DOPAMINE-REGULATING GENES, EXECUTIVE CONTROL, AND THE NETWORK STRUCTURE OF THE HUMAN BRAIN

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By

Evan M. Gordon, B.S.

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Thesis Adviser: Chandan J. Vaidya, Ph.D.

ABSTRACT

Executive control processes, including working memory (WM), attention, and impulse control, rely on brain networks centered in the prefrontal cortex, and especially on dopaminergic signaling in these networks. The function of networks supporting executive processes may thus depend on genetic factors which influence dopaminergic signaling, such as polymorphisms in the dopamine transporter (DAT1) gene. To investigate this possibility, this dissertation describes four studies conducted using functional connectivity magnetic resonance imaging (fcMRI) to examine relationships between WM function, connectivity, DAT1 genotype, and other executive processes. The first study bridges the gap between studies of brain activation during WM and studies of network function at rest. This study demonstrated that networks delineated at rest were the same networks activated during WM, and that the degree of network activation predicted WM accuracy and reaction time. The second study investigated how these networks were persistently altered during WM performance. Results indicated that WM altered functional connectivity within the Task-positive network engaged by the task, as well as between this network and the Default network suppressed by the task. Further, altered connectivities persisted after task completion, suggesting a post-task period of recovery from cognitive effort, and this recovery period obscured relationships between Default connectivity and trait-level inattentiveness. The third study investigated how DAT1 genotype affected functional connectivities between striatum and the prefrontal cortex at rest. Results indicated that
individuals with one copy of the 9-repeat allele (9/10) had stronger connectivity between caudate and a cingulo-opercular network than those homozygous for the 10-repeat allele (10/10), and that these connectivity differences predicted WM ability and trait-level impulsivity. The fourth study investigated how DAT1 genotype affected functional connectivities between Task-positive and Default networks, both at rest and during WM performance. Results indicated that 9/10 individuals had more negative connectivities between Default and Task-positive networks during WM, but not at rest, and that the differences between resting and WM connectivities predicted trait-level inattention and impulsiveness. Taken together, these four studies describe relationships between variation in a dopamine-regulating gene, functional connectivity, and executive function which help demonstrate how functional connectivity serves as an endophenotype between genetic and behavioral variation.
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CHAPTER I: GENERAL INTRODUCTION

Understanding large-scale networks in the human brain is critically important for understanding the neural basis of behavior. In recent years, these networks have been examined using a new neuroimaging measure known as functional connectivity magnetic resonance imaging (fcMRI). This technique, which was first described using positron emission tomography (Friston et al., 1992) and later applied to functional MRI data (Biswal et al., 1995), attempts to describe communications between distant brain regions by examining correlations in the timecourses of brain activity; areas that show strong correlations in activity (termed “functional connectivity”) are conceptualized as communicating with each other, either via direct anatomic connections or indirectly via serial relay, and are thus processing information in a networked fashion. FcMRI thus enables the first investigations of dynamic, large-scale, in vivo networks in the awake human brain, where previously such networks had only been described anatomically in post-mortem tract-tracing studies, at the level of single neurons using electrophysiological recordings of patients undergoing brain surgery, or with poor spatial specificity using EEG.

FcMRI is most frequently measured during the task-free “resting state”, in which subjects are told to relax and try not to think of anything. This technique has allowed the identification of a number of discrete networks in the human brain (Beckmann et al., 2005; De Luca et al., 2006) which are remarkably consistent from one testing session to another (Chen et al., 2008; Van Dijk et al., 2010; Meindl et al., 2009; Shehzad et al., 2009), and which are highly similar across subjects (Van Dijk et al., 2010; Shehzad et al., 2009). The fact that consistent networks can be identified even in the absence of any task is remarkable, and suggests that these networks—termed intrinsic connectivity networks (ICNs)—are not simply a temporary configuration
assumed by the brain to perform a specific task, but rather that they represent the fundamental organizational elements of the human brain.

However, it is not yet fully known whether fcMRI measures of ICNs constitute a good endophenotype, or genetically-linked biomarker, for behavior. A particular measure of neural activity can be termed an endophenotype when it is both highly heritable and predicts individual differences in a specific set of behaviors; such endophenotypes are argued to be causally intermediate between genetics and behavior. Much of the history of cognitive neuroscience has focused on the search for brain-based endophenotypes, as elucidation of endophenotypes not only informs us of the neural mechanisms underlying heritable individual differences in cognitive capacities, but also informs us of the mechanisms disrupted in individuals with heritable neuropsychiatric disorders, which may eventually lead to new treatment possibilities.

To date, investigations of fcMRI measures as possible endophenotypes have been very promising, but relatively sparse (Esslinger et al., 2009; Meyer-Lindenberg, 2009; Smit et al., 2010; Walter et al., 2011; Woodward et al., 2009). This dissertation aims to expand this literature by investigating how functional connectivity can be an endophenotype for processes within the behavioral domain known as executive control.

Executive control is a constellation of processes that support the deployment of cognitive resources in a goal-directed manner; these processes include working memory, attention, inhibitory control, fluency, and task-switching. Establishing endophenotypes of executive control is of particular interest, both because individual differences in the efficacy of these processes are known to be highly heritable (Ando et al., 2001; Anokhin et al., 2003; Kremen et al., 2007; Taylor, 2007; Vasilopoulos et al., 2012) and because these processes are disrupted in highly heritable neuropsychological disorders such as schizophrenia (reviewed in Kerns et al., 2008).
and attention deficit hyperactivity disorder (ADHD; reviewed in Barkley, 1997). This dissertation focuses primarily on investigating fcMRI as an endophenotype for working memory (WM), defined as the temporary maintenance and manipulation of information, because WM is arguably the process most critical for executive function. In an influential model, Miller and Cohen (2001) argued that executive control is the ability to bias neural competition for behavioral expression towards the response most appropriate for a given context; and further, that the maintenance of this bias signal, which allows the continuous mental representation of task goals over intervening time and in the face of distracting information, is achieved by WM processes. The neural bases of these WM processes have been localized to the prefrontal cortex (PFC), where neural firing rates correspond to information maintenance in non-human primates (Goldman-Rakic, 1995), lesions in humans impair WM function (Baddeley and Della Sala, 1996; Shallice, 1982), and fMRI activation supports successful WM performance (reviewed in Tanji and Hoshi, 2008). Further, WM processes are known to critically rely on the function of the mesocortical dopamine (DA) pathway terminating in the striatum and PFC. This pathway provides DA signals that are posited to allow the striatum to “gate” the entry of new information into the PFC. This gating system regulates the alteration and updating of currently maintained bias signals (or goal states) in response to new contextual information, allowing for the selective, context-appropriate updating of goals or information maintained in WM (Miller and Cohen, 2001). Thus, this dissertation will investigate how functional connectivity measures of striatal and PFC networks may serve as an endophenotype for WM function, and how they may be affected by heritable factors that influence DA signaling.

One heritable factor influencing DA signaling is a specific polymorphism in the DAT1 gene. The DAT1 gene codes for the DA transporter (DAT) protein that regulates DA signaling by re-
uptaking DA following its release in the striatum (Madras et al., 2005). This gene has a common polymorphism which appears to influence the expression of DAT in vitro (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005), with greater DAT expression associated with the “10-repeat” allele compared to the “9-repeat” allele. This genetically influenced difference in DAT expression likely leads to differences in DA signaling, with the 9-repeat resulting in elevated DA signaling due to reduced clearance (Madras et al., 2005). There are several reasons to believe that relationships between DA signaling level, functional connectivity, and WM may be particularly affected by this DAT1 polymorphism. First, the 10-repeat DAT1 allele has been associated with worse executive control, including reduced WM performance (Stollstorff et al., 2010), reduced benefits of WM training (Brehmer et al., 2009), increased impulsivity (Gizer and Waldman, 2012) and hyperactivity (Mill et al., 2005), and worse inhibitory control (Cornish et al., 2005). Second, the 10-repeat allele has also been associated with reduced striatal and frontal activation during WM performance (Bertolino et al., 2006, 2008, 2009; Caldu et al., 2007; Stollstorff et al., 2010). Third, this allele has also been associated with ADHD (Yang et al., 2007), a disorder which similarly demonstrates increased impulsivity and reduced WM function (Van De Voorde et al., 2010). Notably, ADHD individuals also demonstrate reduced functional connectivity between the striatum and the PFC (Cubillo et al., 2010; Rubia et al., 2009), as well as elevated connectivity between cortical ICNs incorporating the PFC (Castellanos et al., 2008). This converging evidence suggests that functional connectivity in these circuits may serve as an endophenotype mediating the associations between the DAT1 gene and executive function, including WM. This dissertation provides the first experimental tests of this possibility.
**Dissertation Goals**

In examining the possibility of functional connectivity as a DAT1-modulated endophenotype for WM, this dissertation focused on two primary aspects of the DAT1→connectivity→WM causal chain. First, two studies examined relationships between resting state ICNs and WM-driven brain function. While resting state fcMRI can elucidate fundamental organizational principles of the brain, there are several ways in which we do not fully understand how ICNs measured at rest are related to the execution of a WM task. First, it is not clear whether the ICNs observed during rest are the same networks engaged during tasks such as WM. A seminal study by Smith et al. (2009) showed that regularities in patterns of coactivation across more than 7000 fMRI tasks well matched ICNs derived from the resting state, indicating that in general, ICNs are engaged by tasks. However, no previous work has showed that this is specifically true for a WM task. Here, Chapter II describes a study which used a novel analysis technique to show which specific ICNs are activated or deactivated by performance of an N-back working memory task, which ICNs are sensitive to the working memory “load”, or difficulty of the task, and which ICNs predict performance on the task. Second, it is not clear how the cognitive effort involved in performing a WM task may change the connectivity of ICNs, and whether those changes persist after the WM task is completed. A body of work has emerged showing that task performance can induce changes in functional connectivity that persist beyond the duration of the task and into a subsequent resting state. Such persistent alterations in connectivity have been observed after motor (Albert et al., 2009; Duff et al., 2008; Vahdat et al., 2011), visual (Lewis et al., 2009), language (Grigg and Grady, 2010; Waites et al., 2005), and memory (Tambini et al., 2010) tasks, but not previously after an executive task requiring effortful cognition. Here, Chapter III describes a study which examined how functional connectivity within and between two major
ICNs, the Default and Task-Positive networks, changes from an initial resting state to a WM task, and further, whether that connectivity returns back to baseline levels during a subsequent resting state after task completion or remains persistently altered. This study thus investigates for the first time how the cognitive effort involved in a WM task may persistently alter the network structure of the brain. Together, these two studies shed new light on how resting state ICNs can both predict and be altered by the execution of a WM task, and they indicate that fcMRI measurements of ICNs are likely to be a valid endophenotype of WM.

Second, two studies examined the impact of DAT1 genotype on functional connectivity and executive control. Chapter IV describes a study which measured resting state functional connectivity between the striatum and the prefrontal cortex in healthy adults genotyped for DAT1 and tested whether individuals with two copies of the 10-repeat allele had lower connectivity than individuals with one copy. Subsequently, this study tested whether the striato-frontal functional connectivities which differed by genotype predicted executive control abilities, including WM and impulsivity. Finally, Chapter V describes a study which measured functional connectivity between two PFC-related cortical ICNs in healthy adults genotyped for DAT1 during both a resting state and during performance of a WM task. This study tested whether connectivity was affected by DAT1 genotype, by performance of a WM task, or by interactions between genotype and task performance. The study subsequently tested whether WM-related changes in functional connectivity predicted executive control abilities such as attention and impulsivity. Together, these two studies are the first to examine linkages between the DAT1 gene, functional connectivity, and WM, and they provide convincing evidence of functional connectivity as an endophenotype of WM and other executive function processes.
CHAPTER II: USING SPATIAL MULTIPLE REGRESSION TO IDENTIFY INTRINSIC CONNECTIVITY NETWORKS INVOLVED IN WORKING MEMORY PERFORMANCE


Introduction

Functional magnetic resonance imaging (fMRI) studies of the human brain in a resting state (that is, while subjects are not engaged in a directed task) have demonstrated that spontaneous fluctuations in activity within various distinct regions exhibit a strong temporal correlation, suggesting that the correlated regions form a functional network (Biswal et al., 1995). Several such functional networks have been identified (Beckmann et al., 2005; De Luca et al., 2006), and these networks are spatially consistent across subjects and across testing sessions (Chen et al., 2008; Van Dijk et al., 2010; Meindl et al., 2009; Shehzad et al., 2009). These networks, termed “intrinsic connectivity networks” (ICNs; Seeley et al., 2007) have been posited to represent a fundamental functional organization of the brain (Fox and Raichle, 2007). However, the extent to which this functional network architecture may be relevant to cognition is an open question.

It is well established that there is close correspondence between the spatial composition of ICNs and sets of regions engaged by specific cognitive processes. For example, the Default Mode network is one well-studied ICN comprising the ventral and anterior medial prefrontal cortex, the precuneus/posterior cingulate cortex, and bilateral angular gyrus (Buckner et al., 2008). These same regions are also reliably activated during self-referential processing, such as
autobiographical memory and theory of mind tasks (Spreng et al., 2009), and reliably deactivated during performance of tasks requiring a focus on external stimuli rather than on the self (Raichle et al., 2001). The close correspondence between the spatial extent of the functional connectivity pattern identified during the resting state and the activation/deactivation pattern during task-evoked states suggests that the Default Mode ICN is being engaged or suppressed by task demands. This observation—that an entire ICN may be engaged or suppressed by some task demands—appears to hold true not just for the Default Mode network, but also for other ICNs. Spatial similarities between task activation and resting connectivity have similarly been observed in motor (Biswal et al., 1995), visual (Lowe et al., 1998), and higher-order executive control regions (Fransson, 2006). In a formal analysis of this effect, a close spatial correspondence between ICNs and task-evoked activation patterns was demonstrated between resting-state ICNs identified in a relatively small group of subjects and task-related activation peaks reported in more than 7000 fMRI studies using a variety of tasks (Smith et al., 2009). Such evidence supports the notion that ICNs are functionally specialized to subserve discrete cognitive processes. Specific networks have been identified as serving sensory functions, such as a Visual network, an Auditory network, and a Somatosensory network (Beckmann et al., 2005), while others serve higher functions, such as a Frontoparietal Executive Control network and a cingulo-opercular Set Maintenance network (Dosenbach et al., 2007). This suggests that specific regions may tend to activate together in the same tasks because they are intrinsically linked into a coherent network specialized for discrete forms of information processing.

Complex cognitive functions that involve multiple forms of information processing are likely to draw upon multiple ICNs, each contributing a distinct cognitive process. One complex cognitive function is the short-term maintenance and manipulation of information in the face of
distracting stimuli—commonly referred to as working memory. Working memory is often measured by the N-back task, in which subjects see a continuous sequence of stimuli and are required to press a button whenever a stimulus matches a stimulus they saw $n$ trials ago. This task draws upon several cognitive processes, including visual attention and monitoring of presented stimuli, maintenance of target information in working memory, identification and orienting towards targets when they appear, constant updating of new target information, and suppression of previously relevant but currently irrelevant targets (Owen et al., 2005). These cognitive processes are likely subserved by brain regions comprising multiple ICNs, rather than by a single ICN. Indeed, the regions activated during N-back performance, which include dorsal anterior cingulate cortex, dorsolateral and ventrolateral prefrontal cortex, and inferior parietal lobule (D’Esposito et al., 1998; Owen et al., 2005), are part of two ICNs, the Frontoparietal Control network and the Set Maintenance network. Further, activation within these regions, particularly in the frontal lobe, is known to scale with the “$n$” or working memory load of the N-back task (Braver et al., 1997; Manoach et al., 1997). However, no previous study has tested ICN engagement during the task-evoked state in individual subjects by examining the extent to which the spatial pattern of activation matches ICNs identified in the resting state.

The nature and extent of ICN engagement is known to predict individual differences in cognition. In healthy subjects scanned at rest, there is some variability in the spatial extent and connectivity strength of these networks (Damoiseaux et al., 2006; Mennes et al., 2010). There is also variability in task-evoked functional connectivity strength (Hampson et al., 2006) and the activation/suppression of regions within these networks (Eichele et al., 2008; Weissman et al., 2006). In some networks, this individual variability is associated with differences in performance, as lower Default Mode network suppression and weaker functional connectivity
was associated with more errors and slower performance (Eichele et al., 2008; Hampson et al., 2006; Weissman et al., 2006). Further, altered activation/suppression and connectivity of several networks has been linked to neuropsychological disorders such as Attention Deficit-Hyperactivity Disorder (Cao et al., 2006; Castellanos et al., 2008; Fassbender et al., 2009; Uddin et al., 2008), Autism Spectrum Disorders (Kennedy and Courchesne, 2008; Kennedy et al., 2006), Schizophrenia (Bluhm et al., 2007; Calhoun et al., 2008; Whitfield-Gabrieli et al., 2009), and Alzheimer’s disease (Greicius et al., 2004; Rombouts et al., 2005; Wermke et al., 2008), all of which include symptoms with some form of cognitive impairment. Thus, both normal variation in ICN engagement among healthy subjects and pathological variation among disordered populations are predictive of behavior. Together, this evidence suggests that engagement of ICNs during working memory performance ought to be associated with differences in task performance across individuals.

To summarize, close spatial correspondence between the ICNs observed during rest and the activation patterns observed during tasks suggests that complex cognitive processing may be conceptualized as activation of multiple ICNs delineated during the resting state. This possibility has not been formally tested within individual subjects. In the present study, we tested the hypothesis that a given subject’s pattern of brain activation during working memory corresponds to the engagement of multiple ICNs, which are detected in that subject using functional connectivity analyses at rest. Furthermore, we hypothesized that the degree of network engagement will change as working memory load increases, and that the degree of network engagement will predict performance. Healthy young adults underwent fMRI during the resting state and during performance of an N-back task with 3 levels of working memory load. We used a novel spatial multiple regression technique to compare the spatial extent of activation patterns
observed in individual subjects during the N-back task to subject-level networks derived from an Independent Components Analysis (ICA) of the resting-state data. This technique allows unbiased, hypothesis-free comparisons of task-related activation patterns with all ICNs in the brain. We predicted the following: First, patterns of N-back-evoked activation would be spatially similar to subjects’ ICNs, specifically those ICNs comprising regions known to mediate working memory and cognitive control processes, such as the Frontoparietal Control network and the cingulo-opercular Set Maintenance network. Second, the patterns of deactivation observed would be similar to the Default Mode network observed in those subjects. These results would demonstrate for the first time that individual variability in the shape of ICNs detected at rest is reflected in the variability in activation patterns observed during tasks. Third, as regions within the Frontoparietal Control, cingulo-opercular Set Maintenance, and Default Mode networks are known to be sensitive to working memory load (Braver et al., 1997; McKiernan et al., 2003) and cognitive performance (Perlstein et al., 2003; Weissman et al., 2006), we predicted that the degree of engagement of these networks would be altered by increasing working memory loads and would predict behavioral performance.

Materials and Methods

Participants

Forty-four Georgetown University undergraduates (25 female) ages 18 to 22 years (M ± SD = 19.22 ± 1.17) participated in the study for payment. Informed consent procedures were carried out according to Georgetown University’s Institutional Review Board guidelines. Exclusion criteria included 1) Self-reported use of psychotropic medication (e.g., stimulants, anti-anxiety/depression); 2) Self-reported history of neurological injury or disease, seizure disorder,
psychiatric diagnosis; 3) Contraindications for MRI – e.g., metal implants in the body, dental work involving metal, pregnancy. Four subjects were excluded from analysis due to technical problems during the resting-state scanning session.

**MRI Scanning Protocol**

**N-back Task:** Subjects were scanned during performance of the N-back task, in which nine 30s blocks of task (three blocks each with 1-, 2-, and 3-back trials) alternated with eight 15s blocks of fixation, lasting for 6:26 minutes in total. Each N-back block consisted of nine serially presented consonants appearing for 500ms, with an inter-trial-interval of 2500ms. The N-back load condition (1-, 2-, or 3-back) varied between task blocks, with condition order pseudorandomized using a modified Latin Square. Each block was preceded by a 3000ms screen informing the subject of the condition. Subjects were instructed that, in each n-back condition, they should press a right-hand-held button when the current letter matched the letter n trials ago. Targets were present on 19% of trials; each block contained between one and three targets with target frequency balanced across conditions. No condition contained sequences of stimuli that were appropriate as targets in any other condition. To eliminate learning effects during the scanning session, all subjects had been previously exposed to a variant of the same N-back task with different stimulus sequences. Stimuli were presented using E-Prime software (Psychology Software Tools Inc., Pittsburg, PA).

**Rest:** Subjects were scanned for 5:04 minutes while lying awake with eyes closed. Subjects were told to relax and to not think of anything in particular.

**Image Acquisition:** All images were acquired on a 3.0T MRI Siemens Trio system. For each subject, a high resolution structural scan (MPRAGE) was acquired, followed by functional imaging during the N-back task, followed by functional imaging during the resting state. The
scan parameters were as follows:  

*Structural*—a T1-weighted MPRAGE scan was acquired (TR/TE=2300/2.94ms, TI=900ms, 90 degree flip angle, 1 slab, 160 sagittal slices with a 1.0mm thickness, FOV=256x256mm2, matrix=256x256, resulting in an effective resolution of 1.03mm isotropic voxels).  

*N-back*—193 whole-brain images were acquired using a gradient echo pulse sequence (34 slices, TR = 2000 ms, TE = 30 ms, 256x256mm FOV, 90 degree flip angle, voxel dimensions 4x4x4.2mm).  

*Rest*—150 whole-brain images were acquired using a gradient echo pulse sequence (37 slices, TR = 2000 ms, TE = 30 ms, 192x192mm FOV, 90 degree flip angle, voxel dimensions 3mm isotropic).

**Data Analysis**

**Preprocessing:** The first two images were discarded from both fMRI runs to allow for signal stabilization. The remaining images were processed in SPM5 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (Version 7.0 Mathworks, Inc., Sherborn, MA). For both the Rest and N-back sessions, images were corrected for translational and rotational motion by realigning to the first image of the session. Exclusion criteria for excessive motion was set at 3mm of translation (1 voxel width) in any orthogonal direction or 1° rotation around any one axis. All timepoints in all subjects were found to be below these criteria (actual range: all translation < 1.2mm; all rotation < 1°). Images were then corrected for slice acquisition timing and were coregistered with the high-resolution structural images. The structural images were segmented into separate gray and white matter images, and the gray matter image was normalized into standard MNI space by comparison with a template gray matter image. The normalization parameters used were then applied to the functional images to bring them into MNI space. All images were smoothed using a Gaussian kernel with full-width at half-maximum (FWHM) of 8mm.
Following preprocessing, the following analysis were performed (as illustrated in Figure 1):

![Figure 1: Schematic diagram of spatial multiple regression analysis steps.](image)

(A) For one subject, N-back > Fixation contrasts were computed using a General Linear Model (GLM) to generate a spatial mask of N-back activation. (B) Independent Component Analysis (ICA) was conducted for each subject, as well as for the entire group, to generate group-level and subject-level intrinsic connectivity network (ICN) maps. Subject-level ICNs were identified by spatially correlating with group-level ICNs. (C) For one subject, a spatial multiple regression was conducted in which the spatial pattern of the N-back activation map (dependent variable; from step A) was explained as a linear combination of subject-level ICNs (independent regressors; from step B). Parameter estimates for each network were obtained and fed into later analyses.

**Regions activated and deactivated during N-back task:** First-level analysis was performed using a general linear model as implemented in SPM5. For each subject, four temporal regressors were specified, each one consisting of a boxcar timeseries convolved with a hemodynamic response function. The regressors represented the timecourses of the 1-back, 2-
back, 3-back, and fixation conditions. For each subject, four contrasts were specified: Task > Fixation, representing the regions activated by the average of the three N-back conditions; and 3-back > Fixation, 2-back > Fixation, and 1-back > Fixation, representing regions activated for each load condition.

**Group-level ICNs:** In order to delineate ICNs present in the group, the 150 time points of preprocessed resting data from each subject were temporally concatenated across subjects to create a single data set. An Independent Components Analysis (ICA) was performed on this dataset using the MELODIC toolbox (Beckmann and Smith, 2004) implemented within FSL (Centre for Functional Magnetic Resonance Imaging of the Brain, University of Oxford, London, UK). This ICA algorithm was preset to specify 20 independent components, as this number of components has been shown to divide the brain into recognizable networks (Abou-Elseoud et al., 2010). The ICA thus delineated 20 components in the form of 20 3D Z-score images, in which the Z-score in each voxel represents the degree of covariation with the rest of the network. Group-level components in which the areas of maximal covariation were non-neuronal (e.g., white matter, cerebrospinal fluid, brain edge covariation resulting from head motion) were visually identified and removed from further analysis. The remaining group components were visually inspected for similarities to known brain networks.

**Subject-level ICNs:** In order to discover coherent networks within each subject’s brain, ICA was performed on each subject’s preprocessed resting data, with the algorithm again preset to return 20 independent components. The resulting components represented the networks detectable in each subject’s brain.

A fourier transform of the average timecourse within each of these subject-level networks was conducted to determine the frequencies present in that network. As neuronally-based ICNs
are known to oscillate at frequencies between .01 and .1 Hz (Biswal et al., 1995), we considered any detected network which contained more than 50% of its total signal energy in a range above .1 Hz to be an artifactual network. Such networks were removed from further analysis.

Each of these subject-level networks delineated by the ICA analysis was presumed to be that subject’s version of one of the ICNs identified at the group level. To identify which group-level network best matched each subject-level network, all unthresholded (whole-brain image) subject-level networks were spatially correlated against all unthresholded group-level networks, separately for each subject. The spatial correlation procedure involved using MARSBAR (Brett et al., 2003) to extract the Z-scores of the subject-level network within every voxel in the brain, and then reshaping the 3D matrix of voxel values into a 1D vector. This extraction was then repeated for the group-level network, and a Pearson’s correlation was computed between the subject-level network vector of Z-scores and the group network vector of Z-scores. The resulting $r$ value represents the square root of the percent of variance in the spatial pattern of Z-scores from the individual subject network that can be explained by the spatial pattern of the group-level network. In order to identify each individual subject network as a version of a group-level network, the subject-level network was spatially correlated against all group-level networks; the group-level network with the most positive $r$ value was the group network which explained the highest percentage of the variance in the individual network. This group network was considered the best spatial match for that individual network, and the individual network was thus considered that subject’s version of that group network. Individual networks which did not match any group-level network with an $r$ value of greater than .1 were considered to have no group-level equivalent. Such networks with no group-level match were not included in later analyses. This relatively liberal threshold of $r = .1$ was used in order to preserve individual
variability in network shape.

This analysis allowed comparison of different ICA-delineated networks across different subjects (i.e., subject-level networks from two subjects which matched the same group-level network could be treated as the “same” network in each subject).

