrontal area also increased. They did not rely on error-checking system as much. Healthy subjects seemed to engage the prefrontal areas only when the task was more difficult. However, we cannot distinguish whether healthy subjects were hyper or hypofrontal during the highest memory load.

Figure 3.1 : First latent variable in the one-digit analysis. (a) The singular image identifies the voxels covarying during the one digit task (shown in radiological convention, L = R). (b) The correlational profile describes the correlation among voxels and task. Yellow areas represent regions that are positively correlated with the task. Blue areas represent the inverse correlation.

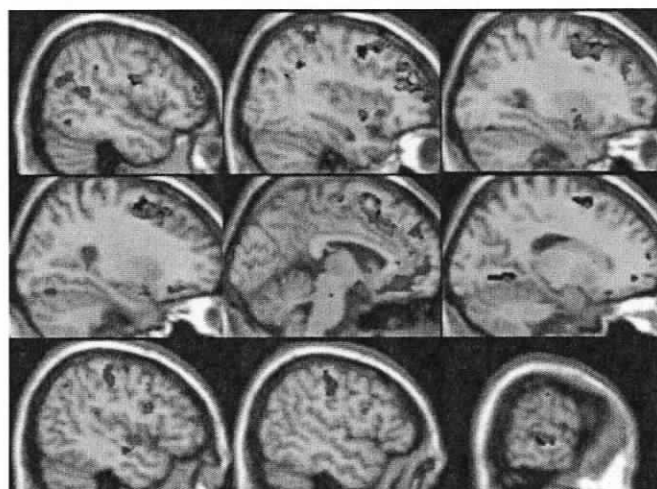


(a)

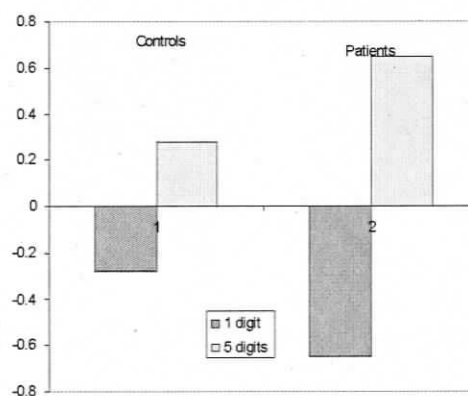


(b)

**Figure 3.2: First latent variable in high and low memory load analysis in the two groups. (a) The singular image identifies voxels activating in higher memory load relative to lower memory load (shown in radiological convention, L = R). (b) The correlational profile describes the correlation among voxels and task in the two groups. The correlational profile shows a pattern that is found in schizophrenic subjects only. Yellow areas represent regions that are active during the higher memory load compared to low memory load in schizophrenic subjects only. Blue areas represent the inverse correlation.**

