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Cortico-striatal Function in Individuals at Genetic Risk for Schizophrenia

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Cortico-striatal Function in Individuals at Genetic Risk for Schizophrenia

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Psychology

by

Dana Wagshal

2012

ABSTRACT OF THE DISSERTATION

Cortico-striatal Function in Individuals at Genetic Risk for Schizophrenia

by

Dana Wagshal

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2012

Professor Barbara Knowlton, Chair

Evidence demonstrates that individuals with schizophrenia have impaired corticostriatal functioning. Non-psychotic relatives of patients with schizophrenia exhibit a subset of the deficits present in schizophrenia patients, suggesting that there are cognitive endophenotypes of this disorder and that these deficits appear to be associated with genetic liability for schizophrenia. In order to determine if a deficit in corticostriatal functioning is an endophenotype for schizophrenia, it is important to investigate the idea that genetic risk for schizophrenia is associated with impairment in striatal dependent tasks. Therefore, we will use both behavioral measures and fMRI to assess differences in performance level and pattern of brain activation associated with different striatal-dependent tasks to investigate the corticostriatal function of relatives of patients with schizophrenia. Both behavioral and fMRI studies will be conducted in

healthy adolescent and adult relatives schizophrenic individuals and controls using cognitive and motor skill learning tasks. The behavioral studies will measure performance and function of two different corticostriatal loops and will investigate the developmental course for the behavior pattern of relatives of schizophrenia patients. The fMRI studies will expand the results of the behavioral studies and determine whether there is a difference in the pattern of brain activation between the adolescent relatives, adolescent controls, adult relatives, and adult controls. This research makes a novel contribution in that it demonstrates that individuals with genetic liability for schizophrenia exhibit deficits in corticostriatal function. Because these individuals do not have the disease, their performance gives insights into whether corticostriatal function is an endophonotype of schizophrenia, independent of the effects of medication and the illness itself and also leads to an increased understanding of the neural mechanisms of schizophrenia and advances the search for the treatment of this severe disorder.

The dissertation of Dana Wagshal is approved.

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University of California, Los Angeles

2012

This dissertation is dedicated to my family, friends, and everyone else that believed in me

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CHAPTER 1: Introduction

Schizophrenia is a psychiatric disorder in which disturbances in thinking and cognition are key features. Patients with schizophrenia show deficits in several cognitive domains, including working memory, attention, executive control, language, and declarative memory (Asarnow, 1999; Gold & Harvey, 1993; Kuperberg & Heckers, 2000; Riley et al., 2000). Non-psychotic relatives of patients with schizophrenia exhibit a subset of these deficits, suggesting that there are cognitive endophenotypes of this disorder and that these deficits appear to be associated with genetic liability for schizophrenia. The cognitive deficits of these individuals with genetic risk to schizophrenia are readily apparent in the domains of memory, executive functioning, attention, and inhibitory control (Asarnow, Steffy, MacCrimmon, & Cleghorn, 1977; Sitskoorn et al., 2004; Szöke et al., 2005; Snitz, Macdonald, & Carter, 2006; Vink et al., 2006).

In parallel, several fMRI studies have revealed abnormal patterns of activation in a number of brain regions in non-psychotic first-degree relatives of patients with schizophrenia. In many of these studies, abnormal activity was detected in the prefrontal and parietal regions while participants were engaged in executive control or working memory tasks. Even when the relatives demonstrated normal task performance, blood oxygen level dependent (BOLD) signal differences suggested an inefficiency in the prefrontal-parietal network compared to controls (Pearlson & Calhoun, 2009). Non-psychotic relatives also exhibit functional striatal abnormalities that are similar to those seen in patients with schizophrenia (an underactivation of striatal regions compared to controls), suggesting that striatal abnormalities may be associated with genetic risk (Vink et al., 2006; Raemaekers, Ramsey, Vink, et al., 2006).

This dissertation will examine skill learning, which is the gradual improvement of performance with practice on a task and is demonstrated by reduced reaction time or increased accuracy in a task, in healthy, non-psychotic siblings of patients with childhood onset schizophrenia (COS) and relatives of patients with adult onset schizophrenia (AOS) compared to controls. Specifically, we will examine 2 subtypes of skill learning, cognitive and motor skill learning. Cognitive skill learning is the improvement of performance in a task that is not motor or perceptual in nature, while motor skill learning is a change in motor performance.

Findings from studies of patients with basal ganglia disorders have shown that the basal ganglia are important for skill learning (Heindel et al., 1989; Knowlton et al., 1996). Neuroimaging studies in healthy subjects have also lent support to this theory (Grafton, Hazeltine, & Ivry, 1995; Rauch et al., 1997; Poldrack & Gabrieli, 2001; Schiltz et al., 2001; Aron et al., 2004; Moody et al., 2004). In addition, there is a longstanding hypothesis that there is a relation between the pathophysiology of schizophrenia and the dysfunction of corticostriatal circuits (Kleist, 1960). Different types of skill learning have been shown to be impaired in schizophrenics, suggesting that different cortico-striatal loops are impaired in schizophrenia. Since schizophrenia is thought to affect the corticostriatal system, studying the impairments and non-impairments of relatives of schizophrenia patients on skill learning tasks will help determine which corticostriatal loops are affected in the disorder.

To assess cognitive skill learning abilities, we will use the Weather Prediction Task (WPT), a probabilistic classification task that has been shown to depend on the integrity of the neostriatum (Knowlton, Mangels, & Squire, 1996; Knowlton, Squire, Paulsen, et al., 1996). This type of nondeclarative learning also activates striatal regions in fMRI (Foerde, Knowlton, & Poldrack, 2006; Poldrack, Clark, Pare-Blagoev, Shohamy, Moyano et al., 2001; Poldrack,

Prabakharan, Seger, & Gabrieli, 1999) and also involves activity in cognitive corticostriatal circuits including the caudate nucleus/dorsoalateral prefrontal cortex (DLPFC) and ventral striatum/orbitofrontal cortex (VS/OFC) (Aron, Shoshamy, Clarck, et al., 2004; Poldrack, Clark, Pare-Blagoev, et al., 2001; Poldrack, Prabhakaran, Seger, et al., 1999). Performance on the WPT is impaired in patients with schizophrenia (Foerde, Poldrack, Knowlton et al., 2008; Keri, Juhasz, Rimanoczy, et al., 2005; Horan, Green, Knowlton, et al., 2008; Weickert, Terrazas, Bigelow, et al., 2002), consistent with the idea that schizophrenia is associated with striatal abnormalities (Buchsbaum, 1990; Buchanan, 1993). In addition to the weather prediction task, patients with schizophrenia show deficits in other cognitive skill learning tasks such as the Tower of Toronto and the Tower of Hanoi (Gimenez, Junque, Perez, et al., 2003; Purdon, Woodword, Lindborg, et al., 2003). However, these tasks demand considerable executive control resources, and thus impairments may reflect additional deficits in these patients. Because of its relatively simple task demands, the WPT may be a more specific task suitable to assess cognitive skill learning that taps corticostriatal dysfunction.

A previous study (Foerde, Knowlton, & Poldrack, 2006) has shown that in healthy controls, the WPT can be learned explicitly using medial temporal lobe circuitry or implicitly using basal ganglia circuitry. It appears that in this task, subjects may be declaratively memorizing cue-outcome associations, while in other circumstances they learn these associations as automatic habits. It may be that the two groups reach similar behavioral endpoints, but the COS and AOS relatives have more difficulty learning the task automatically, and would continue to rely on controlled processing even after extensive practice. By inserting blocks where there is a secondary task the subject must perform, we can assess whether this task affects performance in either group and determine whether automatization has occurred.

Automatization of a skill is the ability to execute a task without the demand for executive control and effortful behavior. A major characteristic of automaticity is that there are no detrimental effects to the skill with the addition of secondary task. There is agreement in the literature that individuals with schizophrenia have a diminished capacity for processing resources than normal controls and thus the ability of individuals with schizophrenia to automate skills is more impaired than controls (Braff & Saccuzzo, 1985; Holzman, 1987; Neuchterlein, 1991). Processing resources are used when an individual is first learning a skill, and with practice, learning becomes automated and makes fewer demands on the available resources (Schneider, Dumais, & Shiffrin, 1984; Asarnow, 1999). The insufficient processing resources that handle higher processing loads can result in cognitive impairment in schizophrenia patients (Asarnow, Granholm, & Sherman, 1991; Asarnow & Sherman, 1984; Gjerde, 1983; Sherman & Asarnow, 1985). This also suggests that patients reach the limits of their available resources at lower processing loads and have fewer available resources than healthy controls (Asarnow, 1999).

Since schizophrenia is a highly genetic disorder, it would be interesting to determine if non-psychotic first-degree relatives of individuals with schizophrenia also possess this deficit by inserting dual-task probe blocks and observe if their performance changes. If performance does not decline when a concurrent task is performed, it would suggest that performance of the WPT is relatively automatic and is based on stimulus-response habits involved in automatic skills.

Though it is well documented that schizophrenia patients have a deficit in cognitive skill learning, there are mixed results in the literature concerning specific types of motor skill learning. Studies using the Pursuit Rotor and Mirror Tracing tasks have demonstrated that patients with schizophrenia show similar learning comparable to controls (Huston & Shakow, 1949; Goldberg et al., 1993; Granholm, Bartzokis, Asarnow, & Marder, 1993; Clare, McKenna,

Mortimer & Baddeley, 1993; Kern, Green, & Wallace, 1997; Weickert et al., 2002; Scherer, Stip, Paquet & Bedard, 2003; Bedard et al., 2000). However, on another measure of motor skill learning, the Serial Reaction Time (SRT) task, there is less agreement in the literature of whether or not patients possess a deficit.

The SRT is a four-alternative spatial choice reaction time task. Unknown the subject, on some sets of trials the stimuli are presented in a particular sequence while on other sets there is a pseudorandom order. The SRT depends on the integrity of the striatum and will be used to assess corticostriatal function. Performance on this task activates striatal regions in fMRI (Grafton, Hazeltine, & Ivry, 1995; Poldrack et al., 2005) that are part of motor corticostriatal circuits including the supplementary motor area (SMA), putamen, globus pallidus, and thalamus (Grafton et al., 1992; Grafton, Hazeltine, & Ivry, 1995; Poldrack et al., 2005).

Schizophrenia patients treated with typical antipsychotics display an impairment in motor skill learning compared to controls (Stevens et al., 2002, Schwartz et al., 2003). However, Foerde et al. (2008) compared performance on the WPT with performance on the SRT in patients who were treated with atypical antipsychotic drugs. There was no impairment in patients on the serial reaction time task despite a large impairment on the WPT. Other studies have also shown that patients with schizophrenia have intact motor skill learning, at least if they are treated with atypical antipsychotic medications that produce few motor side effects (Stevens, Schwarz, Schwarz, et al., 2002). Foerde and colleagues suggested that a corticostriatal loop involved in cognitive skill learning was compromised in schizophrenia while loops that are involved in motor functioning were relatively intact. This dissociation was not due to differences in the psychometric properties of the tasks. Therefore to assess motor skill learning, we will use the SRT. In addition, studies with fMRI have revealed that while schizophrenia patients and controls

perform comparably on the SRT, they do not employ the same brain regions to complete the task, and they show an underactivation of the basal ganglia (Zedkova et al., 2006; Reiss et al., 2006; Purdon et al., 2011).

Given the striking behavioral deficits found in patients with schizophrenia on the WPT but not the SRT, it is possible that deficits in cognitive skill learning are present in individuals with genetic liability for schizophrenia as well. In a recent study (Weickert, Goldberg, Egan, et al., 2010), the performance of patients with schizophrenia was compared to their siblings and controls on the WPT. While the patients exhibited a severe learning deficit consistent with previous work, there was no significant difference between the siblings and controls in terms of performance. However, further analyses suggested that there were subtle differences between these groups. When subjects were divided into good and poor learners, the siblings were disproportionately represented in the poor learner group. One reason why Weickert and colleagues may not have found an overall significant impairment of performance in siblings is that they tested siblings of adult onset patients. We will be testing first-degree relatives of AOS patients, as well as siblings of patients with COS in 2 separate studies. COS is continuous with the adulthood-onset form and it is a more rare and severe variant (Asarnow & Asarnow;, 1994; Nicolson & Rapoport, 1999; Childs & Scriver, 1986). Thus, COS patients may be a more homogeneous population with more salient genetic factors and a stronger genetic predisposition than their adult counterpart, reflecting a possibility that cognitive endophenotypes associated with this liability may be more apparent in this population (Asarnow et al., 2001).

There has only been 1 study thus far to examine SRT performance in relatives and this was done using fMRI. Woodward and colleagues (2007) tested adult siblings and controls and like schizophrenia patients, both groups demonstrated normal performance on the task. However,

compared to controls the relatives demonstrated less activation in the basal ganglia as well. Thus, Woodward et al. (2007) suggested that altered neural activation during the performance of cognitive tasks may be related to the genetic vulnerability for schizophrenia.

These results raise the possibility that genetic risk for schizophrenia is associated with poor learning on the WPT; in contrast, the results from the SRT literature lead us to hypothesize that relatives of COS and AOS patients will not exhibit any behavioral differences from their controls, though there might be difference in the pattern of brain activation. The proposed studies will investigate the idea that genetic risk for schizophrenia is associated with a behavioral impairment in a subset of striatal dependent tasks and that completely, health first-degree relatives will also exhibit a similar corticostrial pathology that is also evident in schizophrenia patients. To the best of our knowledge, the current studies will be the first to examine adolescents with genetic vulnerability to schizophrenia who do not have the disease to determine whether impaired cognitive skill learning is an endophenotype for schizophrenia, and these studies will also expand the conflicting literature of relatives of schizophrenia patients in the motor skill literature. These proposed studies will be able to provide an understanding of the neural basis for the behavioral pattern seen in siblings of schizophrenic patients. This work is very important because it will augment our understanding of the neural basis of schizophrenia and may contribute to the treatment of this disorder.

CHAPTER 2: Deficits in Probabilistic Classification Learning and Liability for Schizophrenia

2.1 Introduction

There are abnormalities in striatal structure and function in patients with schizophrenia. Patients with schizophrenia have structural abnormalities in the basal ganglia (Buchsbaum, 1990) and neurochemical imbalances in corticostriatal circuits (Carlsson and Carlsson, 1990). Consistent with the hypothesis that schizophrenia is associated with striatal abnormalities (Buchsbaum, 1990; Buchanan, 1993) patients with schizophrenia show impaired performance on probabilistic tasks including the weather prediction task (WPT; Weickert et al., 2002; Keri et al., 2005; Foerde et al., 2008; Horan et al., 2008), a task that measures corticostriatal function.

With few exceptions, the patients with schizophrenia included in the above studies were being treated with anti-psychotic medications. Alterations in striatal dopamine neurotransmission is a major mechanism of anti-psychotic drugs (Berke and Hyman, 2000). In prior studies of striatal function in patients with schizophrenia, the effect of schizophrenia on striatal functioning was inextricably confounded with the effects of the anti-psychotic medications used to treat schizophrenia. In the present study we will test the hypothesis that corticostriatal dysfunction is associated with liability to schizophrenia, and is not simply a reflection of anti-psychotic drug treatment, by studying the unaffected siblings of patients with childhood onset of schizophrenia (who were never treated with antipsychotic drugs) with the WPT (Figure 1).

While several studies have found a substantial performance deficit on the WPT in patients with schizophrenia (Weickert et al., 2002; Keri et al., 2005), other studies have demonstrated that the rate of learning is reduced as well. These studies have shown that within 100 and 600 trials of training (Horan et al., 2008; Foerde et al., 2008), there is a diminished rate

of learning during acquisition. Given the striking deficits found in patients with schizophrenia on the WPT, it is possible that these deficits are present in individuals with genetic liability for schizophrenia as well. In a recent study (Weickert et al., 2010), the performance of patients with schizophrenia was compared to their siblings and controls on the WPT. While the patients exhibited a severe learning deficit consistent with previous work, there were no statistically significant performance differences between the controls and siblings. However, further analysis demonstrated that when subjects were divided into good and poor learners, the siblings of schizophrenia patients were disproportionately represented in the poor learner group, suggesting that a subset of the siblings were impaired on this task.

Consistent with Weickert et al. (2010), fMRI studies have revealed an underactivation of striatal regions in non-psychotic first-degree relatives of patients with schizophrenia that are similar to those seen in patients with schizophrenia, suggesting that striatal abnormalities may be associated with liability to schizophrenia (Vink et al., 2006; Raemaekers et al., 2006).

In the present study, we tested siblings of individuals with COS. COS is a severe form of schizophrenia that appears clinically continuous with the adult onset form (Asarnow and Asarnow, 1994; Nicolson and Rappaport, 1999). COS may represent a more homogenous disorder than adult-onset schizophrenia, with a more pronounced genetic risk (Asarnow, et al., 200l). Patients with COS tend to show the same cognitive deficits, but to a greater extent than patients with adult onset of schizophrenia (Asarnow and Kernan 2009). Relatives of patients with COS may have greater genetic liability for schizophrenia than relatives of adult onset patients, and thus may be likely to show the cognitive skill learning deficit present in patients with schizophrenia (Asarnow et al., 2001). If so, it would suggest that a deficit in cognitive skill

learning reflecting corticostriatal dysfunction is associated with liability to schizophrenia and not simply an artifact of anti-psychotic treatment.

Patients with schizophrenia show deficits on other cognitive skill learning tasks such as the Tower of Toronto and the Tower of Hanoi (Gimenez et al., 2003; Purdon et al., 2003). However, these tasks demand considerable executive control, and thus impairments may reflect additional, non-striatal deficits in these patients. Because of its relatively simple task demands, the WPT may be a more specific measure of cognitive skill learning that taps corticostriatal dysfunction. Previous studies have shown that reinforcement learning driven by rewards is impaired in patients with schizophrenia, consistent with corticostriatal dysfunction (Waltz, et al., 2007; See Barch & Dowd, 2010 for a review).

The WPT depends on the integrity of the neostriatum (Knowlton et al., 1996a; Knowlton et al., 1996b) and will be used to assess corticostriatal function. Performance of this task activates striatal regions on fMRI (Poldrack et al., 1999; Poldrack et al., 2001; Foerde et al., 2006) that are part of cognitive corticostriatal circuits including the caudate nucleus/dorsolateral prefrontal cortex (DLPFC) and ventral striatum/orbitofrontal cortex (VS/OFC) (Poldrack et al., 1999; Poldrack et al., 2001; Aron et al., 2004).

In healthy controls, the WPT can be learned explicitly using medial temporal lobe circuitry or implicitly using basal ganglia circuitry (Foerde et al., 2006). The WPT requires participants to learn the probabilistic associations between cues and binary outcomes by attending to visual stimuli presented on a computer screen after which they are provided feedback about the correctness of their response. To probe whether the WPT was learned implicitly, we inserted dual-task tone-counting probe trials to test whether performance was automatic at different points during training. Automatization of a skill refers to the ability to

execute a task without the demand for effortful control. If performance does not decline when a concurrent task is performed, it would suggest that performance on the WPT is relatively automatic and is based on stimulus-response habits involved in automatic skills rather than declarative memory.

To our knowledge this is the first study to investigate the performance of adolescent siblings of COS probands on an implicit learning task. A previous study has demonstrated that healthy young, adolescent control performance on a PCT is comparable to that of healthy young adult controls (Marsh et al., 2004). Based on studies that have compared schizophrenic patients and their first-degree relatives to healthy controls, we hypothesize that the unaffected siblings of COS patients will have an impairment in performance during early learning and after extended training. We also hypothesize that the siblings of COS patients will show a deficit in automaticity revealed by a decrement in dual-task performance after extended training.

2.2 Methods

Participants

Sixteen siblings of COS patients and 87 control participants who were matched in age, education, and gender to the COS siblings participated in the experiment (Table 1). Eight controls and four siblings were excluded from analysis based on computer malfunction or not responding on more than 10% of the trials. All participants provided informed consent according to the procedures approved by the University of California, Los Angeles (UCLA) Human Subjects Committee and were paid for their participation. Siblings of COS probands were recruited based on their previous participation in family studies of COS at UCLA. Families of potential control participants were recruited through online advertisements, flyers, and by

randomly calling families found through a commercially available list of households within a 25-mile radius of UCLA (Survey Sampling Inc., Fairfield, CT, USA). All participants were screened and were excluded if there was a history of prior treatment of psychiatric disorders (including psychosis, attention-deficit hyperactivity disorder, learning disabilities, Tourette's Syndrome) traumatic brain injury, drug or alcohol abuse, or neurological disorders that affect cognitive functioning. Control participants were excluded if a first-degree relative was reported to have been diagnosed with psychosis.

Experimental design

Subjects practiced the WPT for a total of one and a half hours, spanning two days. The second session took place within seven days of the first. On the first day, subjects were assessed for any neurological disorder or psychotic symptoms by a psychiatrist, underwent a neuropsychological battery by completing the Wechsler Abbreviated Scale of Intelligence (WASI) vocabulary and block design subtests (Table 1), and completed 50 trials of the WPT either inside or outside of an MRI scanner depending on whether the participants were in the behavioral or fMRI condition. In the second session, subjects were trained for an additional 800 trials outside the scanner occurring in two sets with an intervening break of 30 minutes where another task (the serial reaction time task) was performed. If the subjects were in the fMRI condition, they also completed 50 trials of the WPT while in the scanner on the second day. At the end of training, subjects' declarative knowledge of cue-outcome associations were tested by asking them to estimate how frequently each outcome occurred for each of the cue combinations.

In the WPT used here (Knowlton et al., 1994) participants are told that they have to predict the weather (sun or rain) based on cues. These cues are probabilistically related to the outcomes. On every trial between 1 and 3 cues (out of 4 possibilities) can appear, yielding 14

possible combinations. The association of the different cues with different probabilities was randomized across participants. The cue strength of each of the 14 resulting stimuli were such that the overall probability associating each cue with sun or rain is 0.756, 0.575, 0.425, and 0.244 across the trials. A response was counted as correct if it matched the outcome most strongly associated with a stimulus; thus, a response could be counted as correct even if feedback reported an incorrect answer (i.e. the feedback presented was associated with the less strongly associated outcome). Therefore, the percentage correct score reflects how well the subjects learned the cue-outcome associations (Marsh et al., 2004).

Secondary Task: On the second day, a secondary task was introduced during trials 81-160 and 641-720. These probe trials were inserted to assess whether WPT performance was unaffected by the addition of a concurrent task and was thus relatively automatic (Foerde et al., 2008). For the secondary task, participants heard high (1000 Hz) and low (500 Hz) pitched tones during the task and had to count the number of high-pitched tones. At the end of each dual task block the subject reported the number of high-pitched tones they counted by entering the number into the computer.

Data Analysis

The WPT data were analyzed using 2-way repeated measures ANOVA. To correct for violations of sphericity, the Huynh-Feldt test was used. We tested a directional hypothesis: the COS siblings would perform worse than healthy, age-matched controls.

The effect of the secondary task was assessed by computing the differences between the average of the trial block immediately before and after the two dual task blocks and the average of the two dual task blocks.

Performance on the declarative knowledge test was assessed by computing the average of the difference between the true and the participant's estimated probability of each outcome for each cue combination. Thus, a lower score would reflect more veridical declarative knowledge of the cue-outcome associations. Chance performance would equal the difference between 50% and the veridical probability for each cue combination.

2.3 Results

We examined performance on the WASI subtests and found significant differences between the groups with the controls performing better on both tests, Vocabulary $t(88) = 5.537$, $P < 0.00$, Blocks $t(86) = 4.550$, $P < 0.00$. For these analyses, one of the COS siblings was not tested on the WASI subtests and two control participants were not tested on the Blocks subtest due to time constraints.

Early Learning

We first examined learning by analyzing accuracy during early learning, which we defined as learning on Day 1 (50 trials) and the first block of Day 2 (80 trials). The greatest improvement in accuracy occurred during these periods. Figure 2 presents accuracy of the two groups during this early learning phase. During the 50 trials of training on Day 1, there was a main effect of group ($F(1, 89) = 3.606$, $P = 0.031$, $MSE = 0.104$, $\eta_p^2 = 0.039$) with significantly better performance by the controls. There was no main effect of block, ($F<1$), but there was a trend for an interaction between group and block ($F(4, 356) = 1.743$, $P = 0.071$, $\eta_p^2 = 0.019$) due to the better learning in the control group. During blocks 2-5 of Day 1, control performance was significantly above chance, t's$(78) > 3.3$, $P < 0.001$, while COS sibling performance did not improve during Day 1 and remained at chance levels, . Within 8 blocks of 10 trials in the first 80 trials on Day 2, there was a trend for a main effect of group ($F(1, 89) = 1.986$, $P = 0.081$) and a

trend for a main effect of trial blocks ($F(7, 623) = 1.548$, $P = 0.074$, $MSE = 0.036$, $\eta_p^2 = 0.017$). There was no significant interaction between trial blocks and group, ($F<1$) suggesting that early during the second day both groups showed learning, although the control group appeared to maintain a higher level of accuracy.

There was a significant main effect of block ($F(4, 356) = 2.911$, $P = 0.014$, $MSE = 54740.379$, $\eta_p^2 = 0.032$) for reaction time during the early training on Day 1 (blocks 1-5 of Figure 2), with reaction times decreasing over trials. The main effect of group and the interaction between block and group were not significant.

Performance after extended training

We next analyzed accuracy during the second day of training. Figure 3 presents accuracy for the two groups during extended training. We did not include the dual task trials in these analyses. We analyzed the Day 2 performance broken into two sessions (the 320 single-task trials before and after a 30 minute break). For the first session, there was no main effect of group ($F<1$), block ($F(3, 267) = 1.369$, $P = 0.127$, $MSE = 0.006$, $\eta_p^2 = 0.015$), or a significant interaction between block and group, ($F<1$). For the second session, there was a main effect of group, ($F(1, 89) = 3.019$, $P = 0.043$, $MSE = 0.063$, $\eta_p^2 = 0.033$). The COS sibling group performed significantly more poorly than controls. There was no main effect of block, or a significant interaction between block and group, (F's<1). In addition, within group t-tests revealed that there was no difference in accuracy for the controls or in the siblings of COS probands between sessions 1 and 2. Table 2 presents the group means and standard deviation for every trial block.

Reaction time was also analyzed for session 1 and session 2 on the second day. There was no significant main effect of group ($F<1$) or block ($F(3, 267) = 1.492$, $P = 0.115$, $MSE =$

29209.533, $\eta_p^2 = 0.016$), and no interaction between block and group ($F<1$), for the first session. During the second session, there was no main effect of group, or an interaction between block and group (F's <1). There was a main effect of block ($F(3, 267) = 2.969$, $P = 0.020$, MSE = 20228.810, $\eta_p^2 = 0.032$), with reaction times decreasing across the session for both groups.

The effect of the secondary task was assessed by computing the differences between the average of the trial block immediately before and after the two dual task blocks and the average of the two dual task blocks. For both groups, there was little cost associated with performing the concurrent task. There was no effect of the dual task on accuracy in either the first session (Mean of controls = 0.64, Mean of siblings = 0.58, $t(89) = 0.051$, $P = 0.480$), or the second session, (Mean of controls = 0.66, Mean of siblings = 0.61, $t(89) = 0.537$, $P = 0.296$). This lack of any cost associated with performance of the secondary task suggests that performance of the PCT was relatively automatic, at least by the 130 trials of training that occurred before the first dual task probe. For reaction time, we calculated difference scores for the dual task trials and the single task trial immediately before and after the two dual surrounding single task trials. When comparing reaction times for single and dual task blocks, there was a nonsignificant trend for subjects in both groups to perform faster during dual task blocks during both the first session ($t(89) = 1.511$, $P = 0.067$) and for the second session ($t(89) = 1.420$, $P = 0.080$).

On the declarative cue estimation test, there was a significant difference between the controls and siblings in terms of the deviation of the subjects' estimates from the true probabilities ($t(85) = 1.738$, $P = 0.043$), revealing that the controls had more explicit knowledge of the cue-outcome associations. In addition, it was not the case that the siblings did not understand the declarative knowledge assessment; further analysis revealed that the siblings performed above chance ($t(8) = 4.296$, $P < 0.01$).

2.4 Discussion

In the present study, siblings of COS patients were impaired on a cognitive skill learning task compared to controls. At the beginning of training, both groups began at chance levels of accuracy. However, controls showed clear learning over the first 50 trials, while the siblings of COS probands did not, and a significant group difference in accuracy emerged during this early training. While the groups did not differ significantly during the first session of Day 2 the siblings of COS probands were significantly less accurate than controls during the second session of Day 2. During this second session, both groups appeared to reach asymptote. There was no evidence of further learning, indicating that the groups differed in their level of asymptotic performance. Even after hundreds of trials, the siblings of individuals with COS were not performing significantly above chance.

By the 130th trial, when the first dual task trials were inserted, there was no dual task effect. The performance of a secondary task had no significant effect on WPT accuracy for either group after 130 trials. Because the concurrent task decreases declarative memory retrieval by occupying working memory, the lack of a dual task effect suggests that subjects were not explicitly recalling information about the associations to perform the task when the probe trials were presented. However, other evidence suggests that learning on the WPT in early trials can be supported by declarative memory. Data provided by computational modeling, studies of amnestic and Parkinson's patients, and pharmacological manipulations suggest that “multiple, distinct cognitive processes contribute to how people predict outcomes” on the WPT (Shohamy et al., 2008). Early in practice healthy subjects can use simple, easily verbalizeable, non-optimal rules, which can be learned in a single episode. This type of learning is impaired in patients with amnesia with medial temporal lobe damage but preserved in patients with mild Parkinson's

disease. As practice continues, subjects use a more optimal, incremental strategy based on error prediction. This strategy is supported by the basal ganglia (Shohamy et al., 2008). The degree to which subjects rely on declarative vs. habit strategies likely depends on the ability to memorize the outcomes of specific trials (Foerde et. al., 2006). Prior research shows that patients with schizophrenia have impaired medial temporal lobe function, and in the present study, the siblings of COS probands exhibited impaired declarative memory for the cue-outcome associations, raising the possibility that the poor performance of the siblings of COS patients early in practice reflects medial temporal lobe dysfunction while their poor performance later in practice reflects basal ganglia dysfunction. We are currently processing fMRI data to see if early in practice there are differences in MTL activation and later in practice differences in basal ganglia activation in siblings of COS patients on the WPT.

Consistent with other studies of first degree relatives of patients with schizophrenia (Hill et al., 2008) in the present study adolescent siblings of patients with COS with schizophrenia performed significantly more poorly than controls on the WASI, a measure of intellectual functioning. The poor performance of relatives of patients on intelligence tests has been interpreted as indicating that poor intellectual function may reflect liability to schizophrenia (Hill et al, 2008). However, it is unlikely that this impairment can explain the deficits in this group on the WPT, in that patients with Alzheimer's disease, who also exhibit impairments on tests of intellectual function, nevertheless perform as well as age-matched control subjects on the WPT (Eldridge et el., 2002).