**ICNs engaged during N-back task performance:** In order to test the hypothesis that each individual’s task activation map can be expressed as a combination of that subject’s ICNs identified during rest, we conducted a spatial multiple regression to explain the spatial pattern of each subject’s unthresholded task-activation map as a linear combination of all of that subject’s unthresholded ICA-derived ICN maps. As in the spatial correlation procedure described earlier, the Z-scores of each individual subject network were extracted within every voxel in the brain and the 3D matrix of voxel values was reshaped into a 1D vector. These vectors were used as regressors against a similarly reshaped vector of the values within the previously calculated Task>Fixation contrast map. Notably, only valid individual-level networks (as determined by the spatial correlation procedure above) were entered as regressors. Thus, different subjects could have different numbers of regressors. However, this variability did not affect subsequent results (see Supplementary Materials).

This analysis returned an $r^2$ value for each subject, reflecting the degree to which that subject’s spatial pattern of task activation can be explained as a combination of ICNs. The analysis also returned a beta value for each network in each subject, reflecting the degree to which the spatial pattern of that particular ICN explained the spatial pattern of task activation—in other words, the degree to which that network was active during the N-back task for that subject. To identify which networks were reliably activated during the N-back task across subjects, one-sample t-tests (testing against the null hypothesis of beta = 0) were conducted for
the beta values in each network, and the results were Bonferroni-corrected for the number of t-tests conducted.

**Effect of working memory load on ICN activation:** To test the prediction that networks important for task performance would be more likely to be activated at higher loads, we repeated the same methods detailed above for each load condition. Thus, spatial multiple regression was used to compare subject-level ICNs to the individual subject’s 1-back > Fixation, 2-back > Fixation, and 3-back > Fixation contrasts. To examine the effect of load on each network, that network’s beta values (activation) from each subject were analyzed with a repeated measures one-way ANOVA, with condition (1-back, 2-back, 3-back) as the within-subjects factor. The critical significance value for the effect of load was determined by Bonferroni-correction for the total number of networks analyzed (and therefore, ANOVAs conducted). For networks with significant load effects, post-hoc t-tests were conducted to determine the direction of load modulation.

**Comparison of ICN activation to task performance:** We hypothesized that individual variability in the degree to which ICNs were activated by the n-back task would predict task performance. Both mean reaction time (RT) for correct target responses and percent accuracy (correct targets minus false alarms) were computed for each subject across all load conditions. To test for effects of network activation on RT, a multiple regression was performed with the beta values for each ICN entered as regressors. As accuracy was at or near ceiling in all conditions, we computed a categorical measure for accuracy by classifying each subject as either performing perfectly across all conditions, or as having committed at least one error in the experiment. To test for effects of network activation on accuracy, a binary logistic multiple regression was performed with the calculated beta values entered as regressors testing for effects
on accuracy (perfect/not perfect). In these regressions, if a subject had no subject-level ICN matching a given group-level ICN above the threshold of $r > .1$, a beta of 0 was used for that network in that subject.

**Results**

**Behavior:** A repeated-measures one-way ANOVA testing for load effects on percent accuracy showed a main effect of load, $F (2,38) = 13.59, p < .001$. Pairwise contrasts indicated that mean accuracy in the 1-back (99.4% ± 2.8%) and 2-back (99.8% ± 1.0%) conditions were near ceiling and did not differ, but both were performed more accurately than the 3-back condition (85.3% ± 19.5%), $p < .001$. However, the 3-back condition was also performed near ceiling level in some subjects, as 22 of the 40 subjects had 100% accuracy in the 3-back condition. Over all task conditions, twenty subjects had 100% accuracy, while twenty subjects made one or more mistakes.

A repeated measures one-way ANOVA on response times (Mean ± SD) showed a main effect of load, $F (2,38) = 15.14, p < .001$. Pairwise contrasts indicated that the 1-back task (518ms ± 150) was performed faster than the 2-back task (575ms ± 174), $t (39) = 2.24, p < .05$; and the 2-back task was performed faster than the 3-back task (684ms ± 268), $t (39) = 3.45, p = .001$ (Figure 2). Overall mean RTs did not differ between the subjects with perfect accuracy and those with at least one error ($p > .1$), indicating that RTs were not being driven by a speed-accuracy tradeoff (for further evidence see Supplementary Materials).
Figure 2: Mean ± SD reaction times (RTs) for the N-back task for three load conditions. RTs differed significantly by load. * indicates differences at p < .05; ** indicates differences at p = .001.

Regions activated and deactivated during N-back task: Figure 3 displays N-back vs Fixation contrast maps for five representative subjects. Visual examination of subjects’ N-back > Fixation contrast maps (thresholded at p < .01 for visualization purposes) suggested that most subjects activated a variety of frontal and parietal regions, commonly including dorsal medial supplementary motor area extending into dorsal anterior cingulate cortex, bilateral premotor cortex, bilateral middle frontal gyrus, bilateral anterior insula, bilateral thalamus, bilateral inferior parietal lobule, and bilateral middle and lateral cerebellum. This pattern of activation—in particular the medial and lateral prefrontal clusters, as well as the lateral parietal clusters—is broadly consistent with those reported in previous studies using the N-back task (D’Esposito et al., 1998; Owen et al., 2005). Examination of the reverse contrasts (Fixation > N-back) suggested that subjects commonly deactivated posterior cingulate cortex extending into precuneus, cuneus, and retrosplenial cortex; ventral medial prefrontal cortex extending into
anterior medial prefrontal cortex; bilateral posterior insula; bilateral angular gyrus; and bilateral ventral and medial temporal cortex including hippocampus, parahippocampal gyrus, and fusiform gyrus. This pattern of deactivation—in particular the posterior cingulate, ventral medial prefrontal, and angular gyrus clusters—is broadly consistent with the “Default Mode network”, a set of brain regions which consistently deactivates compared to baseline conditions across many tasks (Raichle et al., 2001). These activation/deactivation patterns were also observed at the group level (see Figure S1).

**Group-level ICNs:** ICA of all subjects’ concatenated rest datasets delineated 20 spatial networks of coherent activity. Nine networks were identified as deriving from non-neuronal
sources: two from CSF, three from eyeball movement, and four from subject head motion. The remaining 11 networks (Figure 4; Table 1) were labeled based upon visual similarity to past reports. These networks included: a cingulo-opercular Set Maintenance network, a left-lateralized Frontoparietal Control network, and a right-lateralized Frontoparietal Control network (identified by Dosenbach et al., 2007; lateralization shown by Beckmann et al., 2005; Habas et al., 2009; Kiviniemi et al., 2009; Smith et al., 2009; Stevens et al., 2009); a posterior Default Mode network and an anterior Default Mode network (identified by Greicius et al., 2003; anterior-posterior split shown by numerous studies including Abou-Elseoud et al., 2010; Smith et al., 2009; Uddin et al., 2009; Zuo et al., 2010); a Dorsal Attention network (Fox et al., 2006); an Auditory network (Upadhyay et al., 2008); a Visual network (De Luca et al., 2006); a Sensorimotor network (Biswal et al., 1995); a Language network (Hampson et al., 2002); and a medial/lateral temporal cortex network which has not been well-described in previous literature, and which we tentatively name a Memory network.
Figure 4: Eleven ICNs delineated in the resting-state data by the ICA procedure. ICN maps are thresholded at $Z = 4.9$, corresponding to a voxelwise probability of inclusion in the network of 99.9%. Note that the ICN maps were unthresholded when used in the later spatial multiple regression analysis.
<table>
<thead>
<tr>
<th>Network</th>
<th>Peak Coordinates (MNI)</th>
<th>Peak Location</th>
<th>Network</th>
<th>Peak Coordinates (MNI)</th>
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Table 1: ICA-derived resting-state ICNs. Coordinates and location of network “peaks” (voxels of local maximal covariation with the network) are listed.
**Subject-level ICNs:** For each subject-level ICN delineated by the ICA procedure, spatial correlations were conducted with every group-level ICN to determine the best individual-group matches; these subject-level ICNs were labeled as that subject’s version of the group-level ICN. Across subjects, valid subject-level ICN matches (i.e., above the threshold of \( r = .1 \)) were found for 91.8% of the group-level networks. 17 of 40 subjects had subject-level versions of all eleven group-level ICNs; no subject had subject-level versions of fewer than 8 group-level ICNs (i.e., three missing ICNs). The average number of valid subject-level ICNs was 10.1 out of 11 (less than 1 missing). This low variability in regressor number suggests that the number of ICNs present was unlikely to drive any systematic effects on the resulting beta values; and indeed, formal tests revealed no effect of the number of valid ICNs on subsequent results (Supplementary Materials).

Of valid group-individual network matches, the mean \( ± \) SD \( r \) value was \( .42 ± .17 \). Across subjects, the networks with the lowest average \( r \) values were the Memory \( (.24 ± .08) \) and Sensorimotor \( (.29 ± .12) \) networks; all other networks had average \( r \) values between .38 and .63. The Sensorimotor network was the network that most commonly had no valid group-individual matches; it was present in 29 of 40 individuals (72.5%). All other networks were present in at least 34 of 40 individuals (85%).

**ICNs engaged during N-back task performance:** For each subject, spatial multiple regressions were conducted evaluating the spatial pattern of N-back activation as a linear combination of ICNs. The average \( r^2 \) of these regressions across subjects was \( .38 \) (SD=.12; range = .11 to .59). Thus, the linear combination of ICNs explained on average 38% of the variance of the spatial pattern of task-evoked activations.

Beta values from this spatial multiple regression were calculated for each network in each
subject, reflecting the degree to which that network was activated (or deactivated, in the case of a negative beta value) during the N-back task (Figure 5). A one-sample t-test of these beta values was conducted across subjects for each of the 11 networks. Networks for which beta values were significantly different than zero at p<.05 after Bonferroni correction for the 11 t-tests (critical threshold: p < .0045) included the right Frontoparietal Control network (M ± SD = .14 ± .12), the cingulo-opercular Set Maintenance network (.10 ± .12), the left Frontoparietal Control network (.083 ± .14), all of which were significantly activated, as well as the anterior Default Mode network (-.15 ± .16), posterior Default Mode network (-.11 ± .13), and Visual network (-.11 ± .13), which were significantly deactivated. The Dorsal Attention network (.049 ± .11, p=.006 uncorrected) and the Memory network (-.039 ± .099, p=.018 uncorrected) were also respectively activated and deactivated, but did not survive correction for multiple comparisons.

![Figure 5](image)

**Figure 5:** Results of the spatial multiple regression analysis comparing subject-level ICNs to subject-level Task > Fixation contrasts. The graph displays average Beta values across subjects for every network. * indicates that values were significantly different from zero (p < .05, corrected for the number of networks).

**Effect of working memory load on ICN activation:** Three spatial multiple regressions were conducted to determine the degree to which the networks were activated during the 1-back,
2-back, and 3-back conditions (Figure 6). For each network, the resulting beta values for the three conditions were entered into a repeated-measures one-way ANOVA testing for effects of load at p<.05 after Bonferroni correction for 11 ANOVAs (critical threshold: p < .0045). Activation differed significantly among the three levels of load for the Dorsal Attention network (F (2,38) = 6.83), the Set Maintenance network (F (2,38) = 12.20), the left Frontoparietal Control network (F (2,38) = 19.80), and the right Frontoparietal Control network (F (2,38) = 17.27). Effects of load were also observed in the Visual network (F (2,38) = 5.20, p = .01 uncorrected), but this effect did not survive correction for multiple ANOVA tests.

Post-hoc t-tests for the networks modulated by load revealed that, for the Dorsal Attention and both left and right Frontoparietal Control networks, activation was lower during the 1-back than 2-back and 3-back conditions (all ps < .005), but 2-back and 3-back conditions did not differ (all ps > .5). In contrast, the Set Maintenance network showed a graded pattern of activation such that activation was lower during the 1-back than 2-back condition (p = .057), which in turn was lower than activation in the 3-back condition (p = .014). We further examined whether these four load-sensitive networks had activation greater than zero in all three loads by conducting one-sample t-tests on the beta values of each network in each load condition. We found that the Dorsal Attention and left Frontoparietal Control networks had activity greater than zero in 2-back and 3-back conditions (ps < .005), but not in the 1-back condition (ps > .5). The Set Maintenance and right Frontoparietal Control networks had activation greater than zero for all three loads (ps < .005). This suggests that the Set Maintenance and right Frontoparietal Control networks were active under all load conditions, but that the Dorsal Attention and left Frontoparietal Control networks only activated during the more difficult conditions.
Figure 6: Results of the spatial multiple regression analysis assessing effect of working memory load by comparing subject-level ICNs to subject-level 1-Back > Fixation, 2-Back > Fixation, and 3-Back > Fixation contrasts. The graph displays average Beta values across subjects for 11 networks included in the analysis, for each load. Significant effects of load were found in Dorsal Attention, Set Maintenance, and Frontoparietal Control networks. Differences between load conditions obtained from post-hoc tests within those networks are indicated by * (p < .05) and † (p < .10).

**Comparison of ICN activation to task performance:** The beta values for each network from the spatial multiple regression analysis (across load) were entered as regressors in a multiple regression testing for effects of network activation on mean RT across all loads. Only activation of left Frontoparietal Control network predicted RT significantly (p=.033). A post-hoc test showed that left Frontoparietal Control network activation correlated negatively with RT (r (39) = -.31, p = .053; Figure 7), such that subjects with greater left Frontoparietal Control network activation during the N-back task had faster mean RT across all conditions. This effect was not due to any systematic differences between subjects with and without 100% accuracy, as mean RT did not differ between these groups, and the effect was still present at trend level if only perfectly-performing subjects were included (Supplementary Materials). The beta values for each network were also entered as regressors in a binary logistic multiple regression testing for effects of network activation on binary accuracy (perfect or not perfect). Greater activation
of both the Dorsal Attention network (B = 36.9, Wald = 5.44, p=.020) and the left Frontoparietal Control network (B = 9.90, Wald = 4.09, p=.043) significantly predicted a greater likelihood to have perfect accuracy across all conditions.

Figure 7: Correlation between spatial multiple regression Beta values from the left Frontoparietal control network and reaction time.

Discussion

Using a novel spatial multiple regression technique, the present study found that the spatial extent of selective intrinsic connectivity networks (ICN), characterized while subjects were in the resting state, corresponded to their pattern of functional neural engagement during working memory assessed by the N-back task. Of the 11 ICNs included, the patterns of activation best matched the left and right Frontoparietal Control networks and the cingulo-opercular Set Maintenance network, and the pattern of deactivation best matched the anterior and posterior Default Mode networks and the Visual network. Furthermore, the degree of network engagement increased at higher working memory loads, such that increasing load from 1-back to 2-back increased activation of the Dorsal Attention, Set Maintenance, and right and left
Frontoparietal Control networks, and increasing the load from 2-back to 3-back induced additional activation increases in the Set Maintenance network only. Degree of network engagement was associated with performance, such that increased activation of the left Frontoparietal Control and Dorsal Attention networks predicted faster response speed and an increased chance of attaining 100% task accuracy. Default mode network deactivation did not relate to performance. In sum, these results confirm, within individual subjects, the close correspondence between functional organization of resting and task-evoked states that has been previously reported across studies (Smith et al., 2009). Further, these results demonstrate that the degree of task-rest correspondence in individuals is sensitive to cognitive load and performance variability.

**Decomposition of the functional neural architecture of working memory in terms of Intrinsic Connectivity Networks**

The present findings extend current knowledge about similar functional organization between resting and task-evoked states in two ways. First, strong spatial matches between functional neural engagement and ICNs were observed at the individual subject level. In a seminal study by Smith et al. (2009), spatial matches were evaluated at the group level between activation patterns derived from multiple task-evoked studies and single ICNs derived from a separate group of 36 subjects. The present results extend those findings to *individual* subjects, from whom both the task-evoked activation/deactivation patterns and the ICNs were derived. Second, while Smith et al. evaluated matches between task activation patterns and single ICNs, the present findings demonstrate that the combination of *multiple* ICNs explained a large amount of variance in the spatial distribution of activation associated with working memory. A linear combination of ICNs explained between 11% and 59% of the spatial pattern of task-evoked
activation across subjects; the average across all subjects was 38%. By comparison, Smith et al. demonstrated visually convincing spatial correlation matches between task-evoked activation and resting networks in which the resting network explained at least 6.3% of the task activation ($r = .25$), which was considered a minimum threshold for a good spatial match. Thus, the task-rest spatial matches in the present study were well above that minimum threshold. Together, our results show that task-evoked states in individual subjects reflect the simultaneous engagement and disengagement of not just one, but several of the ICNs detected at rest in the same subjects.

The pattern of activation and deactivation during N-back performance was largely consistent with past reports, with one exception. Results showed that the bilateral Frontoparietal Control networks (including dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, and inferior parietal cortex) and the Set Maintenance network (including bilateral anterior insula and dorsal anterior cingulate cortex) were consistently activated by the N-back task. Networks deactivated by the task included the anterior Default Mode network (including ventral and dorsal medial prefrontal cortex and posterior cingulate cortex) and the posterior Default Mode network (including posterior cingulate cortex extending into precuneus and retrosplenial cortex, ventromedial prefrontal cortex, and bilateral angular gyrus). This regional composition of activation (D’Esposito et al., 1998; Owen et al., 2005) and deactivation (McKiernan et al., 2003) is consistent with past studies using the N-back task. An unexpected finding, however, was that deactivations included the Visual network (including most of occipital cortex). Deactivation of visual regions appears contrary to task demands of a visual N-back task. Examination of deactivated regions revealed by the Fixation > N-back contrast showed that cuneus, lateral middle occipital cortex, and lateral inferior occipital cortex were deactivated (Figure S2, yellow circles), but primary visual cortex was not. Thus, only extrastriate portions of the Visual
network that are associated with higher-order visual processing were deactivated. Indeed, such processing is unlikely to be evoked during perception of single letter stimuli used in the present task.

In sum, the present results extend past working memory findings by demonstrating that the observed patterns activation/deactivation can be decomposed in terms of temporally segregated networks, ICNs, that are detected without task demands. However, while these findings seem to suggest that the task evokes entire ICNs to a greater or lesser extent, it is important to note that the present analysis cannot readily discriminate differential engagement of smaller sub-units of ICNs (as demonstrated by the partial deactivation of the visual cortex). Indeed, whether or not a whole ICN is engaged is likely to depend on the demands of specific tasks. This limitation of the analysis is further discussed below (“Advantages and Limitations of Spatial Multiple Regression”).

Effect of Working Memory Load

The Set Maintenance and bilateral Frontoparietal Control ICNs that were engaged during N-back performance were also sensitive to increased load. In addition, activation in the Dorsal Attention network was sensitive to load, although this network was not significantly active in the overall task. Post-hoc analyses indicated that this network was indeed active during the 2- and 3-back conditions, but not activated in the low-load 1-Back condition. Past studies have demonstrated load-sensitivity of individual regions within the ICNs observed here, such as prefrontal, parietal, and anterior cingulate regions (Braver et al., 1997; Callicott et al., 1999; Veltman et al., 2003). Dosenbach and colleagues (Dosenbach et al., 2007, 2008) have argued that the Frontoparietal Control and Set Maintenance networks perform distinct cognitive control operations, with the Frontoparietal Control network adapting to short-term variations in task
requirements and the Set Maintenance network maintaining longer-term task goals and strategies. These cognitive functions are likely to be required at all loads, but to be engaged more strongly in more difficult conditions than in easy conditions such as the 1-Back. The Dorsal Attention network has been argued to control voluntary, top-down orienting of attention and selection of behavior (Corbetta and Shulman, 2002), and specifically to be involved in rehearsal during working memory (Corbetta et al., 2002). Unlike the 2- and 3-Back conditions, the optimal strategy for performing the 1-Back condition does not involve rehearsing the target stimulus, which may explain why this network was not active during the 1-back condition.

These four ICNs were differentially responsive to increased working memory demands. The Set Maintenance network responded linearly to working memory load, such that activation was greater during the 3-back condition than during 2-back, which in turn was greater than during the 1-back condition. In contrast, Frontoparietal Control and Dorsal Attention networks responded to load in a “stepwise” function, such that activation was greater during 2 and 3-back than 1-back, but 2-back and 3-back activation did not differ. In previous studies using a multi-load N-back design, both uniformly linear (Braver et al., 1997; Veltman et al., 2003) and mixed linear and stepwise (Callicott et al., 1999; Cohen et al., 1997; Jonides et al., 1997) load-responses have been reported. Those studies reporting mixed responses found stepwise responses within lateral prefrontal cortex (Callicott et al., 1999; Cohen et al., 1997; Jonides et al., 1997), but linear responses within dorsal anterior cingulate cortex (Callicott et al., 1999). This pattern is similar to that observed here, as lateral prefrontal cortex is part of the Frontoparietal Control network while dorsal anterior cingulate is part of the Set Maintenance network.

Neither the Anterior nor the Posterior Default Mode network was modulated by load. By contrast, McKiernan et al. (2003) demonstrated that deactivation in medial prefrontal but not in
posterior Default Mode regions increased with increasing working memory load. It is possible that the working memory task used by McKiernan et al. (an auditory monitoring task) may be more sensitive in detecting load-related deactivation effects. In addition, effects of load on performance also differed more substantially in the auditory monitoring task than in the present study. Thus, both task and performance characteristics may be important factors in determining deactivation effects.

Relationship with Performance

Of the seven ICNs comprising task-evoked activation and deactivation, only two were associated with N-back performance. Increased activation of the left Frontoparietal Control network predicted both faster reaction time and an increased probability of perfect performance, while increased activation of the Dorsal Attention network predicted an increased probability of perfect performance. Greater right-lateralized (Callicott et al., 2000; Tan et al., 2006) and bilateral (Callicott et al., 1999) lateral prefrontal involvement has previously been associated with superior performance on a spatial N-back paradigm. By contrast, the specifically left-hemispheric involvement of the Frontoparietal Control network in our study likely reflects use of verbal processing required for N-back performance with letter stimuli. While associations between N-back performance and engagement of medial and superior parietal regions comprising the Dorsal Attention network have not been reported in the literature, these regions have been associated with information rehearsal during a delayed response working memory task (Corbetta et al., 2002).

By contrast, no association was found between behavioral performance and activation of the Set Maintenance or Default Mode networks. The Set Maintenance network is posited to mediate maintenance of relatively stable information such as task goals (Dosenbach et al., 2007, 2008)
and has been associated with detection of stimuli which are salient to current goals (Seeley et al., 2007). Further, the anterior cingulate gyrus within this network has been associated with monitoring for responses incompatible with current goals (errors) (Carter et al., 1998). Failure to deactivate the Default Mode network has previously been associated with increased errors (Eichele et al., 2008) and slower reaction times (Weissman et al., 2006) during cognitively demanding tasks, which has been interpreted as Default Mode-related lapses of attention (though this has not been shown in working memory tasks). The N-back task in the present study produced unusually high performance levels (50% of subjects made no errors over the course of the task), suggesting that lapses of attention were infrequent. Few lapses of attention and few errors may have reduced our ability to detect associations with the Default Mode and Set Maintenance ICNs. Use of binary logistic regression allowed detection of ICNs associated with perfect/imperfect performance, but this analysis is still not as sensitive as a linear regression would be, which may explain why no association was found between performance and Default Mode or Set Maintenance networks. These high performance levels may have limited our findings in other ways as well. The high accuracy may have been due at least in part to subjects’ previous exposure to the task, but it may also suggest that these subjects are not representative of the average population in terms of intellectual ability and/or motivation. Further, our findings regarding linear and step-wise effects of load should be considered in the context of this near-ceiling performance. Specifically, it is possible that the nature of ICN engagement depends upon subjects’ subjective experience of increased demands, which may be more challenging to a lower performing sample than ours.

Advantages and Limitations of Spatial Multiple Regression
We employed a novel spatial multiple regression analysis in this study to compare ICNs identified at rest to activation patterns observed during task. In this analysis, the dimensionality of the brain is first reduced from many thousands of voxels to a few ICNs using ICA, a well-established technique for delineating functional brain networks (Beckmann and Smith, 2004), and then these networks are further examined during task-evoked states. Conceptually, using spatial multiple regression involves viewing the task-performing brain as a small collection of ICNs rather than as many thousands of voxels. As the regions within ICNs have very strong intra-network functional connectivity (Seeley et al., 2007), tasks that activate one part of an ICN will likely activate all parts of the ICN, such that all voxels within an ICN will be activated in approximately the same way. Thus, it is reasonable to conduct hypothesis testing in an ICN-wise fashion rather than in a voxel-wise fashion. An advantage of this approach is that fewer statistical tests are conducted relative to a voxel-wise analysis, which eliminates the need to use stringent corrections for multiple comparisons that increase the likelihood of Type II errors. This advantage is well illustrated in the present study. We tested for effects of network activation on response speed and found that increased activity in the left Frontoparietal Control network significantly predicted faster RT (Figure 7). For comparison, we conducted a post-hoc whole-brain voxelwise analysis using RT as a linear regressor. This analysis also showed clusters well-matching the left Frontoparietal Control network (in left dorsolateral prefrontal, ventrolateral prefrontal, and inferior parietal cortex, as well as small clusters in right ventrolateral prefrontal and inferior parietal cortex), but these clusters did not survive a correction for multiple comparisons (Figures S3A and B).

The approach of conducting spatial multiple regression against task is based on observations of group-level task-rest similarities (Fransson, 2006; Smith et al., 2009), and extends that work to
show similarities between task activation and multiple ICNs within individual subjects. Other recent work has similarly demonstrated task-rest associations. Mennes et al. (2010) used a voxel-matched regression method to demonstrate voxels in which task activation was directly predicted by degree of functional connectivity. An advantage of that technique is its ability to examine rest-task relationships in a voxelwise fashion, allowing for direct comparisons of the strength of resting connectivity to task activation. This is not possible with our proposed technique, which compares the shape of resting ICNs to task activation. However, the voxel-matched regression technique is also somewhat limited by its use of only two broad brain networks, the “task-positive” and “task-negative” networks. Thus, the specificity of the obtained connectivity values may not be high in this technique, as they represent connectivity with a broad conglomerate of ICNs.

Multiple regressions in the spatial domain have previously been employed to analyze ICA data using the dual regression technique (Filippini et al., 2009). Dual regression, which involves spatially regressing group-level ICA components against an individual-level functional session and then temporally regressing the resulting timecourses against that functional session, allows the reconstruction of individual components without running ICA on individuals. As such, it represents an alternative to the component-matching procedure we used to identify individual-level ICNs. While the dual regression technique has been shown to have strong test-retest reliability (Zuo et al., 2010), it is as yet unclear how much individual variability is lost by forcing the ICNs from individual subjects to conform to group-level ICNs. Dual regression may thus increase consistency across subjects by reducing individual variability. Importantly, the primary purpose of both the component matching technique used here and the dual regression technique (as well as the back-reconstruction method used in the GIFT software,
is to identify individual versions of group components. Once this identification is performed, the spatial multiple regression against task activation data detailed in this paper may be conducted using the results of any of these techniques. Thus, the technique proposed in the present study is a flexible tool to determine the specifics of task-rest similarities that can be employed in concert with any component identification method.