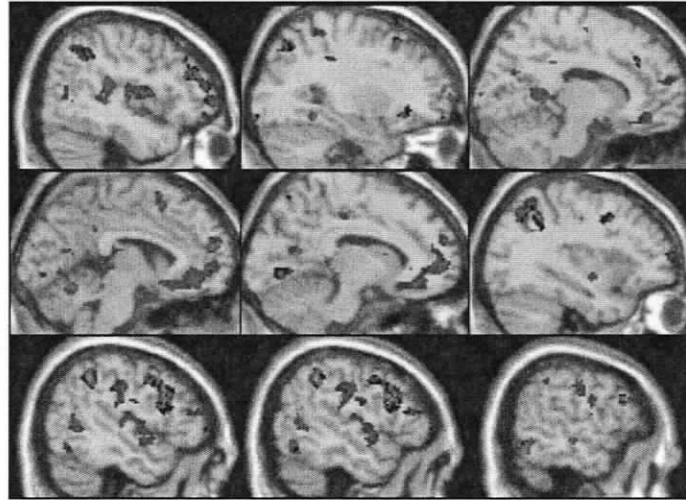


**(a)**

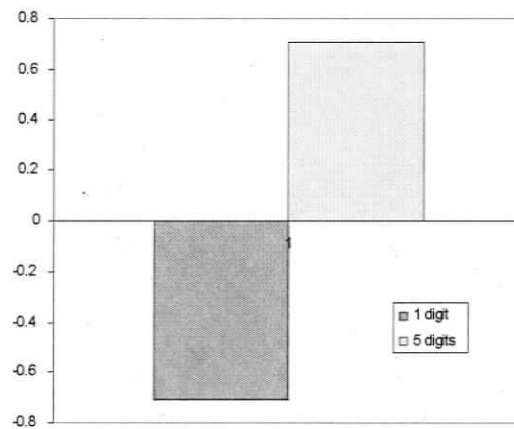


**(b)**

Figure 3.3: First latent variable in high and low memory load analysis in healthy subjects only. (a) The singular image identifies voxels activating in higher memory load relative to lower memory load (shown in radiological convention, L = R). (b). Yellow areas represent regions that are active during the higher memory load compared to low memory load in healthy subjects only. Blue areas represent the inverse correlation.



(a)



(b)

## Conclusion and Future Directions

This research has shown the complexity of cognitive processes in pathological and non-pathological working memory. We found a differential use of many brain structures between schizophrenic and healthy subjects. Significant differences between healthy and schizophrenic subjects were found in networks related to response time (RT). Diagnostic patterns included specific areas in frontal, cingulate, insular, basal ganglia, occipital, parietal, thalamic and temporal regions. Our research also suggests a genetic basis of schizophrenia. Overall multivariate analysis proved to be an appropriate tool as it allows detection of distributed networks (Calhoun et al 2006; Kim et al in preparation; Lloyd in press; McIntosh et al 1996; McIntosh and Lobaugh 2004).

Interestingly, healthy subjects appeared to rely more on error checking systems for easy tasks and fast responses than schizophrenics. Normally, prefrontal areas and the dorsolateral prefrontal cortex are heavily engaged during demanding tasks. On the other hand, schizophrenic subjects relied on the prefrontal areas at both low and high levels of difficulty in memory tasks and in order to be fast. Schizophrenic subjects did not rely as much on error checking systems, suggesting a possible dysfunction in the cingulate gyrus.

The etiology of schizophrenia is still under investigation since a number of genes may be involved and clinical classifications do not lead to consistent classifications; however, brain patterns can provide reliable quantitative traits.

Since antipsychotic drugs affect dopamine transmission and DRD1 regulation, DRD1 has been thought to be one of the candidate genes. The imaging-genetic investigation offered clues that variation in the DRD1 gene may indeed affect the

functional circuitries of schizophrenic patients. There was a difference in the way the two genotypes engaged the neurocircuitry in order to be more accurate. Interestingly, one genotype used more the dorsolateral prefrontal cortex for accurate performance.

Different types of analyses capture different aspects of the circuitries. Univariate analyses detect focal changes. Multivariate analyses give the bird's eye view of the circuitries in the entire brain. Behavioral, clinical and genetic covariates may reveal specific effects on the brain. However, it is important in a long term study to attempt reconciliation and replication among all these different techniques in order to have a consistent understanding of the circuitries. Matching functional circuitries to neuroanatomical circuitries will be a critical step to determine effective circuitries.

With Partial Least Squares method, in order to detect differences between groups it was important to include the use of covariates. Otherwise, BOLD activation patterns (without correlations with any covariates) did not distinguish the two groups. This indicates that the brain is a dynamic organ in which complex activities are distributed, and the action of only a few areas may not explain the underlying cognitive task. Therefore, different types of analysis will need to be conducted in the attempt to capture all the different regions of the brain that are involved in circuit activity. Smaller BOLD signal coherence patterns may be detected by using seed analysis or independent component analysis. Seed analyses, similar to Region of Interest analyses in standard univariate systems, consist of using one brain region's BOLD signal and finding other areas covarying with that BOLD signal. Selecting the correct seed will require a careful study of covariance patterns, a thorough study of raw BOLD signals, ROI in previous studies and clusters identified with task and behavioral multivariate analysis. Larger

datasets will also help to reach higher significance in those cases where the signal is very small. Also, forcing contrasts between different conditions with post-hoc analysis may help capturing group differences.