It was also not the case that the WPT task was too difficult for the adolescent participants in the present study. The WPT task has been shown to be learned relatively well in patients in this age group (Marsh et al., 2004). Moreover, performance in the healthy control group in our

study was comparable to the performance of healthy young adolescent and adults in the Marsh et al. study and in healthy adults in previous work (Knowlton et al., 1994).

We observed a greater deficit in WPT performance in siblings of individuals with schizophrenia than that seen by Weickert et al. (2010). The major difference between the studies is that we tested siblings of patients with COS, while in Weickert et al. (2010) siblings of adult onset patients were tested. The COS siblings may have a greater genetic liability for schizophrenia, and thus cognitive endophenotypes associated with this liability may be more apparent. Another difference between the present study and the study conducted by Weickert and colleagues (2010) is that we tested adolescents rather than adults. It may be that this type of habit learning is at the cusp of development, and thus may be more sensitive to individual differences.

The impairment exhibited by siblings of patients with COS in this study contrasts with the relatively good performance of patients with amnesia or Alzheimer's disease on this task. Although these patient groups exhibit severe deficits in declarative memory, they are able to perform well on the WPT (Knowlton et al., 1994; Eldridge et al., 2002). The performance of the COS siblings seems similar to that of patients with basal ganglia disorders. Similar to the COS siblings in the current study, patients with Huntington's disease exhibit impaired learning and a very low level of asymptotic performance (Knowlton et al., 1996b). The siblings of patients with COS perform comparably to patients with schizophrenia, who also have impaired performance in the WPT and similar tasks. In patients with schizophrenia, there are performance gains early on by the controls and the patients never catch up to the same level even after extended training (Foerde et al., 2008) This close correspondence between the performance of patients with schizophrenia and the unaffected siblings of COS patients supports the hypothesis that deficits in

probabilistic classification are associated with liability to schizophrenia and not a consequence of expression of the disease or medication history.

Genetic liability for schizophrenia involves cognitive impairments as well as functional brain abnormalities. The present results suggest that dysfunction in specific corticostriatal loops may reflect liability for schizophrenia. An important direction for future work is the analysis of striatal function during performance of the WPT using fMRI in first-degree relatives of patients with schizophrenia.

2.5 Figures

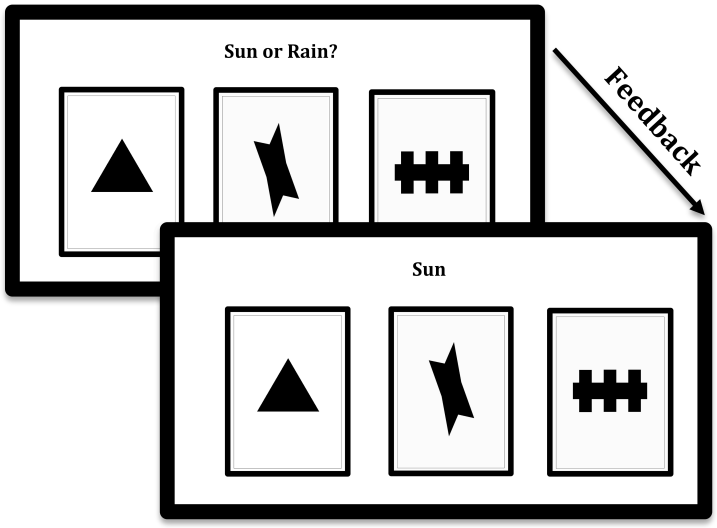

Figure 2.1. Weather Prediction Task. Participants were told to predict the weather (sun or rain) based on cues. On every trial between 1 and 3 cues (out of 4 possibilities) could appear, yielding 14 possible combinations. The cues were probabilistically related to the outcomes. The association of the different cues with different probabilities was randomized across participants. The cue strength of each of the 14 resulting stimuli were such that the overall probability associating each cue with sun or rain was 0.756, 0.575, 0.425, and 0.244 across the task. Since

feedback was probabilistic, a response was considered correct if it matched the outcome most strongly associated with a stimulus, regardless of feedback. Thus, a response could be "correct" even if feedback reported an incorrect answer. Therefore, the percentage correct score reflected how well the subjects learned the cue-outcome associations (Marsh et al., 2004). The cues are shown on the screen for a maximum of 3 seconds, the feedback is shown on the screen for 1 second, and the time between trials is 0.5 seconds. During the secondary task, a subject hears a series of high and low pitch tones during the task and has to count the number of high pitch tones while completing the WPT. Between 1-3 tones are heard during each trial of the secondary task.

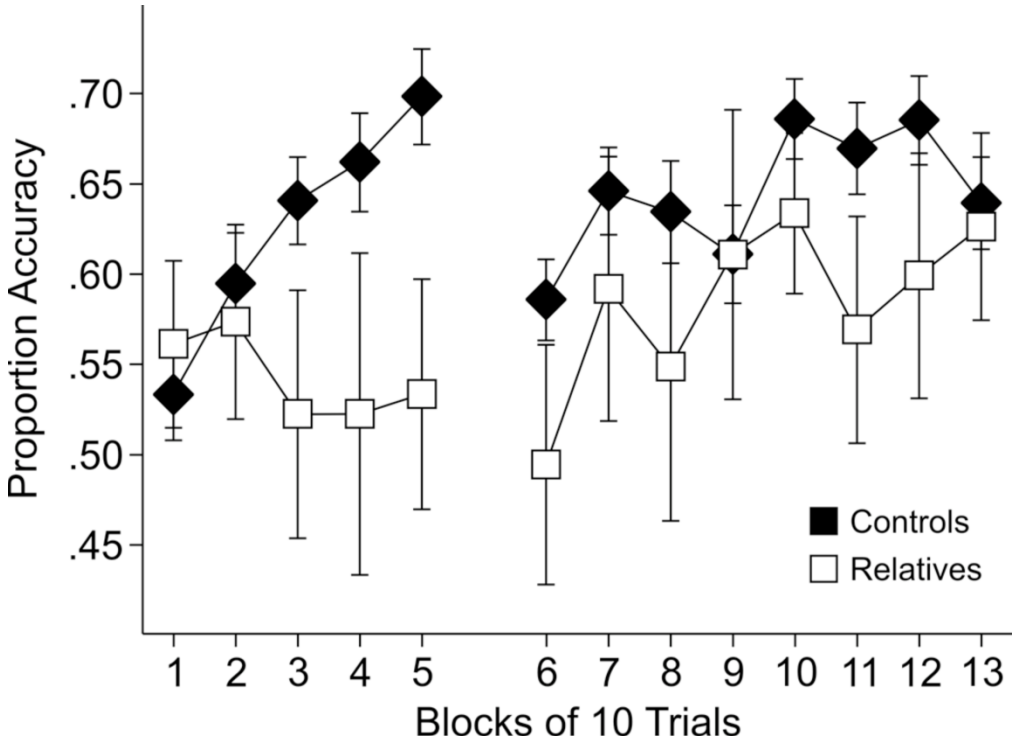

Figure 2.2. WPT Accuracy- Early Training. WPT accuracy of the controls and COS relatives in early training [(Day 1 and the first 80 trials of Day 2 (blocks 6-13 in this graph)]. Error bars represent the standard error of the mean.

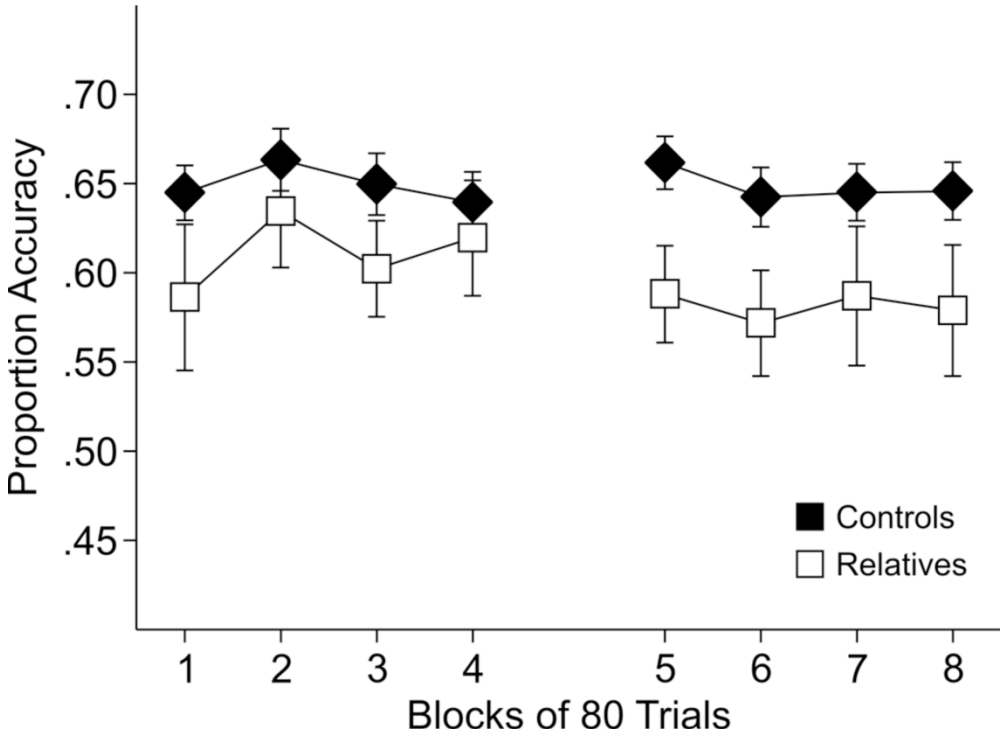

Figure 2.3. WPT Accuracy- Extended Training. WPT accuracy of the controls and COS relatives During Day 2. Only single task trials are depicted. Error bars represent the standard error of the mean.

2.6 Tables

Variable	Controls (n = 79) M	SD	Siblings (n = 12) M	SD
Age	12.76	2.39	12.58	2.19
Education	6.00	2.49	6.75	2.38
Gender*	40/39		6/6	
Vocabulary**	60.53	8.62	45.00	9.44
Blocks***	56.91	7.87	45.00	9.82

Table 2.1. Demographic Table.

*Men/Women, **WASI Vocabulary subtest (missing 1 sibling), ***WASI Block subtest (missing 1 sibling and 2 controls)

		Day 1					Day 2							
Variable	**Blocks**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Mean	Controls	0.53	0.59	0.64	0.66	0.70	0.64	0.66	0.65	0.64	0.66	0.64	0.65	0.65
	Relatives	0.56	0.57	0.52	0.52	0.53	0.59	0.63	0.60	0.62	0.59	0.57	0.59	0.58
SD	Controls	0.22	0.25	0.22	0.24	0.24	0.14	0.16	0.15	0.15	0.13	0.15	0.14	0.14
	Relatives	0.16	0.19	0.24	0.32	0.19	0.14	0.11	0.09	0.11	0.09	0.10	0.14	0.13

Table 2.2. Group Means and Standard Deviations. Group mean of performance of proportion accuracy on the WPT and standard deviation (SD) at each block.

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CHAPTER 3: The Behavioral and Neural Mechanisms of Adolescent and Adult Relatives on the Weather Prediction Task

3.1 General Introduction

Consistent with the hypothesis that the pathophysiology of schizophrenia involves dysfunction of corticostriatal circuits (e.g., Kleist, 1960), patients with schizophrenia perform poorly on cognitive skill learning tasks that tap into a cognitive cortical-striatal network (Gimenez et al., 2003; Schroder, Tittel, Stockert, & Karr, 1996; Purdon, Woodword, Lindborg, & Stip, 2003; Foerde et al., 2008; Weikert et al., 2010). Skill learning is the gradual improvement of performance with practice on a task and is demonstrated by reduced reaction time or increased accuracy. The corticostriatal system has been shown to play an important role in skill learning (Heindel et al., 1989; Knowlton et al., 1996; Doyon et al., 2009; Peigneaux et al., 2000). Neuroimaging studies in healthy subjects have also lent support to this theory (Grafton, Hazeltine, & Ivry, 1995; Rauch et al., 1997; Poldrack & Gabrieli, 2001; Schiltz et al., 2001; Aron et al., 2004; Moody et al., 2004).

One type of cognitive skill learning is the Weather Prediction Task (WPT) (Knowlton, Squire, & Gluck, 1994), a probabilistic classification task that requires participants to learn the probabilistic associations between cues and binary outcomes by attending to visual stimuli presented on a computer screen after which they are provided feedback about the correctness of their response (Figure 1). Performance on the WPT is impaired in patients with schizophrenia (Foerde, Poldrack, Knowlton et al., 2008; Keri, Juhasz, Rimanoczy, et al., 2005; Horan, Green, Knowlton, et al., 2008; Weickert, Terrazas, Bigelow, et al., 2002), consistent with the idea that schizophrenia is associated with striatal abnormalities (Buchsbaum, 1990; Buchanan, 1993). In addition to the weather prediction task, patients with schizophrenia show deficits in other cognitive skill learning tasks such as the Tower of Toronto and the Tower of Hanoi (Gimenez,

Junque, Perez, et al., 2003; Purdon, Woodword, Lindborg, et al., 2003). However, these tasks demand considerable executive control resources, and thus impairments may reflect additional deficits in these patients. Because of its relatively simple task demands, the WPT may be a more specific task suitable to assess cognitive skill learning that taps corticostriatal dysfunction.

While deficits in cognitive skill learning appear to be present in patients with schizophrenia, there is some question about the meaning of those deficits. The patients in studies where cognitive skill learning deficits were detected were receiving anti-psychotic medications to control their psychotic symptoms. Anti-psychotic medications have effects on striatal structure (enlargement of the volume of the basal ganglia) and alter striatal D2 receptors (Paquet et al., 2004; Kumari et al., 2002). It is possible that the anti-psychotic medications patients with schizophrenia received result in impaired striatal function reflected in cognitive skill learning deficits. One way to address this issue is to study the non-psychotic first degree relatives of patients with schizophrenia. These relatives share some of the familial liability to schizophrenia with patients with schizophrenia but since they are not psychotic they are not receiving anti-psychotic medications. Thus, if they show cognitive skill learning deficits, those deficits would appear to reflect familial liability to schizophrenia, not the effects of anti-psychotic medication.

There are two prior studies of cognitive skill learning in first degree relatives of patients with schizophrenia. Weickert et al. (2010) compared adult patients with schizophrenia, their adult siblings, and controls on the WPT. The patients demonstrated a severe learning deficit, while there was no significant difference between the adult siblings and the controls. However, when subjects were separated into good and poor learners the siblings of first degree relatives of patients with schizophrenia were disproportionately in the poor learner group. In a recent study conducted by Wagshal et al. (2012, Under Review), adolescent siblings of patients with

childhood onset schizophrenia (COS) exhibited deficits in the WPT. In this study, controls showed significant learning in the first 50 trials while the sibling did not, and even after extensive training, the COS siblings reached a lower level of asymptotic performance than controls. Thus, the adolescent siblings demonstrated impaired early and late learning on the weather prediction task compared to controls. The siblings of COS patients may have exhibited a greater deficit than the siblings in the Weickert et al. (2010) study because COS appears to be a more severe form of the disorder and may have a more pronounced genetic component. Second, in Wagshal et al., we tested adolescent subjects, and thus it is possible that the impairment in the COS siblings represents a developmental delay.

Given that the data in Wagshal et al. (2012, Under Review) indicated behavioral differences between the groups early in training and during extended training, we were interested in investigating neural and behavioral differences early and late in learning as well. In this series of experiments we examined the neural correlates of fronto-corticostriatal function during skill learning, as well as the extent to which skill learning was automated using a dual task paradigm to detect subtle impairments in skill learning in adolescent siblings of COS patients and in adult first degree relatives of COS and AOS patients to determine if the deficit we found in adolescent siblings was due to a developmental delay (for which it is expected that the adults would not be impaired on the task) or was characteristic of the genetic liability (for which it is expected that the adults would be impaired on the WPT) for schizophrenia.

Experiment 1

3.2 Introduction

The current study is the first to use fMRI to directly study neural circuits that support cognitive skill learning in healthy, adolescent siblings of patients with schizophrenia. The neural

network supporting performance on cognitive skill learning tasks is the striatum (Foerde, Knowlton, & Poldrack, 2006; Poldrack, Clark, Pare-Blagoev, Shohamy, Moyano et al., 2001; Poldrack, Prabakharan, Seger, & Gabrieli, 1999) and the cognitive corticostriatal circuit including the caudate nucleus/dorsoalateral prefrontal cortex (DLPFC) and ventral striatum/orbitofrontal cortex (VS/OFC) (Aron, Shoshamy, Clarck, et al., 2004; Poldrack, Clark, Pare-Blagoev, et al., 2001; Poldrack, Prabhakaran, Seger, et al., 1999). Identifying alterations in the neural networks supporting cognitive skill learning in the siblings of patients with schizophrenia is an important first step in demonstrating that those networks are associated with liability to schizophrenia.

This study examined siblings of childhood onset schizophrenia patients. Patients with COS present with a more severe form of schizophrenia than patients with adult onset, with increased aggregration of schizophrenia spectrum disorders in first degree relatives compared to first degree relatives of patients with adults onset of schizophrenia (Asarnow et al., 2001). First degree relatives of COS probands show neurocognitive impairments similar to those shown by patients with schizophrenia (Asarnow, 1999; Gold & Harvey, 1993; Kuperberg & Heckers, 2000; Riley et al., 2000). Thus, the genetic liability for schizophrneia may be greater in healthy relatives of these patients, and this group may be particularly informative in determining if the behavior and brain abnormalities are markers of schizophrenia without the confound of medication effects.

3.3 Methods

Participants

Sixteen adolescent siblings of COS patients (age range: 8-16) and forty-five adolescent controls (age range: 8-16), who were right handed and were matched in age, education, and

gender to the COS siblings participated in the experiment (Table 1). These subjects were a subset of the participants in our previous behavioral study of WPT learning in siblings of COS patients (Wagshal et al., under review). Twenty controls and six siblings were excluded from analysis based on computer malfunction, not responding on more than 10% of the trials, excessive movement of 3mm or greater, or not completing both days of training. Siblings were recruited through previous participation in family studies of COS at the University of California, Los Angeles (UCLA). Families of potential control subjects were within a 25-mile radius of UCLA were contacted by phone. All participants provided informed consent according to the procedures of the UCLA Institutional Review Board. Potential participants were screened and excluded for reports of prior treatments of psychiatric disorders including psychosis, attention-deficit hyperactivity disorder, learning disabilities, Tourette's Syndrome, traumatic brain injury, drug and alcohol abuse, and neurological disorders that affect cognitive functioning. Control subjects were excluded if a first-degree relative had been reported to have been diagnosed with psychosis.

Task design

Participants were administered the weather prediction task (Knowlton et al., 1994). The MATLAB (The MathWorks, Inc., Natick, MA) Psychophysics Toolbox (Brainard, 1997) version 7.4 was used to present the stimuli and to record responses on an Apple G4 PowerBook using the OSX operating system.

Weather Prediction Task (WPT): The WPT (Knowlton et al., 1994) is a probabilistic classification task that can be learned nondeclaratively, in which the participants are told that they have to predict the weather (sun or rain) based on cues (Figure 1). These cues are probabilistically related to the outcomes. On every task between 1 and 3 cues (out of 4

possibilities) can appear on each trial, yielding 14 combinations. The association of the different cues with different probabilities was randomized across participants. The cue strength of each of the 14 resulting stimuli were such that the overall probability associating each cue with sun or rain is 0.756, 0.575, 0.425, and 0.244 across the trials. A response is counted as correct if it matches the outcome most strongly associated with a stimulus; thus, a response can be counted as correct even if feedback may report an incorrect answer. This task assesses cognitive skill learning because subjects are learning to choose the most associated outcome based on the cues presented. The percentage correct score reflects how well the subjects learn the cue-outcome associations (Marsh, Alexander, Packard, et al., 2004).

The secondary tone counting task was introduced for a small subset of trials (160 trials) during trials 81-160 and 641-720 on the second day during the behavioral training. For the secondary task subjects hear a series of high (100Hz) and low (500Hz) pitch tones and have to count the number of high pitch tones while completing the WPT. These probe trials were inserted to assess whether WPT performance was unaffected by the addition of a concurrent task and was thus relatively automatic (Foerde et al., 2008).

When the subjects were completing the task in the fMRI scanner, the baseline condition, which was interleaved between trials, consisted of the same 3 cues on the screen in which the subjects were instructed to press left. The baseline was the same task for both days of training in the scanner.

Procedure

On the first day participants were screened for neurological or psychiatric disorders and then completed the Wechsler Abbreviated Scale of Intelligence (WASI) vocabulary and block design subtests (Table 1). Afterward the subjects came into the scanner and completed the fMRI

portion of the study in the scanner, which lasted approximately 60 minutes. During the scan, behavioral data and fMRI data were collected simultaneously. Within one week, participants returned and first attended a behavioral testing session that lasted approximately two hours where the subjects were trained for an additional 800 trials of the WPT occurring in two sets with an intervening break of 30 minutes Then after completing 50 trials of the WPT while in the scanner on the second day, subjects' declarative knowledge of cue-outcome associations were tested by asking them to estimate how frequently each outcome occurred for each of the cue combinations.

Imaging procedure:

Scanning was performed on a 3-Tesla Siemens Allegra head-only MRI machine in the Ahmanson-Lovelace Brain Mapping Center and Ronald Reagan Hospital at UCLA. For the functional runs, T2*-weighted echoplanar images (EPI) were collected (34 slices; slice thickness = 4 mm; TR = 2000 ms; TE = 30 ms; voxel size = 3.1 x 3.1 x 4.00 mm; flip angle = 90°; matrix size = 64 x 64; field of view = 200, distance factor = 0%). Structural images were collected as well: a T2-weighted matched-bandwidth high-resolution scan (34 slices; slice thickness = 4 mm; TR = 5000 ms; TE = 33 ms; voxel size = 1.6 x 1.6 x 4.0 mm; flip angle = 90°; matrix size = 128 x 128; field of view = 200; distance factor = 0%) and a magnetization-prepared rapid acquisition gradient echo image (MPRage; 160 sagittal slices; slice thickness, 1 mm; TR = 2300 ms; TE = 2.1 ms; voxel size = 1.3 x 1.3 x 1.0 mm; flip angle = 8°; matrix, 192 x 192; field of view = 256; distance factor = 50%).

Participants viewed stimuli presented on an Apple G4 Powerbook through MRI compatible goggles and responded with an MRI compatible response box connected directly to the computer. Head movement was minimized with foam padding in a standard radiofrequency

single channel head coil. Two volumes were acquired at the beginning of each functional scan to allow equilibration to steady-state and were subsequently excluded from the analysis.

Behavioral data analysis

All data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) 16 (SPSS, Chicago, IL). The variables of interest were reaction time and accuracy. The data were analyzed using a repeated-measures Analysis of Variance (ANOVA) with the Huyhn-Feldt correction for non-sphericity. The control vs. COS sibling group was the between-group subject factor. We tested a directional hypothesis: siblings of COS patients would demonstrate a decrease in activation in the striaturm and other regions compared to healthy, age-matched controls.

Imaging data preprocessing and analysis

Data preprocessing was conducted using FSL version 3.3 (www.fmrib.ox.ac.uk/fsl). Images were motion-corrected using FMRIB's motion correction linear image registration tool (McFLIRT)) using a normalized correlation ratio cost function and linear interpolation (Jenkinson et al., 2002). Brains were extracted using the brain extraction tool (BET; Smith, 2002).

Data analysis was conducted in FSL version 4.1 using the fMRI Expert Analysis Tool (FEAT version 5.98, first at an individual subject-level (using fixed effects model) and then using a mixed-effects model at the group analysis level contrasts. For the individual subject-level analysis, data was spatially smoothed using a 5 mm full-width-half-maximum Gaussian kernel, intensity normalized, and filtered with a nonlinear high-pass filter (Gaussian-weighted least-squares straight line fitting, with sigma = 100.0 sec). A three-step registration process aligned individual participant data into standard Montreal Neurological Institute (MNI) space. EPI

images were registered to the matched-bandwidth image (7 degrees of freedom [DOF]), then to the MPRage image (7 DOF), and finally to MNI space using FMRIB's Linear Image Registration Tool (FLIRT; Jenkinson et al., 2002) using an affine transformation with 12 DOF.

The data were prethresholded using an anatomical mask at the individual subject-level analysis in FEAT. The regions included in the mask came from an a priori hypothesis showing that the hippocampus, striatum, and anterior and posterior cingulate gyrus are differentially affected in schizophrenia patients and siblings (Rametti et al., 2009; Horan et al., 2008; Liddle et al., 2006; Neuhaus et al., 2007; Ogg et al., 2008; McKiernan et al., 2003). The contrast of interest was task versus baseline for early learning on day 1 and also separately for late learning on day 2. Day 1 and day 2 data were combined within subjects using a fixed-effects model. Higher-level analyses using a mixed effects model compared differences between contrast of interest between days within and between groups. Regressors of interest were created using a delta function with trial onset times convolved with a canonical (double gamma) hemodynamic response function, along with the temporal derivative. For all contrasts of interest, Z-statistic images were created using a cluster-level threshold of $z > 2.3$ and a corrected significance threshold of $p < 0.05$ using cluster-based Gaussian random field theory. For all time-series statistical analyses, FMRIB's improved linear model (FILM) with local autocorrelation correction (Woolrich et al., 2001) was used. Regions of interests (ROIs) were identified from regions in the whole brain group-level analysis that passed threshold. We wanted to find areas that were significantly different in activation between siblings and controls during day 1 (the first 50 trials) and day 2 (the last 50 trials, trials 850-900) when the subjects completed the task as compared to baseline (Figures 2 and 3). The ROIs were then defined anatomically and used to extract the percent signal change during task-baseline after early learning on the first day and after the extended learning on the

second day. To calculate the average percent signal change within ROIs of interest, we used FSL Featquery (FEAT version 5.98). ROIs included the left and right caudate, left and right putamen, and the anterior and posterior bilateral cingulate gyrus. These values were then analyzed in a repeated measures ANOVA using SPSS. For the first and second day separately we used a 6 (ROI region) x 2 (Group; controls and siblings) Repeated Measures ANOVA. For directly comparing Day 1 and Day 2, we used a 6 (ROI region) x 2 (Group) x 2 (Day) repeated measures ANOVA. For each individual ROI, we used a 2 (Day) x 2 (Group) repeated measures ANOVA.

3.4 Results

Behavioral Results

Adolescent siblings of COS patients exhibited deficits in a probabilistic classification task. Controls showed significant learning in the first 50 trials while the siblings did not. Consistent with the findings of the larger behavioral study (Wagshal et al., under review) the subset of the siblings of COS probands who participated in the fMRI study reached a lower level of asymptotic performance than controls, even after extensive training. There were also significant differences between the groups in WASI subscales, consistent with previous studies (Vocabulary, $t(32) = 4.165$, $P < 0.001$, and Blocks, $t(30) = 3.279$, $P < 0.003$). For these analyses, one of the siblings of COS probands was not tested on the WASI subtests and two control participants were not tested on the Blocks subtest due to time constraints.

fMRI Results

We explored neural activation during performance of the task for a task-baseline contrast in a whole brain group-level voxel-wise analysis during early and late learning. During early learning (the first 50 trials), in the left and right putamen, right caudate, bilateral posterior cingulate, and the bilateral anterior cingulate gyrus there were regions that were significantly

more active in the siblings of COS probands than in controls (Figure 2; Table 2). During late learning (the last 50 trials on Day 2 after the 800 behavioral trials, trials 850-900), in the left caudate, left putamen, and the bilateral anterior cingulate gyrus there were regions that were significantly more active in controls than in siblings of COS probands (Figure 3; Table 2).

To determine how these areas change in the pattern of brain activation during early and late learning on day 1 and 2 and if there were differences between both the groups, we then conducted an independent anatomical ROI analysis on regions that were significant at the whole brain group-level analysis to further investigate group differences in activation during the two time points. For early learning, there was a main effect of ROI, $F(5, 165) = 19.989$, $p < 0.001$ and a trend for a main effect of group, $F(1, 33) = 1.671$, $p = 0.1025$, with the siblings displaying greater activation overall. There was a strong trend for a significant interaction between ROI and Group, $F(5, 165) = 1.920$, $p = 0.059$. Between subjects t-tests for ROI revealed significantly increased activation during the task for Day 1 within the bilateral anterior cingulate and the bilateral posterior cingulate gyrus for the siblings of COS probands compared to healthy controls, $t(33) = -2.471$, $p = 0.0095$ and $t(33) = -1.785$, $p = 0.0415$ respectfully (Figure 4). For late learning there was a trend for a main effect of ROI, $F(5, 165) = 1.629$, $p = 0.0955$, with the controls demonstrating greater activation in all of the ROIs during this period of later learning. There was no significant main effect of group or an interaction. However between subjects t-test for ROI revealed trends for the controls to show greater activation during the task for the left putamen ($t(33) = 1.651$, $p = 0.0.054$), the bilateral anterior cingulate ($t(33) = 1.608$, $p = 0.0585$), the left caudate ($t(33) = 1.460$, $p = 0.077$), and in the right caudate ($t(33) = 1.289$, $p = 0.103$) (Figure 4).

For early versus late learning, there was a significant main effect of ROI, $F(5, 165) = 13.063$, $p < 0.001$, a significant interaction between ROI and Day, $F(4, 165) = 6.683$, $p < 0.001$, a significant interaction between Day and Group, $F(1, 33) = 3.905$, $p = 0.0285$, and a significant interaction between ROI and Group, $F(5, 165) = 2.371$, $p = 0.035$, with the greatest changes in learning across days in the bilateral anterior cingulate, right putamen, and left and right caudate for the siblings and in the bilateral posterior cingulate for controls. No other main effects or interactions were significant. Within subjects t-test for early verses late learning for the controls showed a significant effect within the bilateral posterior cingulate gyrus (early > late, $t(24) = -2.336$, $p = 0.014$) and in the bilateral anterior cingulate gyrus (early > late, $t(24) = -1.764$, $p = 0.045$), with the controls showing a decrease in activation during early compared to late learning (Figure 4). Within subjects t-tests that examined changes between early learning on Day 1 and late learning on Day 2 for the siblings of COS probands displayed a significant difference across days for the left caudate, $t(9) = 1.852$, $P = 0.0485$, right caudate, $t(9) – 2.051$, $p = 0.035$, right putamen , $t(9) = 1.951$, $P = 0.0415$, and the bilateral anterior cingulate, $t(9) = 2.089$, $P = 0.033$, with an increase in activation for early learning and then a decrease in activation for late learning (Figure 4).