Importantly, despite the advantages offered by spatial multiple regression analysis, this technique is not suitable for testing hypotheses about sub-regions within ICNs. For example, possible dissociations between frontal and parietal regions within the Frontoparietal Control network cannot be identified in the present study because these regions were parceled into the same ICN. The degree of network parcellation can be controlled, as a higher model order ICA will divide the functional networks into smaller sub-networks (Abou-Elseoud et al., 2010; Kiviniemi et al., 2009) and thus allow for more specificity in the spatial multiple regression analysis. We used a model order of 20 in the present study because that model order has been shown to return visually identifiable networks (Abou-Elseoud et al., 2010) similar to those described using seed-based connectivity. However, the large scale of these networks does prevent examination of sub-network effects in the present study. This limitation is illustrated by our finding of deactivation in the Visual ICN during N-back performance, which closer examination showed was limited to extrastriate regions, a subset of the network (Figure S2). These sub-regional differences were not discerned by the spatial multiple regression analysis because the ICA-generated Visual network included both primary and secondary visual cortex. However, while increasing the ICA model order would likely increase the specificity of testing, it would also increase the number of ICNs statistically tested, and thus require more stringent correction. A logical next step in the development of the spatial multiple regression technique
would be to systematically investigate how the number of ICNs delineated in the ICA procedure may affect the specificity and post-correction power of the results. Such an investigation could conceivably identify an “optimal” number of ICNs beyond which the increased spatial specificity does not make up for the loss of power, and could be of great interest for the field.

**Conclusion**

We have demonstrated that a novel analysis, spatial multiple regression, is useful in identifying network-level functional engagement during working memory. The left and right Frontoparietal Control and Set Maintenance networks were activated during the N-back task, while Default Mode and Visual networks were deactivated during the task. Further, activated networks, in addition to the Dorsal Attention network, were sensitive to increased working memory load. The degree of activation of the left Frontoparietal Control and Dorsal Attention networks was associated with performance. The spatial multiple regression approach compares task-states to multiple rest-state connectivity networks, and also allows sensitive examination of relatively subtle effects on network activation by avoiding correction for large numbers of statistical comparisons. In future work, this approach should be promising for testing hypotheses about network-level differences between conditions or populations. As functional disruptions in a number of disorders (e.g., Schizophrenia, Attention Deficit-Hyperactivity Disorder, Autism Spectrum disorders, and Alzheimer’s disease) are hypothesized to include abnormalities at the network level, rather than at the level of the individual voxel or brain region (Broyd et al., 2009), an approach which examines functional activation at the network level may be substantially more sensitive to differences between disordered populations and healthy controls than voxelwise analyses.
CHAPTER III: WORKING MEMORY-RELATED CHANGES IN FUNCTIONAL CONNECTIVITY

PERSIST BEYOND TASK DISENGAGEMENT

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**Introduction**

The functional architecture of the human brain is composed of discrete, large-scale networks with widely spaced nodal regions, which demonstrate highly correlated activity across time. Such correlations, termed “functional connectivity” are evident in low frequency spontaneous BOLD signal fluctuations observed while subjects are resting, a state defined by the absence of directed cognitive engagement. Connectivity measured during rest is said to be “intrinsic” connectivity. However, functional connectivity networks are known to be sensitive to cognitive state, as the strength of connectivity is altered during task engagement relative to the resting state. Specifically, regions engaged by auditory (Arfanakis et al., 2000), visual (Arfanakis et al., 2000; Hampson et al., 2004; Nir et al., 2006), motor (Arfanakis et al., 2000; Jiang et al., 2004), and cognitive (Newton et al., 2011) tasks show altered connectivity during performance of those tasks. These findings indicate that task-evoked cognitive states induce selective changes in the strength of brain networks relative to the resting state.

Two lines of research indicate that task-driven changes in functional connectivity persist beyond the duration of the task and into a subsequent resting state. First, task content affects the strength of connectivity during a subsequent resting state. Resting state connectivity between visual and frontal regions immediately following performance of a task varied depending on
whether the task involved classifying faces or scenes (Stevens et al., 2010). Similarly, precuneus connectivity after a language comprehension task varied depending on the content of the language in that task (Hasson et al., 2009). Second, direct comparisons of resting state functional connectivity before and after task performance reveal marked differences. Such task-driven differences in resting connectivity have been observed after finger tapping (Duff et al., 2008), motor (Albert et al., 2009; Vahdat et al., 2011) and visual (Lewis et al., 2009) learning, language generation (Waites et al., 2005), memory encoding (Tambini et al., 2010), and semantic classification (Grigg and Grady, 2010). These persistent alterations in functional connectivity were observed not only within task-relevant networks (Albert et al., 2009; Tambini et al., 2010; Waites et al., 2005), but also between networks (Duff et al., 2008; Grigg and Grady, 2010; Lewis et al., 2009). Notably, the direction of change in resting-state connectivity was not consistent across these studies, with connectivity after task performance becoming more positive (Albert et al., 2009; Duff et al., 2008; Grigg and Grady, 2010; Tambini et al., 2010; Vahdat et al., 2011; Waites et al., 2005), more negative (Lewis et al., 2009; Vahdat et al., 2011), and less negative (Lewis et al., 2009). Taken together, these results indicate that not only is the network structure of the brain altered during task performance, but that such alterations persist after the task ends, likely in ways specific to the task and connections being tested.

Several characteristics of these persistent connectivity changes remain unknown. First, how effortful cognition affects subsequent resting-state connectivity is not known. An investigation of the resting brain using non-connectivity measures (Barnes et al., 2009) showed that the brain’s oscillatory dynamics were perturbed after engagement of working memory (WM). WM is defined as the temporary maintenance and manipulation of task-relevant information, and it contributes critically to higher order cognitive functioning (Engle et al., 1999). Further, recovery
from this perturbation took longer after greater WM loads, suggesting that effortful cognition requires a subsequent post-task recovery period, which is affected by the degree of that effort. While it is known that engagement of WM alters connectivity within and across the default mode network (DMN) and the task-positive network (TPN) (Bluhm et al., 2011; Fransson and Marrelec, 2008; Fransson, 2006; Chapter V), whether these changes persist after WM disengagement remains to be examined. Second, while connectivity after a task may be altered compared to connectivity before the task, it is not known whether these pre/post task differences are predicted by the strength of connectivity during the task. Third, while the strength of resting state connectivity predicts individual differences in cognitive performance (e.g., Hampson et al., 2010), the stability of those connectivity-cognition relationships is not known. Specifically, it is unknown whether resting state connectivity still predicts individual differences in cognition if the relevant networks have been altered by a preceding task.

The present study addressed these gaps by examining whether altered functional connectivity patterns during WM performance persist following conclusion of that performance. We examined functional connectivity within the DMN and TPN, as well as between the DMN and TPN, during three different scans: a baseline resting state session, an N-back working memory task, and a subsequent resting state session immediately following the task. Based on previous findings that task performance generally alters functional connectivity in a persistent fashion, and that working memory in particular affects connectivity within and between the DMN and TPN, we first hypothesized that these network relationships would be altered during the N-back task relative to baseline, and that alteration would persist during the subsequent resting state scan. Further, the magnitude of the changes in resting state connectivity from baseline to post-task scans should be predicted by the strength of connectivity during the N-back task. Such a
finding would strongly suggest that any persistent changes in resting state functional connectivity are specifically due to the performance of the task. Notably, as the reported direction of change in functional connectivity during N-back tasks has varied widely across previous studies (Bluhm et al., 2011; Fransson and Marrelec, 2008; Fransson, 2006; Chapter V), we have no clear basis for specifying the direction in which connectivity will be persistently altered by the task. Second, while it is known that within-DMN and DMN-TPN resting state connectivity predict task-irrelevant thought (Buckner et al., 2008), attention lapses (Weissman et al., 2006), and mind-wandering (Mason et al., 2007), it is unknown whether such relationships exist if the connectivity has been persistently altered by a working memory task. Thus, we hypothesized that resting state DMN connectivity would correlate with a behavioral measure of inattentiveness, and we examined whether this correlation remains stable across the three states: the initial, “baseline” resting state session, the N-back task, and the subsequent resting state session.

**Materials and Methods**

**Subjects:** 50 Georgetown University undergraduates (35 female; 48 right handed) ages 18 to 22 years (M ± SD = 20.44 ± .90) participated in the study for payment. Informed consent procedures were carried out according to Georgetown University’s Institutional Review Board guidelines. Exclusion criteria included 1) Self-reported use of psychotropic medication (e.g., stimulants, anti-anxiety/depression); 2) Self-reported history of neurological injury or disease, seizure disorder, psychiatric diagnosis; 3) Contraindications for MRI – e.g., metal implants in the body, dental work involving metal, pregnancy.

**Behavioral testing:** Subjects completed the Adult ADHD Self-Report Scale v1.0 (Kessler et al., 2005), which reported trait measures of Inattention and Hyperactivity-Impulsivity. This scale
consists of eighteen questions about subjects’ inattentive and impulsive behaviors consistent with DSM-IV criteria for ADHD. It is scored on a 5-point Likert scale; scores can range from 0 to 36 for each measure. The questionnaire was administered at the time of subject recruitment. Analysis focused on the Inattention score, as the Hyperactivity-Impulsivity measure did not relate to connectivity in past work (Chapter V). Across subjects, mean Inattention scores were 13.58 ± 3.64; these scores were approximately normally distributed across the sample (D’Agostino-Pearson omnibus test statistic = 1.27, p = .47).

**Scanning procedure:** Each scanning session included five scans over the course of thirty minutes. The first scan was a resting state scan (Rest1) lasting 5:04 minutes in which subjects were told to relax with eyes closed and to not think of anything in particular. This was followed by two scans during a simple shape discrimination task (not described here) lasting 5:46 minutes each, and then by acquisition of a high-resolution anatomical image lasting 4:18 minutes. Subjects then underwent an N-back working memory task lasting for 6:26 minutes (task described below) followed immediately by a second resting state scan (Rest2) identical to the Rest1 scan. Note that as the focus of this study is on post-task modification of resting state connectivity, we consider the impact of only the N-back task on the Rest2 scan, as it immediately preceded the Rest2 scan; effects of the shape discrimination task cannot be directly linked with the Rest2 scan as it was separated in time from the Rest2 scan by the intervening N-back task and by the anatomical image acquisition.

The N-back task consisted of nine 30s N-back blocks (three blocks each at 1-, 2-, and 3-back) alternating with eight 15s blocks of fixation. Each N-back block consisted of nine serially presented consonants appearing for 500ms, with an inter-trial-interval of 2500ms. The N-back load condition (1-, 2-, or 3-back) varied between task blocks, with condition order
pseudorandomized using a modified Latin Square. Each block was preceded by a 3000 ms screen informing the subject of the N-back condition. Subjects were instructed to press a hand-held button with their right hand when the current letter matched the letter n trials ago (e.g., for the 2-back condition, subjects see: R V N W N – button-press for N). Targets were present on 19% of trials; each block contained between one and three targets with target frequency balanced across conditions. No condition contained sequences of stimuli that were targets in any other condition. Stimuli were presented using E-Prime (Psychology Software Tools Inc., Pittsburg, PA).

N-back accuracy was calculated as (% targets hit - % false alarms). N-back accuracy was very high (see Supplementary Materials), and therefore was not examined in any further analyses. Reaction time (RT) was calculated as the mean time to respond to targets correctly hit.

fMRI data acquisition: Imaging was performed on a Siemens Trio 3T scanner (Erlangen, Germany). For the resting runs, 152 whole-brain images were acquired using a gradient echo pulse sequence (37 slices, TR = 2000 ms, TE = 30 ms, 192x192mm FOV, 90 degree flip angle, voxel dimensions 3mm isotropic). For the N-back run, 197 whole-brain images were acquired using a gradient echo pulse sequence (34 slices, TR = 2000 ms, TE = 30 ms, 256x256mm FOV, 90 degree flip angle, voxel dimensions 4x4x4.2mm). The first 4 images of each functional run were discarded to allow for signal stabilization. A high resolution T1-weighted structural scan (MPRAGE) was acquired with the parameters: TR/TE=2300/2.94ms, TI=900ms, 90 degree flip angle, 1 slab, 160 sagittal slices with a 1.0mm thickness, FOV=256x256mm2, matrix=256x256, resulting in an effective resolution of 1.03mm isotropic voxels.

were corrected for translational and rotational motion by realigning to the first image of each scanning run (Rest1, N-back, and Rest2). Across all runs, all subjects demonstrated less than 2.0mm of translational motion in any one direction (max translation = 1.25mm) and less than 1.0° of rotation around any one axis (max rotation = .54°). Root mean squared (RMS) motion was further calculated as a summary measure of motion for each subject in each run. RMS motion was .076 ± .23 in the Rest1 run, .085 ± .39 in the Nback run, and .083 ± .31 in the Rest2 run, and paired t-tests indicated that it did not differ between runs (all pairwise ts < 1.8, all ps > .05). Further analyses indicated that the reported results were not caused by effects of motion (see Supplementary Materials).

Images were then slice-time corrected, normalized to an EPI template, and smoothed using a Gaussian kernel with full-width at half-maximum (FWHM) of 8mm. Finally, a band-pass filter was applied to the data in order to restrict signal variation to frequencies between .01 Hz and .1 Hz, corresponding to the frequency range established in the literature for fluctuations in resting state data (Biswal et al., 1995).

**Functional connectivity calculation:**

**Network node creation.** Regions of interest (ROIs) representing nodes of the DMN and TPN networks were created based on a previously published Independent Components Analysis (ICA) of resting state data conducted in an independent sample (N=44) of subjects demographically similar to those in the present study (Chapter II). Two ICA components were identified which together visually matched the canonical DMN, one comprising the anterior aspect of the DMN and one comprising the posterior aspect, and one component (described as the “Set Maintenance” component in Chapter II was identified which visually matched the canonical anticorrelated TPN. ROIs were created as the large clusters (k > 50 voxels) of the identified
components after thresholding the Z-map at Z > 7.0. This resulted in five DMN nodes located in ventral and anterior dorsal medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), precuneus (Prec), and bilateral angular gyrus (lAG, rAG), as well as six TPN nodes located in dorsal anterior cingulate cortex (dACC), bilateral dorsolateral prefrontal cortex (ldLPFC, rdLPFC), bilateral anterior insula (laIns, raIns), and right supramarginal gyrus (rSMG). These nodes well-matched regions strongly correlated and anticorrelated with an independent PCC seed in the present data (see Supplementary Materials; Figure S1), and also had good overlap with regions activated and deactivated by the present N-back task (see Supplementary Materials; Figure S2).

**Nuisance signal identification.** To identify the effects of motion and physiological noise (such as respiration and heart rate) that would be common to all nodes, timecourses approximating these signals were calculated for each scanning run. Physiological noise timecourses were approximated by obtaining signal timecourses from white matter and CSF segmentations of the MPRAGE image (Van Dijk et al., 2010). Motion timecourses were obtained as the six realignment parameter timecourses from the motion correction preprocessing step, expressed as absolute differences from the first timepoint in each of the three translation and rotation directions. Notably, the global signal was not included as a nuisance signal, as regression of the global signal is known to strongly affect observed negative correlations (Chang and Glover, 2009; Fox et al., 2009; Murphy et al., 2009), and recent work suggests that the procedure may reduce the accuracy of connectivity estimates (Saad et al., 2012).

**Functional connectivity calculations:** For each network node in each run, the average node timecourse was calculated across all voxels. Connectivity between two nodes in a given run was calculated by conducting partial correlations between the timecourses of those nodes, while
partialling out the motion and physiological noise timecourses. The resulting $r$ values were converted to Z-scores using Fisher’s transformation in order to increase normality of the distribution, allowing further statistical analysis of correlation strengths. In the N-back run, analysis was restricted to task performance by excluding the 15 s in each fixation block plus 6 subsequent seconds (to allow for hemodynamic response stabilization). The partial correlations were thus assessed on 148 timepoints in each Rest run and 108 timepoints in the N-back run. Results obtained without removal of these fixation blocks are presented in Supplementary Materials; they did not differ from those obtained with removal of the fixation blocks. To further avoid any artificial inflation of the N-back correlations that could be driven by the block structure of the task load conditions, effect of load was partialled out. Load-effect timecourses were obtained by convolving three boxcar timecourses (one for each load condition) with a canonical hemodynamic response, and were partialled out of the N-back connectivity correlations (see Chapter V and Jones et al. (2010) for discussions of the rationale for removing task-load effects).

*Functional connectivities calculated for each run, in each network.* Using this method, for each subject in each run (Rest1, N-back, Rest2), *within-DMN* connectivity calculations were conducted between the PCC node and each other DMN node. *Cross-network DMN-TPN* connectivity calculations were conducted between the PCC node and each TPN node. Finally, we calculated the strength of anticorrelation between each node and the PCC node in the independent dataset. This calculation revealed that the rSMG node was most negatively correlated with the PCC, followed by the raIns node. As the SMG is not usually considered a key TPN node, we selected the raIns as the central TPN node. Therefore, *within-TPN* connectivity calculations were conducted between the raIns node and each other TPN node.
Effects of performing a working memory task on connectivity:

*Changes in functional connectivity across runs.* For the within-DMN connections calculated above, mean connectivity values were entered into a 3 (State: Rest1, N-back, Rest2) X 4 (connections from PCC to other DMN nodes) repeated measures Analysis of Variance (ANOVA). A similar 3 (State) X 6 (connections from PCC to TPN nodes) repeated measures ANOVA was conducted for the cross-network PCC-TPN connections, and a 3 (State) X 5 (connections from rIns to other TPN nodes) repeated measures ANOVA was conducted for the within-TPN connections. In the three ANOVAs, main effects of State tested whether overall connectivity differed across Rest1, N-back, and Rest2 states, and State X connection interactions tested whether state-related differences were present more strongly in some connections than in others. Significant effects were followed up with paired t-tests.

*Effect of task connectivity and performance on subsequent resting state connectivity.* For within-DMN, DMN-TPN, and within-TPN connections, we tested whether connectivity in these connections during the N-back task predicted the subsequent Rest2 connectivity. To reduce multiple comparisons, we calculated average connectivity from the PCC to all other DMN nodes as overall within-DMN connectivity, average connectivity from the PCC to all TPN nodes as overall DMN-TPN connectivity, and average connectivity from the rIns to all other TPN nodes as overall within-TPN connectivity. We then correlated the strength of these overall connectivities during the N-back task to their strength during Rest2. Significant findings were followed up with post-hoc connection-wise analyses to identify connections with the most robust N-back vs Rest2 correlations. Finally, we tested whether the strength of Rest2 connectivity was also predicted by N-back task performance in these nodes by correlating average RT against the strength of Rest2 connectivity.
Correlations between within-DMN connectivity and trait-level Inattention measures:

For each run (Rest1, N-back, Rest2), we tested whether the overall strength of within-DMN connectivity (calculated above) was correlated with trait-level Inattention; significance values were corrected for the three runs tested using Bonferroni correction. We then tested whether the correlations with Inattention differed in the different runs using Meng’s t-test. Significant correlations were followed up with post-hoc nodewise analyses to determine for which nodes the correlations were most robust. Though our a priori interest was in associations between Inattention and within-DMN connectivity, we also tested within-TPN and DMN-TPN connections for completeness.

Results

Effect of performing the N-back task on connectivity

Within-DMN: The State X Node ANOVA revealed a significant main effect of State (F(2,48)=5.12, p=.01), indicating that connectivity between PCC and other DMN nodes differed between Rest1, N-back, and Rest2. Effects are graphed in Figure 1. Overall PCC connectivity with DMN nodes became significantly less positive from the Rest1 run to the N-back run (t(49) = 3.18, p = .003), and then became more positive again from the N-back run to the Rest2 run (t(49) = 2.35, p = .023), with no differences observed between the Rest1 and Rest2 runs (t(49) = .45, p = .65). Further, a significant interaction indicated that the effect of State varied by node (F(6,44)=8.03, p<.001) such that connectivity with the angular gyrus nodes became significantly less positive from the Rest1 run to the N-back run (rAG: t(49) =7.29, p < .001; lAG: t(49) = 3.55, p = .001), and then became significantly less positive again from the N-back run to the Rest2 run in the rAG node (t(49) = 5.94, p < .001) but not the lAG node (t(49) = 1.90, p = .064), with no
differences observed between the Rest1 and Rest2 runs in either node (ts(49)<1.5, ps > .30). In the Prec and mPFC nodes, no differences by state were observed (all ts(49) < 2.0, all ps > .05). These findings indicate that the N-back task altered within-DMN functional connectivity in the AG nodes, but that connectivity returned to baseline after the task was complete.

Figure 1: Default mode network nodes (brain slices) and within-DMN connectivity strength in those nodes (small graphs) and across the whole network (larger bottom graph) measured during the baseline Rest1 scan, the N-back working memory scan, and the post-task Rest2 scan. The seed region for connectivity calculations was the PCC (in green). Unless otherwise indicated, * indicates significant differences from Rest1 at p<.05.
DMN-TPN: The State X Node ANOVA revealed a significant main effect of State (F(2, 48) = 15.82, p < .001). Effects are graphed in Figure 2. Overall PCC connectivity with TPN nodes became significantly less negative from the Rest1 run to the N-back run (t(49) = 4.48, p < .001) and remained less negative in the Rest2 run than it was in the Rest1 run (t(49) = 4.52, p < .001), with no difference observed between the N-back and Rest2 runs (t(49) = .35, p = .72). Further, a significant interaction indicated that the effect of State varied by node (F(10, 40) = 7.00, p < .001) as follows: Connectivity of the PCC with the dACC, raIns, and laIns nodes became less negative from the Rest1 run to the N-back run (ts(49) > 3.50, ps < .001) and remained less negative in the Rest2 run than it was in the Rest1 run (ts(49) > 2.9, ps < .005), with no differences observed between the N-back and Rest2 runs (ts(49) < 1.7, ps > .10). Thus, in these nodes, connectivity became less negative during the N-back run and remained so during the Rest2 run. A similar pattern was observed for the rSMG node, with connectivity becoming less negative from the Rest1 run to the N-back run (t(49) = 8.51, p < .001), though connectivity did become more negative again from the N-back run to the Rest2 run (t(49) = 3.78, p < .001) but still remained less negative in the Rest2 run than it was in the Rest1 run (t(49) = 4.73, p < .001). Thus, in this node connectivity became less negative during the N-back run and only returned partially back to its original baseline in the Rest2 run. By contrast, in the rdlPFC and ldlPFC nodes, connectivity with the PCC did not change from the Rest1 run to the N-back run (ts(49) < .80, ps > .45), but became less negative from the N-back run to the Rest2 run (significantly for the ldlPFC: t(49) = 2.02, p = .049; and at trend level for the rdlPFC: t(49) = 1.70, p = .095) and, critically, remained less negative in the Rest2 run than in the Rest1 run (ts(49) > 2.60, ps < .015). These findings indicate that the N-back task induced PCC connectivity with dACC, alns, and
rSMG TPN nodes to become less negative, and, critically, PCC connectivity with all TPN nodes remained less negative during the post-task resting state relative to the pre-task resting state.

Figure 2: Task-positive network nodes (brain slices) and cross-network DMN-TPN connectivity strength in those nodes (small graphs) and across the whole network (larger bottom graph) measured during the baseline Rest1 scan, the N-back working memory scan, and the post-task Rest2 scan. The seed region for connectivity calculations was the PCC (in green). Unless otherwise indicated, * indicates significant differences from Rest1 at p<.05.
**Within-TPN:** The State X Node ANOVA revealed a significant main effect of State (F(2,48)=6.00, p=.005). Effects are graphed in Figure 3. Overall raIns connectivity with TPN nodes became significantly less positive from the Rest1 run to the N-back run (t(49)=3.15, p=.003) and remained less positive in the Rest2 run than it was in the Rest1 run (t(49)=2.97, p=.005), with no difference observed between the N-back and Rest2 runs (t(49)=1.29, p=.20). Further, a significant interaction indicated that the effect of State varied by node (F(8,42)=4.32, p=.001) as follows: In the laIns and ldlPFC nodes, connectivity with the raIns followed the pattern of the overall network, becoming significantly less positive from the Rest1 run to the N-back run (t(49) > 2.00, p < .05) and remaining less positive in the Rest2 run than it was in the Rest1 run (t(49) > 2.40, p < .02), with no differences observed between the N-back and Rest2 runs (t(49) < 1.0, p > .35). By contrast, in the rSMG node, connectivity with the raIns also became less positive from the Rest1 run to the N-back run (t(49) = 4.98, p < .001), but then became more positive again from the N-back run to the Rest2 run (t(49) = 4.71, p < .001), returning all the way back to baseline such that no difference was observed between the Rest1 and Rest2 runs (t(49) = 1.66, p = .10). Finally, State did not appear to affect connectivities of the raIns with the dACC or rdlPFC nodes, as no significant pairwise connectivity comparisons emerged (t(49) < 2.0, p > .05). These findings indicate that the N-back task induced connectivity between the raIns and laIns, ldlPFC, and rSMG nodes to become less positive, and that connectivities remained less positive in the laIns and ldlPFC nodes during the subsequent resting state than they were in the initial resting state.
Figure 3: Task-positive network nodes (brain slices) and within-TPN connectivity strength in those nodes (small graphs) and across the whole network (larger bottom graph) measured during the baseline Rest1 scan, the N-back working memory scan, and the post-task Rest2 scan. The seed region for connectivity calculations was the right anterior insula (in green). Unless otherwise indicated, * indicates significant differences from Rest1 at p<.05.