The combination of clinical, imaging and genetic study is critical to reach a reliable quantifiable stratification of the disease. Future studies imaging-genetic studies should include the use of genome-wide scans and quantify modulation of gene expression in functional circuitries. So far, most studies have used the candidate gene approach; however, these were found less successful in complex diseases.

### **Final Remarks**

Descartes used to say "*Cogito Ergo Sum*" ("I think therefore I am"). Some scientists believe that we are the product of synaptic processes. Scientific research shows that engaging specific circuitries prompts specific mental functions... then who or what makes us engage those circuitries to begin with?

## References

- Agid O, Mamo D, Ginovart N, et al (2007): Striatal vs extrastriatal dopamine D2 receptors in antipsychotic response--a double-blind PET study in schizophrenia. *Neuropsychopharmacology* 32:1209-15.
- Assaf M, Calhoun VD, Kuzu CH, et al (2006): Neural correlates of the object-recall process in semantic memory. *Psychiatry Res* 147:115-26.
- Barch DM, Carter CS, Braver TS, et al (2001): Selective deficits in prefrontal cortex function in medication-naive patients with schizophrenia. *Arch Gen Psychiatry* 58:280-8.
- Bonilha L, Molnar C, Horner MD, et al (2007): Neurocognitive deficits and prefrontal cortical atrophy in patients with schizophrenia. *Schizophr Res*.
- Brebion G, Amador X, Smith MJ, Gorman JM (1998): Memory impairment and schizophrenia: the role of processing speed. *Schizophr Res* 30:31-9.
- Brown JW, Braver TS (2005): Learned predictions of error likelihood in the anterior cingulate cortex. *Science* 307:1118-21.
- Bullmore E, Brammer M, Williams SC, et al (1996): Statistical methods of estimation and inference for functional MR image analysis. *Magnetic Resonance in Medicine* 35:261-77.
- Calhoun VD, Adali T, Kiehl KA, Astur R, Pekar JJ, Pearlson GD (2006): A method for multitask fMRI data fusion applied to schizophrenia. *Hum Brain Mapp* 27:598-610.
- Callicott JH (2003): An expanded role for functional neuroimaging in schizophrenia. *Curr Opin Neurobiol* 13:256-60.
- Callicott JH, Bertolino A, Mattay VS, et al (2000): Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. *Cereb Cortex* 10:1078-92.
- Callicott JH, Mattay VS, Bertolino A, et al (1999): Physiological characteristics of capacity constraints in working memory as revealed by functional MRI. *Cereb Cortex* 9:20-6.
- Callicott JH, Mattay VS, Verchinski BA, Marenco S, Egan MF, Weinberger DR (2003): Complexity of prefrontal cortical dysfunction in schizophrenia: more than up or down. *Am J Psychiatry* 160:2209-15.
- Camchong J, Dyckman KA, Chapman CE, Yanasak NE, McDowell JE (2006): Basal ganglia-thalamocortical circuitry disruptions in schizophrenia during delayed response tasks. *Biol Psychiatry* 60:235-41.
- Caplan JB, McIntosh AR, De Rosa E (2007): Two distinct functional networks for successful resolution of proactive interference. *Cereb Cortex* 17:1650-63.
- Carter C, Robertson L, Nordahl T, Chaderjian M, Kraft L, O'Shora-Celaya L (1996): Spatial working memory deficits and their relationship to negative symptoms in unmedicated schizophrenia patients. *Biol Psychiatry* 40:930-2.
- Carter CJ (2006): Schizophrenia susceptibility genes converge on interlinked pathways related to glutamatergic transmission and long-term potentiation, oxidative stress and oligodendrocyte viability. *Schizophr Res* 86:1-14.