Individual repeated-measures ANOVAs were also conducted for each ROI. For the left caudate there was a significant main effect of day, $F(1, 33) = 6.097$, $p = 0.0095$, with greater activation on day 1 vs day 2 and a trend for an interaction between Day and Group, $F(1, 33) = 2.131$, $p = 0.077$, with the siblings demonstrating a greater decrease in activation on the second day compared to controls (Figure 4). For the right caudate, there was also a significant main effect of Day, $F(1, 33) = 5.093$, $p = 0.0155$, with greater activation on day 1 vs day 2 and a trend for interaction between Day and Group, $F(1, 33) = 1.829$, $p = 0.0925$, displaying the same

patterns that was found in the left caudate (Figure 4). For the left putamen, there was a significant interaction between Day and Group, $F(1, 33) = 4.004$, $p = 0.027$, with the controls at baseline for day 1 and then an increase from baseline with higher activation on day 2. For the siblings there was higher activation on day 1 compared to day 2 where they significantly drop below baseline activation (Figure 4). For the right putamen there was a similar pattern of activation as the left putamen with a trend for a main effect of Day, $F(1, 33) = 2.201$, $p = 0.0735$, and a trend for an interaction between Day and Group, $F(1, 33) = 2.457$, $p = 0.0635$ (Figure 4). In the anterior cingulate cortex, there was a strong interaction between Group and Day, $F(1, 33) = 8.394$, $p = 0.0035$, which was driven by the increase in activation early in training in the siblings of COS probands and a sharp decrease well below baseline late in training, whereas in the controls there was a marginal increase in anterior cingulate activation across training days (Figure 4).

3.5 Discussion

In the current study, adolescent siblings of COS patients displayed a behavioral impairment on a cognitive skill learning task and exhibited different patterns of neural activation during learning of the task compared to controls. The behavioral data demonstrated group differences in early learning with clear learning by the controls and differences in the level of asymptotic performance even after numerous trials of extended training. The fMRI data revealed that the siblings of COS probands demonstrated increased activation compared to baseline early in training and decreased activation compared to baseline after extensive training in striatal and medial frontal regions, while the controls maintained or marginally increased their level of activation compared to baseline in these regions across days of training. In the posterior cingulate cortex, a different pattern emerged, with controls showing a marked decrease in

activation in the region early in learning, that was not present on the second day, while in the siblings of COS probands, posterior cingulate activation remained near baseline levels throughout training.

Previous neuropsychological and neuroimaging work has shown that the striatum plays an important role in learning in the WPT (Knowlton et al., 1996; Foerde, Knowlton, & Poldrack, 2006; Poldrack, Clark, Pare-Blagoev, Shohamy, Moyano et al., 2001; Poldrack, Prabakharan, Seger, & Gabrieli, 1999). The fact that the control subjects maintained striatal activity across learning is consistent with previous work. While the siblings of COS patients show striatal activity early in training, this activation is decreased significantly later in training. This pattern may reflect a lack of effective utilization of striatal circuits in the siblings of COS patients, which may contribute to their poorer level of asymptotic performance on the WPT.

In the anterior cingulate cortex, there were robust differences in the patterns of activation across training in controls and siblings of COS probands. In the controls, anterior cingulate activation was near baseline early in training, and increased marginally late in training. In the siblings of COS patients, there was substantial activation early in training, but there was a substantial decrease in anterior cingulate activation as training progressed. The anterior cingulate cortex has been implicated in error detection and conflict monitoring (Ridderinkhoff et al., 2004; Kennerley et al., 2006), and the activation early in practice in the sibling group may reflect their greater error rate in this task. While the sibling group continued to make a greater number of errors later in training than controls, the decreased activation in the anterior cingulate cortex at this time point is consistent with findings in patients with schizophrenia and adult relatives of these patients, who have been shown to exhibit decreased anterior cingulate cortex activation in sustained attention tasks (Diwadkar et al., 2011; Sepede et al., 2010; Filbey et al., 2008; Liddle et

al., 2006; Neuhaus et al., 2007). Our results are consistent with the idea that alterations in the executive control functions of the anterior cingulate cortex may be a hallmark of genetic liability for schizophrenenia.

There was also a significant difference in the patterns of activation across training in the posterior cingulate for the two groups. In the controls, there was a decrease in activation relative to baseline during early learning on the first day of training and a return to baseline later in training. This pattern fits with the notion that the posterior cingulate cortex is a part of the "default" network, and is deactivated during performance of demanding tasks (Ogg et al., 2008; McKiernan et al., 2003; Ungar et al., 2010; Sepede et al., 2010**)**. Later in training, when performance was relatively automatic as indicated by the lack of impairment on dual task probe trials, in controls the default network activity returned to baseline levels. Interestingly, in the COS sibling group there was not a significant decrease in activation in the posterior cingulate cortex, at either training stage. It may be that deficient switching between the default and task related networks is associated with genetic risk for schizophrenia in adolescence.

Experiment 2

3.6 Introduction

We also wanted to examine the behavioral performance of first degree adult relatives of schizophrenia (ROS) patients and to determine if adult relatives would replicate the behavioral and neural pattern seen with adolescent siblings of COS patients. Another aim of our current study was to assess whether genetic risk for schizophrenia was associated with deficits in the development of automaticity in the WPT. Therefore, this study investigated the mechanisms and

strategies that adult relatives employed in order to successfully learn the WPT and determined if there were differences in the pattern of brain activation compared to controls.

To examine the extent to which skill learning was automated, we used a dual task paradigm to detect subtle impairments in skill learning. Automatization of a skill is the ability to execute a task without the demand for executive control and effortful behavior. A major characteristic of automaticity is when a skill becomes habit-like and there are no detrimental effects to the skill with the addition of a secondary task. There is agreement in the literature that individuals with schizophrenia have a diminished processing resources (Braff, 1985; Holzman, 1987; Nuechterlein, 1991), and that the ability of individuals with schizophrenia to automate skills is more impaired than controls. The insufficient processing resources that handle higher processing loads can result in cognitive impairment in schizophrenia patients (Asarnow, Granholm, & Sherman, 1991; Asarnow & Sherman, 1984; Gjerde, 1983; Sherman & Asarnow, 1985). This also suggests that patients reach the limits of their available resources at lower processing loads and have fewer available resources than healthy controls (Asarnow, 1999). Processing resources are used when an individual is first learning a skill, and with practice, learning becomes automated and make fewer demands on the available resources (Schneider, Dumais, & Shiffrin, 1984; Asarnow, 1999). Since healthy relatives possess some of the same deficits as patients, it could also be the case that they would be impaired in automating new skills as well.

3.7 Methods

Ten adult ROS patients (age range: 36-48) and thirteen adult controls (age range: 36-50), who were right handed and were matched in age, education, and gender to the ROS patients participated in the experiment (Table 3). Seven controls and four relatives were excluded from

analysis based on computer malfunction, not responding on more than 10% of the trials, excessive movement of 3mm or greater, or not completing both days of training. Relatives and controls were recruited and excluded through the same process as mentioned in experiment 1. The task design, procedure and analysis were the same as in experiment 1.

3.8 Results

Behavioral Results

We examined performance on two WASI subtests (Vocabulary and Block Design) and found significant differences between the groups with the controls performing better on both tests, Vocabulary $t(22) = 3.023$, $p = 0.006$, Block Design $t(25) = 2.545$, $p < 0.017$. For these analyses, three adult relatives were not tested on the Vocabulary subtest due to time constraints. Based on our previous study (Wagshal et al., under review), we separately analyzed performance early and late in WPT training. Early in training the greatest degree of learning occurs. Late in training, we are able to examine the level of asymptotic performance when it is relatively automatic.

Early Learning

We first examined accuracy during early training (first block of 50 trials on Day 1 and the first block of Day 2 (80 trials). Based on Wagshal et al., (Under review), this is the period during which most learning occurs in the controls. Figure 5 presents the accuracy of the two groups during this early learning phase. During the 50 trials of training on Day 1, there was strong trend for a main effect of block, $F(4, 100) = 1.906$, $p = 0.0575$, with both groups improving accuracy across blocks. There was no significant main effect of group or an interaction between block and group. During blocks 2-5 of Day 1, the performance of healthy controls was significantly above chance, t's$(18) > 2.213$, $p < 0.05$. While the ROS patient group's performance increased

throughout the training on Day 1, they performed at a level not significantly above chance (*t's*(7) <1.606, $p > 0.152$) except for block 4, t(7) = 3.322, $p = 0.013$. Within the 8 blocks of 10 trials in the first 80 trials on Day 2, there were no significant main effects of group or block or an interaction between block and group. The relatives' performance was also analyzed for the first 80 trials on Day 2 and they were only significantly above chance for blocks 3 and 4, *t's*(7) > 2.627, $p < 0.035$.

There was a main effect of block, F(4, 100) = 5.327, $p < 0.001$, for reaction time during the early training on Day 1 with reaction time decreasing for both groups and a trend for an interaction between block and group, F(4, 100) = 1.795, p = 0.069, with the relatives demonstrating more of decrease in reaction time during the later blocks of early learning. The main effect of group was not significant ($F < 1$).

Performance after extended training

Figure 6 presents accuracy for the two groups during extended training (Trials 51 through 850 on the second day, excluding those trials in which the subject performed the concurrent tone-counting task). We analyzed Day 2 overall performance and Day 2 performance broken into two sessions (the 320 single-task trials before and after a 30 minute break) in 80 trial blocks. Overall, there was a significant main effect of block with both groups improving across blocks, F(7, 175) = 6.600, $p < 0.001$. There was not a significant main effect of group or an interaction between block and group. When broken down into 2 separate sessions, for session 1, there were no significant main effects of group or block or an interaction between block and group. For session 2, there was a main effect of block with both groups increasing in accuracy across blocks, F(3, 75) = 3.209, $p = 0.014$. During Day 2, both the controls and relatives performed above chance, *t's*(18) > 6.789, $p < 0.001$ and *t's*(7) > 3.709, $p < 0.008$ respectively. In addition, within groups t-

tests revealed that there was an improvement in accuracy for the controls and ROS group between sessions 1 and 2 [$t(18) = -3.024$, $p = 0.007$ and $t(7) = -3.249$, $p = 0.014$ respectively]. These results indicate that unlike in Wagshal et al., (under review), performance continued to improve with extended training in both groups. In the present study, subjects were performing above 75% accuracy, which is a higher level than in our previous study of adolescent subjects.

Reaction time was also analyzed overall for Day 2 as well as separately for session 1 and 2 on the second day. Overall, there was a main effect of block with both groups responding faster on the second block, $F(7, 175) = 2.384$, $p = 0.033$. Within each session, there were no significant effects or interactions between block and/or group.

Secondary Task

The effect of the secondary task was assessed by computing the differences between the average of the trial block immediately before and after the two dual task blocks and the average of the two dual task blocks (Figure 7). This yielded a measure of the cost of dual task performance, with greater numbers indicating greater cost. We examined dual task cost at both time points during the second day of training (after 80 and 640 trials) to examine the development of automaticity in the two groups. A 2 (Group) x 2 (Session) repeated measures ANOVA revealed a significant main effect of Group, $F(1, 25) = 3.304$, $p = 0.0405$, and a significant interaction between Group and Session, $F(1, 25) = 3.953$, $p = 0.029$, being driven by the decrease in performance by the relatives. There was no main effect of Session.

Between subjects t-test revealed a significant difference between the groups during the second session with the ROS group being more adversely affected by the dual task than controls, $t(25) = -3.387$, $p < 0.001$. There was no group difference for the first session. Within groups subjects t-test revealed that there was a significant ($t(18) = 3.049$, $p = 0.0035$) reduction in the

effect of the dual task in the healthy controls between sessions 1 and 2 with the controls getting more automated in the second session. There was no significant difference in the effect of the dual task across sessions for the ROS group. It appears that the controls subjects showed no dual task cost by the second probe test, while the ROS group continued to show a cost of performing a concurrent task.

On the declarative knowledge assessment, there was no significant difference between the controls and relatives in terms of their estimates of the cue-outcome association probabilities. For both groups, subjects exhibited above chance declarative knowledge of the cue-outcome associations. For controls, performance was significantly above chance $t(8) = -2.357$, $p = 0.046$, and it was marginally above chance for the relatives $t(7) = -2.272$, $p = 0.057$.

fMRI Results

We explored neural activation during performance of the task for a task-baseline contrast in a whole brain group-level voxel-wise analysis during early and late learning. There were no regions that passed threshold due to low power. However, an independent anatomical ROI analysis was performed for the regions that encompassed the prethresholded mask for exploratory analysis to thoroughly examine the differences between the groups and the activations associated with learning across days. For early learning there was a significant main effect of ROI, $F(7, 70) = 6.782$, $p = 0.001$. There was no main effect of group or an interaction between group and ROI (Figure 8). Between subjects t-tests for ROI revealed no significant differences between the controls and relatives for any region. For late learning there was a significant main effect of ROI, $F(7, 70) = 2.944$, $p = 0.012$ with the controls displaying greater activation overall. There was not a main effect of group or an interaction between group and

ROI. However between subjects t-test for ROI revealed a trend for the right caudate for a decrease in activation (relatives > controls): $t(10) = 1.421$, $p = 0.093$.

For early versus late learning, there was a significant main effect of ROI, (7, 70) = 7.061, $p < 0.001$. There was a significant main effect of Day, F(1, 10) = 4.022, $p = 0.00365$, with a decrease in activation for most regions on the second day. There was a trend for an interaction between Group and day, F(1, 10) = 2.494, $p = 0.0725$, with the decrease in activation being driven by the relatives, and a trend for interaction between ROI and Day, F(7, 70) = 1.925, $p = 0.0635$. No other main effects or interactions were significant. Within subjects t-test for early verses late learning for the relatives were significant for the left caudate, t(5) = 3.115, $p = 0.013$, the right caudate, t(5) = 3.455, $p = 0.009$, the left putamen, t(5) = 2.065, $p = 0.047$, and a trend for the right putamen, t(5) = 1.677, $p = 0.077$, with an increase in activation for early learning and then decreasing in activation for late learning (Figure 8). There was a significant difference across days of learning for the left hippocampus, t(5) = 2.305, $p = 0.0345$, and bilateral anterior cingulate, t(5) = 2.065, $p = 0.047$, with a decrease in early learning and more of a decrease in activation during late learning for the relatives (Figure 8). There were no significant results for the controls for early compared to late learning (Figure 8).

A repeated-measures ANOVAs were also conducted for each individual ROI. For the left hippocampus there was a trend for a main effect of Day, F(1, 10) = 2.567, $p = 0.07$, with a decrease in activation on Day 1 and more of an decrease in activation of Day 2. There was no main effect of group or an interaction between Group and Day. There were no significant main effects or an interaction for the right hippocampus. For the left caudate there was a main effect of Day, F(1, 10) = 8.229, $p = 0.0085$, and a strong trend for interaction between Group and Day, F(1, 10) = 2.965, $p = 0.058$, with both groups having a slight increase in activation during Day 1

and then a decrease in activation during Day 2 with the relatives having a sharper decrease. For the right caudate there was a significant main effect of Day, $F(1, 10) = 11.471$, $p = 0.0035$, and a significant interaction between Group and Day, $F(1, 10) = 7.511$, $p = 0.0105$, with the controls near baseline for both Day 1 and 2, while the relatives have an increase in activation for Day 1 and a sharp decrease in activation on Day 2. For the left putamen there was a trend for an interaction between Group and Day, $F(1, 10) = 2.740$, $p = 0.0645$, with both groups near baseline during Day 1 and with a slight increase in activation during Day 2 for the controls while for the relatives there was a sharp decrease in activation. For the right putamen there were no significant main effects or an interaction. For the bilateral anterior cingulate there was a significant main effect of Day, $F(1, 10) = 3.583$, $p = 0.044$, with a greater decrease in activation for Day 2 vs. Day 1. There was no main effect of group or an interaction between Group and Day. For the bilateral posterior cingulate there were no main effects or an interaction (Figure 8).

3.9 Discussion

In the current study, behaviorally the ROS patients did not differ from healthy controls in accuracy or reaction time on the WPT either during early or late training on the single-task trials. Though the ROS group did not perform significantly above chance during much of the early learning phase, during the extended training they performed above chance and at the level of the healthy control group.

In contrast, the healthy control and ROS groups were differentially affected in the dual task probe trials inserted during the second day of training. WPT accuracy was adversely affected by the secondary task early in extended training. In contrast, the healthy controls group showed no dual task cost later in extended training while the ROS group continued to show a cost of performing a concurrent task. These results suggest that during learning on the first 80

trials of training that occurred before the first dual task probe block, both groups were not performing the task in an automated manner. However, by the second dual task probe block during the second session (by 640 trials of training), the controls showed no cost of the tone counting task on WPT performance, while the relatives' performance continued to be adversely affected by the secondary task.

The addition of a secondary task decreases declarative memory retrieval by occupying working memory, and results in relying on controlled rather than automatic processing (Foerde, Poldrack, Knowlton, 2006; Logan, 1978; Craik et al., 1996). Data provided by computational modeling, and studies of neuropsychological patients provide support for the idea that learning of the WPT can be supported by different memory systems (Foerde et al., 2006; Knowlton et al., 1994; Knowlton et al., 1996; Gluck et al., 2002). By one view, in healthy young adults with good declarative memory abilities, early in training subjects acquire simple rule-based strategies that rely on the medial temporal lobe, while the basal ganglia support later learning by utilizing stimulus-response associations to learn the task (Shohamy et al., 2008). In situations where declarative memory is compromised (e.g. dual task conditions, aging), performance may be more reliant on the basal ganglia throughout training. It appears that both declarative memorization of cue-outcome associations and implicit learning of stimulus-response associations can support performance on this task. Their contributions vary depending upon stage of practice.

Our results illustrate that while the two groups reach similar behavioral endpoints, the ROS group did not automate to the task as well as the healthy control group and continued to rely on controlled processing even after extensive practice. Our fMRI data demonstrated that the ROS group also displayed different patterns of neural activation during learning of the task. The fMRI data revealed that the adult relatives were at baseline during early learning and then had a

sharp decrease in activation in the striatum, while the controls maintained their same level of activation near baseline across learning. For the bilateral anterior cingulate the relatives demonstrated a slight decrease in activation on the first day and then a sharp decrease in activation during the second day. These results are paralleled but to an even greater extent in the left hippocampus for the relatives.

The significant difference in the brain activation pattern seen across both groups in the striatum signifies a lack of utilization in the relatives during later learning while controls recruited this region to help themselves complete the task. Though the adult relatives were successful in learning the WPT, this pattern of brain activation in the striatum is similar to the first experiment with adolescent siblings of COS patients who displayed impaired learning of the WPT compared to controls.

3.10 General Discussion

These are the first series of studies to examine adolescent COS patients and adult ROS patients in a cognitive skill learning task using fMRI and to demonstrate a dual task effect in the adult relatives. The result of experiment 1 revealed that the adolescent siblings were impaired on the WPT and displayed a different pattern of brain activation with a decrease in activation compared to controls in the anterior cingulate gyrus and in the striatum during later learning, while the controls demonstrated a decrease in activation compared to siblings in the posterior cingulate gyrus during early learning. The results of experiment 2 revealed that the adult relatives performed comparably to controls and demonstrated learning across both days of training. However, the adult relatives were negatively affected by the dual task, suggesting that the adult relatives were not using an automatic strategy to complete the WPT and perhaps they may have been using a compensatory, alternative explicit memory strategy that could potentially

rely on brain structures such as the medial temporal lobe. The fMRI data did not support this theory, though it did reveal a similar pattern of brain activation that was evident in the adolescent siblings, an underactivation of the striatum.

The decrease in activation in the striatum during late learning for both the adolescent siblings and the adult controls may reflect a lack of effective utilization of striatal circuits. With the adolescent siblings, not employing the striatum may have contributed to their poorer level of asymptotic performance on the WPT. But with the adults, not using the striatum suggests that they must have been using another mechanism to compensate for their good performance on the WPT. However, their performance on the dual task trials were impaired with a cost associated with the secondary task, suggesting that subtle deficits in cognitive skill learning are also associated with familial liability to schizophrenia.

The dual task paradigm provides an important compliment to the more traditional methods for characterizing the degree of skill learning. It permits the detection of more subtle deficits in skill learning then can be identified in a single task paradigm. Consistent with a prior study (Granholm, Asarnow & Marder, 1996) that found that with sufficient practice, patients with schizophrenia performed normally on the Multiple Frame Search Task but still showed deficits relative to controls in a dual task condition, suggesting that they had not automated the task as well as controls. This mirrors our results with the adult relatives.

Why did the concurrent task have an adverse effect on the adult ROS patients but not the adolescent siblings of schizophrenia patients (Wagshal et al., Under Review)? In our previous study, we found that both adolescent siblings of patients with COS and controls showed no cost of performing a secondary task. However, the siblings performed significantly worse than the controls. The reason we did not observe a dual task effect in adolescent siblings of COS patients

was that the siblings of COS patients performed at chance level in the single task conditions. This floor effect precluded finding a dual task effect. Showing a dual task effect in this situation would have required them to perform below chance level in the dual task. In addition, it appears that the relatives may be using controlled processing as a compensatory strategy to elevate their performance to the level of controls. The fact that there was no significant difference between the relatives and the controls in terms of declarative knowledge of the cue-outcome associations suggests that the both groups had this information available to contribute to performance.

This paper aimed at providing evidence using behavioral and MRI data to try and elucidate the different strategies and mechanisms by which schizophrenia relatives implement in the WPT. Knowing which strategies and brain regions that schizophrenia patients and their relatives employ can give insights into whether corticostriatal dysfunction is an endophenotype of schizophrenia, independent of the effects of medication and the illness.

3.11 Figures

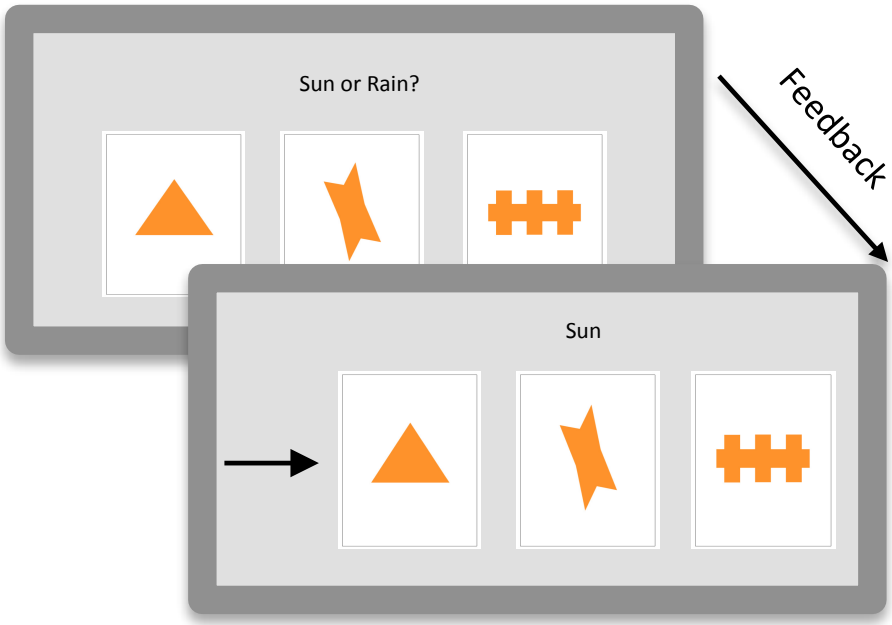

Figure 3.1. Weather Prediction Task. Participants were told to predict the weather (sun or rain) based on cues. On every trial between 1 and 3 cues (out of 4 possibilities) could appear, yielding 14 possible combinations. The cues were probabilistically related to the outcomes. The association of the different cues with different probabilities was randomized across participants. The cue strength of each of the 14 resulting stimuli were such that the overall probability associating each cue with sun or rain was 0.756, 0.575, 0.425, and 0.244 across the task. Since feedback was probabilistic, a response was considered correct if it matched the outcome most strongly associated with a stimulus, regardless of feedback. Thus, a response could be "correct" even if feedback reported an incorrect answer. Therefore, the percentage correct score reflected how well the subjects learned the cue-outcome associations (Marsh et al., 2004). The cues are shown on the screen for a maximum of 3 seconds, the feedback is shown on the screen for 1 second, and the time between trials is 0.5 seconds.

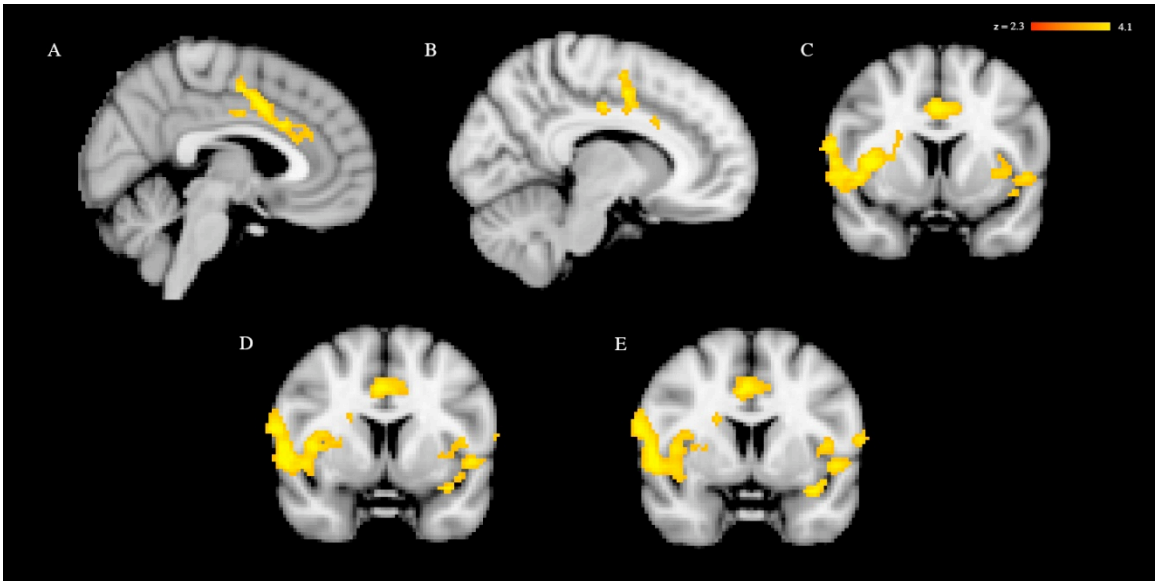

Figure 3.2. fMRI Images during Early Learning on the WPT (adolescents). Early Learning (first 50 trials) in the weather prediction task. Images are from the group-level analysis ($z > 2.3$, cluster-corrected thresholded at $p = 0.05$). A =bilateral anterior cingulate gyrus, B = bilateral posterior cingulate gyrus, C = left putamen, D = right putamen, and E = right caudate for siblings > controls for task vs. baseline.

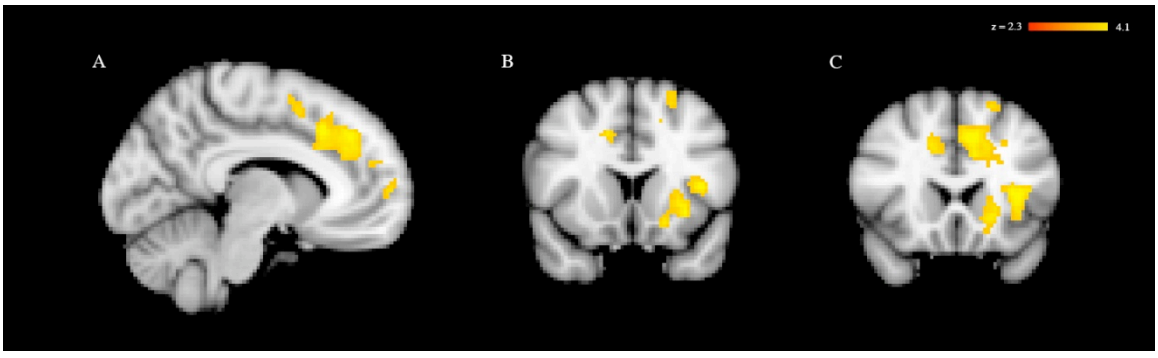

Figure 3.3. fMRI Images during Extended Training on the WPT (adolescents). Extended Training (after 850 additional trials) in the weather prediction task. Images are from the group-

level analysis ($z > 2.3$, cluster-corrected thresholded at $p = 0.05$). A =bilateral anterior cingulate gyrus, B = left putamen, C = left caudate for controls > siblings for task vs. baseline.

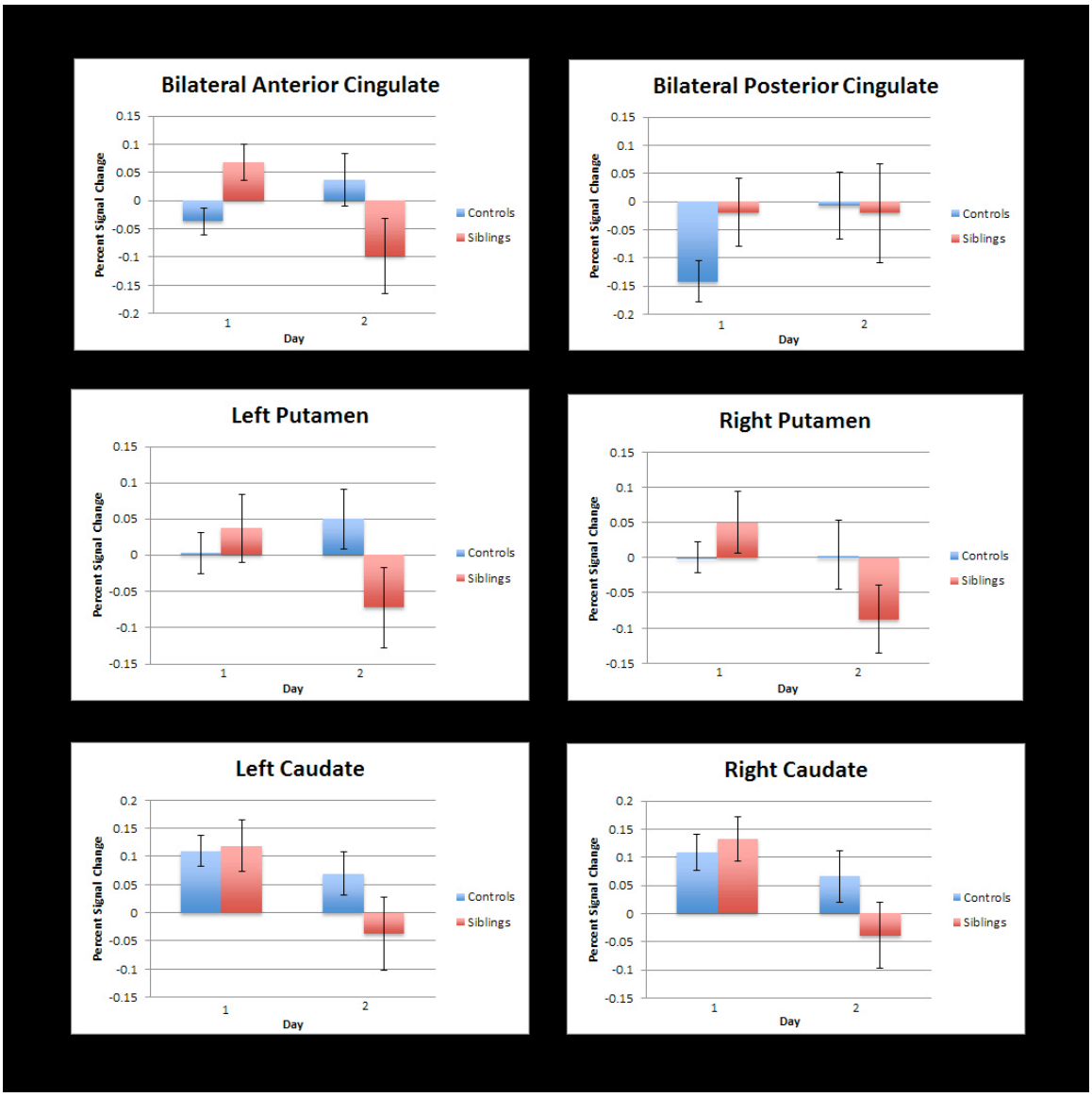

Figure 3.4. ROI graphs of the WPT (adolescents). Percent signal change during the task compared to baseline within the regions that demonstrated areas of significant difference between siblings and controls on day 1 and 2. Error bars represent the standard error of the mean.