Effect of task connectivity and performance on subsequent resting state connectivity

We examined whether the strength of within-DMN, DMN-TPN, or within-TPN connectivity observed during the N-back task predicted the subsequent Rest2 connectivity. We found that N-back connectivity did not predict Rest2 connectivity in within-DMN (r(48) = .07, p = .61) or
DMN-TPN ($r(48) = .20$, $p = .16$) connections, but it did predict Rest2 connectivity in within-TPN connections ($r(48) = .31$, $p = .031$); see Figure 4A. As the previous analyses indicated that within the TPN, persistent post-N-back changes in connectivity were localized to raIns-laIns and raIns-ldIPFC connections, we conducted post-hoc nodewise correlations between N-back and Rest2 connectivity in these two connections. We found that N-back connectivity predicted Rest2 connectivity in the raIns-laIns connection ($r(48) = .34$, $p = .017$; Figure 4B) but not the raIns-ldIPFC ($r(48) = .14$, $p = .34$). We further investigated whether reaction time on the N-back task also predicted subsequent Rest2 connectivity in these two connections. We found that RT predicted Rest2 connectivity in the raIns-laIns connection ($r(48) = -.28$, $p = .05$; Figure 4C), but not in the raIns-ldIPFC connection ($r(48) = .11$, $p = .45$). These findings suggest that the reduction in positive connectivity that persists after the N-back task within the interhemispheric aIns connection is predicted by the strength of the connectivity during the task, as well as by the speed of performance on the task, such that less positive N-back connectivity and slower N-back performance predict less positive resting-state connectivity afterwards.
Figure 4: A) Connectivity during the N-back task predicts subsequent resting-state connectivity within the TPN but not within the DMN or in DMN-TPN connections. B) Post-hoc nodewise analyses show that relationships between N-back connectivity and Rest2 connectivity are strongest in the connection between right and left anterior insula. C) Post-task Rest2 connectivity between right and left anterior insula was predicted by the speed of N-back performance.

**Associations between within-DMN connectivity and trait-level Inattentiveness:**

We examined whether within-DMN connectivity predicted Inattentiveness during the initial resting-state scan, and whether it continued to predict Inattentiveness during and after the N-back task. After correcting for the three correlations conducted, we found that overall connectivity within the DMN did predict Inattentiveness during the Rest1 run ($r(48) = -.36$, $p = .01$) and the N-back task ($r(48) = -.36$, $p = .009$) but not during the Rest2 run ($r(48) = .065$, $p = .65$); see Figure 5A. Meng’s t-tests indicated that this correlation was higher in both the Rest1 ($t(47) =$...
2.90, \( p < .01 \) and N-back runs \( (t(47) = 2.33, \ p < .05) \) than in the Rest2 run, with no differences observed between the Rest1 and N-back runs \( (t(47) = .016, \ p > .9) \). These findings indicate that individuals who were less inattentive demonstrated more positive connectivity within the DMN during the Rest1 and N-back runs, but that the degree of Inattentiveness had no association with resting connectivity after the N-back task. Post-hoc correlations between Inattentive scores and each separate within-DMN connection indicated that increased Inattentiveness was most strongly predicted by a stronger PCC-IAG connection during the Rest1 \( (r(48) = -.31, \ p = .028) \) and N-back \( (r(48) = -.29, \ p = .039) \) runs, but the relationship emerged only at trend level, and, notably, in the opposite direction during the Rest2 run \( (r(48) = .26, \ p = .072) \); see Figure 5B. The PCC-Prec and PCC-mPFC connections also demonstrated negative correlations with Inattentiveness at trend level during the Rest1 and N-back runs \( (rs(48) < -.24, \ ps < .10) \), but no significant correlation during the Rest2 run \( (|rs(48)| < .15, \ ps > .45) \). By contrast, no significant associations emerged between Inattentive scores and within-TPN or DMN-TPN connections.
Discussion

The present study examining the status of functional connectivity networks during working memory (WM) engagement and after its disengagement led to two principal novel findings. First, connectivity with the task-positive network (TPN), both within the network and in cross-network connections to the default mode network (DMN), was altered during N-back task performance relative to the resting state preceding the task. Critically, this altered connectivity persisted following task completion and into the subsequent resting state. Additionally, individuals with a less coordinated TPN during the N-back task demonstrated a less coordinated TPN after the task. Second, associations between DMN connectivity and self-reported...
inattentiveness were robust during the pre-task resting state and during the N-back task, but were eliminated during the post-task resting state. These findings suggest that brain network function is altered during the period immediately following WM performance, with properties that are associated with the preceding cognitive engagement.

**Persistence of working memory-induced functional connectivity**

This study is the first to demonstrate that alterations in functional connectivity during WM task performance can persist beyond the task’s conclusion and into the subsequent resting state. For both within-network connections between the rINs and lateral frontal, parietal, and insular TPN nodes, as well as for cross-network connections between the PCC and TPN nodes, connectivity during the WM task was altered compared to the baseline resting state. These changes largely did not fade away after the task concluded, as connectivity during the immediately following resting state remained altered compared to the baseline resting state. Persistently altered connectivity within the task-relevant network has been previously observed after sensorimotor [finger tapping (Duff et al., 2008), motor learning (Albert et al., 2009; Vahdat et al., 2011), and visual learning (Lewis et al., 2009)], language [language generation (Waites et al., 2005) and semantic classification (Grigg and Grady, 2010)], and episodic memory [face-object pair encoding (Tambini et al., 2010)] tasks. Similar to several of those previous studies (Duff et al., 2008; Grigg and Grady, 2010; Lewis et al., 2009), we also observed pre/post-task connectivity changes between the task-relevant network and the DMN, indicating that both within- and between-network relationships are persistently affected by the performance of a task. The present findings extend this phenomena to WM, a key ability subserving effortful cognitive functions (Engle et al., 1999).
This study is also the first to demonstrate that the strength of resting state functional connectivity observed after completion of a task is predicted by the strength of connectivity during the task itself. Less positive overall connectivity of TPN nodes with the right anterior insula during the N-back task, most prominently with the homologous left anterior insula, predicted less positive connectivity in the same connections after the task, indicating that individuals who had low TPN coordination during the task, compared to other individuals, also had low TPN coordination during the period after the task. By contrast, post-task connectivity in cross-network DMN-TPN connections was not predicted by connectivity during the N-back task, suggesting that effects of the N-back task on cross-network connections may be indirect. The extent to which this relationship is stable will depend upon replication in future studies.

We offer three possible interpretations of the functional significance of the observed findings that we cannot confirm with the present data but which could be tested in future work. First, the persistence of WM-related connectivity changes may reflect a cognitive aftereffect, such that the brain continues to perform the N-back task even after the task stimulation has ceased. However, our results showing selectivity in the persistence of connectivity alterations at the network level (e.g., within-TPN and DMN-TPN but not DMN) and at the connection level (e.g., ralIns with laIns and ldlPFC but not ralIns with dACC and rdPFC) go against this interpretation, as all N-back task-related changes in connectivity ought to have persisted if the brain continued to be similarly engaged in the post-task period. Instead, the specific connections that were persistent—and particularly the persistent connection that was most predictive of individual variation in post-task connectivity, the connection between bilateral anterior insula—has been previously associated with interoceptive processing (Critchley et al., 2004). We speculate that persistent connectivity changes in this connection may reflect persistence of the subjective aspects of an
effortful cognitive experience. This speculation can be tested empirically in the future. Second, the persistence of WM-related connectivity changes may reflect consolidation of the N-back experience. Indeed, studies of resting periods following skill learning (Albert et al., 2009; Duff et al., 2008; Lewis et al., 2009) and memory encoding (Tambini et al., 2010) suggest that post-task connectivity changes reflect consolidation of those experiences in the service of learning. Although subjects were not instructed to learn anything in the present study, any experience has the potential to be a memory and will be consolidated following its conclusion.

Third, persistence of WM-related connectivity may reflect a post-task recovery period following effortful cognition that reflects persistent depletion of cognitive resources. This interpretation is based on the direction of connectivity changes we observed (e.g., less positive connectivity within the TPN and less negative DMN-TPN connectivity during and after the WM task), which have been associated with worse cognitive performance. Less positive connectivity within the TPN predicted reduced executive control abilities (Gordon et al., 2011), and less negative DMN-TPN connectivity was associated with increased inattentiveness and impulsivity (Chapter V), increased trial-to-trial behavioral variability (Kelly et al., 2008) and reduced working memory performance (Hampson et al., 2010; Sala-Llonch et al., 2012). This interpretation also agrees with Barnes et al. (2009), who posited that such a recovery period is required following effortful N-back task performance and that it scales with the effort involved, as they observed that post-task oscillatory alterations returned to baseline about 8 minutes after a 1-back task and about 15 minutes after a 2-back task. In the present task, although accuracy was very high (see Supplementary Materials), verbal reports by subjects indicated that they found the task to be highly effortful. Indeed, we found that subjects who were slower on the N-back task (indicating that more cognitive effort was required for performance) demonstrated less positive
within-TPN connectivities after the completion of the task than subjects who were faster. While this correlation would not have survived a stricter statistical threshold controlling for multiple comparisons, it suggests that it may be worthwhile testing whether the persistent suboptimal network configurations we observed may correspond to the state of depleted cognitive resources and mental fatigue experienced during (Kato et al., 2009; Lorist, 2008) and after (Holtzer et al., 2010; van der Linden et al., 2003) periods of demanding cognition. Unfortunately, our post-task resting state period did not extend long enough to examine whether the timecourse of network recovery to baseline that was observed by Barnes et al. (2009) may generalize to connectivity strength.

The present results may help disambiguate mixed findings of how within- and between-network functional connectivity changes from the resting state to WM task performance. Compared to a resting state, within-TPN connectivity during an N-back task became both more positive (Chapter V; Newton et al., 2011) and less positive (Fransson, 2006; Repovš and Barch, 2012), as in the present study. Within-DMN connectivity both remained unchanged in primary DMN nodes (Bluhm et al., 2011; Chapter V), became less positive (Fransson, 2006; Newton et al., 2011), as in the present study, and became more positive at low loads but less positive at high loads (Repovš and Barch, 2012). Further, cross-network DMN-TPN connectivity became more negative (Fransson, 2006; Repovš and Barch, 2012), more positive (Bluhm et al., 2011), similar to the present study, and changed inconsistently depending on the specific TPN node (Chapter V). One possible explanation for these varied results is that the studies differed in when the resting state session was performed, reflecting a mixture of pre- and post-task connectivity changes. Chapter V and Newton et al. (2011) conducted resting scans after the WM task, while Fransson (2006) counterbalanced scan order across subjects, Bluhm et al. (2011) conducted
resting scans interspersed with the task, and (Repovš and Barch, 2012) did not report the order of scanning. Thus, in all five studies, the resting state scan may have included some remnants of connectivity changes from the preceding WM task for at least some subjects. The present findings thus draw attention to the fact that the order in which resting and task scans are collected may have a substantial impact on measurements of functional connectivity.

**Association of DMN connectivity with individual variation in inattentiveness**

The present study is the first to demonstrate that healthy adults with lower self-reported inattentiveness have more positive functional connectivity within the DMN during a baseline resting state session. Previous work has established that DMN engagement (either strong within-network resting state functional connectivity or strong task-related deactivation) predicts improved performance across a variety of cognitive domains, including self-referential processing and rumination (Zhu et al., 2012), executive function and processing speed (Andrews-Hanna et al., 2007), cognitive control (Eichele et al., 2008; Li et al., 2007), working memory performance (Hampson et al., 2006; Sala-Llonch et al., 2012; Sambataro et al., 2010), and behavioral symptoms of inattentiveness (Brown et al., 2011) and distractibility (Fassbender et al., 2009) in children and adults with Attention Deficit Hyperactivity Disorder (ADHD). The present results show that the association with behavioral inattentiveness extends to healthy young adults who do not meet criteria for ADHD.

Notably, this association with trait-level inattentiveness was observed both in the resting state preceding working memory engagement and during working memory, but not in the resting state following the task. This lack of connectivity-behavior correlations during the post-task session could be explained by the substantial individual variation in post-task resting state network connectivity strengths (as shown by the wide range of Z(r) values in Figure 4A). If post-task
changes in resting state connectivity are due to a recovery period following demanding cognitive effort, as posited above, individual differences in the timing of recovery may function as a source of unexplained variance that would weaken the ability to detect a correlation between behavior and connectivity strength. From a methodological perspective, the fact that resting state connectivity can be altered enough by a previous task to eliminate correlations between connectivity and behavior means that scanning protocols should be designed with care so that resting state connectivity is not contaminated by the effects of previous tasks.

**Caveats**

The following factors ought to be noted, as they may influence comparison of functional connectivity measures during the resting-state and the working memory task. First, the working memory run has one more nuisance regressor (load) than the resting run. Regressing out load is necessary in order to directly compare the resting and working memory runs, as it ensures that connectivity is not being driven by load-related activation differences, as discussed in the Methods section and in previous work (Chapter V). Second, the acquisition voxel size differed between the resting and working memory runs. However, previous work has indicated that differences in voxel size do not affect measures of functional connectivity (Van Dijk et al., 2010). Finally, the design of the present study cannot account for other possible changes in a subject’s cognitive state over the course of the scanning session, which may include increasing fatigue and decreasing alertness. Thus, it is possible that subjects were more drowsy or tired in the second rest than in the initial rest session, which may have driven some portion of the connectivity alterations (though the fact that within-DMN connections were not persistently altered mitigates this concern to some extent). A study design that would have controlled for this (e.g., a control group scanned during Rest1-Rest2-Rest3 or counterbalanced task and rest runs) is
needed in order to definitively determine a causal effect of the WM task on post-task resting state.

**Conclusions**

Functional connectivity within the task-positive network, as well as between the default mode and task-positive networks, was altered during working memory performance compared to a baseline resting state. Most importantly, these alterations in connectivity persisted after disengagement from working memory, during the subsequent resting state. We characterized two properties of this persistently altered connectivity. First, both slower working memory performance and weaker connectivity between two key task-positive nodes during working memory predicted weaker connectivity in that connection after working memory disengagement, suggesting that the putative post-task recovery period was associated with cognitive effort during task performance. Second, while stronger default mode connectivity measured during the baseline resting state and during the working memory task predicted lower inattentiveness, this brain-behavior association disappeared during the immediately following resting state. This suggests that post-task recovery from cognitive effort may obscure connectivity-behavior relationships that are observable before and during a task. These findings have important implications not only for models of how the brain recovers following transient effortful cognition, but also for the design of future studies investigating the resting state and rest-task relationships.
CHAPTER IV: STRIATO-FRONTAL FUNCTIONAL CONNECTIVITY IS SENSITIVE TO DAT1 GENOTYPE AND PREDICTS EXECUTIVE FUNCTION.

Introduction

Executive control is a constellation of processes that support the deployment of cognitive resources in a goal-directed manner; these processes include working memory (WM), defined as the temporary maintenance and on-line manipulation of information, as well as the inhibitory control of impulsivity. Executive control processes depend on the function of striato-thalamo-cortical loops, which are believed to allow the striatum to dynamically gate and/or update information represented in prefrontal cortex (PFC) (Hazy et al., 2007; van Schouwenburg et al., 2010). In humans, the function of these loops can be measured indirectly by assessing functional connectivity in functional magnetic resonance imaging (fMRI) scans, a technique which relies on the correlation of blood oxygen level-dependent (BOLD) signal timecourses across different brain regions to infer the strength of neural communication between those regions. This technique is most commonly conducted during the task-free “resting state”, in which correlations due to neural communication can be disentangled from task-driven coactivation. Using this technique, increased functional connectivity between striatum and prefrontal cortex has been shown to predict faster (Tu et al., 2012) and more accurate WM performance (Klostermann et al., 2012), as well as reduced impulsivity (Cole et al., 2012). However, the genetic bases of the relationship between striato-frontal connectivity and executive control has not been well explored.

Both executive function and striato-frontal connectivity are known to be strongly dependent on dopamine (DA) signaling. Performance of an executive task such as WM induces phasic DA
release (Aalto et al., 2005), which serves as a “gating” signal that regulates access to information being maintained in prefrontal cortex (Braver and Cohen, 2000; Hazy et al., 2007). Both pharmacologic manipulations of DA and genetically-influenced individual differences in baseline DA signaling can have substantial effects on executive control function (Cools and D’Esposito, 2011). Further, several lines of research suggest that striato-frontal connectivity is sensitive to the same effects on DA function that modulate executive function. First, individual differences in DA synthesis capacity in the striatum predicted the strength of striato-frontal connectivity (Klostermann et al., 2012). Second, pharmacologic manipulations which either increase or decrease DA signaling have been shown to increase (Cole et al., 2012; Kelly et al., 2009) or decrease (Cole et al., 2012; Nagano-Saito et al., 2008) connectivity between striatum and PFC, respectively. Third, imaging genetics studies have shown that genetic regulation of DA signaling can impact functional connectivity, as connectivity in healthy adults is affected by polymorphisms of several genes which code for molecular mechanisms that regulate DA, including the catechol-O-methyltransferase gene (COMT: Liu et al., 2010; Sambataro et al., 2009; Tan et al., 2007), the DA receptor D2 gene (DRD2: Blasi et al., 2009; Cohen et al., 2007; Klein et al., 2007; Stelzel et al., 2010), and the DA transporter gene (DAT1: Chapter V; Taurisano et al., 2012). However, only two of these previous studies have investigated effects of these genes on striato-frontal circuitry (Cohen et al., 2007; Stelzel et al., 2010), and neither of these two studies evaluated functional connectivity during the resting state, in which the intrinsic network structure of the brain can measured without contamination by effects of a task. In summary, previous work suggests the possibility of strong causal links between DA signaling, striato-frontal connectivity, and executive function; however, the genetic factors which may influence these links have not been well explored.
Several lines of evidence converge to suggest that a genetic polymorphism of the DAT1 gene may mediate the relationship between DA signaling, striato-frontal connectivity, and executive function. First, the DAT1 gene, which codes for the DA transporter (DAT) protein that regulates DA signaling by re-uptaking DA following its release (Madras et al., 2005), has a variable number of tandem repeats (VNTR) polymorphism in its 3’-untranslated region. The polymorphism’s two most common alleles, with 9 and 10 repeats, appear to influence the expression of DAT in vitro (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005), with greater DAT expression associated with 10-repeat compared to 9-repeat alleles (though in vivo findings have been mixed; Heinz et al., 2000; Jacobsen et al., 2000; Krause et al., 2006). This genotype difference in DAT expression likely leads to differences in DA signaling, with the 9-repeat resulting in elevated DA signaling due to reduced clearance (Madras et al., 2005). Second, compared to individuals with at least one copy of the 9-repeat DAT1 allele, inheritance of two 10-repeat alleles (a 10/10 genotype) has been associated with worse executive control function, including reduced WM performance (Stollstorff et al., 2010), reduced benefits of WM training (Brehmer et al., 2009), increased impulsivity (Gizer and Waldman, 2012) and hyperactivity (Mill et al., 2005), and worse inhibitory control (Cornish et al., 2005). Third, inheritance of two 10-repeat alleles has been associated with differences in brain function during WM, including reduced striatal activation (Stollstorff et al., 2010) and less negative cortico-cortico connectivity that predicted increased impulsivity (Chapter V). Fourth, despite the fact that the DAT protein is highly concentrated in the striatum but only weakly concentrated in PFC (Hall et al., 1999; Madras et al., 2005; Sasaki et al., 2012), 10/10 homozygotes had reduced PFC activation compared to 9-repeat carriers during WM (Bertolino et al., 2006, 2008, 2009; Caldú et al., 2007; Stollstorff et al., 2010). This suggests that DAT1-related effects on DA function in striatum are
affecting PFC, likely via striato-frontal connections. Finally, the 10/10 genotype has been associated with ADHD (Yang et al., 2007), a disorder which also demonstrates increased impulsivity, reduced WM function (Van De Voorde et al., 2010), and reduced striato-frontal connectivity during executive function tasks (Cubillo et al., 2010; Rubia et al., 2009). Despite these suggestive findings, no previous study has investigated whether putative differences in DA function due to DAT1 genotype affect striato-frontal functional connectivity, and whether those connectivity differences affect executive function.

To investigate whether the DAT1 gene affects striato-frontal connectivity and executive function during the resting state, we conducted seed-based functional connectivity analyses in 50 healthy young adults. We defined connectivity seeds based on a previous investigation of striatal functional connectivity (Di Martino et al., 2008), and we conducted a voxelwise analysis to determine whether connectivity with these seeds differed between subjects with a 9/10 genotype and subjects with a 10/10 genotype. Based on previous findings that the 9-repeat allele results in increased striatal DA function, and that increased DA function has been associated with stronger striato-frontal connectivity, we predicted that 9/10 subjects would demonstrate stronger connectivity between the striatum and the PFC than 10/10 subjects. Additionally, subjects self-reported their trait-level impulsivity using the Barratt Impulsiveness Scale (Patton et al., 1995), and subjects’ WM function was tested using an N-back working memory task conducted outside the scanner. We then investigated whether DAT1-related differences in striatal-frontal connectivity were predictive of subjects’ WM function or impulsivity. As stronger striato-frontal connectivity has been associated with superior WM and reduced impulsivity, we predicted that DAT1-related increases in connectivity would be associated with faster and more accurate performance on the N-back task, as well as with lower self-reported trait-level impulsivity.
Materials and Methods

Subjects: One hundred fifty-eight Georgetown University undergraduates aged 18 to 22 years provided saliva samples that were genotyped for DAT1. Subjects were randomly invited to participate in the study for payment from the pools of 10/10 and 9/10 carriers until approximately equal samples were obtained (9/10: n = 24, mean ± SD age = 20.42 ± .85, 7 male; 10/10: n = 26, age = 20.42 ± .96, 8 male). Genotype groups did not differ in either age or gender (ps > .9). Exclusion criteria included self-reports of 1) use of psychotropic medication (e.g., stimulants, SSRIs); 2) overt neurological injury or disease, seizure disorder, psychiatric diagnosis; 3) contraindications for MRI – e.g., presence of metal, pregnancy. All subjects gave informed consent in accordance with guidelines of the Georgetown University Institutional Review Board.

Genotyping: DNA was extracted from Oragene saliva kits (DNA Genotek Inc., Ottawa, Ontario, Canada). The 40 base pair VNTR polymorphism in the 3’ UTR of DAT1 was genotyped by PCR as previously described (Daly et al., 1999) using the following primers; Forward: 5’-TGTGGTGTAGGGAACGGCCTGAG-3’ Reverse: 5’-CTTCCTGGAGGTACCGCTCAAGG-3’. PCR was performed using the AccuprimeTM Taq DNA polymerase system (Invitrogen) with the following PCR program: 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 68°C for 1 min. The PCR products were then run out on a 2% agarose gel stained with ethidium bromide. A 100 bp DNA ladder was then used to identify the various repeat alleles by size: 7-repeat (360bp), 8-repeat (400bp), 9-repeat (440bp), 10-repeat (480bp), and 11-repeat (520bp). Genotyping was successful for 153 of the 158 subjects in the original sample. Observed genotypic frequencies in the sample
were: 10/10 - 60.1%; 9/10 - 33.3%; 9/9 – 5.9%; other, 0.6%.

**Behavioral testing:** All behavioral testing was conducted at the time of genotyping, approximately two months before scanning.

*Trait measure of impulsivity:* Subjects completed the Barratt Impulsiveness Scale version 11 (Patton et al., 1995), which reported trait measures of Impulsiveness (higher score = more impulsive).

*Working memory task:* Subjects performed a computerized N-back task consisting of six 72s N-back blocks which alternated between 2- and 3-back conditions. Each N-back block consisted of twenty-four serially presented consonants appearing for 500ms, with an inter-trial-interval of 2500ms. Each block was preceded by a screen informing the subject of the N-back condition. Subjects were instructed to press the space bar when the current letter matched the letter n trials ago (e.g., for the 2-back condition, subjects see: R V N W N – button-press for N). Targets were present on 21% of trials. Neither condition contained sequences of stimuli that were targets in the other condition. Stimuli were presented using E-Prime (Psychology Software Tools Inc., Pittsburg, PA).

For each condition, accuracy was calculated as % targets identified minus % false alarms. Reaction time (RT) was calculated as the average reaction time to successfully identified targets.

**fMRI data acquisition:** Subjects were scanned for 5:04 minutes during the resting state, in which they were told to relax with eyes closed and to not think of anything in particular. Imaging was performed on a Siemens Trio 3T scanner (Erlangen, Germany). 152 whole-brain images were acquired using a gradient echo pulse sequence (37 slices, TR = 2000 ms, TE = 30 ms, 192x192mm FOV, 90 degree flip angle, voxel dimensions 3mm isotropic). The first 4 images of the resting run were discarded to allow for signal stabilization. Additionally, a high resolution
T1-weighted structural scan (MPRAGE) was acquired with the parameters: TR/TE=2300/2.94ms, TI=900ms, 90 degree flip angle, 1 slab, 160 sagittal slices with a 1.0mm thickness, FOV=256x256mm2, matrix=256x256, resulting in an effective resolution of 1.03mm isotropic voxels.

**Image preprocessing:** Using SPM8 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (Version 7.10 Mathworks, Inc., Sherborn, MA), images were corrected for translational and rotational motion by realigning to the first image of the scan. Across all runs, all subjects demonstrated less than 2.0mm of translational motion in any one direction (max translation = 1.25mm) and less than 1.0° of rotation around any one axis (max rotation = .54°). Images were then slice-time corrected, normalized to an EPI template, resampled every 2mm, and smoothed using a Gaussian kernel with full-width at half-maximum (FWHM) of 8mm. A band-pass filter was applied to the data in order to restrict signal variation to frequencies between .01 Hz and .1 Hz, corresponding to the frequency range established in the literature for fluctuations in resting state data (Biswal et al., 1995). Finally, because even small amounts of motion (<2mm) can affect measures of functional connectivity (Van Dijk et al., 2012; Power et al., 2012), we employed the “scrubbing” technique from Power et al. (2012) to eliminate contributions of motion. For each timepoint, framewise displacement (FD) was calculated as the distance moved relative to the previous timepoint, and scans with FD>.5mm were removed from further analysis. The percent of scans removed by scrubbing was very low on average (mean ± SD: 3.43% ± 6.70%), and the number of scans removed did not differ by genotype (9/10: 3.24% ± 6.64%; 10/10: 3.61% ± 6.87%; t(48) = .20, p = .75) and was not correlated with any N-back or Impulsivity behavioral measure (absolute value of all r(48)s < .17; all ps > .25). Forty-seven of the 50 subjects retained at least 85% of their scans. The remaining
three subjects (one 9/10; two 10/10) retained 77.7%, 70.9%, and 68.9% of their scans, representing at least 3:45 min of scanning time in all subjects.

**Functional connectivity calculation:**

_**Striatal seed creation.**_ Using Marsbar (Brett et al., 2003), bilateral striatal seeds were created as spheres of radius 6mm centered around four of the six left/right mirrored coordinates used by Di Martino et al. (2008). These bilateral seeds were in the dorsal caudate (DC), inferior ventral striatum (VSi), ventral rostral putamen (VRP), and dorsal caudal putamen (DCP). Seed timecourses were calculated as the average timecourse of all voxels in each seed. Di Martino et al. (2008) also reported connectivity from superior ventral striatum and dorsal caudal putamen, but found that these connectivity patterns were very similar to those in the nearby VSi and DCP, respectively; as a result, we excluded these two (arguably redundant) seeds from our analyses. Seeds are shown in Figure 1, left column.