- Castner SA, Williams GV, Goldman-Rakic PS (2000): Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. *Science* 287:2020-2.
- Cheung V, Cheung C, McAlonan GM, et al (2007): A diffusion tensor imaging study of structural dysconnectivity in never-medicated, first-episode schizophrenia. *Psychol Med*:1-9.
- Cichon S, Nothen MM, Erdmann J, Propping P (1994): Detection of four polymorphic sites in the human dopamine D1 receptor gene (DRD1). *Hum Mol Genet* 3:209.
- Della-Maggiore V, Sekuler AB, Grady CL, Bennett PJ, Sekuler R, McIntosh AR (2000): Corticolimbic interactions associated with performance on a short-term memory task are modified by age. *J Neurosci* 20:8410-6.
- Fleming K, Goldberg TE, Binks S, Randolph C, Gold JM, Weinberger DR (1997): Visuospatial working memory in patients with schizophrenia. *Biol Psychiatry* 41:43-9.
- Friston KJ, Ashburner J, Frith CD, Poline J-B, Heather JD, Frackowiak RSJ (1995): Spatial registration and normalization of images. *Human Brain Mapping* 2:165-189.
- Glenthøj A, Glenthøj BY, Mackeprang T, et al (2007): Basal ganglia volumes in drug-naive first-episode schizophrenia patients before and after short-term treatment with either a typical or an atypical antipsychotic drug. *Psychiatry Res* 154:199-208.
- Goeree R, Farahati F, Burke N, et al (2005): The economic burden of schizophrenia in Canada in 2004. *Curr Med Res Opin* 21:2017-28.
- Goldberg TE, Weinberger DR (1995): Thought disorder, working memory and attention: interrelationships and the effects of neuroleptic medications. *Int Clin Psychopharmacol* 10 Suppl 3:99-104.
- Goldman-Rakic PS (1995): Toward a circuit model of working memory and the guidance of voluntary motor action. In Houk JC, Davis JL, Beiser DG (eds), *Models of information processing in the basal ganglia*. Cambridge: Massachusetts Institute of Technology, pp 131-48.
- Goldman-Rakic PS (1999): The physiological approach: functional architecture of working memory and disordered cognition in schizophrenia. *Biol Psychiatry* 46:650-61.
- Grober E, Sliwinski M (1991): Development and validation of a model for estimating premorbid verbal intelligence in the elderly. *J Clin Exp Neuropsychol* 13:933-49.
- Hirvonen J, van Erp TG, Huttunen J, et al (2006): Brain dopamine d1 receptors in twins discordant for schizophrenia. *Am J Psychiatry* 163:1747-53.
- Honey GD, Bullmore ET, Sharma T (2002): De-coupling of cognitive performance and cerebral functional response during working memory in schizophrenia. *Schizophr Res* 53:45-56.
- Iritani S (2007): Neuropathology of schizophrenia: a mini review. *Neuropathology* 27:604-8.
- Johnson MR, Morris NA, Astur RS, et al (2006): A functional magnetic resonance imaging study of working memory abnormalities in schizophrenia. *Biol Psychiatry* 60:11-21.

- Karlsgodt KH, Glahn DC, van Erp TG, et al (2007): The relationship between performance and fMRI signal during working memory in patients with schizophrenia, unaffected co-twins, and control subjects. *Schizophr Res* 89:191-7.
- Kim D, Andreasen NC, Belger A, et al (in preparation): An analysis of the fMRI Multisite Function BIRN and MIND studies shows a unique pattern in the modulation of functional networks related to working memory system in schizophrenia. *Schizophr Res*.
- Koch K, Wagner G, Nenadic I, et al (2008): Fronto-striatal hypoactivation during correct information retrieval in patients with schizophrenia: An fMRI study. *Neuroscience*.
- Lahiri DK, Nurnberger JI, Jr. (1991): A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 19:5444.
- Lloyd D (in press): Through a Glass Darkly: Schizophrenia and Functional Brain Imaging. *Philosophy, Psychiatry, and Psychology*.
- Maccabe JH, Simon H, Zanelli JW, Walwyn R, McDonald CD, Murray RM (2005): Saccadic distractibility is elevated in schizophrenia patients, but not in their unaffected relatives. *Psychol Med* 35:1727-36.
- Manoach D, Schlag G, Siewert B, et al (1997): Prefrontal cortex fMRI signal changes are correlated with working memory load. *Neuroreport* 8:545-549.
- Manoach DS (2003): Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. *Schizophr Res* 60:285-98.
- Manoach DS, Gollub RL, Benson ES, et al (2000): Schizophrenic subjects show aberrant fMRI activation of dorsolateral prefrontal cortex and basal ganglia during working memory performance. *Biol Psychiatry* 48:99-109.
- Manoach DS, Press DZ, Thangaraj V, et al (1999): Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. *Biol Psychiatry* 45:1128-37.
- Martin JH (2003): *Neuroanatomy Text and Atlas*, Mc-Graw-Hill Medical ed.
- McIntosh AR (2000): Towards a network theory of cognition. *Neural Netw* 13:861-70.
- McIntosh AR, Bookstein FL, Haxby JV, Grady CL (1996): Spatial pattern analysis of functional brain images using partial least squares. *Neuroimage* 3:143-57.
- McIntosh AR, Chau WK, Protzner AB (2004): Spatiotemporal analysis of event-related fMRI data using partial least squares. *Neuroimage* 23:764-75.
- McIntosh AR, Lobaugh NJ (2004): Partial least squares analysis of neuroimaging data: applications and advances. *Neuroimage* 23 Suppl 1:S250-63.
- Melle I, Larsen TK, Haahr U, et al (2004): Reducing the duration of untreated first-episode psychosis: effects on clinical presentation. *Arch Gen Psychiatry* 61:143-50.
- Murray GK, Corlett PR, Clark L, et al (2008): Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Mol Psychiatry* 13:267-276.
- Nakamura M, Salisbury DF, Hirayasu Y, et al (2007): Neocortical gray matter volume in first-episode schizophrenia and first-episode affective psychosis: a cross-sectional and longitudinal MRI study. *Biol Psychiatry* 62:773-83.
- Nichols TE, Holmes AP (2002): Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp* 15:1-25.