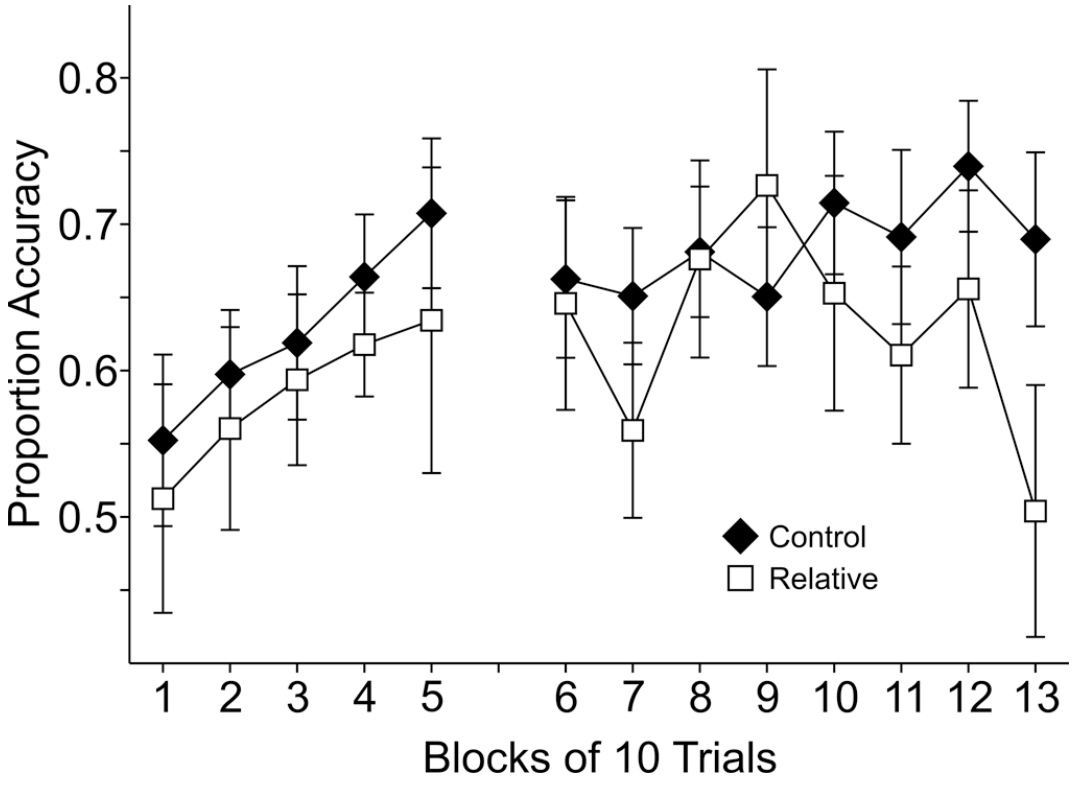

Figure 3.5. WPT Accuracy- Early Training (adults). WPT accuracy of the controls and COS relatives in early training [(Day 1 and the first 80 trials of Day 2 (blocks 6-13 in this graph)]. Error bars represent the standard error of the mean.

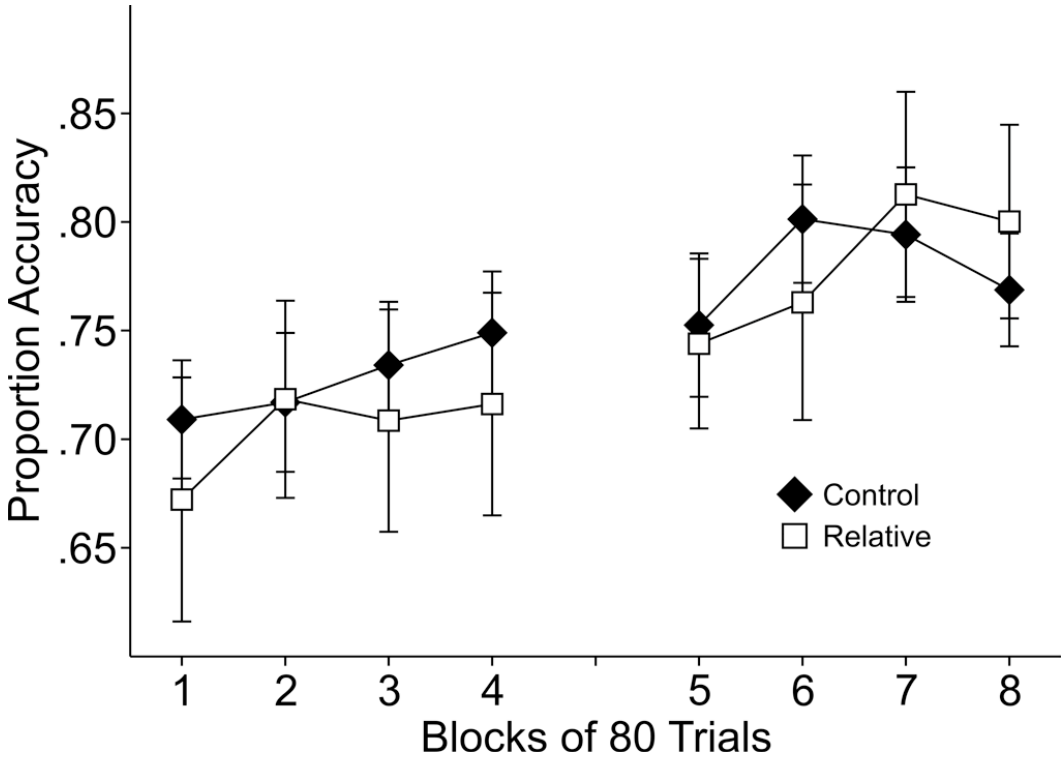

Figure 3.6. WPT Accuracy- Extended Training (adults). WPT accuracy of the controls and COS relatives During Day 2. Only single task trials are depicted. Error bars represent the standard error of the mean.

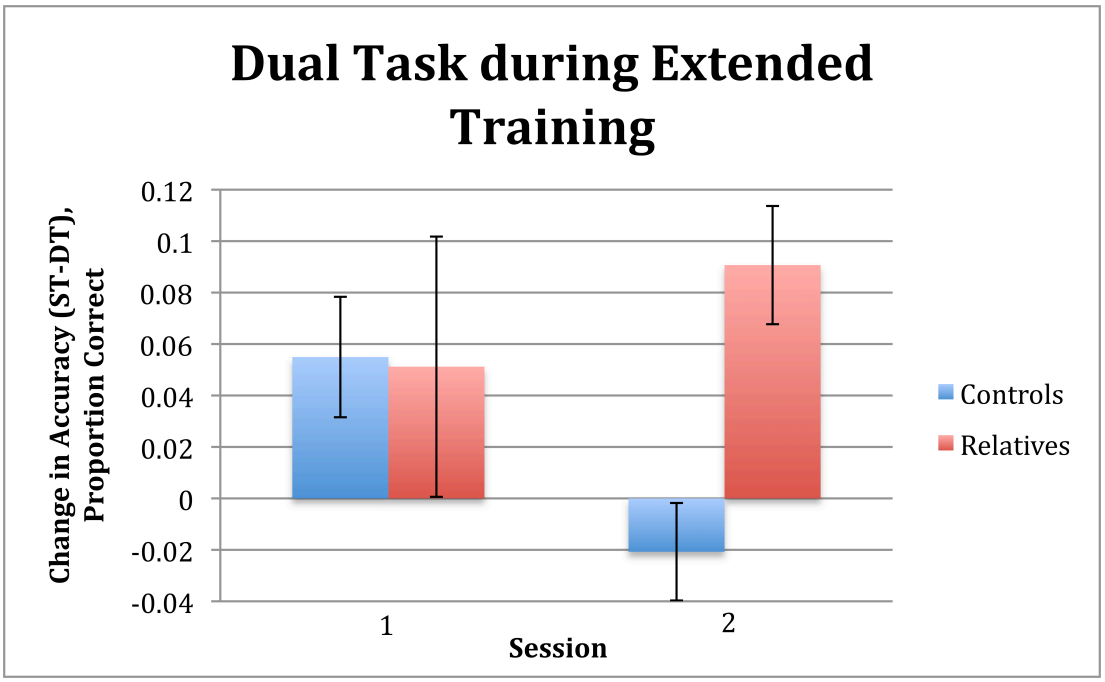

Figure 3.7. Dual Task of the WPT (adults). Performance on the WPT during the dual task effect for accuracy on the second day during sessions 1 and 2. Note that a positive number indicates worse performance on the task.

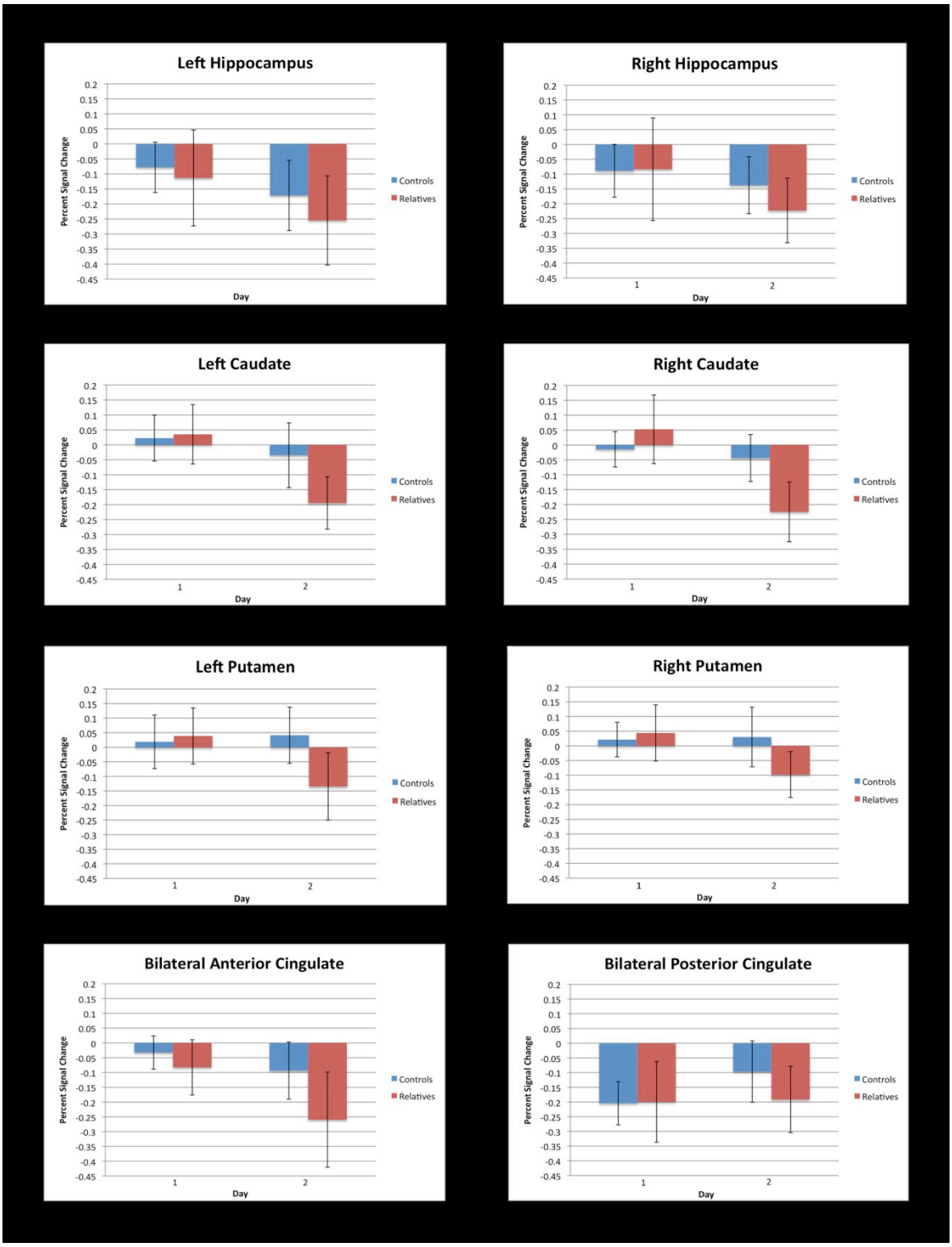
Left Hippocampus
Right Hippocampus
Left Caudate
Right Caudate
Left Putamen
Right Putamen
Bilateral Anterior Cingulate
Bilateral Posterior Cingulate
Percent Signal Change
Day
Controls
Relatives

Figure 3.8. ROI graph of the WPT (adults). Percent signal change during the task compared to baseline within the regions that demonstrated areas of significant difference between siblings and controls on day 1 and 2. Error bars represent the standard error of the mean.

3.12 Tables

	Controls (n = 25)		Siblings (n = 10)	
Variable	**M**	**SD**	**M**	**SD**
Age	12.76	2.59	12.60	2.32
Education	6.76	2.76	6.80	2.53
Gender*	15/10		5/5	
Vocabulary**	59.16	10.723	42.67	8.382
Blocks***	56.91	7.663	46.56	8.974

Table 3.1. Demographic Table (adolescents).

*Men/Women, **WASI Vocabulary subtest (missing 1 sibling), ***WASI Block subtest (missing 1 sibling and 2 controls)

Region	Coordinate (mm) X	Y	Z	Max Z-Value
Day 1 for siblings > controls				
Bilateral Anterior Cingulate	43	65	56	3.839
Bilateral Posterior Cingulate	40	55	56	2.974
Left Putamen	60	67	36	2.676
Right Putamen	32	66	40	2.725
Right Caudate	35	65	47	2.841
Day 2 for controls > siblings				
Bilateral Anterior Cingulate	49	73	51	2.772
Left Putamen	58	70	35	3.010
Left Caudate	53	73	33	2.806

Table 3.2. Voxel-wise Region Coordinates (adolescents). Brain regions that showed significant activation at the group-level analysis for task vs. baseline. Z-statistic maps were whole-brain cluster-corrected at Z > 2.3, P = 0.05. There were no significant regions for Day 1 controls > sibs and Day 2 siblings > controls.

	Controls (n = 6)		**Relatives (n = 6)**	
Variable	**M**	**SD**	**M**	**SD**
Age	42.17	5.269	42.17	4.579
Education	15.17	1.835	12.17	5.193
Gender*	1/5		1/5	
Vocabulary**	59.50	6.979	45.33	6.506
Blocks	64.33	4.676	45.83	9.704

Table 3.3. Demographic Table (adults).

*Men/Women, **WASI Vocabulary subtest (missing 3 relatives)

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CHAPTER 4: Adolescent Relatives in a Motor Skill Learning Task

4.1 Motor Skill Learning in Adolescents with Genetic Vulnerability for Schizophrenia

4.1.1 Introduction

Schizophrenia is a debilitating mental disorder characterized by disruptions in thought processes and the expression of reality. Schizophrenia patients are impaired in numerous domains such as in working memory, attention, executive control, language, and declarative memory (Asarnow, 1999; Gold & Harvey, 1993; Kuperberg & Heckers, 2000; Riley et al., 2000). One such domain, skill learning has been shown to be differentially affected in patients. It is well documented that schizophrenia patients have a deficit in cognitive skill learning (Keri, Juhasz, Rimanoczy, et al., 2005; Horan, Green, Knowlton, et al., 2008; Weickert, Terrazas, Bigelow, et al., 2002), but there are mixed results in the literature concerning specific types of motor skill learning.

Studies using the Pursuit Rotor and Mirror Tracing tasks have demonstrated that patients with schizophrenia show similar learning comparable to controls (Huston & Shakow, 1949; Goldberg et al., 1993; Granholm, Bartzokis, Asarnow, & Marder, 1993; Clare, McKenna, Mortimer & Baddeley, 1993; Kern, Green, & Wallace, 1997; Weickert et al., 2002; Scherer, Stip, Paquet & Bedard, 2003; Bedard et al., 2000). However, on another measure of motor skill learning, the Serial Reaction Time (SRT) task, there is less agreement in the literature of whether or not patients possess a deficit.

The SRT is a four-alternative spatial choice reaction time task. Unknown the subject, on some sets of trials the stimuli are presented in a particular sequence while on other sets there is a pseudorandom order (Figure 1). The SRT depends on the integrity of the striatum and will be

used to assess corticostriatal function. Performance on this task activates striatal regions on fMRI (Grafton, Hazeltine, & Ivry, 1995; Poldrack et al., 2005) that are part of motor corticostriatal circuits including the supplementary motor area (SMA), putamen, globus pallidus, and thalamus (Grafton et al., 1992; Grafton, Hazeltine, & Ivry, 1995; Poldrack et al., 2005).

Schizophrenia patients treated with typical antipsychotics display an impairment in motor skill learning compared to controls (Stevens et al., 2002, Schwartz et al., 2003). However, Foerde et al. (2008) compared performance on a cognitive skill learning task with performance on the SRT in patients who were treated with atypical antipsychotic drugs. There was no impairment in patients on the serial reaction time task despite a large impairment on the cognitive skill learning task. Other studies have also shown that patients with schizophrenia have intact motor skill learning, at least if they are treated with atypical antipsychotic medications that produce few motor side effects (Perry, Light, Davis, & Braff, 2000; Stevens, Schwarz, Schwarz, et al., 2002). One major difference between typical and atypical antipsychotics is the higher incidence of extrapyramidal side effects, commonly observed in patients taking typical antipsychotics (Foerde et al., 2008). Therefore it is possible that the deficits seen in patients taking typical antipsychotics is due to the type of medication and not the disease. Therefore, Foerde and colleagues suggested that the corticostriatal loop involved in cognitive skill learning was compromised in schizophrenia while loops that are involved in motor functioning were relatively intact. This dissociation was not due to differences in the psychometric properties of the tasks.

Nevertheless, there are confounds to studying schizophrenia patients due to the fact that it is not possible to separate the effects of medication and disease severity on striatal dysfunction in studies where schizophrenia patients are treated with antipsychotic drugs. Thus, the deficits

evident in schizophrenia patients on skill learning could be due to the medicational related effects on performance or may be due to the fact that patients with the most severe symptoms and who have possibly more severe striatal dysfunction may receive higher doses of medication. Thus, to untangle these factors one can either use drug naïve patients or genetically vulnerable individuals.

This study investigates the performance of siblings of childhood onset schizophrenia (COS) patients and controls on the serial reaction time task to determine if there is a difference in learning of the task. COS is a severe form of schizophrenia that appears clinically continuous with the adult onset (Asarnow &Asarnow, 1994; Nicolson & Rappaport, 1999). Patients with COS tend to show the same cognitive deficits, but to a greater extent than patients with adult onset of schizophrenia (Asarnow & Kernan 2009). Based on the Foerde et al. (2008) study, we predict no differences in the siblings of COS probands on performance on the SRT compared to controls.

In addition, to probe whether the SRT was learned implicitly, we inserted dual-task tone-counting probe trials to test whether performance was automatic at different points during training. Automatization of a skill refers to the ability to execute a task without the demand for effortful control. If performance does not decline when a secondary task is performed, it would suggest that performance on the SRT is relatively automatic and is based on stimulus-response habits involved in automatic skills rather than declarative memory. Therefore, based on studies that have compared schizophrenic patients and their first-degree relatives to healthy controls, we also hypothesize that the siblings of COS patients will demonstrate a deficit in automaticity indicated by a decrement in dual-task performance after extended training.

4.1.2 Methods

Participants

Fifteen adolescent siblings of COS patients and Ninety-two adolescent controls who were matched in age, gender, and education to the siblings participated in the experiment (Table 1). Twenty-nine controls and six siblings were excluded from analysis based on computer malfunction or not responding on more than 10% of the trials or not being at least 75% accurate on the task. All participants provided informed consent according to the procedures approved by the University of California, Los Angeles (UCLA) Human Subjects Committee and were paid for their participation. Siblings of COS probands and controls were recruited and excluded through the same process stated in the first chapter.

Experimental design

Subjects practiced the SRT for a total of one and a half hours, spanning two days. The second session took place within seven days of the first. On the first day, subjects were assessed for any neurological disorder or psychotic symptoms by a psychiatrist, underwent a neuropsychological battery by completing the Wechsler Abbreviated Scale of Intelligence (WASI) vocabulary and block design subtests (Table 1), and then completed 192 trials of the SRT (half sequenced, half random) either inside or outside of an MRI scanner depending on whether the participants were in the behavioral or fMRI condition. In the second session, subjects were trained for an additional 3120 trials (half sequenced, half random) outside the scanner occurring in two sets with an intervening break of 30 minutes where another task (the weather prediction task) was performed. If the subjects were in the fMRI condition, they also completed 192 trials of the SRT while in the scanner on the second day. At the end of training, subjects' declarative knowledge of cue-outcome associations were tested by asking them to estimate their knowledge of the 14-item sequenced pattern.

The Serial Reaction Time (SRT) task (Nissen & Bullemer, 1987) involves a visual target appearing in one of 4 screen locations. Participants have to respond as quickly and accurately as possible to the target by pressing one of four spatially compatible buttons. On some sets of trials there is a sequential order of the target (however, the participants do not know this) while on others there is a pseudorandom order of the target. With practice, participants generally respond more quickly to the sequence than the pseudorandom order. The amount of improvement in reaction time by this implicit sequence is a measure of motor skill learning

Secondary Task: On the second day, a secondary task was introduced during trials 213-410 and 2761-3000. These probe trials were inserted to assess whether SRT performance was unaffected by the addition of a concurrent task and was thus relatively automatic (Foerde et al., 2008). For the secondary task, participants heard high (1000 Hz) and low (500 Hz) pitched tones during the task and had to count the number of high-pitched tones. At the end of each dual task block the subject reported the number of high-pitched tones they counted by entering the number into the computer.

Data Analysis

The SRT data were analyzed using 2-way repeated measures ANOVA for the benefit to determine if learning occurred. The benefit is the difference between the random and sequenced trials, and thus, a positive number indicates learning. To correct for violations of sphericity, the Huynh-Feldt test was used. The effect of the secondary task was assessed by computing the differences between the average of the trial block immediately before and after the two dual task blocks and the average of the two dual task blocks.

Performance on the declarative knowledge test was assessed by determining the correct number of items of the sequence in a row the subject successfully wrote down to obtain an

estimate of the participants knowledge of the 14-item sequenced pattern. Thus, a higher score would reflect more veridical declarative knowledge of the pattern. Chance performance would equal how many items of the sequence a subject would get in a row by guessing a random pattern. Chance was calculated through Monte Carlo simulations based on 20 sequences that were generated by a random number generator. The average of the correct sequences in these 20 simulations was calculated with a value of 3.33 and this was designated as chance performance.

4.1.3 Results

We examined performance on the WASI subtests and found significant differences between the groups with the controls performing better on both tests, Vocabulary $t(68) = 5.659$, $p < 0.001$, Blocks $t(66) = 4.023$, $p < 0.001$. For these analyses, two controls were missing on the vocabulary subtest and 3 controls and 1 sibling for the blocks subtest due to time constraints (Table 1).

Early Learning

We first examined learning by analyzing the median reaction time benefit from sequence trials for early learning, which were the first 192 trials on the first day. The benefit is the difference between the median reaction time on trials with the pseudorandom order and the sequenced trials. Figure 2 presents the reaction time benefit of the two groups during this early learning phase. During the 192 trials of training on Day 1, there was a main effect of block, $F(7, 490) = 2.569$, $p = 0.019$, with both groups gaining more of a benefit across blocks. There was also a trend for an interaction between Group and Block, $F(7, 490) = 1.748$, $p = 0.109$, with the siblings gaining more of a benefit by the end of the last block. This was confirmed with a post-hoc t-test that showed that it was the last block of benefit with the siblings driving this trend, $t(70) = -3.133$, $p = 0.003$. There was no significant main effect of group.

Figure 5 presents the proportion accuracy benefit of the two groups during the early learning phase. There were no main effects or an interaction between Group and Block for accuracy during the early training on the first day.

Performance after extended training

We next analyzed the benefit of reaction time during the second day of training. Figure 3 presents the reaction time for the two groups during the extended training. We did not include the dual task trials in these analyses. We analyzed Day 2 overall performance and Day 2 performance broken into two sessions (the 1320 single-task trials before and after a 30 minute break). Overall, there were no significant main effects or an interaction between group and block. When broken down into 2 separate sessions, for session 1 there was a trend for an interaction between group and block for the controls to have more benefit across blocks, $F(10, 700) = 1.716$, $p = 0.107$. A post-hoc between subjects t-test revealed that the benefit of block 8, $t(70) = 2.583$, $p = 0.012$, was driving the trend for the interaction, as well as a trend for the benefit of block 1, $t(70) = -1.821$, $p = 0.073$. There were no significant main effects. For session 2, there were no significant main effects or an interaction between group and block. In addition, within groups t-test revealed that there was no difference in reaction time for both groups between sessions 1 and 2.

Figure 6 presents the benefit for the proportion accuracy for the two groups during the extended training. Proportion accuracy was also analyzed overall for Day 2 as well as separately for session 1 and 2 on the second day. Overall, there were no significant main effects or an interaction between group and block. When broken down into 2 separate sessions, for session 1, there was a trend for a significant main effect of group with the siblings being more accurate across block, $F(1, 70) = 2.904$, $p = 0.093$. There was no main effect of block or an interaction

between group and block. For session 2, there were no significant main effects or an interaction between group and block. Furthermore, within groups t-test demonstrated that there was no difference in accuracy between session for the siblings but for the controls there was a trend for the controls to be less accurate in session 2, $t(62) = 1.921$, $p = 0.059$.

The effect of the secondary task was assessed by computing the average of the trial block immediately before and after the two dual task blocks and the average of the two dual task blocks (Figure 4). A positive score for the benefit is good, reflecting a faster reaction time for the sequenced trials. A 2 (session) x 2 (group) repeated measures ANOVA revealed a trend for an interaction between session and group, $F(1, 70) = 0.0925$, for the siblings to be affected by the dual task for both sessions while the controls are affected only during the second session. Between subjects t-test revealed a significant difference between the groups during the single task trials in session 1 with the siblings having more of a benefit during the single task trials than controls, $t(70) = -1.802$, $p = 0.038$, for the single task trials immediately surrounding the dual task trials. Within subjects t-test across sessions for the controls revealed a significant difference for the single task trials with more of a benefit during the second session, $t(62) = -2.605$, $p = 0.0055$, and a significant different between the single task trials and the dual task trials for session 2 with the dual task having more of a negative effect on benefit, $t(62) = 4.165$, $p < 0.001$. For the siblings there were no significant differences.

For accuracy we also calculate the dual task effect by computing the average of the single task trial immediately before and after the two dual surrounding single task trials (Figure 7). A 2 (session) x 2 (group) repeated measures ANOVA revealed no main effects or an interaction between session and group. Between subjects t-test revealed no significant difference between the groups for either the single or dual task trials. Within subjects groups t-test revealed a

difference in accuracy for the single task trials across sessions with the controls having more of a benefit of accuracy during session 2, $t(62) = 2.401$, $p = 0.0095$. There was also a trend for the controls to have more of a benefit for the dual task for session 2 as well, $t(62) = 1.302$, $p = 0.099$. There were no significant differences across sessions for the siblings. To determine if the groups were above chance on the dual task, a one-sample within subjects t-test was computed for each group. The controls were above chance for the single and dual task trials used to compute the dual task effect during both sessions, $t(62) = -2.879$, $p = 0.0025$ and $t(62) = -4.604$, $p < 0.001$ respectively. The siblings had a trend to be above chance during the first session of the dual task, $t(8) = -1.578$, $p = 0.0725$. However, the siblings were not significantly above chance during the single task trials and the second session of the dual task, *t's*$(8) < -1.085$, $p > 0.155$, but they were not affected by the dual task because they had a negative score for the benefit, which signifies learning due to their more accurate performance during the sequenced trials compared to the random trials.

On the declarative knowledge assessment (Figure 8), a between subjects t-test revealed that there was not a significant difference between the controls and relatives of their knowledge of the sequenced pattern. However, when comparing chance performance and if they possessed explicit knowledge of the sequence, the results demonstrated that the controls had explicit knowledge of the sequenced pattern and were significantly above chance, $t(62) = 3.072$, $p = 0.0015$, while the siblings were not above chance and did not possess explicit knowledge of the sequenced pattern.

4.1.4 Discussion

In the current study, there was no difference in performance of the siblings of COS patients compared to controls for either early or extended training and both groups demonstrated clear

learning across trials. Dual task probes were also inserted during the extended training to determine whether the groups were relying on controlled or automatic processing. While the results revealed no group differences on the dual task trials, there was a difference for the controls for session 2 between the single and dual task trials with the dual task having more of a negative effect on benefit. However, the controls still possessed a positive score for the benefit for the dual task trials during session 2, reflecting learning due to a faster reaction time for the sequenced compared to random trials.