_**Nuisance signal identification.**_ To identify the effects of motion and physiological noise (such as respiration and heart rate) that would be common to all voxels, timecourses approximating these signals were calculated. Physiological noise timecourses were approximated by obtaining signal timecourses from white matter and CSF segmentations of the MPRAGE image (Van Dijk et al., 2010). Motion timecourses were obtained as the six realignment parameter timecourses from the motion correction preprocessing step, expressed as absolute differences from the first timepoint in each of the three translation and rotation directions. Notably, the global signal was not included as a nuisance signal, as recent work suggests that regression of the global signal may reduce the accuracy of connectivity estimates (Saad et al., 2012).

_Voxelwise intrinsic functional connectivity calculation._ For each subject, partial correlations
were conducted between each seed’s timecourse and the timecourses of every voxel in the brain, while partialling out the motion and physiological noise timecourses. The resulting $r$ values were converted to $Z$-scores using Fisher’s transformation in order to increase normality of the distribution, allowing further statistical analysis of correlation strengths. This produced a brain map of the strength of intrinsic connectivity with each seed.

**Statistical analysis:**

*Overall connectivity.* For each seed in each genotype group, we identified overall patterns of connectivity with the seed by entering subjects’ individual connectivity maps into a voxelwise one-sample $t$-test using SPM8. Results were thresholded at $p<.001$ uncorrected for display purposes.

*DAT1 effects on connectivity.* For each seed, we tested for effects of *DAT1* on striatal functional connectivity by entering each subject’s connectivity map into a voxelwise two-sample $t$-test using SPM8. Results were corrected for multiple comparisons (including both the number of voxels and the number of seed maps tested) at $p<.05$ using Monte-Carlo simulation (Ward, 2000), which established the correction threshold at $p<.001$, $k=64$.

**Association between *DAT1*-modulated functional connectivity and executive function:**

To determine whether striatal connectivity with any *DAT1*-modulated region predicted executive function, we examined correlations between *DAT1*-modulated striatal connectivity and our behavioral measures. Regions of interest (ROIs) were created as the distinct clusters of voxels in which striatal connectivity significantly differed by *DAT1* genotype. For each of these ROIs, each subject’s mean connectivity strength was calculated between the striatal seed and all voxels in the ROI. For each behavioral measure (Impulsiveness, 2- and 3-back accuracy, and 2- and 3-back RT), we conducted a hierarchical stepwise multiple regression which attempted to explain
the variance in that measure with the variance in striatal connectivity strengths in each ROI. Significant associations were further examined using post-hoc bivariate correlations.

**Results**

**Behavioral measures:**

*Trait-level Impulsiveness*: Across all subjects, the trait-level measure of Impulsiveness was 55.66 ± 7.39. Genotype groups did not differ in Impulsiveness (9/10: 54.00 ± 6.32; 10/10: 57.19 ± 8.06; t(48) = 1.55, p = .13).

*Working memory performance*: Across all subjects, working memory accuracy was higher in the 2-back condition (91.0% ± 9.5%) than in the 3-back condition (80.0% ± 18.5%; t(49) = 4.47, p<.001). Accuracy did not differ between genotype groups in either the 2-back condition (9/10: 92.8% ± 6.8%; 10/10: 89.3% ± 11.3%; t(48) = 1.30, p = .20) or the 3-back condition (9/10: 83.3% ± 18.4%; 10/10: 76.8% ± 18.3%; t(48)=1.26, p = .22).

Similarly, RT across all subjects was faster in the 2-back condition (574.0 ± 151.8 ms) than in the 3-back condition (685.9 ± 241.7 ms; t(49) = 5.83, p<.001). However, RT did not differ between genotype groups in either the 2-back condition (9/10: 554.2 ± 139.4 ms; 10/10: 592.3 ± 163.0 ms; t(48) = .89, p = .38) or the 3-back condition (9/10: 656.8 ± 221.4 ms; 10/10: 712.7 ± 260.4 ms; t(48) = .81, p = .42).

**Striatal connectivity networks**: Striatal connectivity networks in each group are shown in Figure 1, middle columns. Across groups, common patterns of connectivity emerged that were very similar to those observed by Di Martino et al. (2008) using the same seeds, as follows:

*DC seed*: The bilateral DC seed was strongly connected to perigenual and dorsal anterior cingulate extending into medial prefrontal cortex and supplementary motor area, bilateral
anterior insula extending into bilateral inferior frontal gyrus, and bilateral posterior middle frontal gyrus. Small clusters of high connectivity also emerged in bilateral angular gyrus, bilateral anterior thalamus, bilateral rostral putamen, left middle temporal gyrus, and dorsal pons.

**VSi seed:** The bilateral VSi seed was strongly connected to subgenual and anterior cingulate extending into ventral and anteromedial prefrontal cortex, and posterior cingulate extending into ventral putamen. Small clusters of high connectivity also emerged in middle cingulate cortex, anteromedial thalamus, bilateral anterior middle temporal gyrus, and bilateral temporal pole.

**DCP seed:** The bilateral DCP seed was strongly connected to bilateral insula extending into superior temporal gyrus, bilateral primary motor cortex, middle cingulate extending into supplementary motor area, and bilateral supramarginal gyrus. Small clusters of high connectivity also emerged in bilateral middle frontal gyrus, bilateral inferior frontal gyrus, bilateral central thalamus, bilateral posterior middle temporal gyrus, and bilateral cerebellum.

**VRP seed:** The bilateral VRP seed was strongly connected to dorsal anterior and middle cingulate extending into supplementary motor area, bilateral anterior insula, bilateral caudate, bilateral anterior middle frontal gyrus, and bilateral supramarginal gyrus. Small clusters of high connectivity also emerged in bilateral primary motor cortex, bilateral anterior and central thalamus, and bilateral posterior middle temporal gyrus.

**Effects of DAT1 genotype on striatal connectivity:** For each seed, we examined whether DAT1 genotype groups differed in functional connectivity with that seed. Results are shown in Table I and Figure 1, right column.

**DC seed:** 9/10 subjects demonstrated significantly greater functional connectivity with the DC than 10/10 subjects in dorsal anterior cingulate, supplementary motor area, bilateral anterior insula, and left anterior middle frontal gyrus. 10/10 subjects did not demonstrate greater
functional connectivity with DC than 9/10 subjects in any region.

*VSi seed:* 9/10 subjects demonstrated significantly greater functional connectivity with the VSi than 10/10 subjects in two clusters within left inferior frontal gyrus. 10/10 subjects did not demonstrate greater functional connectivity with VSi than 9/10 subjects in any region.

*Putamen seeds:* No effects of DAT1 were observed on functional connectivity with either the DCP or the VRP seeds.

In summary, subjects with the 9/10 DAT1 genotype demonstrated increased connectivity between the caudate (but not the putamen) and various regions in the frontal cortex. Subjects with the 10/10 genotype did not demonstrate increased striatal connectivity with any region.

<table>
<thead>
<tr>
<th>Seed</th>
<th>9/10 Connectivity</th>
<th>10/10 Connectivity</th>
<th>9/10 &gt; 10/10 Connectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td><img src="image1" alt="Connectivity Images" /></td>
<td><img src="image2" alt="Connectivity Images" /></td>
<td><img src="image3" alt="Connectivity Images" /></td>
</tr>
<tr>
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<td><img src="image5" alt="Connectivity Images" /></td>
<td><img src="image6" alt="Connectivity Images" /></td>
</tr>
<tr>
<td>DCP</td>
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<td><img src="image8" alt="Connectivity Images" /></td>
<td><img src="image9" alt="Connectivity Images" /></td>
</tr>
<tr>
<td>VRP</td>
<td><img src="image10" alt="Connectivity Images" /></td>
<td><img src="image11" alt="Connectivity Images" /></td>
<td><img src="image12" alt="Connectivity Images" /></td>
</tr>
</tbody>
</table>

Figure 1: T-tests of connectivities from the four bilateral striatal seeds (first column) reveals regions which showed strong connectivity across all subjects with the 9/10 (second column) and 10/10 (third column) genotypes. Two-sample t-tests (fourth column) reveal regions in which 9/10 individuals had significantly stronger connectivity with the DC and the VSi than 10/10.
individuals. No region demonstrated greater connectivity for 10/10 than 9/10 individuals, and no effects of DAT1 were found for DCP- or VRP-seeded connectivity.

<table>
<thead>
<tr>
<th>Cluster Location</th>
<th>Peak Coordinates</th>
<th>Peak T value</th>
<th>Cluster Size (voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed: Dorsal Caudate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/10 &gt; 10/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal anterior cingulate</td>
<td>6, 24, 28</td>
<td>5.54</td>
<td>530</td>
</tr>
<tr>
<td>Right anterior insula</td>
<td>42, 10, -2</td>
<td>4.43</td>
<td>164</td>
</tr>
<tr>
<td>Supplementary motor area</td>
<td>6, -6, 66</td>
<td>3.90</td>
<td>121</td>
</tr>
<tr>
<td>Left anterior insula</td>
<td>-36, 18, 6</td>
<td>3.71</td>
<td>69</td>
</tr>
<tr>
<td>Left middle frontal gyrus</td>
<td>-40, 46, 26</td>
<td>3.45</td>
<td>84</td>
</tr>
</tbody>
</table>

| Seed: Inferior Ventral Striatum |                  |              |                       |
| 9/10 > 10/10                    |                  |              |                       |
| Left posterior inferior         | -52, 12, 28      | 4.91         | 92                    |
| frontal gyrus                   |                  |              |                       |
| Left anterior inferior          | -36, 44, 6       | 4.52         | 201                   |
| frontal gyrus                   |                  |              |                       |

Table I: Clusters demonstrating differences in striatum-seeded connectivity between 9/10 and 10/10 subjects

**Associations between DAT1-modulated functional connectivity and executive function:**

We created ROIs from the seven clusters found to be significant in the analysis above, and within those clusters calculated the average strength of connectivity with the appropriate striatal seed. For each behavioral measure, we then conducted a stepwise multiple regression model to examine whether any of these connectivity values predict the behavior. Results of the analysis can be seen in Figure 2.

**Impulsiveness:** Reduced Impulsiveness was predicted by increased connectivity between VSi and posterior inferior frontal gyrus ($r(48) = -0.32, p = 0.026$).

**Working memory performance:** In the 2-back condition, increased accuracy was predicted by increased connectivity between DC and right anterior insula ($r(48) = 0.33, p = 0.018$), while faster RT was predicted by increased connectivity between DC and dorsal anterior cingulate cortex.
(r(48) = -.31, p = .029). In the 3-back condition, both increased accuracy (r(48) = .32, p = .022) and faster RT (r(48) = -.32, p = .023) were predicted by increased connectivity between DC and left anterior middle frontal gyrus.

In summary, several of the striato-frontal connections that were shown to be stronger in 9/10 than in 10/10 individuals were correlated with behavior. In all cases, increased striato-frontal connectivity—which was present in the 9/10 genotype—predicted superior executive function.
Figure 2: *DAT1*-modulated functional connectivity predicted behavior. A) The strength of functional connectivity from DC to various PFC regions predicted 2- and 3-back accuracies (top row) and reaction times (bottom row). B) The strength of functional connectivity from VSi to left posterior IFG predicted self-reported Impulsiveness. RaIns: right anterior insula; L aMFG: left anterior middle frontal gyrus; dACC: dorsal anterior cingulate cortex; L pIFG: left posterior inferior frontal gyrus.
**Discussion**

The primary novel finding of this study was that a polymorphism of the dopamine (DA) transporter gene (*DAT1*), which affects synaptic dopamine reuptake in the striatum, influenced resting-state functional connectivity between the caudate and the prefrontal cortex, which in turn predicted working memory function and trait-level impulsivity.

*DAT1* effects on striato-frontal functional connectivity

This study is the first to demonstrate effects of the *DAT1* genotype on striato-frontal functional connectivity, and the first to demonstrate any effect of *DAT1* on resting-state functional connectivity. As hypothesized, individuals with one copy of the 9-repeat *DAT1* allele demonstrated greater striato-frontal connectivity than those with two copies of the 10-repeat allele. As *DAT1* is most strongly expressed in the striatum (Hall et al., 1999; Madras et al., 2005; Sasaki et al., 2012), and the 9-repeat allele has been linked to greater DA signaling via reduced synaptic DA clearance compared to the 10-repeat allele (Madras et al., 2005), this finding suggests that *DAT1*-related differences in striatal DA signaling affect the communication between striatum and PFC, which may help explain how previous work has found *DAT1* effects in PFC (Bertolino et al., 2006, 2008, 2009; Caldú et al., 2007; Chapter V; Stollstorff et al., 2010) despite the relatively low expression of *DAT1* in that region (Hall et al., 1999; Madras et al., 2005; Sasaki et al., 2012). This finding extends previous work demonstrating that striato-frontal connectivity is increased by a variety of factors which increase DA signaling, including increased striatal DA synthesis capacity (Klostermann et al., 2012), L-dopa administration (Cole et al., 2012; Kelly et al., 2009), and cabergoline administration (Cohen et al., 2007, though this effect depended on the DA receptor D2 gene); and that it is decreased by factors which reduce
DA signaling, including haloperidol (Cole et al., 2012) and a DA depletion procedure (Nagano-Saito et al., 2008).

Subjects with a 9/10 genotype had greater resting-state connectivity than 10/10 subjects between subregions of the caudate and a set of regions which strongly overlaps with a previously-described Cingulo-opercular functional network. This network, which is commonly identified in independent component analyses (ICA) of resting-state data (e.g., Beckmann et al., 2005; Damoiseaux et al., 2006; Chapter II), demonstrates strong intrinsic connectivity between a variety of regions, including dorsal anterior cingulate cortex extending into medial supplementary motor area, bilateral anterior insula, bilateral anterior middle and inferior frontal gyrus, and bilateral supramarginal gyrus (the only region of this network not sensitive to DAT1 genotype in the present study). The present finding, that connectivity between striatum and the Cingulo-opercular network is sensitive to DA function, converges with work by Cole et al. (2012) showing that pharmacological manipulations of DA affect connectivity between ventral striatum and the Cingulo-opercular network.

The dependence of these caudate to Cingulo-opercular connections on DAT1 genotype may reveal why the caudate is sometimes (e.g., Abou Elseoud et al., 2011) but not always identified as part of the Cingulo-opercular network; further, it explains why the overall dorsal and ventral caudate connectivity patterns observed in Di Martino et al. (2008) do not correspond well to the Cingulo-opercular network: 9/10 subjects have strong connectivity between caudate and cingulo-opercular regions, but 10/10 subjects do not (Figure 1). Indeed, some 10/10 subjects demonstrated strong negative connectivity values in these connections (see Figure 2, x-axes), indicating a robust functional segregation rather than integration, as generally seen in the 9/10 subjects. When connectivity strengths are examined without taking genotype into account, as in
previous work, these divergent values average to be near zero, preventing the caudate from being identified as part of the network.

It is notable that DAT1 genotype had strong effects on connectivity seeded from the caudate, but no significant effects on connectivity seeded from the putamen. DAT1 is known to be expressed in both caudate and putamen (Hall et al., 1999; Sasaki et al., 2012), and previous work has shown that pharmacologic manipulations of DA do affect putamen-PFC connectivity (Cohen et al., 2007; Nagano-Saito et al., 2008), though other work has failed to show any DA-related effects on putamen connectivity (Cole et al., 2012; Klostermann et al., 2012), as in the present study, or has shown DA-related effects only in connections between putamen and cerebellum (Kelly et al., 2009). Notably, the studies which observed DA-related effects on putamen-PFC connectivity were conducted during performance of a cognitive task (Cohen et al., 2007, reversal learning; Nagano-Saito et al., 2008, Wisconsin Card Sorting Task), while two of the studies which observed no such effect were conducted during the resting state (Cole et al., 2012; Kelly et al., 2009), like the present study. We speculate that putamen-PFC connectivities may be affected by differences in DA function only when connectivity is measured during a task requiring DA release rather than during the resting state.

*Striato-frontal connectivity predicts executive function*

The present findings indicate that stronger resting-state connectivity between the caudate and these Cingulo-opercular regions predicted better executive control performance. The Cingulo-opercular network has been repeatedly linked to executive functions (Laird et al., 2011), including processing salient information (Seeley et al., 2007), maintaining task sets (Dosenbach et al., 2007, 2008), and inhibitory control (Smith et al., 2009), and it is consistently activated across a wide variety of N-back working memory studies (Owen et al., 2005). Further, the
caudate has been characterized as the striatal nucleus most relevant to inhibitory control (Boehler et al., 2010; Cropley et al., 2006; Li et al., 2008; Menon et al., 2001) and working memory (Moore et al., 2012; White, 2009). Thus, it is not surprising that interactions between these regions should predict executive function.

This study is the first to show that stronger connectivity between dorsal caudate and Cingulo-opercular regions predicted both faster and more accurate working memory performance. Previous work has shown that N-back performance can be predicted by the strength of resting-state connectivity between Cingulo-opercular regions and the putamen (Tu et al., 2012), as well as by task-induced connectivity between the dorsal caudate and the lateral prefrontal cortex (Klostermann et al., 2012). The present findings extend this work to demonstrate that stronger resting-state dorsal caudate to Cingulo-opercular connections, which are affected by the DAT1 gene, predict better N-back performance.

We also found that stronger connectivity between ventral striatum and lateral prefrontal cortex predicted reduced impulsivity, a finding which converges well with previous work. The ventral striatal region, and particularly the nucleus accumbens (which likely overlaps with the VS1 seed used in the present study), is critically involved in the regulation of impulsive choices, and DA effects on this region are known to affect impulsivity (see Basar et al., 2010 for a review). Better control of impulsive behavior, as measured using questionnaires (as in the current study), response inhibition tasks, and delay discounting tasks, has been associated with both higher integrity of white matter tracts connecting ventral striatal and prefrontal regions (Konrad et al., 2010; Liston et al., 2006; Peper et al., 2012) and with stronger functional connectivity between ventral striatal and prefrontal regions (Cole et al., 2012; Davis et al., 2012; Kayser et al., 2012). The present findings extend this work to show that the ventral striatal to PFC connections
which mediate control of impulsivity are also affected by the *DAT1* gene.

*DAT1*→*connectivity*→*executive control linkages*

While a body of previous work has identified three distinct lines of research which, taken together, suggest a causal pathway between the *DAT1* gene, striato-frontal connectivity, and executive function, this study is the first to show direct linkages between these factors. First, the 10-repeat *DAT1* allele is associated with reduced benefits of WM training (Brehmer et al., 2009) and increased impulsivity (Gizer and Waldman, 2012). Second, the 10-repeat allele is also associated with reduced striatal DA function (Madras et al., 2005) which is important because reduced striatal DA function (Klostermann et al., 2012) and pharmacological manipulations which would be expected to reduce striatal DA function (Cole et al., 2012; Nagano-Saito et al., 2008) have been associated with reduced striato-frontal connectivity. Third, reduced striato-frontal connectivity has been associated with reduced WM function (Klostermann et al., 2012; Tu et al., 2012) and increased impulsivity (Cole et al., 2012). Together, these previous findings suggest the possibility of a causal pathway linking *DAT1* to striato-frontal connectivity and executive function, but the present study is the first to demonstrate this complete pathway. Notably, a parallel body of work investigating Attention Deficit Hyperactivity Disorder (ADHD) also suggests such a causal pathway. The 10/10 *DAT1* genotype has been associated with ADHD (Yang et al., 2007), a disorder that is defined in part by increased impulsivity and that demonstrates reduced working memory (Willcutt et al., 2005). ADHD individuals also demonstrate reduced striato-frontal connectivity (Cubillo et al., 2010; Rubia et al., 2009). The present study brings together these suggestive associations by demonstrating that the *DAT1* genotype does affect striato-frontal connectivity, and that the connectivity patterns that differ by genotype are predictive of WM function and impulsiveness. The fact that this linkage is present
in healthy individuals without ADHD indicates that striato-frontal connectivity may be an endophenotype for the reduced executive function observed in ADHD individuals. This suggestion further contributes to an emerging theme in the literature that functional connectivity serves as an endophenotype for a variety of gene-behavior relationships (Esslinger et al., 2009; Chapter V; Meyer-Lindenberg, 2009; Smit et al., 2010; Walter et al., 2011; Woodward et al., 2009).

It is notable that this DAT1→connectivity→executive control linkage emerged despite the fact that the genotype groups did not demonstrate differences in WM or impulsiveness. This negative finding agrees with previous work which has not consistently identified DAT1 effects on executive function even in large samples, suggesting that neuroimaging-based measures, such as the striato-frontal connectivity effects identified here, may be needed to serve as endophenotypes linking DAT1 to behavior (Blanchard et al., 2011).

**Conclusions**

Individuals with a 9/10 DAT1 genotype demonstrated stronger resting-state functional connectivity between dorsal/ventral caudate seeds and the frontal nodes of a Cingulo-opercular network than individuals with a 10/10 genotype. This stronger striato-frontal connectivity predicted faster working memory performance, superior working memory accuracy, and a lower level of self-reported impulsivity, even though no differences in these factors were observed between the genotype groups. These findings suggest that the strength of resting-state striato-frontal connectivity may serve as an endophenotype between the DAT1 gene and executive function.
CHAPTER V: EFFECT OF DOPAMINE TRANSPORTER GENOTYPE ON INTRINSIC FUNCTIONAL CONNECTIVITY DEPENDS ON COGNITIVE STATE

This chapter has been published as: Gordon EM, Stollstorff M, Devaney JM, Bean S, Vaidya CJ (2012): Effect of dopamine transporter genotype on intrinsic functional connectivity depends on cognitive state. Cerebral Cortex 22(9):2182–2196.

Introduction

The functional architecture of the human brain is composed of distinct networks whose regions show correlated activity across time (Bullmore and Sporns, 2009). This network organization exists regardless of cognitive state, as the same networks that demonstrate correlated activity during task performance (Esposito et al., 2006; Fransson and Marrelec, 2008; Fransson, 2006) also show correlated activity at low frequencies (< .08 Hz) during the task-free “resting state” (Beckmann et al., 2005). Further, the spatial composition of these networks matches patterns of regions that are activated by various tasks (Chapter II; Smith et al., 2009). For example, correlated network activity is seen both between bilateral auditory cortex and between bilateral visual cortex (Beckmann et al., 2005), regions also activated by auditory and visual tasks, respectively. Further, several networks include regions that are activated by complex cognitive tasks (e.g., working memory), while others include regions that are deactivated during those same tasks; these have been termed “Task-Positive” and “Task-Negative” (or default mode) networks, respectively (Fox et al., 2005). This inverse relationship in activation between Task-Positive networks (TPN) and Task-Negative networks (TNN) during task performance is also reflected in the temporal relationship between these networks, as they
are anticorrelated during the resting state (Fox et al., 2005). The regional composition and temporal relationships within and between networks (termed functional connectivity) are posited to be established by repeated functional co-activation over a lifetime (Dosenbach et al., 2007).

The strength of functional network connectivity appears to be a key determinant of cognitive abilities. First, the strength of within-network functional connectivity (between nodes of a single network) predicts cognitive performance. Stronger connectivity between major TNN nodes (medial prefrontal cortex and posterior cingulate cortex) is associated with superior working memory performance (Hampson et al., 2006, 2010; Sambataro et al., 2010) as well as superior processing speed, memory, and executive function (Andrews-Hanna et al., 2007). Further, stronger connectivity between major TPN nodes (left and right lateral prefrontal cortex) is associated with superior processing speed and executive function (Gordon et al., 2011). Second, the degree of anticorrelation between TPN and TNN impacts cognition, as individuals who have more negative TPN-TNN correlations demonstrated reduced trial-to-trial behavioral variability (Kelly et al., 2008) and superior working memory performance (Hampson et al., 2010). This negative or reduced cross-network connectivity is thought to reflect reduced interference across networks (Kelly et al., 2008). Third, many neuropsychiatric disorders associated with cognitive deficits demonstrate atypical connectivity patterns. Reduced connectivity within the TNN has been observed in Attention Deficit Hyperactivity Disorder (ADHD) (Castellanos et al., 2008), Autism Spectrum Disorders (Kennedy et al., 2006), Schizophrenia (Bluhm et al., 2007), and Alzheimer’s disease (Greicius et al., 2004). Elevated or less negative cross-network connectivity between TPN and TNN has been observed in ADHD (Castellanos et al., 2008) and Schizophrenia (Whitfield-Gabrieli et al., 2009). Together, these findings suggest that integrity of cognition depends upon optimal within- and between-network functional connectivity.
What factors may determine the nature of within- and between-network relationships? One likely candidate is the neurotransmitter dopamine (DA), as exogenous manipulations of DA affect functional connectivity. In healthy volunteers, DA depletion reduced resting state connectivity between striatum and the TPN and disrupted the relationship between connectivity and speed of executive task performance (Nagano-Saito et al., 2008). Administration of a DA agonist altered resting state striatal connectivity such that it was increased with TPN and motor networks, but reduced with TNN (Kelly et al., 2009). While the behavioral significance of these findings cannot be determined, as subjects were not performing a task, these findings indicate that altered DA levels have widespread effects on temporal relationships of networks. In ADHD, a disorder characterized by DA dysfunction, administration of stimulant medications that enhance DA signaling normalized connectivity between cortical and striatal/cerebellar regions (Rubia et al., 2009), as well as between TPN and TNN (Peterson et al., 2009), such that it was similar to control children. Together, these results suggest that DA function is important for regulating cross-network functional relationships.

Similar to experimental manipulations of exogenous DA, it is possible that endogenous inter-individual variation in DA function is associated with functional connectivity differences between individuals. One endogenous source of DA variation is a widely studied genetic polymorphism, the variable number of tandem repeats (VNTR) in the 3’-untranslated region of the DAT1 gene coding for the DA transporter (DAT), a protein that regulates DA signaling by re-uptaking DA following its release (Madras et al., 2005). The DAT1 gene’s two most common alleles, 9 and 10 repeats, appear to influence the expression of DAT in vitro (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005), with greater striatal DAT expression associated with 10-repeat compared to 9-repeat alleles (though in vivo findings have been mixed; Heinz et al., 2000;
Jacobsen et al., 2000; Krause et al., 2006). Inheritance of two copies of the 10-repeat allele (10/10) has been associated with ADHD (Yang et al., 2007), a disorder defined by reduced executive function (Willcutt et al., 2005). Executive functioning was reduced in healthy adults with a 10/10 genotype, as they showed worse inhibitory performance (Caldú et al., 2007) and reduced benefits of working memory training despite similar baseline performance (Brehmer et al., 2009) relative to 9/10 heterozygotes.