- Pierrot-Deseilligny C, Rivaud S, Gaymard B, Muri R, Vermersch AI (1995): Cortical control of saccades. *Ann Neurol* 37:557-67.
- Poline JB, Worsley KJ, Holmes AP, Frackowiak RS, Friston KJ (1995): Estimating smoothness in statistical parametric maps: variability of p values. *Journal of Computer Assisted Tomography* 19:788-96.
- Potkin S, Turner J, Lee H, et al (2008): A multi-site fMRI study of working memory in schizophrenia: An FBIRN report. Society for Neuroscience Conference.
- Potkin SG, Basile VS, Jin Y, et al (2003): D1 receptor alleles predict PET metabolic correlates of clinical response to clozapine. *Mol Psychiatry* 8:109-13.
- Ragland JD, Yoon J, Minzenberg MJ, Carter CS (2007): Neuroimaging of cognitive disability in schizophrenia: search for a pathophysiological mechanism. *Int Rev Psychiatry* 19:417-27.
- Schneider F, Habel U, Reske M, et al (2007): Neural correlates of working memory dysfunction in first-episode schizophrenia patients: an fMRI multi-center study. *Schizophr Res* 89:198-210.
- Snitz BE, MacDonald A, 3rd, Cohen JD, Cho RY, Becker T, Carter CS (2005): Lateral and medial hypofrontality in first-episode schizophrenia: functional activity in a medication-naive state and effects of short-term atypical antipsychotic treatment. *Am J Psychiatry* 162:2322-9.
- Spengler D, Trillenberg P, Sprenger A, et al (2006): Evidence from increased anticipation of predictive saccades for a dysfunction of fronto-striatal circuits in obsessive-compulsive disorder. *Psychiatry Res* 143:77-88.
- Thermenos HW, Goldstein JM, Buka SL, et al (2005): The effect of working memory performance on functional MRI in schizophrenia. *Schizophr Res* 74:179-94.
- Turner JA, Smyth P, Macciardi F, Fallon JH, Kennedy JL, Potkin SG (2006): Imaging phenotypes and genotypes in schizophrenia. *Neuroinformatics* 4:21-49.
- Uttl B (2002): North American Adult Reading Test: age norms, reliability, and validity. *J Clin Exp Neuropsychol* 24:1123-37.
- Van Snellenberg JX, Torres IJ, Thornton AE (2006): Functional neuroimaging of working memory in schizophrenia: task performance as a moderating variable. *Neuropsychology* 20:497-510.
- WHO (2001): The World Health Report.
- Williams GV, Castner SA (2006): Under the curve: critical issues for elucidating D1 receptor function in working memory. *Neuroscience* 139:263-76.