For the dual task of accuracy, there were no differences between the groups on single and dual task trials. The results also revealed that the controls were above chance during the dual task and were not affected by the dual task for accuracy. However, while the siblings were not significantly above chance during the dual task for accuracy, they were not affected by the dual task, evidenced by a negative score for the benefit, which signifies learning due to a more accurate performance during the sequenced trials compared to the random trials.

Consistent with other studies of first degree relatives of patients with schizophrenia (Hill et al., 2008), in the present study adolescent siblings of patients with COS performed significantly more poorly than controls on the WASI, a measure of intellectual functioning. The poor performance of relatives of patients on intelligence tests has been interpreted as indicating that poor intellectual function may reflect liability to schizophrenia (Hill et al, 2008). Though the adolescent siblings performed poorly on the WASI, they were unimpaired in the task, which parallel results found in another patient group, patients with Korsakoff's amnesia, who also exhibit impairments on tests of intellectual function but perform as well as age-matched control subjects on the SRT (Nissen & Bullemer 1987; Nissen et al., 1989; Van Tilborg et al., 2011).

Our results demonstrate that siblings of schizophrenia patients were not impaired on the SRT, supporting similar findings with schizophrenia patients taking atypical antipsychotics (Foerde et al., 2008; Perry et al., 2000; Stevens et al., 2002). This is in contrast to other basal ganglia disorders, such as Parkinson's and Huntington's disease, in which patients display a deficit in the task (Doyon et al., 1997; Pascual-Leone et al., 1993; Knopman & Nissen, 1991; Willingham et al., 1996).

Studies with fMRI have revealed that while schizophrenia patients and controls perform comparably on the SRT, they do not employ the same brain regions to complete the task (Zedkova et al., 2006; Reiss et al., 2006; Purdon et al., 2011). However, there have been no studies that have examined learning of the SRT in COS patients or relatives. Though there were no differences in performance of the task in the two groups, an important direction for future studies is to employ fMRI to determine if the healthy, unaffected siblings of COS patients are utilizing the same brain structures as controls to successfully complete the task, and if not, to determine what brain structures they are using to compensate for their successful learning of the SRT. Understanding what brain structures are involved in order to complete tasks that schizophrenia patients and their relatives are impaired and unimpaired on is a first step to aid in the understanding of the neural mechanism of the disease and to find treatments for this severe disorder.

4.1.5 Figures

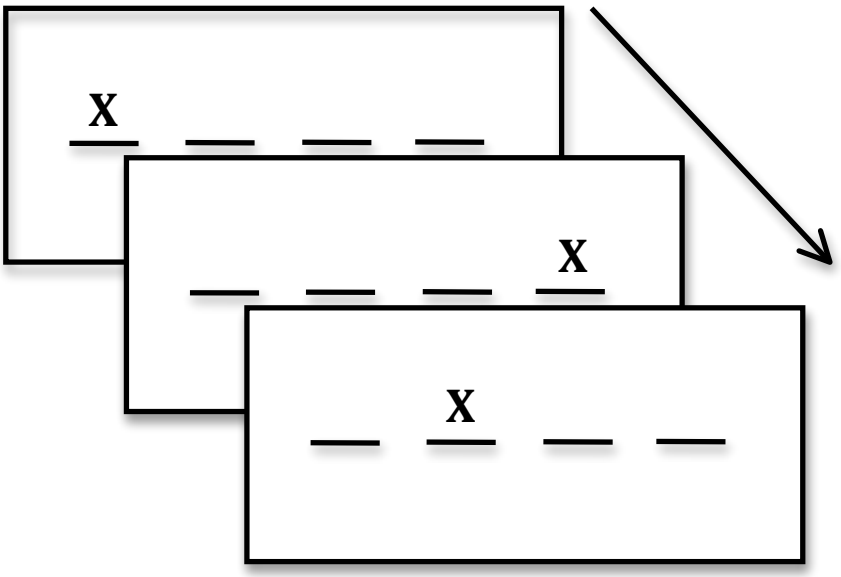

Figure 4.1.1. Serial Reaction Time Task. The SRT involves a visual target appearing in one of 4 screen locations. Participants have to respond as quickly and accurately as possible to the target by pressing one of four spatially compatible buttons. On some sets of trials there is a sequential order of the target (however, the participants do not know this) while on others there is a pseudorandom order of the target.

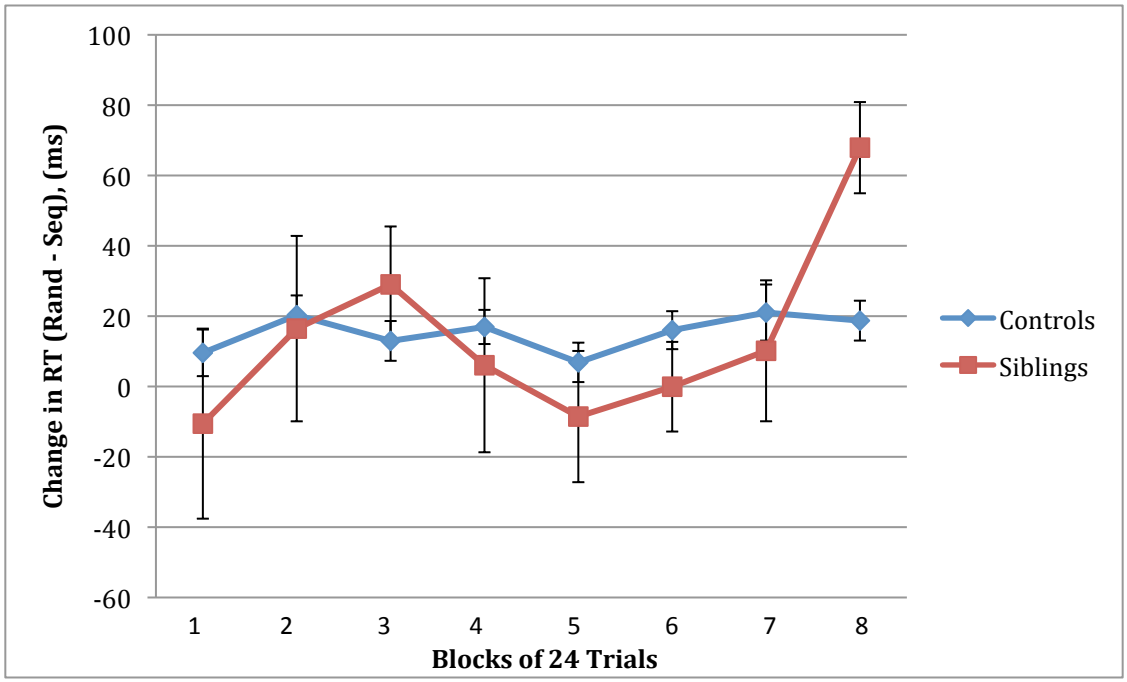

Figure 4.1.2. SRT Reaction Time- Early Training. SRT benefit (random – sequence trials) of reaction time in early training during Day 1. Error bars represent the standard error of the mean.

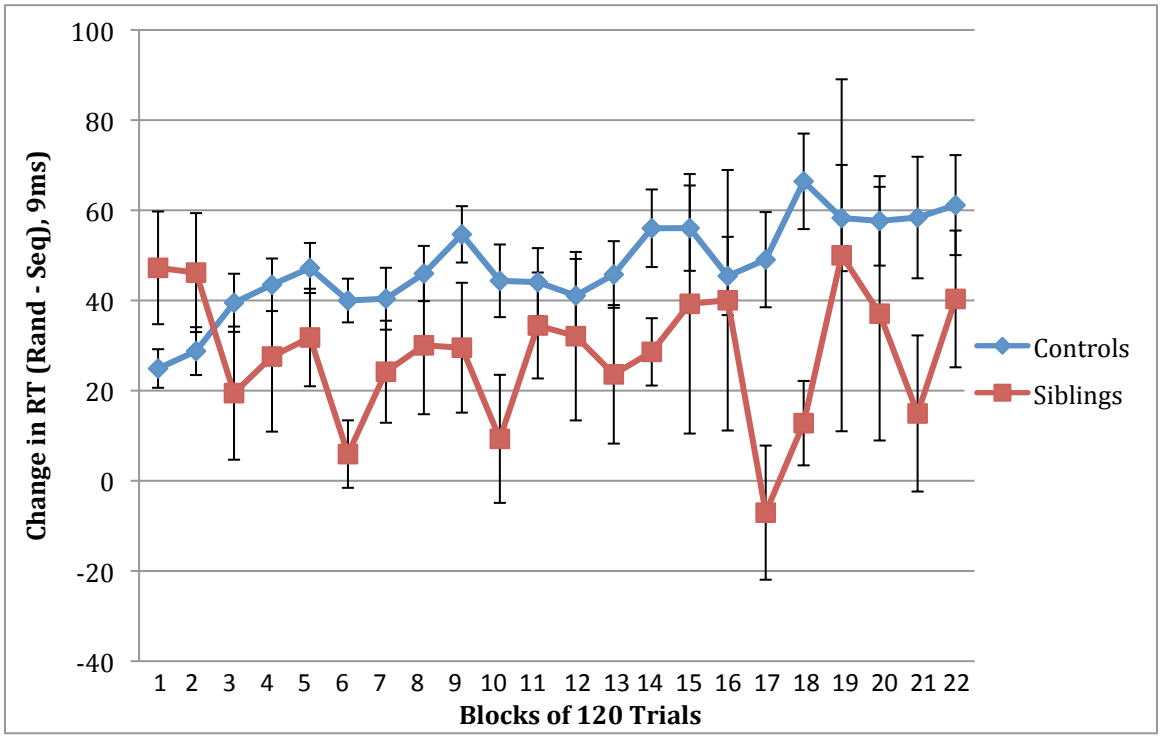

Figure 4.1.3. SRT Reaction Time – Extended Training. SRT benefit (random – sequence trials) of reaction time in the extended training during Day 2. Error bars represent the standard error of the mean.

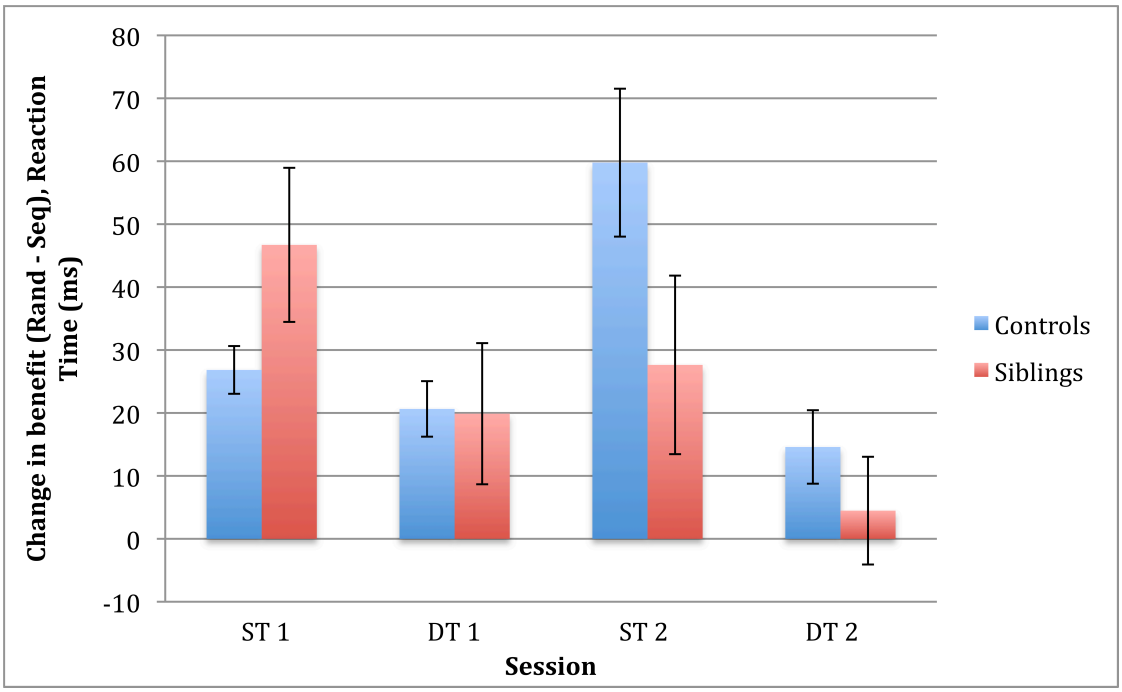

Figure 4.1.4. Dual Task of the SRT- Reaction Time. Error bars represent the standard error of the mean.

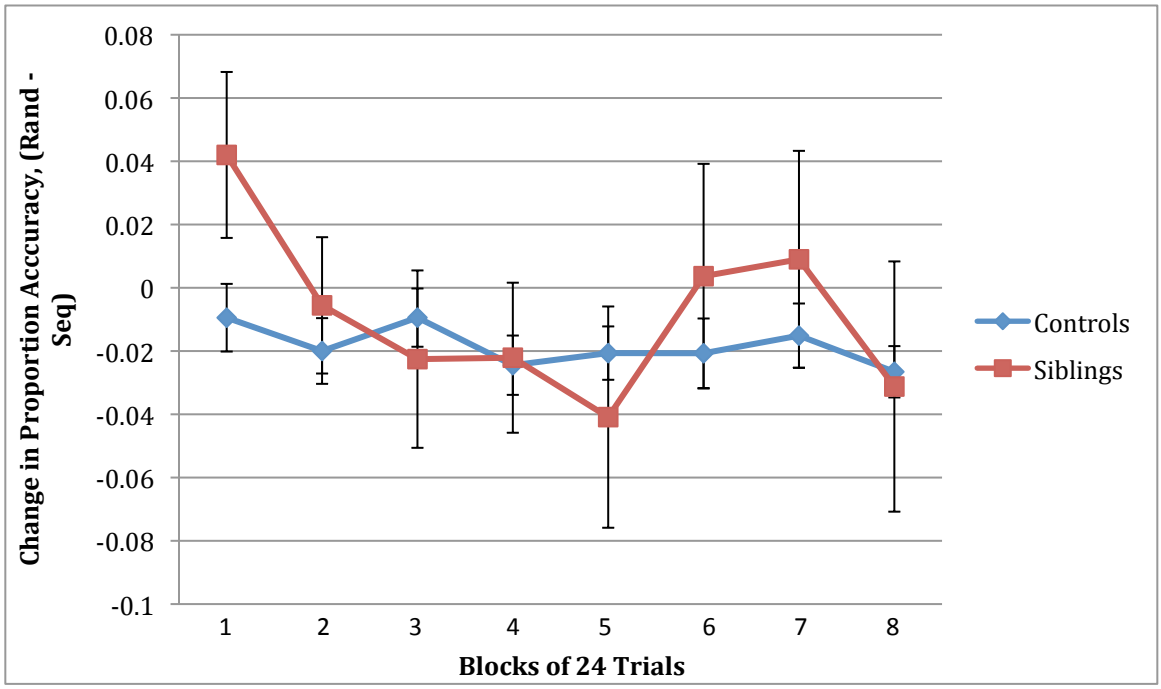

Figure 4.1.5. SRT Accuracy- Early Training. SRT benefit (random – sequence trials) of accuracy in early training during Day 1. Error bars represent the standard error of the mean.

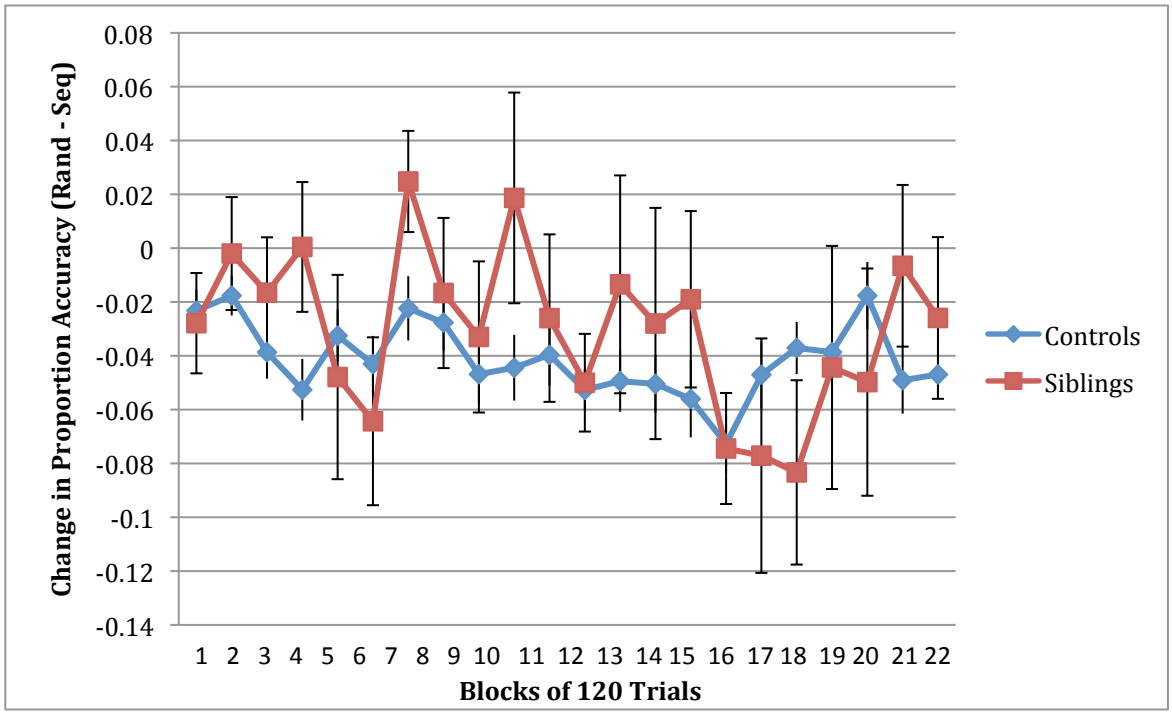

Figure 4.1.6. SRT Accuracy- Extended Training. SRT benefit (random – sequence trials) of accuracy in the extended training during Day 2. Error bars represent the standard error of the mean.

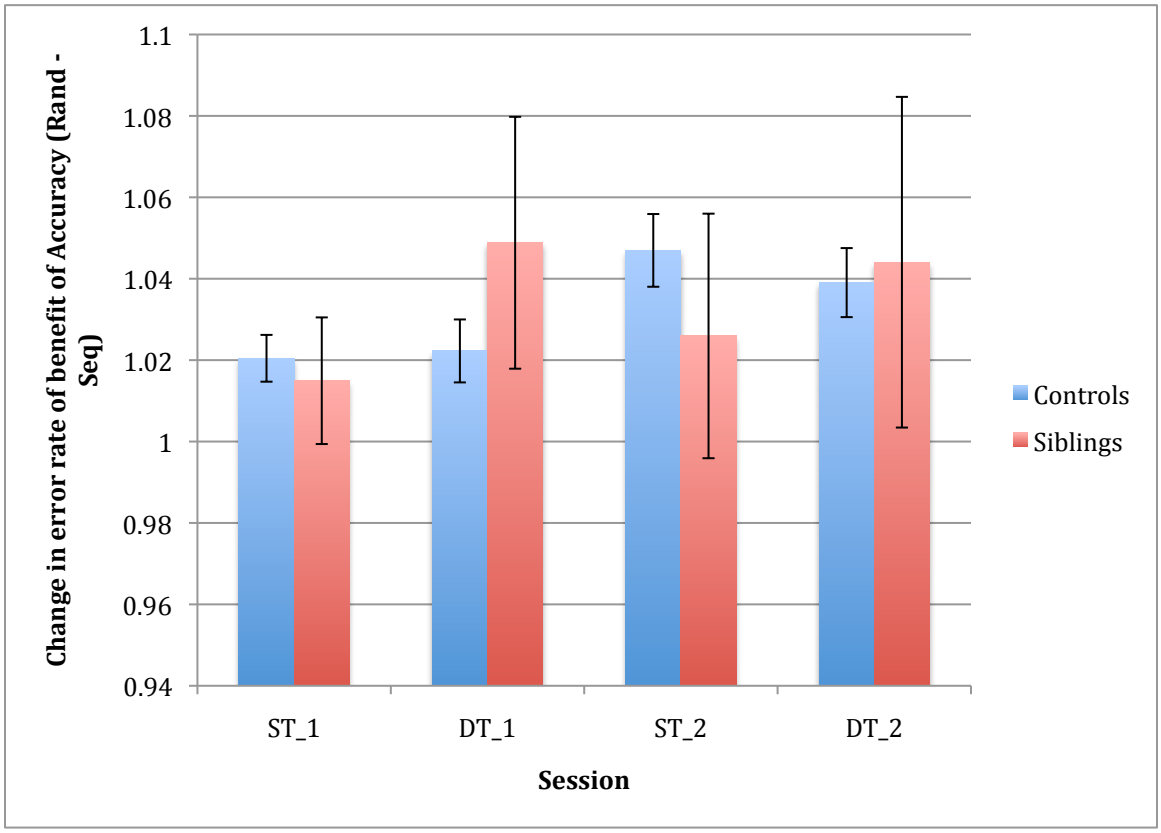

Figure 4.1.7. Dual Task of the SRT- Accuracy. Error bars represent the standard error of the mean.

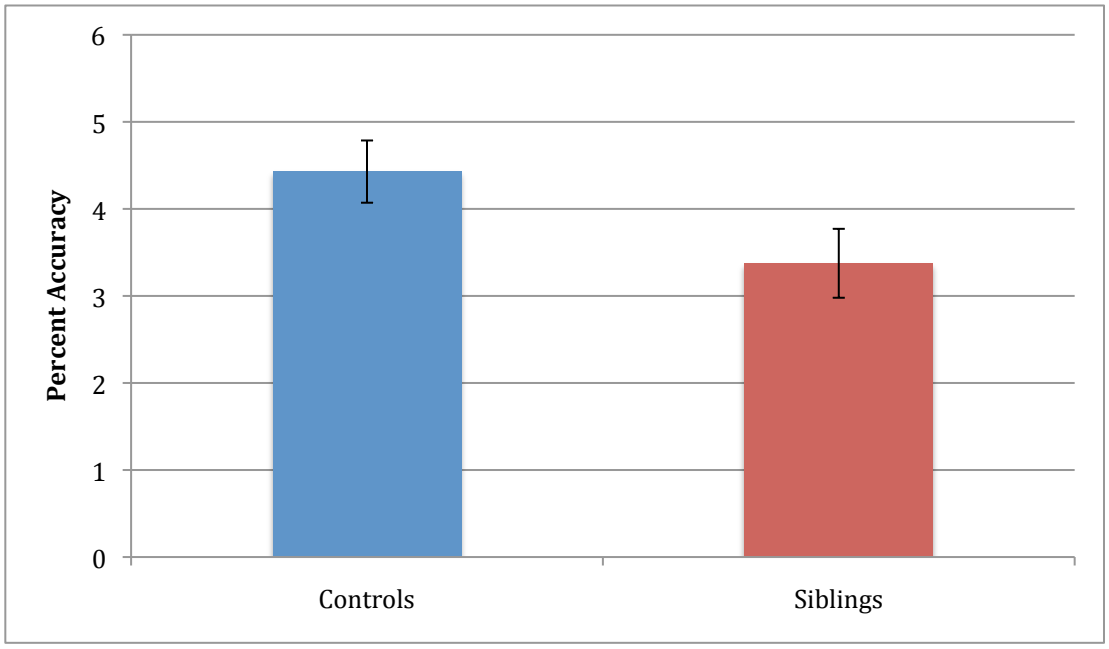

Figure 4.1.8. SRT Declarative Knowledge Questionnaire. Declarative Knowledge Assessment of the 14-item sequence. Error bars represent the standard error of the mean.

4.1.6 Table

	Controls (n = 63)		Siblings (n = 9)	
Variable	**M**	**SD**	**M**	**SD**
Age	13.46	2.03	13.00	1.66
Gender*	33/30		4/5	
Education	7.52	2.15	7.00	2.06
Vocabulary**	60.11	8.52	42.89	8.57
Blocks***	57.27	7.17	45.62	11.15

Table 4.1.1. Demographic Table.

*Men/Women, ** WASI Vocabulary subtest (missing 2 controls), *** WASI Blocks subtest (missing 3 controls and 1 sibling)

4.1.7 References

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4.2 Motor Skill Learning in Adolescent Siblings of Childhood Onset Schizophrenia Patients using fMRI

4.2.1 Introduction

Skill learning is the gradual improvement of performance with practice on a task and is demonstrated by reduced reaction time or increased accuracy in a task. There are different types of skill learning, such as motor, perceptual, and cognitive skill learning. Findings from studies of patients with basal ganglia disorders have shown that the basal ganglia are important for skill learning (Heindel et al., 1989; Knowlton et al., 1996a). Neuroimaging studies in healthy subjects have also lent support to this theory (Grafton, Hazeltine, & Ivry, 1995; Rauch et al., 1997; Poldrack, Prabakharan, Seger & Gabrieli, 1999; Schiltz et al., 2001; Aron et al., 2004; Moody et al., 2004).

There has been disagreement in the literature if individuals with schizophrenia possess a deficit in motor skill learning. Studies using the Pursuit Rotor and Mirror Tracing tasks have demonstrated that patients with schizophrenia show similar learning comparable to controls (Huston & Shakow, 1949; Goldberg et al., 1993; Granholm, Bartzokis, Asarnow, & Marder, 1993; Clare, McKenna, Mortimer & Baddeley, 1993; Kern, Green, & Wallace, 1997; Weickert et al., 2002; Scherer, Stip, Paquet & Bedard, 2003; Bedard et al., 2000). However, on another

measure of motor skill learning, the Serial Reaction Time (SRT) task, there is less agreement in the literature of whether or not patients possess a deficit.

The SRT is a four-alternative spatial choice reaction time task in which subjects are instructed to identify the location of a target as quickly and accurately as possible by pressing the response key that corresponds to the location of the target. Unknown the subject, on some sets of trials the stimuli are presented in a particular sequence while on other sets there is a pseudorandom order (Figure 1). The SRT depends on the integrity of the striatum and will be used to assess corticostriatal function. Performance on this task activates striatal regions on functional magnetic resonance imaging (fMRI) (Grafton, Hazeltine, & Ivry, 1995; Poldrack et al., 2005) that are part of motor corticostriatal circuits including the supplementary motor area (SMA), putamen, globus pallidus, and thalamus (Grafton et al., 1992; Grafton, Hazeltine, & Ivry, 1995; Poldrack et al., 2005). Patient studies with dorsal striatum dysfunction also support the idea that the basal ganglia are important to successfully learn the SRT (Gomez Beldarrain et al., 1998; Gomez Belderrain et al., 1999; Gomez Belderrain et al., 2002; Knopman & Nissen, 1991; Pascual-Leone et al., 1993).

Schizophrenia patients treated with typical antipsychotics display an impairment in motor skill learning compared to controls Stevens et al., 2002, Schwartz et al., 2003; Green et al., 1997). However, Foerde et al. (2008) compared performance on a cognitive skill learning task with performance on the SRT in patients who were treated with atypical antipsychotic drugs. There was no impairment in patients on the serial reaction time task despite a large impairment on the cognitive skill learning task. Other studies have also shown that patients with schizophrenia have intact motor skill learning, at least if they are treated with atypical antipsychotic medications that produce few motor side effects (Perry, Light, Davis, & Braff,

2000; Stevens, Schwarz, Schwarz, et al., 2002). Some major differences that can account for the disparity between typical and atypical antipsychotics is the higher incidence of extrapyramidal side effects suggesting striatal dysfunction, commonly observed in patients taking typical antipsychotics (Foerde et al., 2008), and the fact that typical antipsychotics are potent striatal D2 receptor antagonists and greater impair skill learning than atypical antipsychotics (Exner et al., 2006; Kumari et al., 2002; Woodward et al., 2007; Green et al., 1997). Therefore it is possible that the deficits seen in patients taking typical antipsychotics is due to the type of medication and not the disease.

One method of avoiding a confound of medication is to study relatives of schizophrenia patients. Relatives of individuals with schizophrenia possess some of the same impairments as patients (Asarnow, 1999; Gold & Harvey, 1993; Kuperberg & Heckers, 2000; Riley et al., 2000). Thus, similar deficits in healthy, unaffected relatives can provide insight into the underlying mechanisms that are related to the genetic vulnerability for schizophrenia and can help determine if the impairments observed in patients are related to genetic liability for the disease.

Studies with fMRI have revealed that while schizophrenia patients and controls perform comparably on the SRT, they do not employ the same brain regions to complete the task (Zedkova et al., 2006; Reiss et al., 2006; Purdon et al., 2011). The Zedkova et al. (2006) and Reiss et al. (2006) studies examined schizophrenia patients taking atypical antipsychotic medication while completing the SRT in the scanner and demonstrated that while both groups successfully completed the task, the patients failed to activate the striatum, specifically the caudate, while performing the task. There was also abnormal activity in the premotor cortex, anterior cingulate, and in the temporal and parietal lobe. Another study (Purdon et al., 2011) looked at unmedicated first-episode schizophrenia patients and controls with the SRT in the

scanner. In their results they found comparable behavioral performance of the task of patients compared to controls; however, they had slightly divergent imaging results than previous studies, and demonstrated differences in activation for the patients with less activation in the middle frontal cortex and increased activation in the superior temporal cortex, which they suggest belies a more focal dysfunction in the middle frontal cortex before the onset of treatment (Purdon et al., 2011).

There has been only 1 study thus far to examine the behavioral and neural mechanisms of relatives of schizophrenia patients and controls of the SRT using fMRI (Woodward et al., 2007). Woodward and colleagues tested 12 healthy adult siblings and controls. Both groups demonstrated normal performance on the task. However, compared to controls the siblings demonstrated less activation in frontal and parietal regions as well as the basal ganglia. Thus, they suggested that altered neural activation during the performance of cognitive tasks may be related to the genetic vulnerability for schizophrenia (Woodward et al., 2007).

Thus far there have been no studies that have examined performance of the SRT in childhood onset schizophreni (COS) patients or relatives, much less any studies involving adolescents. Therefore, to our knowledge this study is the first to explore the neural correlates of skill learning in adolescent siblings of COS patients. COS is a severe form of schizophrenia that appears clinically continuous with the adult onset form (Asarnow & Asarnow, 1994; Nicolson & Rappaport, 1999). COS may represent a more homogenous disorder than adult-onset schizophrenia, with a more pronounced genetic risk (Asarnow, et al., 200l). Relatives of patients with COS may have greater genetic liability for schizophrenia than relatives of adult onset patients (Asarnow et al., 2001), and thus may be more likely to show neural activation pattern differences than adult onset relatives. Based on the Woodward et al. (2007) study, we expect to

find no differences in performance of the task in the two groups. However, we do hypothesize that there will be a difference in the pattern of brain activation that the siblings use to compensate in order to successfully learn the SRT task.