In addition to behavioral effects, differences in DAT expression have also been associated with differences in the functional engagement of brain regions important for executive function. First, higher striatal DAT concentration was associated with less deactivation of TNN regions in healthy adults during visual attention (Tomasi et al., 2009). Second, 10/10 homozygotes had reduced activation compared to 9-repeat carriers in the Task-Positive lateral prefrontal cortex during working memory (Bertolino et al., 2006, 2008, 2009; Caldú et al., 2007; Stollstorff et al., 2010) and response inhibition (Congdon et al., 2009); reduced activation in the Task-Positive striatum during response inhibition (Congdon et al., 2009) and reward processing (Dreher et al., 2009; Forbes et al., 2009); and reduced deactivation in Task-Negative medial prefrontal cortex during working memory (Brown et al., 2011). These findings indicate that putative differences in DAT expression induce individual variations in both behavior and related brain activation. Whether DAT1 influences network connectivity is unknown.

Here we investigated functional connectivity within and between TPN and TNN during the resting state and during performance of an N-back working memory task in healthy 9/10 and 10/10 carriers. We first identified intrinsic connectivity networks in the resting state, confirmed that network nodes overlapped with activation during the working memory state, and then examined effects of DAT1 and cognitive state on functional connectivity between these nodes.
Our goals were two-fold. First, we examined whether functional connectivity differs by DAT1. As the 10/10 genotype has been associated with ADHD (Yang et al., 2007), we predicted that 10/10 homozygotes would demonstrate connectivity patterns similar to that observed in ADHD—that is, reduced connectivity within TNN and increased (i.e. less negative) connectivity between TPN and TNN (Castellanos et al., 2008). Second, we examined whether subjects’ cognitive state (resting vs. working memory task) modulates connectivity, and whether this interacts with DAT1. Working memory demands alter functional connectivity relative to rest (Fransson, 2006), both within networks (increases within TPN) and across networks (decreases between TPN and TNN). We expected to replicate these findings. Third, we examined whether DAT1 and cognitive state would interact to modulate functional connectivity. As working memory demands increase DA release (Aalto et al., 2005), differences in DA regulation associated with DAT1 ought to be magnified during working memory relative to rest, yielding a DAT1 X cognitive state interaction on functional connectivity. Finally, as DAT1 has been shown to affect executive control, we examined whether DAT1 X cognitive state interactions in functional connectivity were associated with individual differences in executive control traits.

**Materials and Methods**

**Subjects:** Two hundred ninety-six Georgetown University undergraduates aged 18 to 22 years provided saliva samples that were genotyped for DAT1. Eighty-one subjects were randomly invited from the pool of 10/10 and 9/10 carriers to participate (9/10: n = 37; 10/10: n = 44). Exclusion criteria included self-reports of 1) use of psychotropic medication (e.g., stimulants, SSRIs); 2) overt neurological injury or disease, seizure disorder, psychiatric diagnosis; 3) contraindications for MRI – e.g., presence of metal, pregnancy. Four subjects (two
per genotype) were excluded from analysis due to technical problems during scanning. The final sample included 35 9/10 heterozygotes (mean ± SD age = 20.37 ± .96; 14 male) and 42 10/10 homozygotes (mean ± SD age = 20.26 ± 1.14; 14 male). Groups did not differ in either age or gender (ps > .4). All subjects gave informed consent in accordance with guidelines of the Georgetown University Institutional Review Board.

Genotyping: DNA was extracted from Oragene saliva kits (DNA Genotek Inc., Ottawa, Ontario, Canada). The 40 base pair VNTR polymorphism in the 3’ UTR of DAT1 was genotyped by PCR as previously described (Daly et al., 1999) using the following primers: Forward: 5’-TGTGGTGTAGGGAACGGCTGAG-3’ Reverse: 5’-CTTCCTGGAGGTCAACGGCCTGAG-3’. PCR was performed using the AccuprimeTM Taq DNA polymerase system (Invitrogen) with the following PCR program: 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 68°C for 1 min. The PCR products were then run out on a 2% agarose gel stained with ethidium bromide. A 100 bp DNA ladder was then used to identify the various repeat alleles by size: 7-repeat (360bp), 8-repeat (400bp), 9-repeat (440bp), 10-repeat (480bp), and 11-repeat (520bp). Genotyping was successful for 286 of the 296 subjects in the original sample. Observed genotypic frequencies in the sample were: 10/10 - 59.1%; 9/10 - 29.4%; 9/9 – 8.7%; other, 2.8%.

Behavioral testing: Subjects completed the Adult ADHD Self-Report Scale v1.0 (Kessler et al., 2005) and the Barratt Impulsiveness Scale version 11 (Patton et al., 1995).

Scanning procedure: Subjects were scanned during performance of an N-back task and during rest. The N-back task lasted for 6:26 minutes and consisted of nine 30s N-back blocks (three blocks each at 1-, 2-, and 3-back) alternating with eight 15s blocks of fixation. Each N-back block consisted of nine serially presented consonants appearing for 500ms, with an inter-
trial-interval of 2500ms. The N-back load condition (1-, 2-, or 3-back) varied between task blocks, with condition order pseudorandomized using a modified Latin Square. Each block was preceded by a 3000 ms screen informing the subject of the N-back condition. Subjects were instructed to press a hand-held button with their right hand when the current letter matched the letter n trials ago (e.g., for the 2-back condition, subjects see: R V N W N – button-press for N). Targets were present on 19% of trials; each block contained between one and three targets with target frequency balanced across conditions. No condition contained sequences of stimuli that were targets in any other condition. Stimuli were presented using E-Prime (Psychology Software Tools Inc., Pittsburg, PA). The resting scan was always conducted immediately following the conclusion of the N-back task. For the resting run, which lasted 5:04 minutes, subjects were told to relax with eyes closed and to not think of anything in particular.

**fMRI data acquisition:** Imaging was performed on a Siemens Trio 3T scanner (Erlangen, Germany). A high resolution T1-weighted structural scan (MPRAGE) was acquired with the parameters: TR/TE=2300/2.94ms, TI=900ms, 90 degree flip angle, 1 slab, 160 sagittal slices with a 1.0mm thickness, FOV=256x256mm2, matrix=256x256, resulting in an effective resolution of 1.03mm isotropic voxels. For the N-back run, 197 whole-brain images were acquired using a gradient echo pulse sequence (34 slices, TR = 2000 ms, TE = 30 ms, 256x256mm FOV, 90 degree flip angle, voxel dimensions 4x4x4.2mm). For the resting run, 152 whole-brain images were acquired using a gradient echo pulse sequence (37 slices, TR = 2000 ms, TE = 30 ms, 192x192mm FOV, 90 degree flip angle, voxel dimensions 3mm isotropic). The first 4 images of each functional run were discarded to allow for signal stabilization.

**Image Preprocessing:** Using SPM8 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (Version 7.10 Mathworks, Inc., Sherborn, MA), images
were corrected for translational and rotational motion by realigning to the first image of the session, for each run. All subjects demonstrated less than 2.0mm of translational motion in any one direction (max translation = 1.25mm). One subject demonstrated a transient large rotational motion in the first three TRs of the N-back run; these TRs were removed from further analyses. Subsequently, all subjects demonstrated less than 2° of rotation around any one axis (max rotation = 1.44°). A two-sample t-test showed that genotype groups did not differ in maximum motion in any of the three translational or three rotational directions (all ps > .15). Images were slice-time corrected, normalized to an EPI template, and smoothed using a Gaussian kernel with full-width at half-maximum (FWHM) of 8mm. For connectivity analyses, a band-pass filter was applied to the resting and working memory data in order to restrict signal variation to frequencies between .01 Hz and .1 Hz, corresponding to the frequency range established in the literature for fluctuations in resting state data (Biswal et al., 1995).

**Identification of brain regions activated and deactivated during the N-back task:** First-level analysis was performed using a general linear model as implemented in SPM8. For each subject, three temporal regressors consisting of boxcar timeseries convolved with a hemodynamic response function were specified: one representing the presence of the Fixation cross, one representing the presence of the N-back task, and one representing the effect of load (constructed by reproducing the N-back regressor and parametrically varying the boxcar height according to the load condition). For each subject, Task>Fixation and Fixation>Task contrasts were specified to delineate regions activated and deactivated during the N-back task after removing contributions of the N-back load condition (this was done for consistency with the connectivity analysis—see below). For group averaging, one-sample t-tests were conducted for both contrasts at p < .05, corrected for multiple comparisons using Family Wise Error (FWE).
**Identification of functional networks during rest:** A group-level Independent Components Analysis (ICA) was performed on the preprocessed, filtered resting state images using the MELODIC toolbox (Beckmann and Smith, 2004) implemented within FSL (Centre for Functional Magnetic Resonance Imaging of the Brain, University of Oxford, London, UK). The preprocessed, filtered resting data from all subjects was temporally concatenated to create a single timecourse, and a probabilistic independent components analysis was performed on this timecourse using the MELODIC toolbox, allowing the program to select the optimal number of components to generate. Within each component, MELODIC generated Z-scores for each voxel by generating a mixture model combining a “noise” Gaussian function with two gamma functions modeling “active” voxels and estimating the probability of a given voxel’s intensity fitting the gamma functions rather than the background noise Gaussian function (Beckmann and Smith, 2004).

The ICA delineated 20 components in the form of 3D Z-score images. Components in which the areas of maximal covariation were non-neuronal (e.g., white matter, cerebrospinal fluid, brain edge covariation resulting from head motion) were visually identified (see Kiviniemi et al., 2009) and removed from further analysis. The remaining group components were visually identified based on similarities to known brain networks. These components were identified as Task-Positive networks (TPN) or Task-Negative networks (TNN) based on similarity to networks identified by Fox et al. (2005), or as “Task-Neutral” based on a lack of similarity to those networks.

**Region of interest (ROI) creation:** For each Task-Positive and Task-Negative network, the largest clusters of covariation were delineated, and the voxel of peak network connectivity (i.e. with peak Z-score values in the ICA-generated images) within each cluster was identified as a
network “node” from which functional connectivity analysis should be conducted. To restrict analysis to nodes that were modulated by the N-back task, peak voxels were discarded from further analysis if they did not fall within regions activated or deactivated in the group-level Task>Fixation contrasts. The remaining nodes thus represent regions that were both activated by the working memory task and maximally connected within intrinsic connectivity networks. Spherical regions of interest (ROIs) with radius 6mm were created centered on each of these node voxels using MARSBAR (Brett et al., 2003) and were labeled based on the general anatomical location of the node voxels (as in Duvernoy and Bourgouin, 1999); these ROIs were used for all further connectivity analyses.

**Removal of nuisance signals:** To minimize the effects of motion, load (within the N-back run), and physiological noise (such as respiration and heart rate) that would be common to all ROIs, timecourses approximating these signals were regressed out of each voxel. Physiological noise regressors were approximated by obtaining signal timecourses from white matter and CSF segmentations of the MPRAGE image (Van Dijk et al., 2010). Motion regressors were obtained as the six realignment parameter timecourses from the motion correction preprocessing step. For the N-back run, load-effect regressors were obtained by convolving three boxcar timecourses (one for each load condition) with a canonical hemodynamic response. The effect of load was regressed out because the manipulation of load in the N-back paradigm was expected to drive substantial and systematic activation differences in many brain regions, including both task-positive regions (Braver et al., 1997; Callicott et al., 1999; Veltman et al., 2003) and task-negative regions (McKiernan et al., 2003). If the load structure of the task was not regressed out, these large activation differences would artificially inflate functional connectivities, such that even regions with no moment-to-moment correlations would appear functionally connected.
because they were both driven by load effects over the course of the task (see Jones et al., 2010 for further discussion of this point).

The regression of nuisance signals was conducted separately for each run, and the post-regression residual voxel timecourses were used for all further analysis.

**Functional connectivity analysis:** For the rest and N-back runs separately, residual voxel timecourses were averaged within each ROI, and mean residual timecourses from all ROIs were then correlated against each other in a pairwise fashion to assess functional connectivity. In the N-back run, analysis was restricted to task performance by excluding the 15 seconds in each fixation block plus six subsequent seconds (to allow for hemodynamic response stabilization). The correlations were thus performed on 148 resting timepoints and 108 N-back timepoints. The resulting Pearson’s $r$ values (from each ROI pair, for each subject in each run) were converted to normally-distributed Z-scores using Fisher’s transformation in order to allow further analysis of correlation strengths.

To assess effects of genotype and cognitive state on each functional connection between ROI pairs, a 2 X 2 $DAT1$ (10/10, 9/10; between subjects) X state (resting state, N-back state; within subjects) ANOVA was conducted on the connectivity between each ROI pair using the LinStats software package within Matlab (http://www.mathworks.com/matlabcentral/fileexchange/29876-linstats). For each ANOVA model, an F-test was performed testing the overall fit of the model against a null model (intercept only), and the resulting model fit p-values were tested for significance at p<.05 after Bonferroni correction for the number of ANOVA models (corrected alpha=.000416). ANOVA models that significantly fit the data were subsequently examined for interaction effects and main effects.

**Correlation with executive traits:** To examine whether connectivity affected by $DAT1$ and
state were also associated with executive control traits, we calculated the state-related change in connectivity (N-back state – resting state) for each subject in each of the functional connections showing a significant DAT1 X state interaction. For each behavioral measure (Inattention and Hyperactivity from the ADHD Self-Report Scale and Impulsivity from the Barratt Impulsiveness Scale) separately, we conducted a stepwise multiple regression to examine whether the calculated changes in connectivity predicted individual differences in the behavioral trait.

Results

Behavior

Rating Scales: On the ADHD Self-Report Scale, Inattention scores were marginally higher in 10/10 than 9/10 subjects (9/10: 13.78±3.74; 10/10: 15.63±5.01; t(79)=1.84, p=.069), but scores did not differ on the Hyperactive/Impulsive subscale (9/10: 11.78±4.31; 10/10: 12.23±5.20; p>.6). Barratt Impulsiveness Scale ratings were also marginally higher in 10/10 than 9/10 subjects (9/10: 55.89±6.79; 10/10: 59.30±9.88; t(79)=1.77, p=.080).

N-back Task Performance: Mean reaction time (RT) for correct N-back target responses and N-back percent accuracy (% hits - % false alarms) were computed for each subject. Genotype groups did not differ in mean RT (9/10: 582 ms ± 172 ms; 10/10: 534 ms ± 145 ms; p=.18). Both groups performed near ceiling and did not differ on either accuracy (9/10: 95.4% ± 5.5%, 10/10: 96.0% ± 6.6%; p=.66) or on the number of subjects in each group with perfect accuracy (9/10 = 18; 10/10 = 25, p=.46).

Identification of brain regions activated and deactivated by the N-back task

Group averages of activated (N-back > Fixation) and deactivated (Fixation > N-back) regions are shown in Figure 1. Activated regions included bilateral dorsolateral and ventrolateral
prefrontal cortex, anterior insula, lateral parietal cortex, medial supplementary motor area, and
globus pallidus. Deactivated regions included ventromedial prefrontal cortex and perigenual
anterior cingulate, anterior medial prefrontal cortex, and medial parietal cortex (including
posterior cingulate and precuneus), as well as bilateral fusiform gyrus, hippocampus/amygdala,
posterior insula, anterior middle and superior temporal gyri, and lateral/superior occipital cortex
extending into bilateral angular gyrus. These activation/deactivation patterns did not vary by
*DAT1* genotype (see Supplemental Material I).

Figure 1: Group average of Task>Fixation (in red) and Fixation>Task (dark blue) contrasts
(*p*<.05, FWE-corrected).

**Identification of functional networks**

Twenty components were delineated by Independent Components Analysis of the resting
state data. Of these, five were visually identified as Task-Positive networks (TPN) or Task-
Negative networks (TNN) based on similarity to past reports (Fox et al., 2005), including: a
cingulo-opercular Salience network, a left-lateralized Frontoparietal Control network (lFPC), a
right-lateralized Frontoparietal Control network (rFPC), a parietal-based bilateral Dorsal
Attention network, and a bilateral Striatal network, which were classified as TPN; as well as a
posterior Default Mode network (pDMN) and an anterior Default Mode network (aDMN), which
were classified as TNN (see Figure 2). Additionally, six networks were identified which did not
well-match TPN or TNN; these networks, many of which were similar in appearance to
previously delineated networks (Kiviniemi et al., 2009), were labeled “Task-Neutral” (see Figure
S1). These included networks with high connectivity in auditory cortex; in primary visual cortex; in sensorimotor cortex; in left-lateralized language regions; in medial posterior and middle cingulate cortex; and in bilateral superior temporal and inferior frontal cortex. As these networks are not relevant to our predictions, they were not included in further analysis. The remaining seven components were identified as deriving from non-neuronal sources (Kiviniemi et al., 2009) and were thus excluded from analysis: CSF (1), white matter (1), and subject head motion (5).

Identification of TPN and TNN “nodes” (as described in Methods) resulted in 1-4 node regions of interest (ROIs) for each network (Table 1 and Figure 2, green circles).

Figure 2: Seven networks delineated in the resting-state data by the ICA procedure. Network maps are thresholded for visual purposes at Z=15.0. Label shadings indicate visual categorization of each network: light gray shading—Task-Positive networks; dark gray shading—Task-Negative networks. ROIs used in connectivity analyses are overlaid on top.
<table>
<thead>
<tr>
<th>Network Type</th>
<th>Network</th>
<th>ROI center (MNI coordinates)</th>
<th>ROI location</th>
<th>ROI abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Task-Positive</td>
<td>Right Frontoparietal Control (rFPC)</td>
<td>46 14 44</td>
<td>right posterior dorsolateral PFC</td>
<td>r pdlPFC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34 58 4</td>
<td>right ventrolateral PFC</td>
<td>r vlPFC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46 -54 48</td>
<td>right posterior inferior parietal lobule</td>
<td>r pIPL</td>
</tr>
<tr>
<td></td>
<td>Left Frontoparietal Control (IFPC)</td>
<td>-50 18 28</td>
<td>left posterior dorsolateral PFC</td>
<td>l pdlPFC</td>
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<tr>
<td></td>
<td>Dorsal Attention</td>
<td>42 -38 52</td>
<td>right anterior inferior parietal lobule</td>
<td>r aIPL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-38 -46 56</td>
<td>left anterior inferior parietal lobule</td>
<td>l aIPL</td>
</tr>
<tr>
<td></td>
<td>Salience</td>
<td>34 46 28</td>
<td>right anterior dorsolateral PFC</td>
<td>r adlPFC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-30 46 28</td>
<td>left anterior dorsolateral PFC</td>
<td>l adlPFC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46 14 -8</td>
<td>right anterior insula</td>
<td>r alns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-42 10 -4</td>
<td>left anterior insula</td>
<td>l alns</td>
</tr>
<tr>
<td></td>
<td>Striatum</td>
<td>10 -2 4</td>
<td>right striatum</td>
<td>r Striatum</td>
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<tr>
<td></td>
<td></td>
<td>-10 -6 8</td>
<td>left striatum</td>
<td>l Striatum</td>
</tr>
<tr>
<td>Task-Negative</td>
<td>Anterior Default Mode (aDMN)</td>
<td>-2 50 -4</td>
<td>ventromedial prefrontal cortex</td>
<td>vmPFC</td>
</tr>
<tr>
<td></td>
<td>Posterior Default Mode (pDMN)</td>
<td>-2 -58 20</td>
<td>posterior cingulate</td>
<td>PCC(^1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42 -74 28</td>
<td>right angular gyrus</td>
<td>r AG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-38 -82 32</td>
<td>left angular gyrus</td>
<td>l AG</td>
</tr>
</tbody>
</table>

Table 1: Locations of ROIs constructed around peaks of maximal covariation in the resting-state data, within Task-Positive and Task-Negative networks

**Functional Connectivity within and between Task-Positive and Task-Negative networks**

To display the correlational structure of TPN and TNN, we created connectivity matrices by averaging across subjects’ Z-transformed correlation coefficients for each connection, within each genotype group and condition. These matrices are presented in Figure 3. To statistically test for effects of \textit{DAT1} and cognitive state, subjects’ Z-transformed correlation coefficients in each pairwise connection were subjected to a \textit{DAT1} (9/10, 10/10) X cognitive state (Nback, Rest) mixed ANOVA. Bonferroni correction was conducted at p<.05 for the number of ANOVA

\(^1\) Both the pDMN and the aDMN networks contained a node in posterior cingulate cortex (PCC). Because these two nodes overlapped, the aDMN PCC node (centered at peak voxel [6 -58 20]) was discarded from further analysis (as it was not in the anterior portion of the network). There was no other overlap between ROIs.
models. The following significant effects emerged:

**Main effects of cognitive state.** Significant main effects of cognitive state were found in

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**Figure 3:** Correlation matrices indicating Z-transformed $r$ values for each genotype group in each state. Hot colors indicate positive connectivity between ROIs; cool colors indicate negative connectivity. Shadings of ROI labels indicate network categorization: light gray shading—Task-Positive networks; dark gray shading—Task-Negative networks. The green dotted line demarcates cross-network (Task-Negative to Task-Positive) connections from within-network connections.
thirty-eight TPN to TPN connections and thirteen TPN to TNN connections (Fs(1,77) ranged from 16.07 to 71.65). No significant TNN to TNN connections reached significance. In all TPN to TPN connections, as well as in connections between TNN and the TPN Salience network, connectivity was higher during the N-back task than during rest. By contrast, in connections between TNN and the TPN FPC/Dorsal Attention networks, connectivity was higher during rest than during the N-back task (see Figure 4). Thus, as predicted, working memory demands strengthened connectivity between most nodes of TPN. However, contrary to predictions, the hypothesis that working memory would reduce TPN to TNN connectivity was only supported for the FPC and Dorsal Attention TPN, and not for the Salience TPN.
Figure 4: Matrix indicating ROI pairs in which significant main effects of state were observed on connectivity, after correction for multiple comparisons at the model level. Hot colors indicate increased connectivity during the N-back task compared to Rest; cool colors indicate increased connectivity during Rest compared to the N-back task. Shadings of ROI labels indicate network categorization: light gray shading—Task-Positive networks; dark gray shading—Task-Negative networks. The green dotted line demarcates cross-network (Task-Negative to Task-Positive) connections from within-network connections.

Main effects of DAT1. Significant main effects of DAT1 were found in four TPN to TPN connections and six TPN to TNN connections (Fs(1,77) ranged from 4.49 to 11.27), but not in any TNN to TNN connections. In all of connections showing effects, the 10/10 group exhibited greater connectivity than the 9/10 group (see Figure 5). The TPN to TPN connections included one connection between bilateral frontal nodes of the FPC networks (L pdIPFC to R pdIPFC), two connections between bilateral parietal nodes of the Dorsal Attention network and a frontal node of the Salience network (L adIPFC to R and L aIPL), and one connection between Dorsal Attention and Striatal networks (L Striatum to R aIPL). The TPN to TNN connections (Figure 5, within green line) included two between DMN and FPC networks (PCC and vmPFC vs R pIPL), two between DMN networks and the Dorsal Attention network (PCC and vmPFC vs L aIPL), and two between the pDMN network and bilateral insular nodes of the Salience network (L AG vs R aIns and L aINS).
Figure 5: Matrix indicating ROI pairs in which significant main effects of DAT were observed on connectivity, after correction for multiple comparisons at the model level. All significant effects were found to be driven by greater connectivity in 10/10 subjects than in 9/10 subjects (as indicated by hot color shading). Shadings of ROI labels indicate network categorization: light gray shading—Task-Positive networks; dark gray shading—Task-Negative networks. The green dotted line demarcates cross-network (Task-Negative to Task-Positive) connections from within-network connections.

**DAT1 X Cognitive state interaction.** Significant interaction between DAT1 and cognitive state (Figure 6A) was observed in six connections, all between TPN and TNN nodes (Fs(1,75) ranged from 4.33 to 8.47). Four of these were between TNN and the TPN rFPC network (vmPFC and PCC vs R pdIPFC; vmPFC and PCC vs R pIPL). The other two interactions were observed between the TNN aDMN and the TPN Dorsal Attention network (vmPFC vs L aIPL) and
between the TNN pDMN and the TPN Salience network (L AG vs R aINS). No significant interactions were observed in any TPN to TPN or TNN to TNN connections.

Two-sample t-tests evaluating effects of DAT1 on connectivity in each cognitive state (Figure 6B) revealed that in each connection, the interaction was due to significantly greater connectivity in 10/10 than in 9/10 subjects during the N-back task (ts(75) > 2.72, ps < .008), but not during rest (ps > .25). Paired t-tests evaluating effects of cognitive state in each group separately revealed that state effects on connectivity varied by network. In the five connections between TNN and the TPN FPC/Dorsal Attention nodes, 9/10 subjects demonstrated reduced connectivity during the N-back task compared to rest, but 10/10 connectivity was unchanged. By contrast, in the connection between the TNN left angular gyrus node and the TPN right anterior insula node, 10/10 subjects demonstrated increased connectivity during the N-back task compared to rest, but 9/10 connectivity was unchanged.

Figure 6: A) Matrix indicating ROI pairs in which significant DAT1 X state interaction effects were observed on connectivity, after correction for multiple comparisons at the model level. Shadings of ROI labels indicate network categorization: light gray shading—Task-Positive networks; dark gray shading—Task-Negative networks. The green dotted line demarcates cross-
network (Task-Negative to Task-Positive) connections from within-network connections. B) Connectivity values by DAT and state for each significant ROI pair in (A). Error bars represent SE.

In sum, performing the N-back task increased connectivity in several TPN to TPN and TNN to Salience network connections compared to rest, but decreased connectivity in several connections between TNN and FPC/Dorsal Attention networks in all subjects. Further, regardless of cognitive state, individuals with the 10/10 genotype showed greater connectivity in various regions of the brain, both within TPN and between TPN and TNN, than individuals with the 9/10 genotype. DAT1 differences depended upon cognitive state only in cross-network TPN to TNN connections, such that connectivity was higher in 10/10 than 9/10 subjects during working memory but not during rest. Overall, these results support two posed hypotheses: 1) that 10/10 subjects would demonstrate elevated Task-Positive to Task-Negative connectivity, and 2) that these genotype differences in cross-network connectivity would be enhanced during working memory performance.

**Association between functional connectivity and executive traits**

Stepwise multiple regressions were conducted evaluating whether state-related connectivity changes within connections showing DAT1 X state interactions predicted individual differences in behavioral measures of executive traits. These regressions revealed one TPN to TNN connection—between the L aIPL node of the Dorsal Attention network and the vmPFC node of the aDMN—which significantly predicted both Inattention from the ADHD Self-Report Scale (F(1,75) = 5.45, model R = .26, p = .022, Figure 7A) and Impulsivity from the Barratt Impulsiveness Scale (F(1,75) = 5.84, model R = .27, p = .018, Figure 7C), such that increased connectivity was associated with increased Inattention and Impulsivity. Hyperactivity scores were not predicted by connectivity changes within any connection (ps > .4).
To determine the extent to which these associations differed by DAT1 genotype, we examined these relationships for each genotype group separately. Correlations were statistically significant in the 10/10 group for both Inattention (R = .32, p = .038) and Impulsivity (R = .33, p = .031), but not in the 9/10 group (ps > .65) (Figure 7 B and D).