4.2.2 Methods

Participants

Fifteen adolescent siblings of COS probands (age range: 8-16) and forty-seven adolescent controls (age range: 8-16), who were right handed and were matched in age, education, and gender to the siblings of COS paticipants participated in the experiment (Table 1). These subjects were a subset of the participants in our previous behavioral study of SRT learning in siblings of COS patients (Wagshal et al., unpublished). Twenty-nine controls and seven siblings were excluded from analysis based on computer malfunction, not responding on more than 10% of the trials, being less than 75% accurate, excessive movement of 3mm or greater, or not completing both days of training. Siblings and controls were recruited through the same processes stated in Chapter 1.

Task design

Participants were administered the serial reaction time task (Nissen & Bullemer, 1987). The MATLAB (The MathWorks, Inc., Natick, MA) Psychophysics Toolbox (Brainard, 1997) version 7.4 was used to present the stimuli and to record responses on an Apple G4 PowerBook using the OSX operating system.

Serial Reaction Time (SRT) Task: The Serial Reaction Time (SRT) task (Nissen & Bullemer, 1987) involves a visual target appearing in one of 4 screen locations. Participants have to respond as quickly and accurately as possible to the target by pressing one of four spatially compatible buttons. On some sets of trials there is a sequential order of the target (however, the

participants do not know this) while on others there is a pseudorandom order of the target. With practice, participants generally respond more quickly to the sequence than the pseudorandom order. The amount of improvement in reaction time by this implicit sequence is a measure of motor skill learning.

When the subjects were completing the task in the fMRI scanner, the baseline condition, which was interleaved between trials, consisted of a fixation cross in which the subjects were instructed to look at when it appeared. The baseline was the same task for both days of training in the scanner. For analysis Day 1 was compared to Day 1 baseline and Day 2 was compared to Day 2 baseline.

Procedure

On the first day participants were screened for neurological or psychiatric disorders and then completed a neuropsychological battery including the Wechsler Abbreviated Scale of Intelligence (WASI) vocabulary and block design subtests (Table 1). Afterward the subjects came into the scanner and completed the fMRI portion of the study in the scanner, which lasted approximately 60 minutes. During the scan, behavioral data and fMRI data were collected simultaneously. Within one week, participants returned and first attended a behavioral testing session that lasted approximately two hours where the subjects were trained for an additional 3120 trials (half sequenced, half random) trials of the SRT occurring in two sets with an intervening break of 30 minutes where another task (the weather prediction task) was performed. Then after completing 192 trials of the SRT (half sequenced, half random) while in the scanner on the second day, subjects' declarative knowledge of cue-outcome associations were tested by asking them to guess the 14-item sequenced pattern.

Imaging procedure:

Scanning was performed on a 3-Tesla Siemens Allegra head-only MRI machine in the Ahmanson-Lovelace Brain Mapping Center and Ronald Reagan Hospital at UCLA. For the functional runs, T2*-weighted echoplanar images (EPI) were collected (34 slices; slice thickness = 4 mm; TR = 2000 ms; TE = 30 ms; voxel size = 3.1 x 3.1 x 4.00 mm; flip angle = 90°; matrix size = 64 x 64; field of view = 200, distance factor = 0%). Structural images were collected as well: a T2-weighted matched-bandwidth high-resolution scan (34 slices; slice thickness = 4 mm; TR = 5000 ms; TE = 33 ms; voxel size = 1.6 x 1.6 x 4.0 mm; flip angle = 90°; matrix size = 128 x 128; field of view = 200; distance factor = 0%) and a magnetization-prepared rapid acquisition gradient echo image (MPRage; 160 sagittal slices; slice thickness, 1 mm; TR = 2300 ms; TE = 2.1 ms; voxel size = 1.3 x 1.3 x 1.0 mm; flip angle = 8°; matrix, 192 x 192; field of view = 256; distance factor = 50%).

Participants viewed stimuli presented on an Apple G4 Powerbook through MRI compatible goggles and responded with an MRI compatible response box connected directly to the computer. Head movement was minimized with foam padding in a standard radiofrequency single channel head coil. Two volumes were acquired at the beginning of each functional scan to allow equilibration to steady-state and were subsequently excluded from the analysis.

Behavioral data analysis

All data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) 16 (SPSS, Chicago, IL). The variables of interest were reaction time and accuracy. The data were analyzed using a repeated-measures Analysis of Variance (ANOVA) with the Huyhn-Feldt correction for non-sphericity. The control vs. siblings of COS probands group was the between-group subject factor.

Performance on the declarative knowledge test was assessed by the same process stated in the previous behavioral study of SRT learning in siblings of COS patients (Wagshal et al., unpublished).

Imaging data preprocessing and analysis

Data preprocessing was conducted using FSL version 3.3 (www.fmrib.ox.ac.uk/fsl). Images were motion-corrected using FMRIB's motion correction linear image registration tool (McFLIRT) using a normalized correlation ratio cost function and linear interpolation (Jenkinson et al., 2002). Brains were extracted using the brain extraction tool (BET; Smith, 2002).

Data analysis was conducted in FSL version 4.1 using the fMRI Expert Analysis Tool (FEAT version 5.98, first at an individual subject-level (using fixed effects model) and then using a mixed-effects model at the group analysis level contrasts. For the individual subject-level analysis, data was spatially smoothed using a 8 mm full-width-half-maximum Gaussian kernel, intensity normalized, and filtered with a nonlinear high-pass filter (Gaussian-weighted least-squares straight line fitting, with sigma = 100.0 sec). A three-step registration process aligned individual participant data into standard Montreal Neurological Institute (MNI) space. EPI images were registered to the matched-bandwidth image (7 degrees of freedom [DOF]), then to the MPRage image (7 DOF), and finally to MNI space using FMRIB's Linear Image Registration Tool (FLIRT; Jenkinson et al., 2002) using an affine transformation with 12 DOF.

The data were prethresholded using an anatomical mask at the individual subject-level analysis in FEAT. The regions included in the mask came from an a priori hypothesis showing that the hippocampus, striatum, globus pallidus, supplementary motor area (SMA), inferior frontal gyrus, middle frontal gyrus, superior frontal gyrus, supramarginal gyrus, precuneus parietal cortex, and anterior cingulate gyrus are differentially affected in schizophrenia patients

and relatives (Woodward et al., 2007; Purdon et al., 2011; Zedkova et al., 2006; Reiss et al., 2006; Kumari et al., 2002). The contrast of interest was task versus baseline for early learning on day 1 and also separately for late learning on day 2. Day 1 and day 2 data were combined within subjects using a fixed-effects model. Higher-level analyses using a mixed effects model compared differences between contrast of interest between days within and between groups. Regressors of interest were created using a delta function with trial onset times convolved with a canonical (double gamma) hemodynamic response function, along with the temporal derivative. For all contrasts of interest, Z-statistic images were created using a cluster-level threshold of $z > 2.3$ and a corrected significance threshold of $p < 0.05$ using cluster-based Gaussian random field theory. For all time-series statistical analyses, FMRIB's improved linear model (FILM) with local autocorrelation correction (Woolrich et al., 2001) was used. Regions of interests (ROIs) were identified from regions in the whole brain group-level analysis that passed threshold. We wanted to find areas that were significantly different in activation between siblings and controls during day 1 and day 2 when the subjects completed the task (Figure 9). The ROIs were then defined anatomically and used to extract the percent signal change during for 2 contrasts, sequence – random and sequence-baseline after early learning on the first day and after the extended learning on the second day. To calculate the average percent signal change within ROIs of interest, we used FSL Featquery (FEAT version 5.98). ROIs included the left hippocampus, left and right caudate, left and right putamen, left and right globus pallidus, and the left and right thalamus. These values were then analyzed in a repeated measures ANOVA using SPSS. For the first and second day separately we used a 9 (ROI region) x 2 (Group; controls and siblings) Repeated Measures ANOVA. For directly comparing Day 1 and Day 2, we used a 9 (ROI

region) x 2 (Group) x 2 (Day) repeated measures ANOVA. For each individual ROI, we used a 2 (Day) x 2 (Group) repeated measures ANOVA.

4.2.3 Results

Behavioral Results

We examined performance on the WASI subtests and found significant differences between the groups for both subtests with the controls performing better, Vocabulary $t(24) = 3.555$, $p < 0.001$ and Blocks $t(23) = 2.614$, $p = 0.031$. For these analyses, 1 sibling was not tested on the Blocks subtest due to time constraints (Table 1).

Early Learning

We first examined learning by analyzing the median reaction time benefit from sequence trials for early learning, which were the first 192 trials (half sequenced, half random) on the first day. The benefit is the difference between the median reaction time on trials with the pseudorandom order and the sequenced trials. Figure 2 presents the reaction time benefit of the two groups during this early learning phase. During the 192 trials of training on Day 1, there was a strong trend for a main effect of block, $F(7, 168) = 2.274$, $p = 0.058$, for benefit to increase across trials.

Figure 3 presents the proportion accuracy benefit of the two groups during the early learning phase. There were no main effects or an interaction between Group and Block for accuracy during the early training on the first day.

Performance after extended training

We next analyzed the benefit of reaction time in the scanner during the second day of training for the last 192 trials of training after the subjects had already completed 3120 trials (half sequenced, half random) of previous training earlier that day behaviorally outside of the

scanner. Figure 4 presents the reaction time for the two groups during the extended training. During the 50 trials of training on Day 2, there was a trend for a benefit across blocks, $F(7, 168) = 1.899$, $p = 0.083$. There was no main effect of group or an interaction between group and block.

Figure 5 presents the proportion accuracy of the benefit of the two groups after the extended training in the scanner on the second day. There was a significant main effect of group, $F(1, 24) = 6.157$, $p = 0.020$, and a significant interaction between group and block, $F(7, 168) = 2.362$, $p = 0.027$, with the siblings having more accuracy during learning on the benefit trials.

On the declarative knowledge assessment, a between subjects t-test revealed that there was not a significant difference between the controls and relatives of their knowledge of the sequenced pattern. In addition, when comparing chance performance to determine if both groups possessed explicit knowledge of the sequence, the results demonstrated that the controls had a trend to have explicit knowledge of the pattern, $t(17) = 1.569$, $p = 0.0675$, while the siblings did not acquire explicit knowledge of the sequenced pattern (Figure 6). For these analyses, 1 sibling was not tested on the knowledge questionnaire due to time constraints.

fMRI Results

We explored neural activation during performance of the task for a sequence-random contrast in a whole brain group-level voxel-wise analysis during early and late learning. There were no regions that passed threshold due to low power caused by a lack of subjects for the sibling group. However, an anatomical ROI analysis was performed for the regions that encompassed the prethresholded mask for exploratory analysis. For early and late learning there were no regions that were significant during the ROI analysis for sequence specific learning.

Due to a lack of significance in the sequence specific learning, we also explored the pattern of brain activation during performance of the task for a sequence-baseline contrast in a whole brain group-level voxel-wise analysis during early and late learning. Regions in the basal ganglia, hippocampus, thalamus, SMA, supramarginal gyrus, precuneus parietal cortex, superior frontal gyrus, and middle frontal gyrus passed threshold. However, ROI analyses were only conducted in regions that were different among controls and siblings, which were regions in the basal ganglia (caudate, putamen, and globus pallidus), hippocampus, and thalamus.

During early learning (the first 192 trials), there was a significant main effect of ROI, $F(8, 192) = 6.847$, $p < 0.001$, with the siblings displaying greater activation overall. There was no main effect of group or an interaction between ROI and group for early learning. For later learning (the last 192 trials after 3120 behavioral trials, trials 3121-3112), there was a significant main effect of ROI, $F(8, 192) = 4.719$, $p = 0.001$, with the controls displaying greater activation overall. There was no main effect of group or an interaction between ROI and group for learning after the extended training. For early versus late learning, there was a significant main effect of ROI, $F(8, 192) = 6.767$, $p < 0.001$ and a significant interaction between ROI and day, $F(8, 192) = 4.177$, $p = 0.002$. No other main effects or interactions were significant. Within subjects t-test for early verses late learning for the both groups did not reveal a significant ROI region that was driving the interaction.

Individual repeated-measures ANOVAs were also conducted for each ROI. There were no significant main effects or an interaction between group and day for the left hippocampus, right caudate, left and right putamen, left and right globus pallidus, and left and right thalamus. For the left caudate, there was a significant main effect of day with an overall increase in activation on day 1 compared to day 2, $F(1, 24) = 4.611$, $p = 0.042$. There was also a trend for an

interaction between group and day, $F(1, 24) = 2.688$, $p = 0.114$, with the siblings really driving the interaction with an increase in activation on the first day and a decrease in activation towards baseline on the second day while the controls maintain their increased level of activation across days (Figure 9).

4.2.4 Discussion

Results of this study revealed that behaviorally both the siblings and the controls performed comparably on the SRT and demonstrated a benefit of sequence specific learning in which their reaction time on the sequence trials were faster than on the random trials. However, the fMRI results in the whole brain group level analysis indicated no differences in the pattern of brain activation for sequence specific learning for siblings compared to controls or vice versa. Results from the sequence-baseline contrast indicated that overall the adolescent siblings displayed a different pattern of neural activation during learning of the task. The controls continued to maintain a similar increase in activation across learning while the siblings displayed more of an increase in learning compared to controls on the first day and then displayed a decrease in their level of activation below the controls on the second day. This pattern for the controls and siblings was significant in the left caudate and were also visually similar in the other ROI regions examined (Figure 9).

There have only been a few neuroimaging studies that have examined SRT learning in schizophrenia patients and only 1 study thus far that has examined relatives of schizophrenia patients (Zedkova et al., 2006; Reiss et al., 2006; Purdon et al., 2011; Woodward et al., 2007). The behavioral results from these studies mirrored ours in that there were no behavioral differences between the groups when completing the SRT. However, the pattern of brain activation for the patients and for the relatives differed from the controls.

The Zedkova et al. (2006) and Reiss et al. (2006) studies examined schizophrenia patients taking atypical antipsychotic medication while completing the SRT in the scanner and demonstrated that while both groups successfully completed the task, the patients failed to activate the striatum, specifically the caudate, while performing the task. Zedkova et al. (2006) also saw underactivation of frontal regions and an excess of activation in the superior temporal cortex, anterior cingulate cortex, and globus pallidus, while Reiss et al. (2006) also saw activation in the anterior cingulate cortex and dorsolateral prefrontal cortex that correlated to improved performance on the task. These results suggest that schizophrenia patients might use other regions to complete the task in order to compensate for the underactivation of the striatum.

In addition, Woodward and colleagues (2007) tested healthy adult siblings and controls that demonstrated normal performance on the task. However, compared to controls the adult siblings demonstrated less activation the basal ganglia as well. Our results further support these previous studies by revealing significantly less activation in the left caudate for the siblings compared to controls. However, due to a lack of power caused by a small sample size in the siblings, we were not able to demonstrate more brain regions that were significantly different from controls. Nevertheless, the pattern of activation during early and late learning looks very promising in these regions (Figure 9) and the addition of more siblings could potentially add more power to find a significant difference in these regions between the groups.

This is the first study to examine adolescent siblings of COS patients in a motor skill learning task employing fMRI methods. Future studies need to replicate this finding of an underactivation of the caudate of the siblings versus controls, as well as to investigate the pattern of brain activation of adult relatives to determine if there is also an underactivation of the basal ganglia that Woodward et al. (2007) found in order to verify that relatives of schizophrenia

patients display similar patterns of brain activation comparable to schizophrenia patients. Future studies with an increase in the number adolescent siblings and adult relatives tested might also reveal other regions, such as in the frontal and parietal cortex, that exhibit abnormal activity compared to controls that previous studies have also demonstrated (Zedkova et al., 2006; Reiss et al., 2006; Purdon et al., 2011; Woodward et al., 2007). Other regions that differ in their pattern of activation might be compensatory mechanisms in which the patients and relatives use in order to successfully complete the task while underutilizing the basal ganglia. It would also be interesting to determine if there are developmental differences in relatives of schizophrenic individuals by comparing the activation of brain regions that adolescent and adult relatives implement to ascertain if these groups utilize different regions to facilitate successful learning of the SRT. Understanding the neural mechanisms of implicit learning will help target future treatments to develop successful strategies to help overcome or prevent impairments due to the disease.

4.2.5 Figures

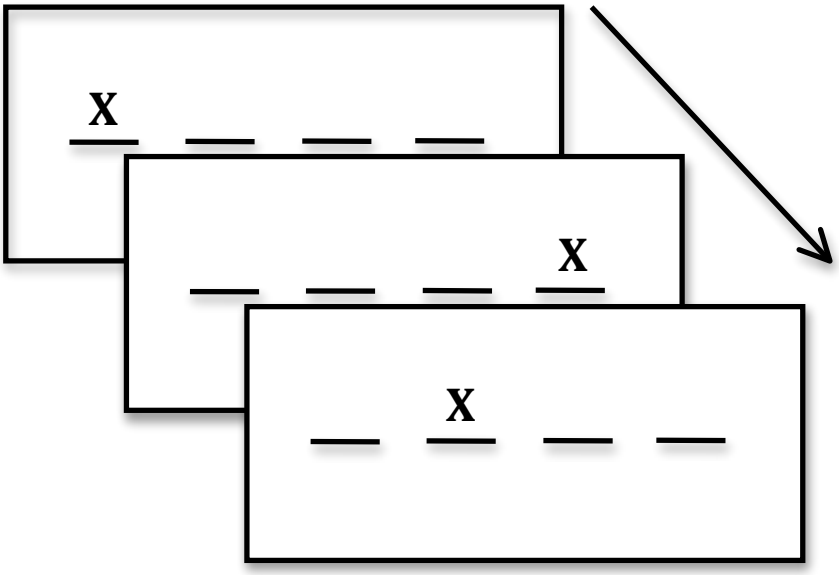

Figure 4.2.1. Serial Reaction Time Task. The SRT involves a visual target appearing in one of 4 screen locations. Participants have to respond as quickly and accurately as possible to the target by pressing one of four spatially compatible buttons. On some sets of trials there is a sequential order of the target (however, the participants do not know this) while on others there is a pseudorandom order of the target.

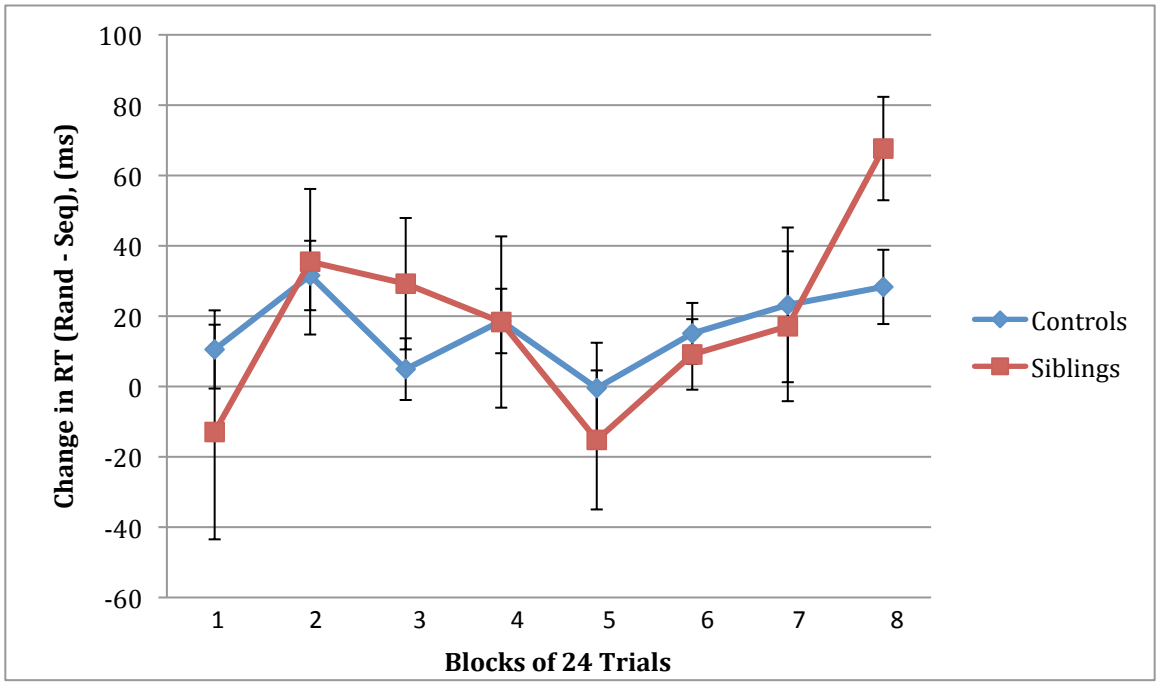

Figure 4.2.2. SRT Reaction Time- Early Learning. Error bars represent the standard error of the mean.

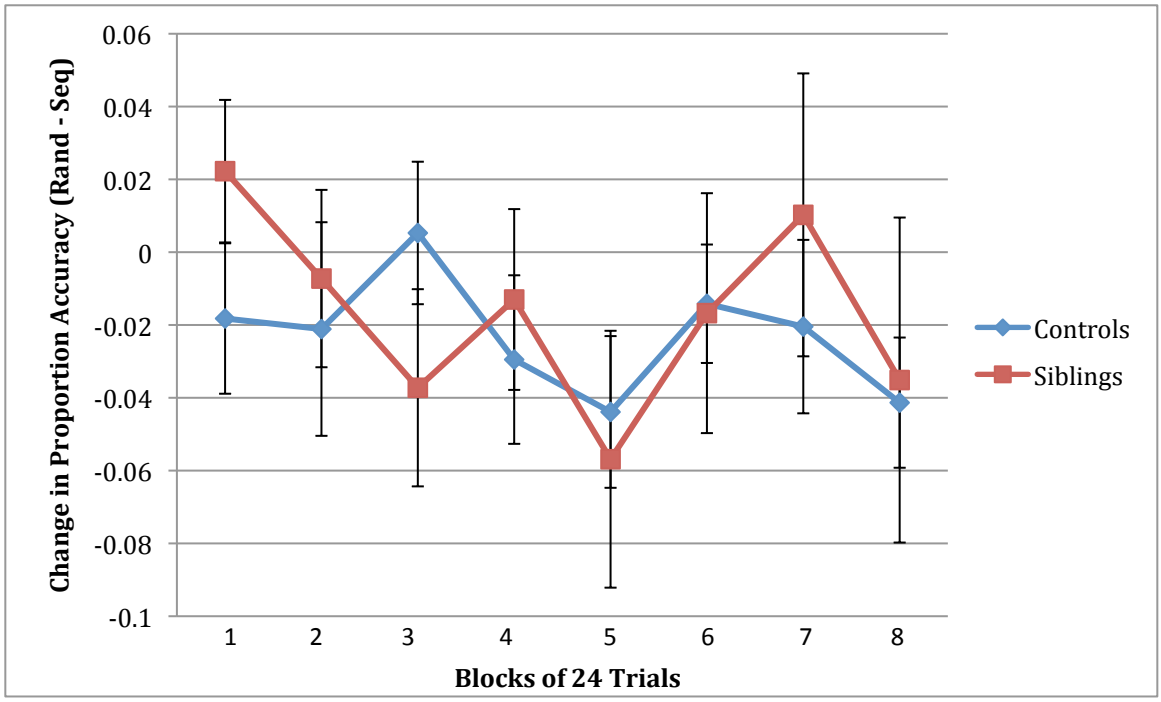

Figure 4.2.3. SRT Accuracy- Early Training. Error bars represent the standard error of the mean.

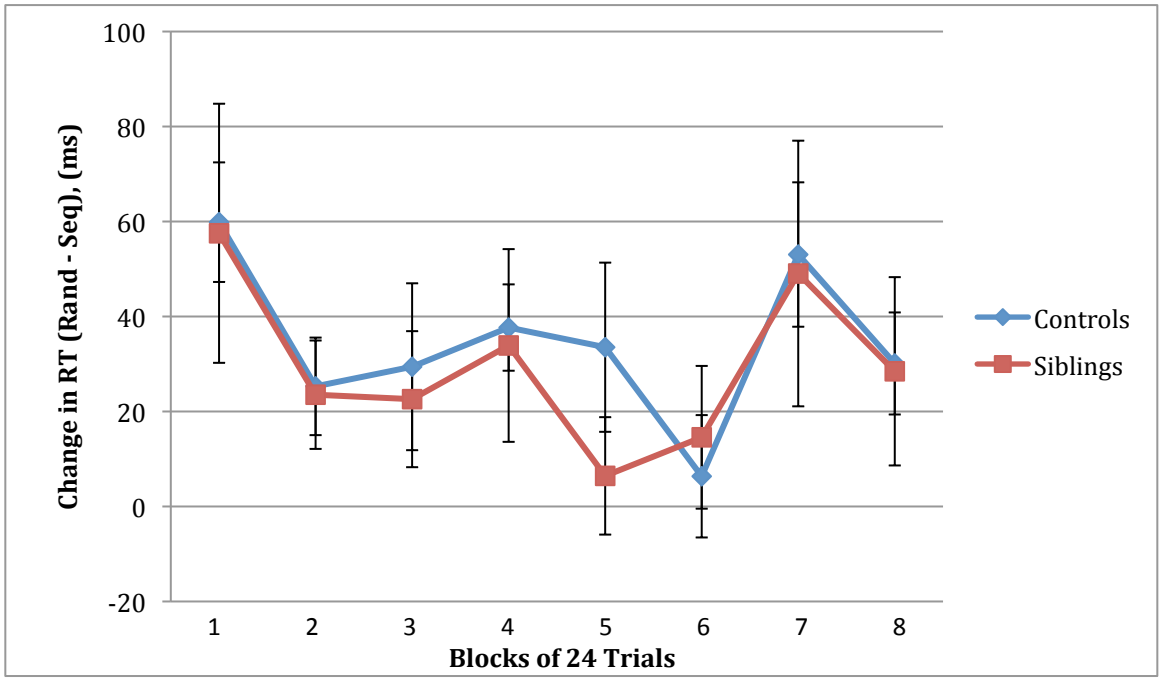

Figure 4.2.4. SRT Reaction Time Extended Training. Error bars represent the standard error of the mean.

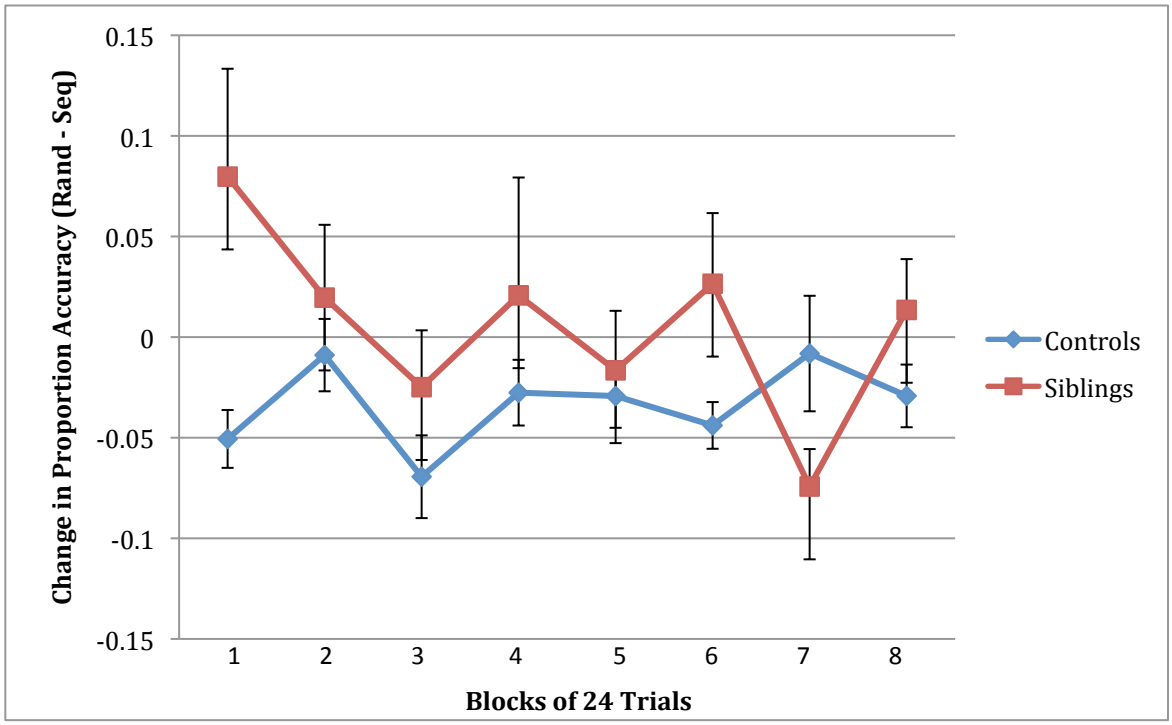

Figure 4.2.5. SRT Accuracy Extended Training. Error bars represent the standard error of the mean.

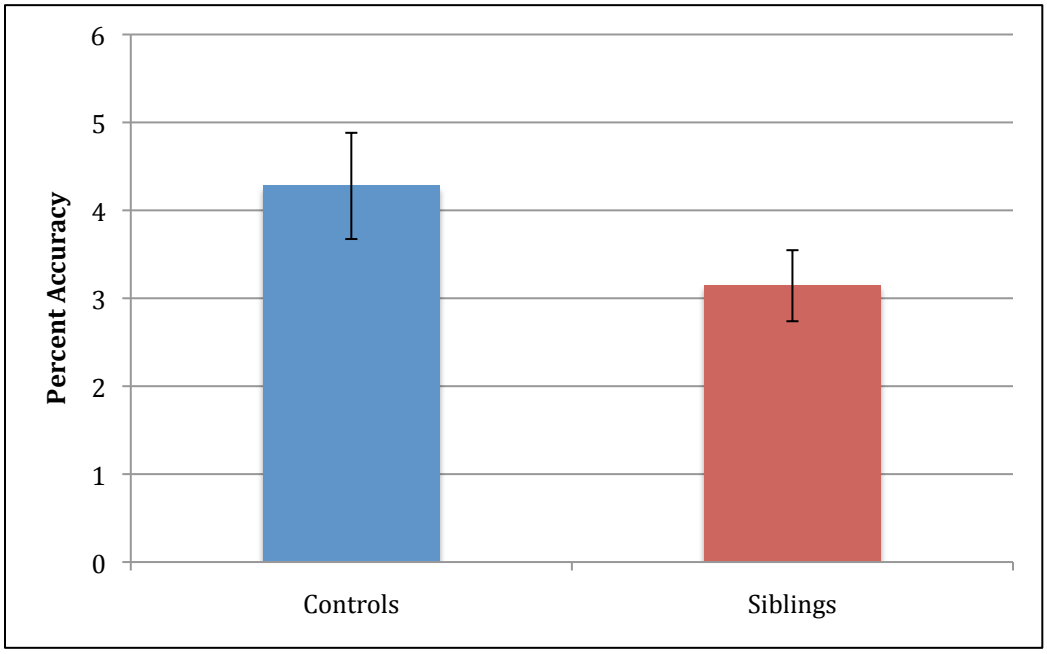

Figure 4.2.6. SRT Declarative Knowledge Questionnaire. Error bars represent the standard error of the mean.

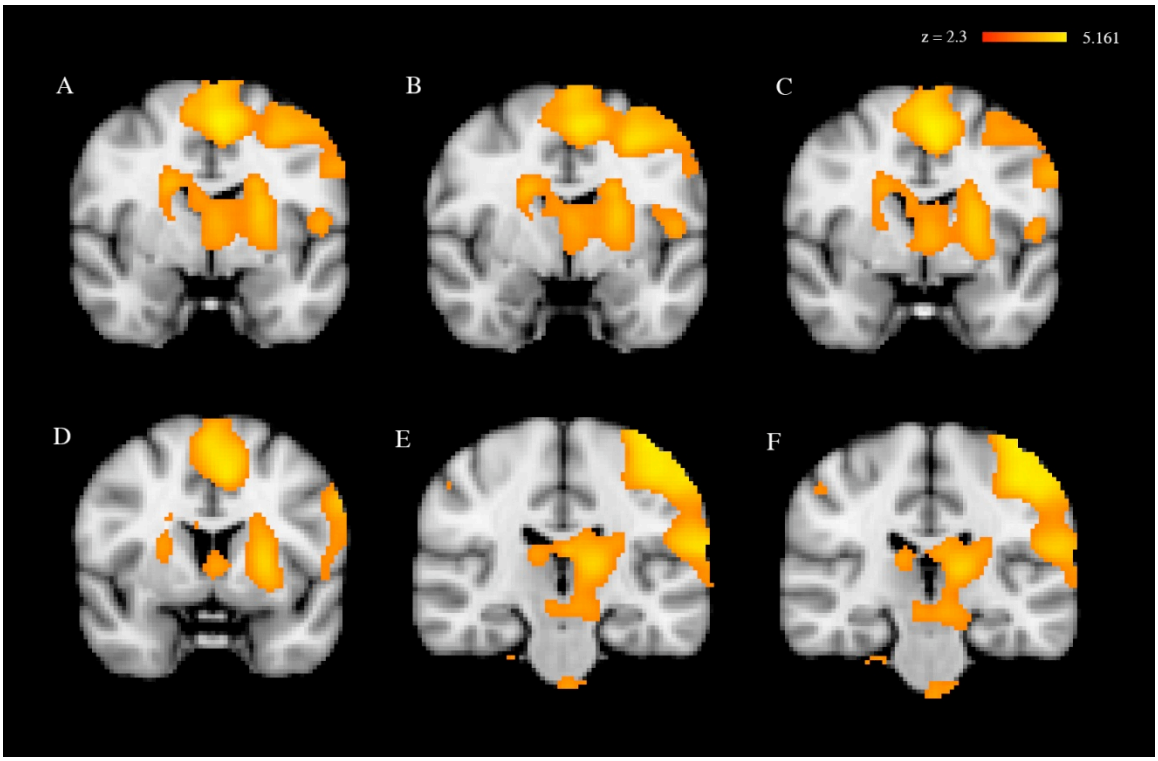

Figure 4.2.7. fMRI Images during Early Training on the SRT. Early training (first 192 trials) in the serial reaction time task. Images are from the group-level analysis ($z > 2.3$ cluster-corrected thresholded at $p = 0.05$). A = left caudate, B = right caudate, C = left putamen, D = right putamen and left globus pallidus, E = left thalamus, and F = right thalamus for controls sequence > baseline.

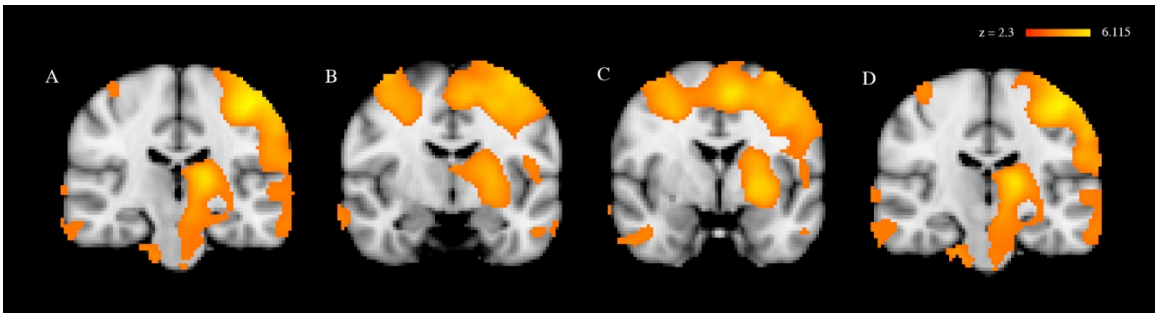

Figure 4.2.8. fMRI Images during Extended Training on the SRT. Extended training (after 3312 trials) in the serial reaction time task. Images are from the group-level analysis ($z > 2.3$ cluster-corrected thresholded at $p = 0.05$). A = left hippocampus, B = left caudate, C = left putamen and left globus pallidus, and D = left thalamus for controls sequence > baseline.

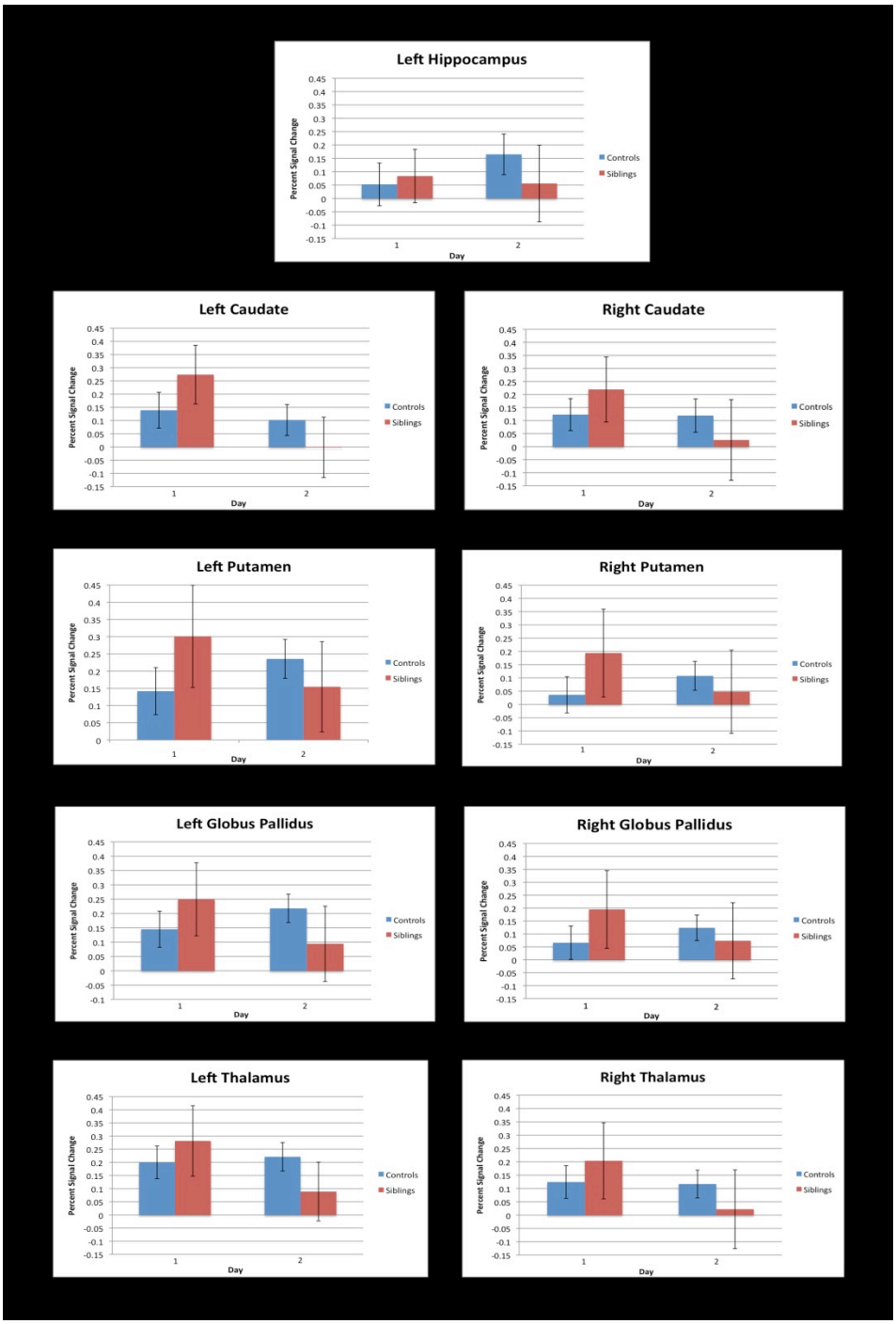

Figure 9. ROI graphs of the SRT. Percent signal change during the task for sequence versus baseline within regions that were examined in the ROI analysis. The left caudate was the only region to demonstrate a significant difference between siblings and controls on day 1 and 2. Error bars represent the standard error of the mean.

4.2.6 Tables

Variable	Controls (n = 18) M	SD	Siblings (n = 8) M	SD
Age	13.56	2.31	12.88	1.73
Gender*	10/8		4/4	
Education	7.50	2.53	6.88	2.17
Vocabulary	59.22	11.67	42.62	9.12
Blocks**	56.50	7.88	45.86	12.02

Table 4.1.1. Demographic Table.

*Men/Women, ** WASI Blocks subtest (missing 1 sibling)

Region	Coordinate (mm) X	Y	Z	Max Z-Value
Day 1 for controls for sequence > baseline				
Left Caudate	54	62	48	2.982
Right Caudate	36	61	49	3.442
Left Putamen	57	63	41	3.881
Right Putamen	33	65	42	2.781
Left Globus Pallidus	54	65	37	2.752
Left Thalamus	52	52	41	4.133
Right Thalamus	38	51	43	2.933
Day 2 for controls for sequence > baseline				
Left Hippocampus	56	52	28	2.492
Left Caudate	52	59	45	2.655
Left Putamen	57	62	35	4.932
Left Globus Pallidus	56	62	35	4.897
Left Thalamus	53	53	41	5.364

Table 4.2.2. Voxel-wise Region Coordinates. Brain regions that showed significant activation at the group-level analysis for sequence-baseline. Z-statistical maps were whole-brain cluster corrected at $Z > 2.3$, $p = 0.05$. There were no significant regions for Day 1 controls > siblings, siblings > controls, or siblings > baseline and Day 2 controls > siblings, siblings > controls, or siblings > baseline.

4.2.7 References

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CHAPTER 5: Adult Relatives in a Motor Skill Learning Task

5.1 Motor Skill Learning in Adult Relatives with Genetic Vulnerability for Schizophrenia

5.1.1 Introduction

Schizophrenia is a devastating disorder characterized by varied, heterogeneous symptoms. While there has been a consensus in the literature demonstrating a deficit in the cognitive domain in areas such as working memory, attention, executive control, language, and declarative memory (Asarnow, 1999; Gold & Harvey, 1993; Kuperberg & Heckers, 2000; Riley et al., 2000), there has been more contention in skill learning. Skill learning is the gradual improvement of performance with practice on a task over the course of many trials and is demonstrated by reduced reaction time or increased accuracy in a task. Skill learning is dependent on the striatum (Knowlton et al., 1996a; Grafton, Hazeltine, & Ivry, 1995; Poldrack, Prabhakaran, Seger, & Gabrieli, 1999; Aron et al., 2004; Moody et al., 2004), unlike other types of memory that are hippocampal-dependent. Moreover, there are also structural abnormalities in the basal ganglia in schizophrenia patients as well (Buchsbaum, 1990; Buchanan, 1993).

There is a longstanding hypothesis that there is a relation between the pathophysiology of schizophrenia and the dysfunction of corticostriatal circuits (Kleist, 1960). Corticostriatal circuitry is characterized by several distinct anatomically and functionally separate loops (Alexander, DeLong, & Strick, 1986). Different types of skill learning have been shown to be impaired in schizophrenics, suggesting that different corticostriatal loops are impaired in schizophrenia. A study by Foerde et al. (2008) demonstrated this phenomenon. They hypothesized that if different corticostriatal loops were impaired in schizophrenia, then patients would demonstrate varied performance and dysfunction across different tasks that depended on

different loops. They examined the different corticostriatal loops by comparing motor and cognitive skill learning in schizophrenia patients. Motor skill learning has been shown to be supported more by the supplementary motor area (SMA), putamen, globus pallidus, and thalamus (Grafton, Hazelton, & Ivry, 1995; Poldrack et al., 2005; Grafton et al., 1992), while cognitive skill learning more by the caudate nucleus, dorsolateral prefrontal cortex, ventral striatum, and orbitofrontal cortex (Poldrack, Prabhakaran, Seger, & Gabrieli, 1999; Poldrack et al., 2001; Aron et al., 2004). In comparing both types of skill learning, Foerde and colleagues could directly determine if both corticostriatal circuits were compromised schizophrenia patients or if just one of them were compromised when compared to healthy control participants. Their results demonstrated impaired cognitive skill learning but unimpaired motor skill learning in patients, suggesting that the corticostriatal loops involved in motor skill learning were relatively intact but that the loops involved in cognitive skill learning were impaired.

While the results of Foerde et al. (2008) have been in agreement with other studies that have examined cognitive skill learning deficits in schizophrenia patients, there have been discrepancies concerning motor skill learning in schizophrenia patients. One major reason for these discrepancies could be due to medication effects of typical antipsychotics that alter striatal D_2 functioning. Patients taking atypical antipsychotics have demonstrated normal learning on a motor skill task, the serial reaction time (SRT) task (Nissen & Bullemer, 1987) compared to controls (Perry et al., 2000; Stevens et al., 2002), while patients taking typical antipsychotics have demonstrated impairment on the SRT compared to controls (Schwartz et al., 2003; Stevens et al., 2002). Studies have implicated altered D_2 functioning caused by typical antipsychotics, suggesting that typical antipsychotics may cause motor skill learning deficits that are evident in

schizophrenia patients rather than a dysfunction of the motor corticostriatal pathway (Paquet et al., 2004; Kumari et al., 2002).

One method of getting around the confound of medication effects is to study healthy relatives of schizophrenia patients. Schizophrenia is a highly genetic disorder and non-psychotic relatives of schizophrenia patients exhibit a subset of the deficits found in schizophrenia patients, suggesting that there are cognitive endophenotypes of this disorder and that these deficits appear to be associated with genetic liability for schizophrenia. The cognitive deficits of these individuals with genetic risk to schizophrenia are readily apparent in the domains of memory, executive functioning, attention, inhibitory control, and cognitive skill learning (Asarnow, Steffy, MacCrimmon, & Cleghorn, 1977; Sitskoorn et al., 2004; Szöke et al., 2005; Snitz, Macdonald, & Carter, 2006; Vink et al., 2006; Wagshal et al. under review). Non-psychotic relatives also exhibit functional striatal abnormalities that are similar to those seen in patients with schizophrenia (an underactivation of striatal regions compared to controls), suggesting that striatal abnormalities may be associated with genetic risk (Vink et al., 2006; Raemaekers, Ramsey, Vink, et al., 2006).

The present study investigated the performance of adult relatives of schizophrenia (ROS) patients compared to healthy controls on the serial reaction time task to determine if healthy, adult relatives of schizophrenia patients, who demonstrate no psychotic symptoms, would show intact motor skill learning based on the Foerde et al. (2008) study. In addition, prior studies have also demonstrated a difficulty in automating new skills and impaired dual task performance in schizophrenia patients compared to controls (Granholm, Asarnow, & Marder, 1996; Braff, 1985; Holzman, 1987; Nuechterlein, 1991; but see Foerde et al., 2008). Automatization of a skill is the ability to execute a task without the demand for executive control and effortful behavior. A

major characteristic of automaticity is when a response to a skill becomes habit-like and there are no detrimental effects to the skill with the addition of secondary tasks intrusions. Therefore, to determine whether the SRT learning was automated, we inserted dual-task tone-counting probe trials to test whether performance was automatic at different points during training. Based on studies that have compared schizophrenic patients and their first-degree relatives to healthy controls, we also hypothesize that the ROS group would demonstrate a deficit in automaticity indicated by a decrement in dual-task performance after extended training.

5.1.2 Methods

Participants

Eleven adult ROS patients and twenty-three adolescent controls who were matched in age and gender to the siblings participated in the experiment (Table 1). Three controls and one relative were excluded from analysis based on computer malfunction or not responding on more than 10% of the trials or not being at least 75% accurate on the task. The relatives and controls were recruited and excluded through the process stated in Chapter 1. The task design, procedure, and analysis were the same as in the previous behavioral study of SRT learning in siblings of COS patients (Wagshal et al., unpublished).

5.1.3 Results

We examined performance on the WASI subtests and found significant differences between the groups with the controls performing better on both tests, Vocabulary , $t(23) = 2.460$, $p = 0.011$, and Blocks, $t(28) = 2.753$, $p = 0.005$. For these analyses, data were missing for 5 relatives on the vocabulary subtest due to time constraints (Table 1).

Early Learning

We first examined learning by analyzing the median reaction time benefit from sequence trials for early learning, which were the first 192 trials on the first day, in a 8 (block) x 2 (Group: controls and relatives) repeated measures ANOVA. The benefit is the difference between the median reaction time on trials with the pseudorandom order and the sequenced trials. Figure 2 presents the reaction time benefit of the two groups during this early learning phase. During the 192 trials of training on Day 1 there was no main effect of group or block or a significant interaction between block and group. A between subjects t-test analysis was performed for the last 3 blocks during early training. There was a significant difference between the groups for the 6^{th} and 8^{th} block, $t(28) = -2.066$, $p = 0.048$ and $t(28) = -2.364$, $p = 0.025$, with an increase in benefit for the relatives.

Figure 3 presents the proportion accuracy benefit of the two groups during the early learning phase. There were no main effects or an interaction between group and block for accuracy during the early training on the first day.

Performance after extended training

We next analyzed the benefit of reaction time during the second day of training. Figure 4 presents the reaction time for the two groups during the extended training. We did not include the dual task trials in these analyses. We analyzed Day 2 overall performance in a 22 (block) x 2 (Group: controls and relatives) repeated measures ANOVA and Day 2 performance broken into two sessions (the 1320 single-task trials before and after a 30 minute break) in a 11 (block) x 2 (Group: controls and relatives) repeated measures ANOVA. Overall, there was no main effect of group or block or a significant interaction between block and group during the extended training. When broken down into 2 separate sessions, for session 1 there was no main effect of group or block or a significant interaction between block and group. For session 2 there was a trend for an

interaction between group and block with the controls gaining more of a benefit early on during the second session but then later during the second session towards the end of training both groups are comparable in their benefit, $F(10, 280) = 1.612$, $p = 0.108$. A post-hoc between subjects t-test revealed that there were no blocks that were significantly driving the trend for the interaction but block 7 for the relatives, $t(28) = 1.565$, $p = 0.129$, was driving the trend for the interaction, as well as a trend for block 13 for the controls, $t(28) = 1.600$, $p = 0.121$. There were no significant main effects of group or block during the second session. In addition, within groups t-test revealed that there was no difference in reaction time for the relatives between sessions 1 and 2, while for the controls there was a trend for a difference between session with an increase in benefit for the second session compared to the first, $t(19) = -1.828$, $p = 0.083$.

Figure 5 presents the benefit for the proportion accuracy for the two groups during the extended training. Proportion accuracy was also analyzed overall for Day 2 as well as separately for session 1 and 2 on the second day. Overall, there was no main effect of group or block or an interaction between group and block. Similarly, when broken down into 2 separate sessions, for both sessions there was no main effect of group or block or an interaction between group and block. A within groups t-test demonstrated that there was no difference in accuracy between sessions for both groups.

The effect of the secondary task was assessed by computing the average of the trial block immediately before and after the two dual task blocks and the average of the two dual task blocks (Figure 6). A positive score for the benefit is good, reflecting a faster reaction time for the sequenced trials. A 2 (session) x 2 (group) repeated measures ANOVA revealed no main effects or an interaction between session and group. In addition, between subjects t-test revealed no differences between the groups for the single and dual task trials. Within subjects t-test across

sessions for the controls revealed a significant difference between the single task trials across session with the controls gaining more of a benefit during the second session, $t(19) = -2.557$ $p = 0.0095$. There was also a trend for a difference between the dual task trials across session with the controls gaining more of a benefit during the second session, $t(19) = -1.523$, $p = 0.072$, and a trend for a difference between the single task trials and dual task trials during the second session for a greater benefit during the single task trials, $t(19) = 1.342$, $p = 0.0975$. For the relatives there was a significant difference for the single task and dual task trials for the first session with a benefit for the single task trials, $t(9) = 2.004$, $p = 0.038$. There was a trend between the single and dual task trials during the second session with a benefit for the single task trials, $t(9) = 1.391$, $p = 0.099$.

For accuracy we also calculate the dual task effect by computing the average of the single task trial immediately before and after the two dual surrounding single task trials (Figure 7). A 2 (session) x 2 (group) repeated measures ANOVA revealed a significant main effect of session, $F(1, 28) = 5.156$, $p = 0.0155$, with an increase in benefit of the dual task during the first session and then a negative effect of the dual task during the second session. Between subjects t-test revealed a significant difference between the groups for the dual task blocks during the second session with relatives incurring a cost for accuracy, $t(28) = -2.017$, $p = 0.0265$. There was also a trend for a difference between the groups for the single task trials during the second session with the controls having a benefit for accuracy, $t(28) = -1.418$, $p = 0.0885$. Within subjects t-test demonstrated a trend for the controls for a difference between the single and dual task trials during the second session with the controls having more of a benefit during the single task trials, $t(19) = -1.349$, $p = 0.0965$. For the relatives there was a strong trend for a difference for the dual task across session for the relatives decreasing in benefit during the second session, $t(9) = -1.736$,

p = 0.0585, and trend for a difference between the single and dual task trials during the second session for a benefit in the single task trials, t(9) = -1.661, p = 0.0655. To determine if the groups were above chance on the dual task during the probes, a one-sample within subjects t-test was computed for each group. For the second session for the single task trials, the controls were significantly above chance, t(19) = -2.981, p = 0.004. There was a trend for the dual task during the second session for the relatives to be significantly different from chance with a negative cost of the dual task, t(9) = 1.644, p = 0.0675. The controls were not significantly above chance during single task trials during the first session and during the dual task trials, *t's*(19) < -1.151, p > 0.132, and the relatives were not significantly above chance during the single task trials and the first session of the dual task, *t's*(9) < -1.225, p > 0.126. Though both groups were not significantly above chance during these trials, they were not affected by the dual task because they had a negative score or a score close to zero for the benefit, which signifies learning due to their more accurate performance during the sequenced trials compared to the random trials.

On the declarative knowledge assessment, a between subjects t-test revealed no differences between the controls and relatives. When comparing chance performance and if they possessed explicit knowledge of the sequence, the results demonstrated that both groups were not above chance and did not possess explicit knowledge of the sequenced pattern, suggesting learning was implicit. For these analyses data was missing for 3 relatives and 3 controls due to time constraints.

5.1.4 Discussion

In the present study, there was no difference in performance between the adult ROS patients and controls for either early or extended training. Dual task probes were also inserted during the extended training to determine whether the groups were relying on controlled or

automatic processing. For reaction time there were no group differences on the dual task trials and there was no cost associated with the dual task. Moreover, there was also a trend for the controls to gain more of a benefit during the dual task trials for the second session compared to the first. Though for the relatives when comparing the single and dual task trials, the single task trials possessed more of a benefit compared to the dual task trials in both sessions.

For accuracy, there was a significant difference between the groups with the dual task trials during the second session, which was really driven by a negative benefit of the dual task trials for the relatives, suggesting a dual task cost during these trials. This was further supported by a trend level difference from chance for the relatives to incur a negative dual task effect. However, there was no effect of the dual task associated with the other single or dual task trials for the relatives or for the controls, though there was more of a benefit for the single task trials compared to the dual task trials in the second session for both groups.

Consistent with other studies of first degree ROS patients (Hill et al., 2008), in the present study adolescent siblings of patients with COS performed significantly more poorly than controls on the WASI, a measure of intellectual functioning. The poor performance of ROS patients on intelligence tests has been interpreted as indicating that poor intellectual function may reflect liability to schizophrenia (Hill et al, 2008). Though the adolescent siblings performed poorly on the WASI, they were unimpaired in the task, which paralleled results found in another patient group, patients with Korsakoff's amnesia, who also exhibit impairments on tests of intellectual function but perform as well as age-matched control subjects on the SRT (Nissen & Bullemer 1987; Nissen et al., 1989; Van Tilborg et al., 2011).

The results from this study reveal no impairment on the SRT for ROS patients, supporting similar findings from schizophrenia patients who were administered atypical

antipsychotics (Foerde et al., 2008; Perry et al., 2000; Stevens et al., 2002). In addition, our hypothesis of a dual task effect was not supported and demonstrated a similar result found by Foerde and colleagues (2008) in which there was no significant effect of the secondary task on performance. Our results were also consistent with a previous study examining adolescent siblings of individuals with childhood onset schizophrenia who also demonstrated no impairment on the SRT or a dual task effect with the addition of the secondary task (Wagshal et al., unpublished). These results are in contrast to another type of skill learning, in which patients and relatives display a deficit in cognitive skill learning tasks (Foerde et al., 2008; Gimenez et al., 2003; Schroder, Tittel, Stockert, & Karr, 1996; Purdon, Woodword, Lindborg, & Stip, 2003, Weickert et al., 2010; Wagshal et al., under review; Wagshal et al., in preparation).

Future studies comparing the pattern of brain activation of ROS individuals need to be carried out to further elucidate the integrity of the corticostriatal loops. Several functional magnetic resonance imaging (fMRI) studies have revealed abnormal patterns of activation in a number of brain regions in non-psychotic first-degree relatives of patients with schizophrenia. In many of these studies, abnormal activity was detected in the prefrontal and parietal regions while participants were engaged in executive control or working memory tasks. Even when the relatives demonstrated normal task performance, blood oxygen level dependent (BOLD) signal differences suggested an inefficiency in the prefrontal-parietal network compared to controls (Pearlson & Calhoun, 2009). Non-psychotic relatives also exhibit functional striatal abnormalities that are similar to those seen in patients with schizophrenia (an underactivation of striatal regions compared to controls), suggesting that striatal abnormalities may be associated with genetic risk (Vink et al., 2006; Raemaekers, Ramsey, Vink, et al., 2006).

Though there was no difference in the behavioral performance on the SRT with the adult relatives and controls there could be a difference in the pattern of brain activation associated with the task. Studies with schizophrenia patients and controls support this theory with patients failing to activate the striatum, specifically the caudate, while performing the task (Zedkova et al., 2006; Reiss et al., 2006; Purdon et al., 2011). There has been only 1 study thus far to examine the behavioral and neural mechanisms of ROS patients and controls of the SRT using fMRI. Both groups demonstrated normal performance on the task. However, compared to controls the relatives demonstrated less activation in frontal and parietal regions as well as the basal ganglia. Thus, they suggested that altered neural activation during the performance of cognitive tasks may be related to the genetic vulnerability for schizophrenia (Woodward et al., 2007).

In a previous study, we investigated SRT performance with fMRI in adolescent siblings of childhood onset schizophrenia patients and controls. Both groups demonstrated comparable behavioral performance but altered neural activation while completing the task in the scanner. Overall, the siblings revealed increased activity early in learning and a decrease in activity during later learning, while the controls maintained their increased level of activation during learning. In addition, there was an underactivation in the left caudate for siblings compared to controls; this pattern was evident in other basal ganglia structures and the thalamus as well, adding further support to Woodward et al. (2007). While we demonstrated an underactivation in the caudate for adolescent siblings, it would be worthwhile to replicate this finding in adult relatives as well in order to gain a better understanding of how schizophrenia patients and relatives successfully complete motor skill learning tasks.

5.1.5 Figures

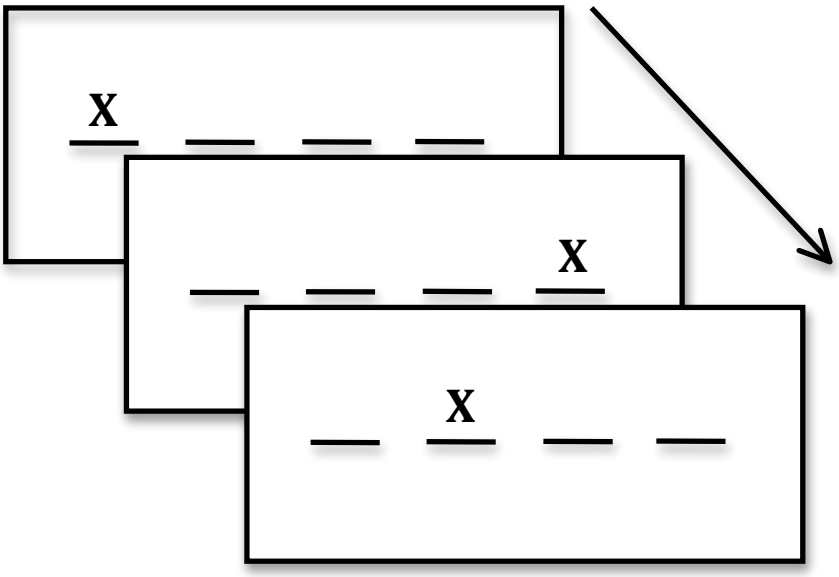

Figure 5.1.1. Serial Reaction Time Task. The SRT involves a visual target appearing in one of 4 screen locations. Participants have to respond as quickly and accurately as possible to the target by pressing one of four spatially compatible buttons. On some sets of trials there is a sequential order of the target (however, the participants do not know this) while on others there is a pseudorandom order of the target.