Thus, the degree to which the N-back task induced increases in cross-network connectivity between vmPFC and L aIPL was associated with self-reported inattention and impulsivity, and these associations were strongest in the 10/10 genotype group.

Figure 7: Associations between behavioral traits of executive control and the change in vmPFC to L aIPL connectivity from the Rest scan to the N-back task. A) Significant correlation between Inattention, as measured by the ADHD Self-Report Rating Scale, and the change in connectivity. B) The correlation between connectivity and Inattention was nonsignificant in 9/10 subjects (top)
but significant in 10/10 subjects (bottom). C) Significant correlation between Impulsivity, as measured by the Barratt Impulsiveness Scale, and the change in connectivity. D) The correlation between connectivity and Impulsivity was nonsignificant in 9/10 subjects (top) but significant in 10/10 subjects (bottom).

**Discussion**

The primary novel finding from this study was that a polymorphism of the dopamine transporter gene, which regulates synaptic dopamine, influenced cross-network functional connectivity, which in turn was associated with behavioral traits associated with executive dysfunction. Regardless of cognitive state, connections between frontal, parietal, and striatal nodes of Task-Positive networks (TPN) and Task-Negative networks (TNN) showed higher functional connectivity in 10/10 homozygotes than in 9/10 heterozygotes. However, performance of a working memory task modulated genotype differences selectively, such that connectivity between TPN and TNN was higher in 10/10 than 9/10 subjects during working memory, but not during rest. Such elevated cross-network connectivity has been thought to signify cross-network interference, suggesting inefficient cognition. Indeed, the magnitude of elevated cross-network connectivity was positively correlated with self-reported inattention and impulsivity in the present study. This association was primarily driven by the 10/10 homozygotes, who also had marginally higher scores on those measures than the 9/10 heterozygotes, despite both groups performing equally well, with high accuracy, on the working memory task. Further, we also replicated a previously reported finding that engagement in working memory strengthened connectivity within TPN nodes and reduced some cross-network TPN to TNN connectivities. By linking a gene regulating synaptic dopamine to a phenotype characterized by inefficient executive function, our primary findings validate cross-network connectivity as an endophenotype of executive dysfunction.
Two methodological considerations are important for interpreting these results. First, our N-back paradigm varied working memory load by including 1-back, 2-back, and 3-back blocks. As our primary hypotheses concerned effects of DAT1 and cognitive state, these load effects were regressed out, thereby ensuring that observed genotype differences were not driven by differential response to load. Thus, the results represent effects on connectivity during working memory performance, without including effects due to variability associated with changing demands between the 1-back, 2-back, and 3-back conditions. Importantly, analyses conducted without regressing out load resulted in a similar pattern of DAT1 effects on connectivity, with DAT1 effects observed primarily in cross-network connections (Figures S3 and S4), suggesting that reported DAT1 differences were not driven by the load manipulation. (The main effect of state was affected by load—see next section).

Second, we interpret all results from the present study in relative terms (more or less negative connectivity in different groups/states) rather than in absolute terms (negative or positive connectivity), as interpreting the negative connectivity sometimes observed in cross-network connections is ambiguous. While negative cross-network correlations may reflect competitive or mutually antagonistic network relationships (Fox et al., 2005), recent work has shown that the emergence of negative connectivity strongly depends on the processing steps used, as regression of the global signal can introduce widespread and (arguably) artifactual negative connectivity (Chang and Glover, 2009; Fox et al., 2009; Murphy et al., 2009). Therefore, we used alternate processing steps, including regression of motion parameters and signal from white matter / cerebro-spinal fluid, which have been proposed as a middle ground (Van Dijk et al., 2010). While these steps are known to reduce the appearance of negative connectivity, it is not possible
to determine definitively whether remaining negative connectivity actually represents antagonistic relationships (Chang and Glover, 2009).

**Effect of Cognitive State on Connectivity**

The present examination of the effect of working memory on functional connectivity partially replicates past findings. In support of the hypothesis that working memory would increase connectivity within TPN compared to rest, we found that connectivity indeed increased within TPN. However, the hypothesis that working memory would decrease connectivity between TPN and TNN was only partially supported, as we found connectivity decreases between TNN and the TPN Frontoparietal Control / Dorsal Attention networks, but connectivity increases between TNN and the TPN Salience network.

Previous work has shown that, when compared to a resting state, performance of lower-level sensory/motor tasks increased connectivity within the regions engaged by the task, whether that task was auditory (e.g., listening to speech, Arfanakis et al., 2000), visual (e.g., watching a flashing checkerboard, Arfanakis et al., 2000; Hampson et al., 2004; Nir et al., 2006), or motor (e.g., finger tapping, Arfanakis et al., 2000; Jiang et al., 2004). These findings suggest a general principle that connectivity during task-evoked states specifically increases within activated regions. Similarly, performance of a higher-level cognitive task such as the N-back working memory task increased connectivity within the same Task-Positive regions (Fransson, 2006) that are nominally activated during working memory (Owen et al., 2005). This finding was replicated in the present study. However, Fransson (2006) also found that working memory decreased connectivity between TPN and TNN regions, which in the present study was found to be true only for the Frontoparietal Control and Dorsal Attention TPN, but not for the Salience TPN. The most likely explanation for this discrepancy is that we removed effects of load, which was not
done by Fransson (2006). Increasing working memory load is known to parametrically activate TPN regions (Braver et al., 1997; Veltman et al., 2003) and deactivate TNN regions (McKiernan et al., 2003). If not removed from consideration in the functional connectivity analysis, these activation changes could artificially drive the connectivity analysis, such that decreased connectivity might reflect opposing effects of task condition on activation on a timeframe the length of a condition block, rather than decreased temporal synchronization on a second-to-second timeframe (Jones et al., 2010). This could reduce the ability to detect positive association between TPN and TNN. Indeed, examination of effects of cognitive state without removal of load (Supplementary Material II) showed results similar to Fransson (2006): connectivity within TPN increased, while connectivity between TNN and the TPN Frontoparietal Control networks decreased, and the connectivity increases between TNN and the TPN Salience network almost completely disappeared (Figure S2).

**Effect of DAT1 Genotype on Connectivity**

Our study is the first to demonstrate effects of the DAT1 genotype on functional connectivity, either during a task or during the resting state. As hypothesized, DAT1 genotype affected connectivity, and those effects also depended upon cognitive state. Three main findings emerged from this examination of DAT1 effects. First, regardless of cognitive state, subjects with the 10/10 genotype demonstrated greater connectivity than 9/10 subjects in connections within TPN, as well as in connections between TPN and TNN. Second, cognitive state selectively modulated DAT1 differences in connections between TPN and TNN, such that higher connectivity in 10/10 than 9/10 groups was observed during working memory engagement but not during the resting state. Third, within one cross-network TPN to TNN connection between ventromedial
prefrontal cortex and lateral parietal cortex, the state-related connectivity increases predicted self-reported inattention and impulsivity in everyday behavior, especially in 10/10 subjects.

DAT1 differences in connectivity emerged regardless of cognitive state in connections between various TPN (such as between right and left Frontoparietal Control networks, between Salience and Dorsal Attention networks, and between Striatal and Dorsal Attention networks), as well as in connections between TPN and TNN (such as between Default Mode and right Frontoparietal Control, Dorsal Attention, and Salience networks). In all of these connections, 10/10 subjects had higher functional connectivity than 9/10 subjects across resting and N-back scans. In order to gain insight into these overall genotype differences, we examined patterns of connectivity effects in both cognitive states to determine whether they were true main effects or whether they suggested interactive effects of DAT1 and state (Supplemental Material III). Notably, the pattern of mean connectivities in the cross-network TPN to TNN connections resembled interactive effects similar to the significant DAT1 X state interactions discussed below. Thus, these effects may be interpreted as weak interactions that may require a higher sample size to reach significance. By contrast, TPN to TPN connections appeared to exhibit true main effects, with higher connectivity in 10/10 than 9/10 subjects during both the task and rest states. Overall differences by DAT1 in connectivity in bilateral frontal, frontal-parietal, and striatal-parietal connections within TPN suggest baseline differences in the communication of information across these regions that is sensitive to dopaminergic differences. It remains to be seen whether these overall connectivity differences are replicated in future DAT1 investigations.

Performance of a working memory task was found to modulate the effect of DAT1 genotype on connectivity only in cross-network connections between TPN and TNN. These included connections between the Default Mode network and the right Frontoparietal Control, Dorsal
Attention, and Salience networks. Such elevated connectivity between TPN and TNN has been interpreted as indicating more interference or reduced segregation of the networks (Kelly et al., 2008); thus, in the present study, carriers of the 10/10 DAT1 genotype demonstrated reduced segregation of TPN and TNN, particularly during a cognitive state that is associated with increased dopamine release. There is growing evidence suggesting that such reduced TPN-TNN segregation is associated with inefficient cognition. Specifically, higher connectivity between TPN and TNN has been linked to increased trial-to-trial response variability (Kelly et al., 2008) and reduced working memory performance (Hampson et al., 2010). Increased interference from TNN has also been linked to task-irrelevant thought (Buckner et al., 2008), attention lapses (Weissman et al., 2006), and mind-wandering (Mason et al., 2007). Such behaviors are known consequences of reduced executive function. These behavioral effects (Castellanos et al., 2005; Klein et al., 2006; Willcutt et al., 2005) as well as elevated cross-network TPN to TNN connectivity (Castellanos et al., 2008) also characterize ADHD, a disorder defined by symptoms of inattention and impulsivity. Therefore, elevated cross-network connectivity is believed to reflect increased interference between networks that may induce task-irrelevant thoughts, resulting in inattention and impulsivity, which in turn yields inefficient cognitive processing (Sonuga-Barke and Castellanos, 2007). Indeed, in the present study, greater working memory-related increases in cross-network TPN to TNN connectivity predicted increased self-reported behaviors of inattention and impulsivity in everyday life, and this relationship was stronger (and significant) in 10/10 subjects. The 10/10 genotype, which has been associated with ADHD (Yang et al., 2007), has also been associated with inefficient executive function, even in populations without a diagnosis of ADHD. Worse performance was observed on tasks of inhibitory control in healthy 10/10 adults (Caldú et al., 2007) and children (Cornish et al., 2005;
Loo et al., 2003) relative to their 9/10 peers. Further, hyperactivity, a defining behavior of childhood ADHD, was higher in 10/10 than 9/10 children (Mill et al., 2005). Similarly in the present study, inattentiveness and impulsivity, which are associated with adult ADHD, tended to be higher in 10/10 than 9/10 subjects. Thus, our findings in healthy subjects demonstrate that cross-network connectivity increases are both associated with 10/10 homozygosity—a genotype linked to ADHD—and predict ADHD-like behaviors in that group. These findings validate the proposal of elevated cross-network connectivity as an endophenotype of ADHD (Castellanos et al., 2008; Sonuga-Barke and Castellanos, 2007) and further contribute to an emerging theme in the literature that functional connectivity serves as an endophenotype for a variety of gene-behavior relationships (Esslinger et al., 2009; Meyer-Lindenberg, 2009; Smit et al., 2010; Walter et al., 2011; Woodward et al., 2009).

For connections between TPN and TNN, the nature of DAT1 effects on connectivity varied by network. In frontal-parietal and parietal-parietal connections between TNN and the TPN Frontoparietal Control / Dorsal Attention networks, the 9/10 group demonstrated reduced connectivity during working memory compared to rest, but minimal change was observed in the 10/10 group. By contrast, in a parietal-insular connection between TNN and the TPN Salience network, the 10/10 group demonstrated elevated connectivity during working memory compared to rest, but minimal change was observed in the 9/10 group. Thus, it appears that during working memory, 10/10 subjects demonstrate an increase in cross-network interference of the Salience network, along with a failure to reduce cross-network interference of the Frontoparietal Control and Dorsal Attention networks. In light of past findings, the observed pattern of results suggests that differences in segregation of TPN during working memory are associated with DAT1 genotype. The Dorsal Attention network, which primarily includes regions along the intraparietal
sulcus, has been argued to control voluntary, top-down orienting of attention and selection of behavior (Corbetta and Shulman, 2002) and specifically to be involved in rehearsal during working memory (Corbetta et al., 2002), while the Frontoparietal Control networks, which include lateral frontal and parietal regions, are believed to initiate and adjust executive control processes (Dosenbach et al., 2007, 2008; Seeley et al., 2007; Vincent et al., 2008). During working memory, these functions, which are primarily relevant to immediate task goals, are likely to be strongly segregated from the TNN, which process task-irrelevant thought (Fox et al., 2005; Spreng et al., 2010). By contrast, the Salience network, which includes anterior insula, anterior middle frontal gyrus, and dorsal anterior cingulate, is believed to maintain longer-term task goals and process stimuli salient to those goals (Dosenbach et al., 2007, 2008; Seeley et al., 2007). Based on the involvement in this canonical network of the dorsal anterior cingulate, which is known to perform monitoring processes (Carter and Van Veen, 2007), as well as the involvement of the anterior insula, which is believed to mediate dynamic interactions between brain networks (Menon and Uddin, 2010), we speculate that increased TNN to Salience connectivity during working memory may reflect an increased need to detect TNN-based cross-network interference and segregate the networks appropriately. In this context, the failure to reduce connectivity between TNN and Frontoparietal Control / Dorsal Attention networks in 10/10 subjects reflects a lack of segregation between networks, and those subjects’ increased connectivity between TNN and Salience networks reflects increased effort needed to prevent this lack of segregation from interfering with task performance.

The mechanism by which DAT1 may influence differences in connectivity is not clear. DAT1 genotype affects expression of dopamine transporter (DAT), as higher expression is associated with the 10-repeat allele (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005), likely
.leading to genotype differences in DA signaling (Madras et al., 2005). In light of observations of
greater phasic DA release during working memory than during rest (Aalto et al., 2005), DAT1-
related differences may be enhanced during working memory, as more DA would be available in
the synapse to be reuptaken at differential rates (depending on genotype); this likely explains
why the effects of DAT1 on cross-network connections emerged most strongly during working
memory. DAT concentrations are highest in striatum (Hall et al., 1999; Madras et al., 2005),
moderate in parietal cortex (Lewis et al., 2001) and relatively low in prefrontal cortex (Karoum
et al., 1994). Therefore, DAT1 effects on brain function should be strong within striatum and
reduced within prefrontal and parietal cortex—yet the present study, and past fMRI studies
(Bertolino et al., 2006, 2009; Caldú et al., 2007; Stollstorff et al., 2010), found effects of DAT1
in prefrontal and parietal cortex. This is consistent with positron emission tomography (PET)
studies, which have found that the degree of DAT expression (Tomasi et al., 2009) and dopamine
synthesis (Braskie et al., 2011) within the striatum predicts cortical activation. We speculate that
these DAT1 effects on cortico-cortical connectivity might be driven by the degree to which the
striatum “gates” communication between different networks; as suggested by Braskie et al.
(2011), this gating is likely enabled through the striatal-palladial-thalamic-cortico loops which
innervate both prefrontal and parietal cortex (Alexander et al., 1986; Schmahmann and Pandya,
2006). Both neurocomputational models (Hazy et al., 2007) and imaging evidence (van
Schouwenburg et al., 2010) suggest that the striatum plays a causal role in allowing or
preventing (“gating”) information transfer between cortical regions. Thus, increased cross-
network TPN to TNN interference observed in 10/10 DAT1 subjects could be due to lower
striatal DA function reducing the ability of the striatum to gate information transfer between
networks. While DAT1 effects were observed on connectivity between striatum and the parietal
Dorsal Attention network, these effects were insensitive to state, suggesting that this connection is unlikely to be a gating signal mediating the DAT1 X cognitive state interaction effects on cross-network connectivity. We speculate that the gating effect may be causally “upstream” of network connectivity effects, and so undetectable using connectivity assessed via pairwise correlations. Future investigations using more complex connectivity measures such as dynamic causal modeling might profitably investigate this question.

Limitations

While associations between cross-network connectivity and trait-level measures of executive function were successfully observed, this study was notably limited in its inability to investigate relationships between DAT1, connectivity, and behavioral performance on the N-back task, as overall N-back accuracy was very close to ceiling (mean±SD = 96%±5.8%), and likely as a result, the genotype groups did not differ in accuracy in any condition (ps >.15). This lack of difference is not unusual: while deficits in 10/10 subjects have been observed on the N-back task in children (Stollstorff et al., 2010), such deficits have not been found in adults (Bertolino et al., 2006, 2008, 2009; Blanchard et al., 2011; Caldú et al., 2007).

Associations with connectivity were observed with Barratt Impulsiveness Scale scores but not with the Hyperactive/Impulsive scores of the ADHD Self-report Rating Scale. This is likely because the ADHD Scale is designed to assess the presence of ADHD in adults with a limited number of questions, and therefore may not be sensitive to individual variation in hyperactivity/impulsivity within a nonclinical population. By contrast, the Barratt Scale is designed to assess impulsivity in nonclinical populations within several domains. By this interpretation, the fact that connectivity did correlate with Inattention on the same clinically-
oriented ADHD Scale suggests a particularly strong relationship between connectivity and inattentiveness.

We regressed out the effect of the N-back load condition to avoid contaminating the N-back connectivity analysis with load-related coactivations. However, using a block design prevented us from being able to model and remove effects of coactivation in individual trials; nor could we model and remove error trials (though errors were sparse in both genotype groups). The extent to which the inclusion of these trial-by-trial coactivations and errors may be altering our functional connectivity results is unknown.

**Conclusion**

The present results, which show that cross-network connectivity is sensitive to a genetic polymorphism important for regulating synaptic dopamine, provide an endophenotype for inefficient executive function, as reflected in higher impulsivity and inattention. This finding has important implications for cognitive disorders associated with dopamine dysregulation, such as schizophrenia and, especially, ADHD. Future studies are required to address the mechanisms by which dopamine transporter expression may lead to elevated cross-network connectivity between networks engaged and suppressed during externally oriented cognitive engagement.
CHAPTER VI: GENERAL DISCUSSION

This dissertation presented four studies exploring how intrinsic connectivity networks (ICNs) measured during the task-free resting state are related to working memory (WM) performance, dopamine (DA) function, and trait-level executive control in healthy adults. To address the dissertation’s first goal of investigating relationships between resting state ICNs and WM-driven brain function, Chapter II demonstrated concordance between the ICNs delineated during the resting state and those active during performance of an N-back WM task, and showed how knowledge of resting state ICNs could help identify correlations between task activation and WM performance. Further, Chapter III demonstrated that ICNs are altered during WM performance, and that those alterations persist into a subsequent resting state, which affects the ability to observe correlations between connectivity strength and trait-level inattentiveness. Together, these two studies illustrated how resting state ICNs are both engaged by and are altered by WM, and that those ICNs predict multiple executive function processes, including both WM and inattentiveness. These findings suggest that resting-state measures of ICNs can serve as an endophenotype for WM and other executive control functions.

To address the dissertation’s second goal of examining the impact of DAT1 genotype on functional connectivity and executive control, Chapter IV demonstrated that a common polymorphism in the DAT1 gene affects resting state connectivity between the striatum and the prefrontal cortex, and that the affected connectivity strengths predict N-back performance and trait-level impulsiveness. Further, Chapter V demonstrated that the same polymorphism affects cross-network connectivity between “Default” and “Task-positive” ICNs during an N-back WM task, but not during the resting state, and that the degree to which these connectivities change
during the task predicts trait-level inattentiveness and impulsivity. Together, these two studies demonstrated the first linkages between the *DAT1* gene, functional connectivity of striato-cortical and cortico-cortical networks, and multiple executive function processes, including WM, inattentiveness, and impulsivity. In sum, these four studies indicate that ICNs are engaged by WM, are altered by WM, predict both WM capacity and trait-level executive function, and are influenced by *DAT1*. These findings thus strongly suggest functional connectivity as an endophenotype that is causally intermediate between the *DAT1* gene and multiple executive processes such as WM, attention, and control of impulsiveness.

**Implications for Future Studies**

*Implications for studies of working memory*

Chapter III demonstrates that alterations of functional connectivity driven by performance of a WM task can persist after the conclusion of that task. These persistent connectivity alterations were tentatively hypothesized to represent a recovery period following the strong cognitive effort required to perform the task. Future work may profitably test this hypothesis by extending the present experiment in two ways. First, if the post-task connectivities are truly affected by cognitive effort, then manipulating the degree of cognitive effort should affect those post-task connectivity changes. An effective approach would be a within-subjects design in which each subject is scanned while performing a highly challenging task between two resting scans, as well as while performing an unchallenging task between two other resting scans. Second, as discussed in Chapter III, if post-task connectivity changes reflect a recovery period, then it would be expected that this recovery period would be transient, and thus that the changes in connectivity would eventually return to pre-task baseline levels, as shown by Barnes et al. (2009) using
oscillatory brain dynamics. The possibility of a return to baseline of the task-altered functional connectivities could be examined by extending the post-task resting state scan to 20 minutes. These experimental manipulations would help to further conceptualize the post-task alterations in resting state functional connectivity observed in Chapter III.

*Implications for studies of DAT1*

The findings from Chapters IV and V showing that a polymorphism of the *DAT1* gene can affect both striato-frontal and cortico-cortical connectivities may explain how previous work has found effects of *DAT1* in cortex, even though the gene is not strongly expressed in cortex. *DAT1* is primarily expressed in striatum, with only low concentrations of DAT found in cortex (Hall et al., 1999; Madras et al., 2005; Sasaki et al., 2012). However, *DAT1* has been shown to affect WM-related activation in the purely cortical Task-Positive network (Bertolino et al., 2006, 2008, 2009; Caldú et al., 2007; Stollstorff et al., 2010) as well as deactivation in the cortical Default network (Brown et al., 2011). Similarly, other measures of striatal DA function have been shown to impact cortical function, as both DAT availability in the striatum (Tomasi et al., 2009) and striatal DA synthesis capacity (Braskie et al., 2011) have been shown to predict Default network activation, while striatal D1 receptor density has been shown to predict functional connectivity between the Default and Task-Positive networks (Rieckmann et al., 2011). The present work indicating that *DAT1* genotype affects both connectivity between striatum and Task-Positive PFC regions as well as connectivity between Task-Positive and Default networks suggests a possible explanation: that effects of striatal DA function on cortical activation and connectivity may be “downstream” effects of altered striato-prefrontal connectivity. In this conception, differences in the *DAT1* gene directly affect the function of the striatum, but not the cortex. This
altered striatal function influences how the striatum communicates with Task-Positive PFC regions, and in turn, this DAT1-modulated communication influences how these regions communicate with Default network regions in both PFC and posterior cortex, resulting in widespread effects of genotype on connectivity even outside the regions directly influenced by the expressed gene. This possibility has not been explicitly tested, but may be explored in future work using analysis techniques that can explore “effective”, or causally directed, connectivity, such as dynamic causal modeling (Friston et al., 2003). Effective connectivity analyses could more directly test whether DAT1-related differences in cortico-cortical connectivity are causally influenced by differences in striato-cortical connectivity. If verified, this explanation would illustrate how perturbations of one aspect of a brain system may be transmitted via downstream effects to alter a wide variety of brain networks—a possibility not considered by many studies that examine effects on single a priori brain regions and connections.

The studies presented here may profitably be extended to study how DAT1 influences other executive processes that may involve different aspects of the DA system. For example, the results from Chapter IV indicate the presence of relationships between DAT1, resting state connectivity, and trait-level impulsivity. A delay discounting task can measure impulsivity by assessing the control of impulses driven by the immediacy of reward stimuli. Delay discounting tasks are known to involve the ventral striatum (Hariri et al., 2006) and to be sensitive to DA function (Koffarnus et al., 2011), and performance on these tasks correlates with functional connectivity between the ventral striatum and prefrontal cortex (Kayser et al., 2012). It would thus be very informative to examine how DAT1-dependent connectivity relationships may be altered during performance of a delay-discounting task, using methods similar to those in
Chapter V, and then to examine whether those alterations persist after task conclusion, as in Chapter III.

**Implications for studies of other genes**

By helping to validate functional connectivity as an endophenotype, this work paves the way for future investigations exploring associations between polymorphisms in other genes known to be important for cognition, variation in physiologically plausible connectivity circuits, and individual differences in behavior. For example, similar to the *DAT1* gene investigated in the present study, the catechol-*O*-methyltransferase (*COMT*) gene has a common polymorphism that reduces synaptic DA signaling in prefrontal cortex, has been linked to a neuropsychiatric disorder (schizophrenia; Wonodi et al., 2003), impairs WM and other prefrontal-dependent executive functions (Bruder et al., 2005), and affects functional connectivity of prefrontal cortex (Liu et al., 2010; Sambataro et al., 2009). It thus seems plausible that functional connectivity measures may serve as endophenotypes between the *COMT* gene and the disrupted WM seen in schizophrenia, but this has not been comprehensively investigated. By carefully elucidating all aspects of the relationship between genes, functional connectivity, and behavior, the present studies help provide a blueprint that future investigations can follow in order to identify fcMRI endophenotypes mediating relationships between behavior and *COMT*, or any other genetic polymorphisms of interest.

Beyond single-gene effects, it will soon be important to investigate endophenotypes that are sensitive to multiple genetic factors, and functional connectivity measures are likely to be sensitive in this way. Currently, a substantial proportion of imaging genetics work investigates only a few well-studied genes, such as the *DAT1* gene examined in the present work, the *COMT*
gene (e.g., Bertolino et al., 2006), the brain-derived neurotrophic factor (BDNF) gene (e.g., Egan et al., 2003), and the serotonin transporter gene promoter region (e.g., Pezawas et al., 2005). While examining a few well-studied genes is informative, it is unlikely that these few polymorphisms constitute the entire repertoire of genetic influences on neural function and cognition. Indeed, with the advent of relatively cheap genotyping technologies for single polymorphisms, and the promise of affordable whole-genome sequencing in the not-too-distant future, it is likely that soon the field will see an explosion of behaviorally relevant genetic polymorphisms, many of which will have slightly different but strongly overlapping (or interacting!) effects on neural function. Because measuring functional connectivity can potentially provide information about the connection between any two regions in the brain, it results in unusually rich datasets that may be used to explore the combined effects of these genetic factors. For example, many behaviorally relevant polymorphisms are already known to affect the DA system, including those in DAT1, COMT, the DA receptor D2 gene, the DA receptor D4 gene, the MAO-A gene, and others. Importantly, anatomical knowledge of the locations in which these genes are expressed—such as that DAT1 and DRD2 are primarily expressed in striatum, while COMT is primarily expressed in prefrontal cortex—can help provide a priori information about network connections that may be affected by the genes. When this knowledge is applied to a large enough dataset that comprehensively measures the network structures of individual human brains, the effects of many different DA-modulating genes may be incorporated into an overall model of DA network function that can be empirically tested using functional connectivity.

*Implications for studies of ADHD*
Chapters IV and V demonstrate relationships between the 10/10 allele of the DAT1 gene, reduced striato-frontal and increased Default-Task Positive functional connectivity, and worse function of executive processes including WM, impulsivity, and inattentiveness. A natural extension of these studies would be to conduct similar investigations of the effect of the DAT1 gene in adults with Attention Deficit Hyperactivity Disorder (ADHD), as ADHD is a disorder that is associated with the 10/10 genotype (Yang et al., 2007), demonstrates reduced striato-frontal (Cubillo et al., 2010; Rubia et al., 2009) and Default-Task Positive (Castellanos et al., 2008) connectivity, exhibits impaired WM function (Willcutt et al., 2005), and is defined by worse impulsivity and inattention. The striking similarity in neural and behavioral alterations observed here in 10/10 individuals to those previously observed in ADHD individuals strongly suggests that altered functional connectivity in these striato-frontal and Default-Task positive circuits is an endophenotype partially mediating the relationship between the DAT1 gene and ADHD-like behaviors. However, while the association between the 10/10 DAT1 genotype and ADHD is significant, it is not very strong (Odds ratio = 1.17; Yang et al., 2007), indicating that a substantial portion of the population variability in ADHD cannot be explained by DAT1 genotype. It would thus be very informative to use techniques similar to those in Chapter IV and V in order to examine how DAT1 affects network connectivities in genotyped adults with and without ADHD, and how those connectivities predict individual differences in executive function. This would help to disentangle effects of DAT1 on ADHD-like connectivity and behaviors from other unknown effects.

The validation of functional connectivity as an endophenotype between DAT1 and executive function elucidated by this work also suggests that changes in functional connectivity may be a valid outcome measure for certain treatments, including treatment of ADHD. ADHD is
commonly treated using stimulant medications that strongly affect the DA system in order to improve executive functioning. While ADHD individuals demonstrate reduced striato-frontal connectivity, like the 10/10 DAT1 genotype individuals in the present studies, those connectivity alterations are normalized by administration of stimulant medication (Peterson et al., 2009). The present findings suggest that these stimulant-related improvements in connectivity observed in ADHD individuals may be correcting the abnormal connectivities induced by genetic factors such as DAT1. More generally, it can be argued that functional connectivity circuits that have been shown to be endophenotypes of disorder-related genetic polymorphisms may be good targets for treatment.

**Conclusions**

Studies of intrinsic functional connectivity have generated a great deal of recent excitement in the cognitive neuroscience field for several reasons. First, the technique can be used in many populations that would not be able to perform a task for activation-based fMRI studies, such as catatonic patients, sleeping infants, or even in utero fetuses. Second, the technique newly provides the ability to examine in vivo network level brain function that is not driven by the performance of a task, but instead reflects the brain’s intrinsic network architecture. The four studies presented in this dissertation, which described how intrinsic functional connectivity networks are related to working memory function, trait-level executive control, and a genetic polymorphism regulating dopamine signaling, help provide a new reason for enthusiasm about functional connectivity, as they demonstrate the potential for this technique to be a valuable tool
in the search for genetically-influenced neural underpinnings of executive function and dysfunction.
APPENDIX

Supplemental Material from Chapter II

Figure S1: Group-level N-back > fixation (red-yellow) and Fixation > N-back contrasts (blue-green) conducted in SPM5. Individual subject β contrast maps were entered into one-sample t-tests against a null hypothesis of zero. Results were thresholded at p<.05, FWE corrected for multiple comparisons.

Figure S2: Group-level Fixation > N-back contrast conducted in SPM5. Individual subject Fixation > N-back β contrast maps were entered into a one-sample t-test against a null hypothesis of zero. Results were thresholded at p<.05, FWE corrected for multiple comparisons. Note N-back related deactivation in secondary visual regions (yellow circles).
**Speed-accuracy relationships:** The high accuracy rates observed in the current study may result from a speed-accuracy tradeoff, which could alter the interpretation of faster RTs as indicating superior performance, and confuse the interpretation of ICN-RT relationships. A speed-accuracy tradeoff is unlikely, as subjects with perfect accuracy were not slower than subjects who made mistakes (main text). To further demonstrate that speed-accuracy tradeoffs do not affect the current findings, three post-hoc analyses were performed. First, we tested whether RTs differed for any individual load condition between perfect subjects and subjects who made mistakes. For each subject, mean RT was calculated in each load condition. For each load, two-sample t-tests were conducted testing for RT differences between subjects who were perfect across all conditions and subjects who made at least one mistake. No RT differences were found in any load (ps > .1), suggesting that subjects who made no mistakes did not perform well just because they responded slower. Second, we tested whether higher accuracy in subjects who made mistakes was associated with slower RT. For the twenty subjects who made at least one mistake, a correlation was conducted between overall accuracy and overall mean RT. This correlation was not significant (r = .12, p > .6), indicating no consistent relationship between speed and accuracy even when subjects were below ceiling. Third, as the inclusion of error trials in the block design might theoretically alter patterns of RT and induce artificial relationships between RT and brain measures, we tested whether the association between RT and IFPC network activation existed even in subjects with perfect accuracy. For the twenty subjects with perfect accuracy, IFPC β values were correlated with overall mean RT. Even with this reduction in power, the negative correlation previously observed (main text) remained present at trend level (r = -.38, p = .096), suggesting that the association between RT and IFPC network activation was present even when no errors existed to affect RT values. In summary, no speed-
accuracy tradeoffs were observed in the present study, and exclusion of subjects who made errors did not alter the association between RT and IFPC activation.

Figure S3: Group-level effects of mean reaction time (RT) on task-related activation conducted in SPM5. Individual subject N-back > Fixation β contrast maps were entered into a linear regression, using the mean RT from each subject as a regressor of interest. (A) At a threshold of $p<.05$ uncorrected, RT is predicted by activity in left-hemisphere lateral frontal and inferior parietal regions. (B) Correction for multiple comparisons eliminates the ability to detect this effect.

Effects of regressor number on Spatial Multiple Regression: Individual subjects’ ICNs were identified by matching their ICA components to ICNs from a group-level ICA. Using this procedure, subjects were commonly “missing” one or more individual versions of the group ICNs. These missing ICNs could not be used as regressors in the subsequent spatial multiple regression against task activation; thus, some subjects had a full complement of 11 regressors, while others had as few as 8 regressors. In theory, these variations could systematically affect the β values resulting from the regression. To test whether different numbers of regressors was a
source of potential bias in the resulting β values, we conducted two analyses. First, a one-way ANOVA for each network was conducted to test whether number of components significantly affected β values for that network (ANOVAs were used rather than correlations because the number of components could only be one of four discontinuous values: 8, 9, 10, and 11). The effect of component number was nonsignificant for every network (ps > .09), indicating no β differences between subjects with 8, 9, 10, or 11 regressors for any of the networks. Second, the group linear regression testing for effects of β values on RT, as well as the group logistic regression testing for effects of β values on accuracy and accuracy (main text), were repeated with the number of valid ICNs for each subject included as a regressor of no interest. The results reported in the main text did not change. RT was predicted only by lFPC engagement (p=.037), and accuracy was still predicted by engagement of lFPC (p=.044) and Dorsal Attention (p=.021).

In summary, it appears that no systematic bias was introduced by using different numbers of regressors for each subject.

**Supplemental Material from Chapter III**

**N-back performance**

N-back accuracy was very high both overall (95.5% ± 5.7%) and in each load condition (1-Back: 97.6% ± 5.9%; 2-Back: 99.0% ± 4.0%; 3-Back: 89.5% ± 14.2%). Paired t-tests revealed that accuracy was lower in the 3-back condition than in both the 2-back condition (t(49)=4.83, p<.001) and the 1-back condition (t(49)=3.95, p<.001), with no differences between the 1- and 2-back conditions (t(49)=1.46, p=.15). However, 22 of the 50 subjects made no mistakes in any load condition. Because this ceiling-level performance would preclude variance in performance, accuracy was not included in any further analyses.
For each load, mean reaction times (RT) were calculated for all correct responses. Paired t-tests revealed that 1-back RT (494ms ± 123ms) was lower than 2-back RT (527 ± 146, t(49)=2.13, p<.05), while 2-back RT was significantly lower than 3-back RT (599ms ± 236ms, t(49)=3.21, p<.005).

Identification of PCC-seeded resting state networks in the present data

To determine whether the independently derived a priori ROIs overlapped with the present subjects’ DMN and TPN regions, we identified the DMN and TPN in the Rest1 runs of the present subjects using voxelwise correlations with a posterior cingulate (PCC) seed. A PCC seed region of interest (ROI) was created using Marsbar (Brett et al., 2003) as a sphere of radius 6mm centered around the coordinates [-3 -53 27], which was the MNI-transformed voxel of maximal DMN connectivity reported by De Luca et al. (2006). For each subject’s initial resting state run, partial correlations were conducted between the PCC seed timecourse and the timecourses of every voxel in the brain, while partialling out motion and physiological noise timecourses. The resulting r values were converted to Z-scores using Fisher’s transformation. This produced a brain map of intrinsic connectivity strength with the PCC during the initial resting state. The resulting PCC connectivity maps from all subjects were entered into a one-sample T-test (p < .05, FWE corrected) testing for voxels positively connected to the PCC, and another testing for voxels negatively connected to the PCC. This analysis generated a map of positive connections with PCC, which can be interpreted as the DMN, as well as a map of negative connections with PCC, which can be interpreted as the TPN.

The one-sample t-test revealed widespread positive connectivity within the classic DMN (Greicius and Menon, 2004; Raichle et al., 2001), including large clusters within PCC/Precuneus extending into retrosplenial and medial temporal cortex, ventral and anterior dorsal medial
prefrontal cortex extending into ventral striatum and into bilateral superior frontal gyrus, bilateral angular gyrus, and bilateral lateral temporal cortex (Figure S1A, red). Negative connectivity with the PCC was observed in classic Task-Positive regions (Fox et al., 2005), including in bilateral middle frontal gyrus, bilateral anterior insula extending into inferior frontal gyrus, bilateral supramarginal gyrus, and dorsal anterior cingulate cortex extending into medial supplementary motor area (Figure S1B, red). Notably, the identified DMN and TPN had good spatial overlap with the ICA-derived DMN and TPN node ROIs used in the present study (Figure S1A-B, blue).

Figure S1: A) Overlap between a priori DMN nodes used in the present study in blue, and regions strongly correlated with a spherical posterior cingulate seed (from De Luca et al. 2006), in red. B) Overlap between a priori TPN nodes used in the present study, in blue, and regions strongly anticorrelated with a spherical posterior cingulate seed (from De Luca et al. 2006), in red.
Identification of brain regions activated and deactivated during the N-back task

First-level analysis was performed using a general linear model as implemented in SPM8. For each subject, four temporal regressors were specified, each one consisting of a boxcar timeseries convolved with a hemodynamic response function. The regressors represented the timecourses of the 1-back, 2-back, 3-back, and fixation conditions. For each subject, Task>Fixation (i.e. [1Back+2Back+3Back]>Fixation) and Fixation>Task contrasts were specified to delineate regions activated and deactivated during the task, regardless of N-back condition. For group averaging, a one-sample t-test was conducted for each contrast at p < .05, Family-Wise Error corrected for multiple comparisons.

Figure S2 displays group averages of activated (N-back > Fixation; red-yellow scale) and deactivated (Fixation > N-back; blue-green scale) regions. Activated regions had good overlap with the TPN node ROIs used in the present study and included bilateral middle frontal gyrus, anterior insula, premotor cortex, lateral parietal cortex, medial dorsal anterior cingulate extending into supplementary motor area, and globus pallidus. Deactivated regions had good overlap with the DMN node ROIs used in the present study and included ventromedial prefrontal cortex and perigenual anterior cingulate, anterior medial prefrontal cortex, and medial parietal cortex (including posterior cingulate and precuneus), as well as bilateral fusiform gyrus, hippocampus/amygdala, posterior insula, anterior middle and superior temporal gyri, and lateral/superior occipital cortex extending into angular gyrus.
Examining whether motion was associated with reported functional connectivity and behavioral trait effects.

While motion in our subjects was minimal across all runs, recent work has shown that even small movements can alter functional connectivity measurements (Power et al., 2012). However, multiple pieces of evidence suggest that the effects of performing a working memory task on functional connectivity we observed were not artifactually driven by motion. First, as described in the main text, the root mean square (RMS) summary measure of motion did not significantly differ between runs (Rest1 vs Nback: t(49) = 1.71, p = .09; Nback vs Rest2: t(49) = .25, p = .80; Rest1 vs Rest2: t(49) = 1.8, p = .08). Second, we correlated RMS measures in each run against all functional connectivity measures in that run. None of the 60 correlations were significant at p<.05, False Discovery Rate corrected for multiple comparisons, indicating that there were no reliable relationships between motion and functional connectivity. Finally, we correlated the RMS measures of motion from each run against the behavioral Inattention scores and found no significant relationships (Rest1 RMS vs Inattention: r = -.25, p=.08; Nback RMS vs Inattention: r
= -.19, p=.18; Rest2 RMS vs Inattention: r = -.15, p=.30), indicating that motion was likely unrelated to the connectivity-behavior relationships observed in Rest1.

**Examining whether removal of timepoints from the N-back run drives reported effects on functional connectivity.**

To restrict connectivity analyses during the blocked N-back task to only those times during which the task was being performed, timepoints during and surrounding the fixation blocks were removed from analysis, resulting in only 108 timepoints of the N-back run being analyzed, compared to 148 timepoints from the two resting-state runs. As both the N-back and Rest2 runs were found to show altered functional connectivities compared to the Rest1 run, it is unlikely that this removal of timepoints from the N-back run artifactually drove these findings, as no such removal was conducted for the Rest2 run. To further demonstrate that this removal of timepoints was not responsible for the primary findings of the study, we re-calculated functional connectivity measures from the N-back task without removing the timepoints associated with the task’s fixation blocks. As in the main text, State X Node ANOVAs were then conducted on the connectivities from each run (Rest1, N-back without removal of timepoints, and Rest2) within the same ROIs.

**Within-DMN:** As in the primary analysis, both a main effect of State (F(2,48) = 4.03, p = .024) and a State X node interaction (F(6,44) = 8.34, p < .001) emerged. Overall PCC connectivity with the DMN nodes became significantly less positive from the Rest1 run to the N-back run (t(49)=2.81, p = .007), and then returned to be more positive in the Rest2 run than in the N-back run (t(49) = 2.02, p = .049), with no differences between the Rest1 and Rest2 runs (t(49) = .45, p = .65)—the same pattern as demonstrated in the primary analysis. As in the primary analysis, the interaction effect was driven by significant connectivity alterations during the N-
back task in the two AG nodes, in which connectivity became significantly less positive from the 
Rest1 run to the N-back run (rAG: t(49) = 7.27, p < .001; lAG: t(49) = 3.14, p = .003), and then 
became significantly less positive again from the N-back run to the Rest2 run in the rAG node 
(t(49) = 6.21, p < .001) but not the lAG node (t(49) = 1.50, p = .14), with no differences observed 
between the Rest1 and Rest2 runs in either node (t(49) < 1.5, ps > .30). The mPFC node showed 
an effect in the opposite direction during the N-back task, with connectivity becoming more 
positive during the N-back task than during the Rest1 run (t(49) = 2.43, p = .019), but no change 
between the N-back and Rest2 runs (t(49) = 1.26, p = .21). In the Prec node, no differences by 
state were observed (all t(49) < 2.0, all ps > .10).

**DMN-TPN:** As in the primary analysis, both a main effect of State (F(2,48) = 12.62, p < 
.001) and a State X Node interaction (F(10,40) = 8.00, p < .001) emerged. Overall PCC 
connectivity with the TPN nodes became significantly less negative from the Rest1 run to the N-
back run (t(49) = 3.65, p = .001), and remained less negative in the Rest2 run than it was in the 
Rest1 run (t(49) = 4.52, p < .001), with no difference observed between the N-back and Rest2 runs 
(t(49) = .59, p = .56)—the same pattern as demonstrated in the primary analysis. As in the primary 
analysis, the interaction effect was driven by different behavior among the nodes across runs as 
follows: In the dACC, raIns, and laIns nodes, connectivity became less negative from the Rest1 
run to the N-back run (t(49) > 2.66, ps < .015) and remained less negative in the Rest2 run than 
it was in the Rest1 run (t(49) > 2.9, ps < .005), with no differences observed between the N-
back and Rest2 runs (t(49) < 1.2, ps > .20). A similar pattern was observed in the rSMG node, 
with connectivity becoming less negative from the Rest1 run to the N-back run (t(49) = 8.05, p < 
.001), though connectivity did become more negative again from the N-back run to the Rest2 run 
(t(49) = 3.82, p < .001) but still remained less negative in the Rest2 run than it was in the Rest1
run \((t(49) = 4.73, \ p < .001)\). In the rdlPFC and ldlPFC nodes, connectivity did not change from the Rest1 run to the N-back run \((t(49) < .60, \ ps > .50)\), but became less negative from the N-back run to the Rest2 run \((t(49) > .28, \ ps < .01)\) and remained less negative in the Rest2 run than in the Rest1 run \((t(49) > 2.60, \ ps < .015)\). This pattern of results exactly duplicates the results from the primary analysis.

**Within-TPN:** As in the primary analysis, both a main effect of State \((F(2,48) = 6.78, \ p = .003)\) and a State X Node interaction \((F(8,42) = 4.70, \ p < .001)\) emerged. Overall raIns connectivity with TPN nodes became significantly less positive from the Rest1 run to the N-back run \((t(49)=3.14, \ p=.001)\) and remained less positive in the Rest2 run than it was in the Rest1 run \((t(49)=2.97, \ p=.005)\), with no difference observed between the N-back and Rest2 runs \((t(49)=1.59, \ p=.12)\)—the same pattern as demonstrated in the primary analysis. As in the primary analysis, the interaction was driven by different behavior among the nodes across runs as follows: In the laIns node, connectivity followed the pattern of the overall network, becoming significantly less positive from the Rest1 run to the N-back run \((t(49) = 2.18, \ p = .034)\), with no differences observed between the N-back and Rest2 runs \((t(49) = .21, \ p= .83)\). By contrast, in the rSMG and dACC node, connectivity also became less positive from the Rest1 run to the N-back run \((ts(49) > 3.25, \ ps < .005)\), but then became more positive again from the N-back run to the Rest2 run \((ts(49) > 2.80, \ ps < .01)\), returning all the way back to baseline such that no difference was observed between the Rest1 and Rest2 runs \((ts(49) < 2.0, \ ps > .05)\). Finally, State did not appear to affect connectivities in the ldlPFC or rdlPFC nodes, as no significant pairwise connectivity comparisons emerged \((ts(49) < 2.0, \ ps > .05)\).

In summary, including the fixation block timepoints in the analyses of N-back connectivity altered those connectivity estimates only slightly, which altered a few of the effects observed in
individual nodes. This is not surprising, as those N-back connectivity estimates now also include data from periods during which no task is being performed, which is very likely to alter the measured connectivity values. Importantly, the findings from the primary analyses—that across all nodes, performance of the N-back task induces less negative DMN-TPN connectivity and less positive within-TPN connectivity, and that those altered connectivity values do not return back to baseline during a post-task resting state, are not affected by the inclusion of the additional timepoints. Thus, these results demonstrate that the primary findings of this study were not artifactually driven by the removal of the fixation block timepoints from the N-back task.

**Supplemental Material from Chapter V**

![Image](image_url)

**Figure S1:** Six “Task-Neutral” networks delineated in the resting-state data by the ICA procedure. Network maps are visually thresholded at $Z = 15.0$. 
I. Activation of network nodes

We do not think that the differential connectivity demonstrated in the two DAT1 genotype groups during the N-back task was driven by differential task- or load-driven activation, as we conducted the connectivity analyses 1) only within the task blocks, and 2) after regressing out effects of working memory load (see main text). However, we tested for the possibility of DAT1 differences in activation by entering N-back > Fixation contrasts from each subject into a two-sample t-test comparing 10/10 and 9/10 groups. No voxels survived Family-Wise correction, \( p < .05 \), for either 10/10 > 9/10 or for 9/10 > 10/10. To minimize Type-II error, we subsequently interrogated each subject’s N-back > Fixation contrast within each of the 19 network node ROIs (as defined in main text), averaging across voxels to obtain the mean N-back-related activation within the ROI. For each ROI, each subject’s mean activation was entered into a two-sample t-test testing for effects of genotype, both for 10/10 > 9/10 and for 9/10 > 10/10. No effects of genotype were found for any ROI (all uncorrected \( p s > .05 \)). Thus, as working memory-related activation did not differ by DAT1 in this sample, activation differences cannot be driving the observed connectivity effects.

II. Connectivity analysis without regression of working memory load effects

To examine whether observed connectivity effects were altered by the preprocessing step of removing variation due to the working memory load manipulation of the N-back task, we reanalyzed the data without this step. All analysis methods were identical to those described in the main text, except that the load parameter regressors (three boxcar timecourses convolved with the hemodynamic response) were not included as effects of no interest when regressing out nuisance signals.
Main effects of State: As in the analysis presented in the main text, main effects of cognitive state were observed in numerous TPN to TPN and TPN to TNN connections (Fs(1,75) ranged from 5.48 to 107.60; see Figure S2). Indeed, all forty-seven TPN to TPN connections and TNN to Frontoparietal Control / Dorsal Attention connections which demonstrated significant effects of cognitive state when regressing out working memory load effects in the main text also demonstrated those effects when the regression was not performed. As in the main text analysis, connectivity in the TPN to TPN connections was uniformly greater during working memory than during rest, and connectivity in TNN to Frontoparietal Control / Dorsal Attention connections was uniformly reduced during working memory compared to Rest. These results suggest that working memory-related connectivity increases in TPN to TPN connections, as well as connectivity decreases in TNN to Frontoparietal Control / Dorsal Attention connections, are not dependent on the regression of working memory load. Further, a TNN to Striatal connection also emerged (vmPFC to R Striatum), with a similar direction of effect as the TNN to Frontoparietal Control / Dorsal Attention connections (Rest > N-back). Notably divergent from the initial main text analysis, only one TNN to Salience network connection emerged (L AG vs R aIns) demonstrating an opposite effect of state (Rest > N-back). Thus, the working memory-related connectivity increases between TNN and Salience networks observed in the main text disappear almost completely when working memory load effects are not removed. These results are consistent with findings by Fransson (2006), who showed cross-network connectivity decreases in lateral frontal and parietal regions during working memory, and who also did not regress out effects of working memory load.
**Figure S2:** Matrix indicating ROI pairs in which significant main effects of State were observed on connectivity, when working memory load was not regressed out of the data. Increased connectivity between ROIs during the N-back task compared to rest is indicated by hot color shading; decreased connectivity is indicated by cool color shading. The green dotted line demarcates cross-network (Task-Negative to Task-Positive) connections from within-network connections.

**Main effects of DAT1:** As in the analyses presented in the main text, main effects of DAT1 on functional connectivity were observed primarily in cross-network TPN to TNN connections, with three TPN to TPN connections also demonstrating DAT1 effects (Fs(1,75) ranged from 4.03 to 14.51; see Figure S3). As in the main text analysis, all significant connections emerged due to increased connectivity in 10/10 subjects compared to 9/10 subjects. All six TPN to TNN connections which demonstrated main effects of DAT1 in the main text when regressing out
working memory load effects remained significant when load effects were not removed, while two TPN to TPN connections (L adLPFC vs R and L aIPL) showing effects in the main text also remained significant. One additional TPN to TNN connection emerged as significant in the present analysis when load effects were not removed (PCC vs R vlPFC), and while two TPN to TPN connections which had been significant in the main text analysis no longer demonstrated significant DAT1 effects (R vs L pdLPFC, L Striatum vs R aIPL), one additional TaskTPN to TPN connection emerged as significant (L Striatum vs L aIPL).

Figure S3: Matrix indicating ROI pairs in which significant main effects of DAT1 were observed on connectivity, when working memory load was not regressed out of the data. Increased connectivity between ROIs for 10/10 subjects compared to 9/10 subjects is indicated by hot color shading; decreased 10/10 connectivity is indicated by cool color shading. The green dotted line
demarcates cross-network (Task-Negative to Task-Positive) connections from within-network connections.

**DAT1 X State Interaction effects:** As in the analyses presented in the main text, interactions between DAT1 and cognitive state were observed primarily in cross-network TPN to TNN connections, though several TPN to TPN connections also demonstrated interactions (Fs(1,75) ranged from 4.02 to 13.46; see Figure S4). For the cross-network interactions, all interactions were all driven by greater connectivity during working memory in the 10/10 group than in the 9/10 group, but no group differences during Rest, as in the main text. All six connections which demonstrated interaction effects in the main text when regressing out working memory load effects remained significant when load effects were not removed. Additionally, interactions emerged in two other TPN to TNN connections (vmPFC vs R vlPFC and L AG vs L aIPL), in which the pattern of the interaction was the same as described previously, and three other TPN to TPN connections (R pdlPFC vs R adlPFC, R vlPFC vs R pIPL, and L Striatum vs R aIns), in which the interactions were inconsistent in direction.
In sum, main and interaction effects involving \textit{DAT1} strongly converged between the main text analysis, in which load effects were regressed out, and the present analysis, in which this regression was not performed. This suggests that the observed \textit{DAT1} effects are not dependent on the regression of working memory load.
III. Characterization of DAT1 main effects

Although main effects of DAT1 emerged in ten different connections (Figure 5, main text), four of these connections also demonstrated DAT1 X State interactions (Figure 6, main text), suggesting that some of these DAT1 main effects may actually have been driven by interactions. To gain additional insight into these main effects, we examined the mean connectivity strength in each genotype group, in each cognitive state (Figure S5). Within each cognitive state, post-hoc t-tests were conducted testing for effects of DAT1. These tests revealed that, in all TPN to TNN connections, 10/10 subjects demonstrated greater connectivity than 9/10 subjects during the N-back task (ps < .05), but the groups were not different during the Resting state (ps > .10). This suggests that these cross-network “main effects” of DAT1 resemble weak interaction effects. By contrast, the TPN to TPN connections showed inconsistent patterns. Within two connections (L adIPFC vs R aIPL, L Striatum vs R aIPL), 10/10s demonstrated at least a trend toward greater connectivity during both Rest and N-back (ps < .10); within another (L adIPFC vs L aIPL), 10/10s demonstrated greater connectivity during Rest (p = .027) but not during N-back (p = .21); and within a fourth (L vs R pdIPFC), 10/10s demonstrated a trend towards greater connectivity during N-back (p = .06) but not during Rest (p = .12).
Figure S5: Connectivity values by DAT and State for each significant ROI pair in Figure 5, main text. Error bars represent SE. * indicates significant difference between genotypes at p<.05; † indicates trend-level difference between genotypes at p<.10.
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wide supported psychosis risk variant on neural activation during a theory-of-mind task.