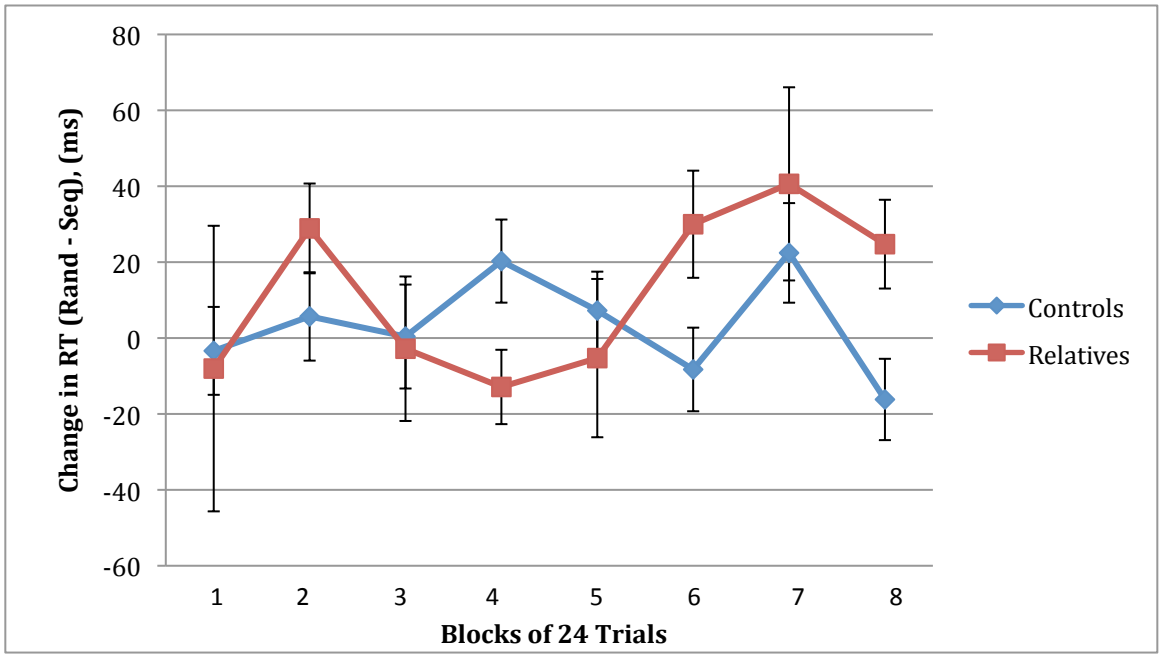

Figure 5.1.2. SRT Reaction Time- Early Training. SRT benefit (random – sequence trials) of reaction time in early training during Day 1. Error bars represent the standard error of the mean.

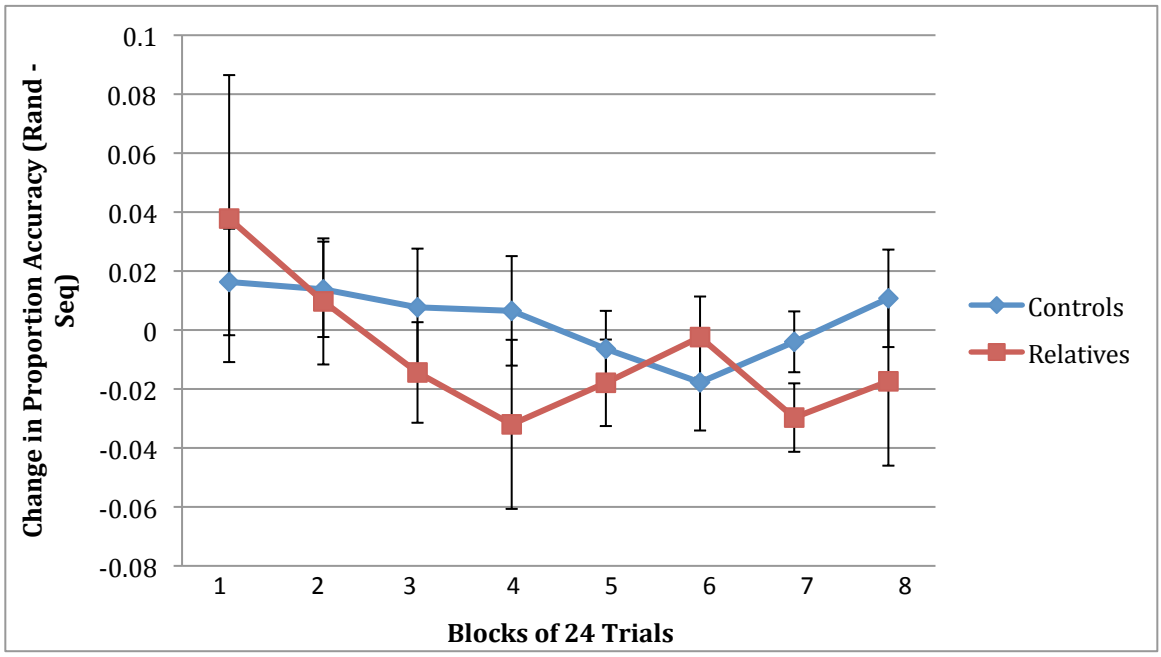

Figure 5.1.3. SRT Accuracy- Early Training. SRT benefit (random – sequence trials) of accuracy in early training during Day 1. Error bars represent the standard error of the mean.

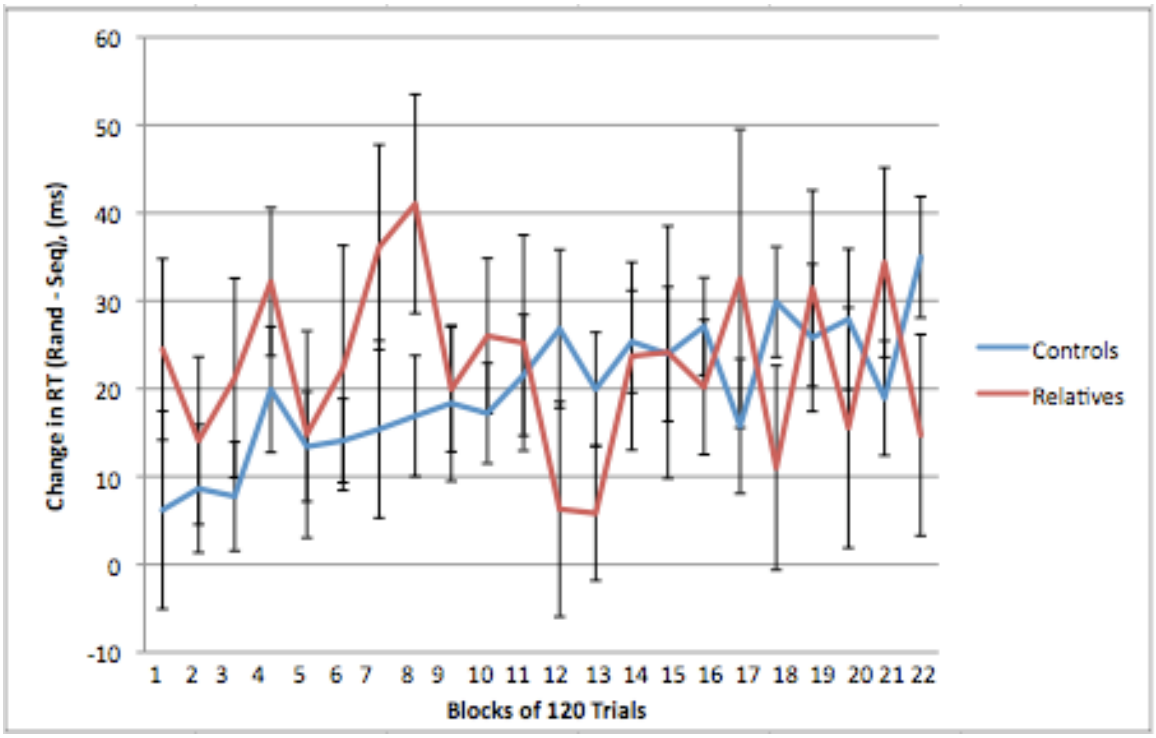

Figure 5.1.4. SRT Reaction Time Extended Training. SRT benefit (random – sequence trials) of reaction time in the extended training during Day 2. Error bars represent the standard error of the mean.

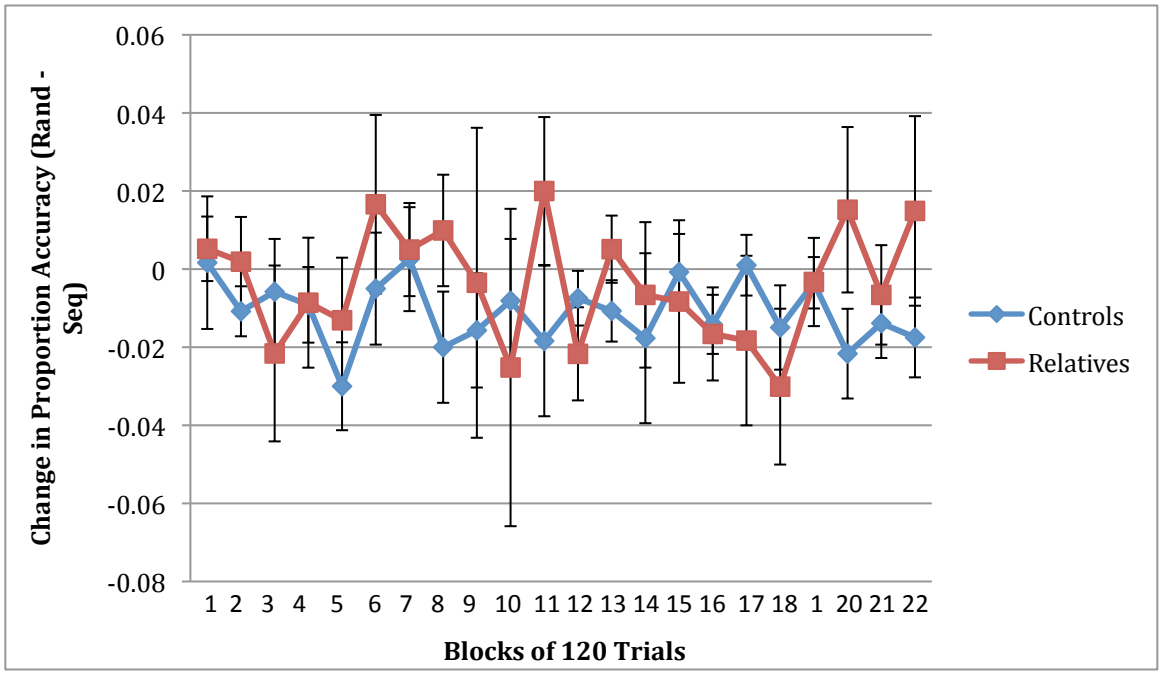

Figure 5.1.5. SRT Accuracy Extended Training. SRT benefit (random – sequence trials) of accuracy in the extended training during Day 2. Error bars represent the standard error of the mean.

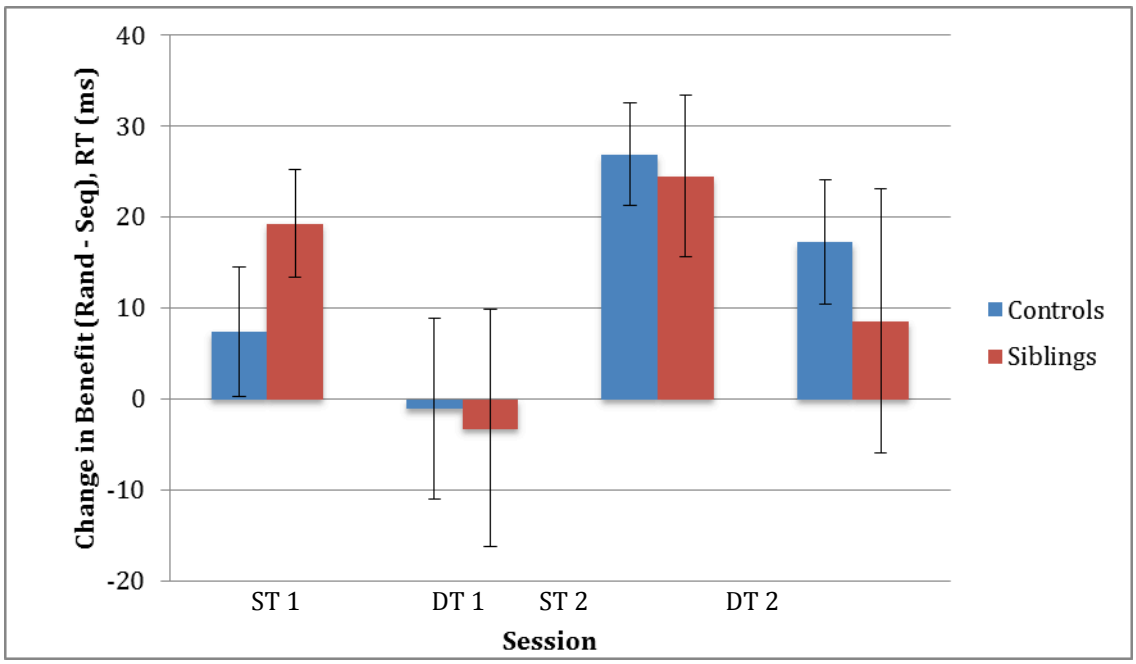

Figure 5.1.6. Dual Task of the SRT- Reaction Time. Error bars represent the standard error of the mean.

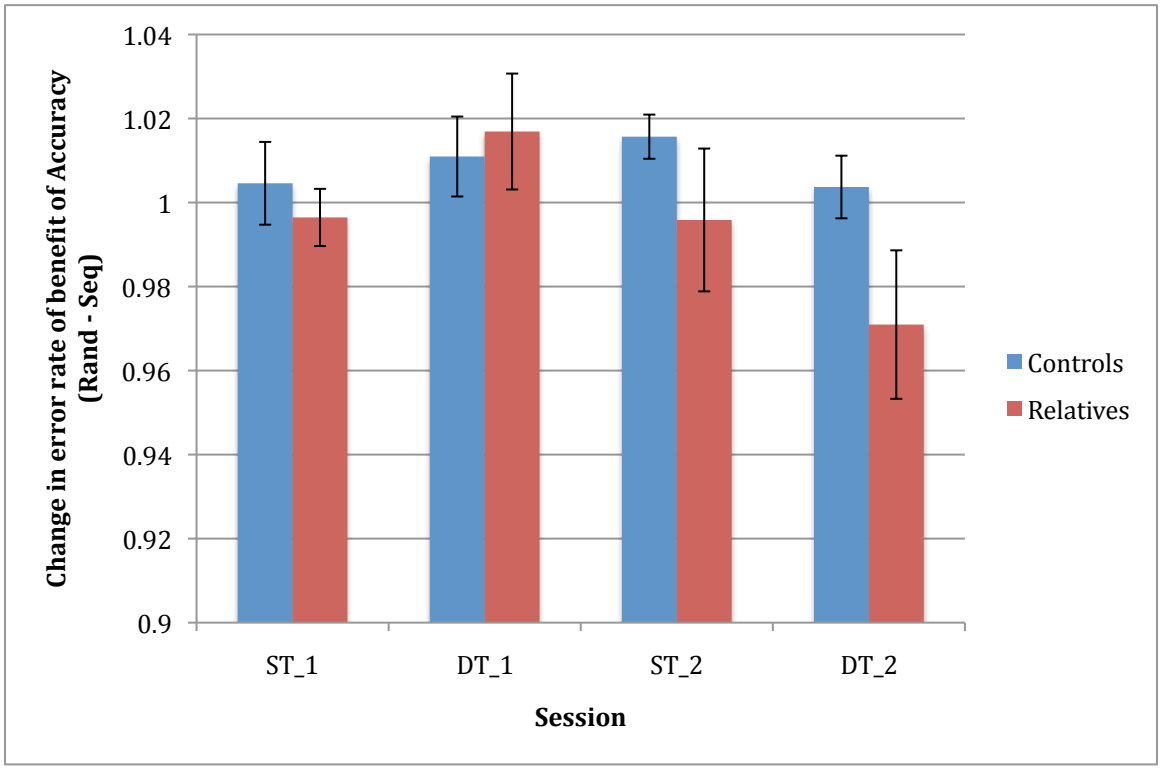

Figure 5.1.7. Dual Task of the SRT- Accuracy. SRT during the dual task during the extended training for error rate (1-proportion accuracy). Error bars represent the standard error of the mean.

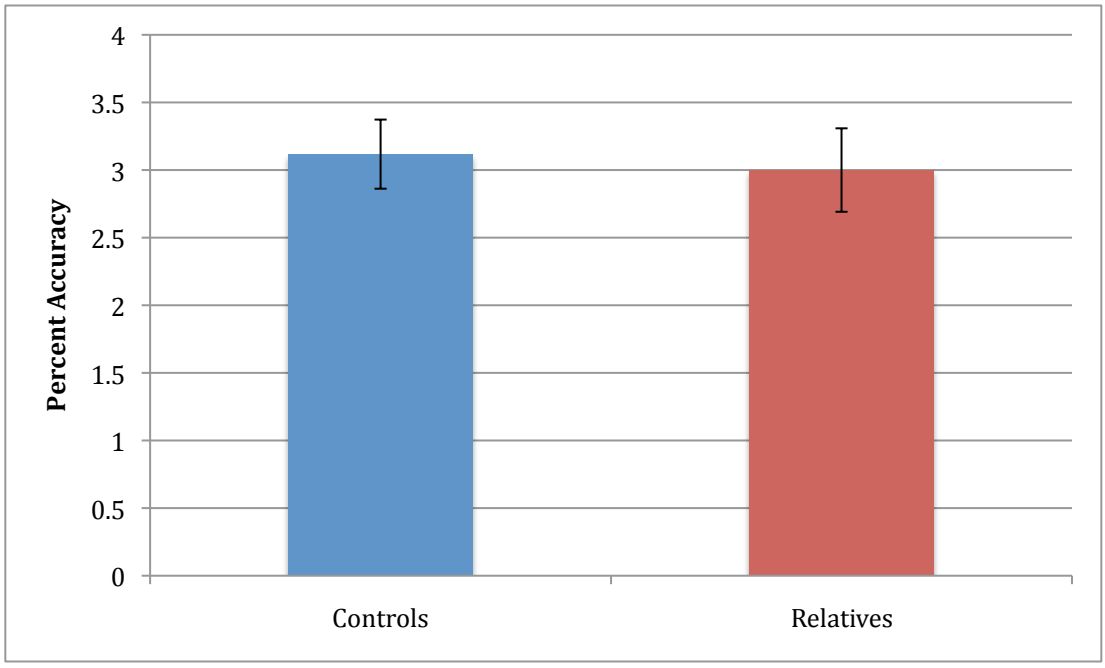

Figure 5.1.8. SRT Declarative Knowledge Questionnaire. Error bars represent the standard error of the mean.

5.1.6 Table

Variable	Controls (n = 20) M	SD	Relatives (n = 10) M	SD
Age	40.85	5.07	39.90	6.28
Gender*	4/16		3/7	
Vocab**	53.85	9.53	42.20	9.20
Blocks	54.50	11.21	43.20	9.16

Table 5.1.1. Demographic Table.

*Men/Women, ** WASI vocabulary subtest (missing 5 relatives)

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5.2 Motor Skill Learning in Adult Relatives of Schizophrenia Patients using fMRI

5.2.1 Introduction

Schizophrenia is a debilitating disorder that has widespread, negative effects in various aspects of a patient's daily life and function. Individuals with schizophrenia have numerous deficits, specifically in the cognitive domain, in areas such as working memory, attention, executive control, language, and declarative memory (Asarnow, 1999; Gold & Harvey, 1993; Kuperber & Heckers, 2000; Riley et al., 2000). Skill learning is another domain in which schizophrenia patients have been reported to possess a deficit. There has been extensive evidence of a cognitive skill learning deficit in patients in tasks such as the weather prediction task (Foerde, Poldrack, Knowlton et al., 2008; Keri, Juhasz, Rimanoczy, et al., 2005; Horan, Green, Knowlton, et al., 2008; Weickert, Terrazas, Bigelow, et al., 2002) and the Tower of Toronto and Tower of Hanoi (Gimenez, Junque, Perez, et al., 2003; Purdon, Woodward, Lindborg, et al., 2003). Conversely, there have been mixed results in the motor skill learning literature. In the past when prescribed to take medication to help alleviate symptoms of the disorder, patients were given typical antipsychotics and were reported to have an impairment in motor skill learning (Stevens et al., 2002, Reiss et al., 2006; Zedkova et al., 2006). However, with the advent of atypical antipsychotic medication, patients have been reported to acquire motor skill learning comparable to controls (Foerde et al., 2008; Perry, Light, Davis, & Braff, 2000; Stevens et al., 2002). A major reason for a lack of impairment when taking atypical antipsychotics could be that antipsychotics produce few motor side effects than typical antipsychotics, and the fact that typical antipsychotics are potent striatal D2 receptor antagonists and thus, greater impair skill learning than atypical antipsychotics (Exner et al., 2006; Kumari et al., 2002; Woodward et al., 2007; Green et al., 1997).

A subset of the deficits of schizophrenic patients is also present in non-affected relatives of schizophrenia (ROS) patients. Some of these deficits in the healthy relatives are in areas such as memory, executive function, attention, and inhibitory control (Asarnow, Steffy, MacCrimmon, & Cleghorn, 1977; Sitskoorn et al., 2004; Szöke et al., 2005; Snitz, Macdonald, & Carter, 2006; Vink et al., 2006). We have shown previously that adolescent siblings of childhood onset schizophrenia patients also possess an impairment in cognitive skill learning, while adult relatives do not (Wagshal et al., under review, Wagshal et al., in preparation). Weickert et al. (2010) also showed a similar lack of impairment for adult relatives as well. However, not much is known about ROS patients on motor skill learning, specifically on the serial reaction time (SRT) task (Figure 1).

The SRT is a four-alternative spatial choice reaction time task in which subjects are instructed to identify the location of a target as quickly and accurately as possible by pressing the response key that corresponds to the location of the target. Unknown the subject, on some sets of trials the stimuli are presented in a particular sequence while on other sets there is a pseudorandom order. The measure of motor skill learning is the improvement in reaction time on the sequenced trials compared to random trials. One study published thus far (Woodward et al., 2007) has found that adult siblings behave like schizophrenia patients in that there is no deficit in performance. Our previous behavioral studies with adolescent siblings (Wagshal et al., unpublished) and adult relatives (Wagshal et al., unpublished) also support this conclusion.

Studies with functional magnetic resonance imaging (fMRI) have revealed that while schizophrenia patients and controls perform comparably on the SRT, they do not employ the same brain regions to complete the task. Both Zedkova et al. (2006) and Reiss et al. (2006) demonstrated that individuals with schizophrenia fail to activate the striatum while completing

the SRT. However, there has been mixed results about other brain regions with respect to underactivation versus overactivation compared to controls (Zedkova et al., 2006; Reiss et al., 2006; Purdon et al., 2011).

In parallel, several fMRI studies have revealed abnormal patterns of activation in a number of brain regions in healthy ROS patients. In many of these studies, abnormal activity was detected in the prefrontal and parietal regions while participants were engaged in executive control or working memory tasks. Non-psychotic relatives also exhibit functional striatal abnormalities in the basal ganglia that are similar to those seen in patients with schizophrenia (an underactivation of striatal regions compared to controls), suggesting that striatal abnormalities may be associated with genetic risk (Vink et al., 2006; Raemaekers, Ramsey, Vink, et al., 2006). The only fMRI study with ROS patients demonstrated normal performance on the task, but compared to controls the relatives demonstrated less activation in frontal and parietal regions as well as in the basal ganglia (Woodward et al., 2007). Thus, they suggested that altered neural activation during the performance of cognitive tasks may be related to the genetic vulnerability for schizophrenia.

Our study will use the SRT to discern any impairment of the corticostrial loops that are involved when learning this task. The SRT depends on the integrity of the striatum and will be used to assess corticostriatal function. Performance on this task activates striatal regions on fMRI (Grafton, Hazeltine, & Ivry, 1995; Poldrack et al., 2005) that are part of motor corticostriatal circuits including the supplementary motor area (SMA), putamen, globus pallidus, and thalamus (Grafton et al., 1992; Grafton, Hazeltine, & Ivry, 1995; Poldrack et al., 2005). Patient studies with dorsal striatum dysfunction also support the idea that the basal ganglia are important to successfully learn the SRT (Gomez Beldarrain et al., 1998; Gomez Belderrain et al., 1999;

Gomez Belderrain et al., 2002; Knopman & Nissen, 1991; Pascual-Leone et al., 1993). Since striatial dysfunction is a hallmark of schizophrenia patients (Buchsbaum, 1990; Buchanan, 1993) and successful learning of the SRT depends on striatum, this task will be instrumental in examining if corticostriatal function is intact in ROS patients on this motor task and will help determine if the pattern of brain activation in the adult relatives is similar to controls or if it supports the preexisting schizophrenia patient and relative literature.

5.2.2 Methods

Participants

Eleven adult ROS patients (age range: 36-48) and thirteen adult controls (age range: 36-47), who were right handed and were matched in age, education, and gender to the adult relatives participated in the experiment (Table 1). These subjects were a subset of the participants in our previous behavioral study of SRT learning in adults of schizophrenia patients (Wagshal et al., unpublished). Seven controls and three relatives were excluded from analysis based on computer malfunction, not responding on more than 10% of the trials, being less than 75% accurate, excessive movement of 3mm or greater, or not completing both days of training. The task design, procedure, and analysis were the same as in the fMRI study of SRT learning in adolescent siblings of COS patients (Wagshal et al., unpublished) except we also conducted an exploratory analysis with a fixed effects model at the group level voxel-wise analysis for a sequence-random contrast with a lowered p value, threshold at $p = 0.01$ instead of $p = 0.05$, to really pick up any trend level differences between the groups in other regions that could be significant with the addition of more subjects.

5.2.3 Results

Behavioral Results

We examined performance on the WASI subtests and found significant differences between the groups for the Blocks subtest with the controls performing better, $t(12) = 4.181$, $p = 0.001$. There was no significant difference between the groups for the Vocabulary subtest. For these analyses, 5 relatives were not tested on the Vocabulary subtest due to time constraints (Table 1).

Early Learning

We first examined learning by analyzing the median reaction time benefit from sequence trials for early learning, which were the first 192 trials (half sequenced, half random) on the first day. The benefit is the difference between the median reaction time on trials with the pseudorandom order and the sequenced trials. Figure 2 presents the reaction time benefit of the two groups during this early learning phase. During the 192 trials of training on Day 1, there was no main effect of group or of block or an interaction between group and block.

Figure 3 presents the proportion accuracy benefit of the two groups during the early learning phase. There was a trend for a main effect of block, $F(7, 84) = 2.133$, $p = 0.085$, for both groups to gain more of a benefit across blocks. There was no main effect of group or an interaction between group and block for accuracy during early training on the first day.

Performance after extended training

We next analyzed the benefit of reaction time in the scanner during the second day of training for the last 192 trials of training after the subjects had already completed 3120 trials (half sequenced, half random) of previous training earlier that day behaviorally outside of the scanner. Figure 4 presents the reaction time for the two groups during the extended training. During the 192 trials of training on Day 2, there was no main effect of group or of block or an interaction between group and block. Figure 5 presents the proportion accuracy of the benefit of

the two groups after the extended training in the scanner on the second day. There was no main effect of group or of block or an interaction between group and block.

On the declarative knowledge assessment, a between subjects t-test revealed that there was not a significant difference between the controls and relatives of their knowledge of the sequenced pattern (Figure 6). In addition, when comparing chance performance to determine if both groups possessed explicit knowledge of the sequence, neither the relatives nor controls possessed explicit knowledge of the sequence. For these analyses, 1 relative and 1 control were not tested on the knowledge questionnaire due to time constraints.

fMRI Results

We explored neural activation during performance of the task for a sequence-random contrast in a whole brain group-level voxel-wise analysis during early and late learning. During early learning (the first 192 trials), there were no regions that passed threshold. During late learning (the last 192 trials on the second day after the 3120 trials of behavioral training, trials 3121-3312), the left caudate, left putamen, left globus pallidus, and left thalamus were significantly more active in relatives compared to baseline (Figure 7; Table 2). To determine how these areas change in the pattern of brain activation during early and late learning on day 1 and 2 and if there were differences between both the groups, we then conducted an independent anatomical ROI analysis on regions that were significant at the whole brain group-level analysis. For early and late learning there were no regions that were significant during the ROI analysis for sequence specific learning.

Due to a lack of subjects, we also explored the pattern of brain activation during performance of the task for a sequence-random contrast in a whole brain group-level voxel-wise analysis during early and late learning using a fixed-effects model to obtain trend level

differences between the groups in regions that could be significant with the addition of more subjects. During early learning the bilateral precuneus parietal cortex passed threshold and was significantly more active for relatives compared to controls (Figure 8). During late learning, regions in the left and right caudate, left and right putamen, left globus pallidus, and in the bilateral anterior cingulate demonstrated greater activity in the relatives compared to controls (Figure 9).

During early learning (the first 192 trials), there were no significant main effects or an interaction. For later learning (the last 192 trials after 3120 behavioral trials, trials 3121-3112), there was a significant main effect of group, $F(1, 12) = 4.903$, $p = 0.047$, with the relatives displaying more activation than the controls and a trend for a main effect of ROI, $F(6, 72) = 2.034$, $p = 0.125$. There was no interaction between ROI and group for learning after the extended training. Between subjects t-tests for ROI revealed significant difference between the groups for more activation in the relatives compared to controls in the left putamen, $t(12) = -2.243$, $p = 0.045$, right putamen, $t(12) = -2.214$, $p = 0.047$, and left globus pallidus, $t(12) = -2.204$, $p = 0.048$, and trend level differences between the groups in the left caudate, $t(12) = -2.107$, $p = 0.057$, anterior cingulate, $t(12) = -1.924$, $p = 0.078$, right caudate, $t(12) = -1.780$, $p = 0.100$, and precuneus cortex, $t(12) = -1.734$, $p = 0.109$ (relatives > controls; Figure 10). For early versus late learning, there was a significant main effect of ROI, $F(6, 72) = 2.706$, $p = 0.048$, and a trend for a main effect of group, $F(1, 12) = 4.021$, $p = 0.068$, with the relatives having more of an activation than controls. The interaction was not significant. Within subjects t-test for early verses late learning for the both groups did not reveal any differences.

Individual repeated-measures ANOVAs were also conducted for each ROI. For the left putamen, there was a significant group difference with a greater increase in activation in the

relatives compared to the controls $F(1, 12) = 5.595$, $p = 0.036$. For the left and right caudate [$F(1, 12) = 2.796$, $p = 0.120$ and $F(1, 12) = 3.422$], right putamen [$F(1, 12) = 4.334$, $p = 0.059$], left globus pallidus [$F(1, 12) = 4.160$, $p = 0.064$], and the bilateral anterior cingulate gyrus [$F(1, 12) = 2.476$, $p = 0.145$] there was a trend for a main effect of group with the relatives demonstrating an increase in activation compared to the controls across both days of learning (Figure 10).

5.2.4 Discussion

The results of this study revealed that behaviorally both the relatives and the controls performed comparably on the SRT. For the fMRI data, the voxel-wise whole brain analysis demonstrated significant differences in later learning with an increase in the left caudate, left putamen, left globus pallidus, and left thalamus for the relatives but not for controls when compared to baseline in the mixed effects model. Due to a lack of subjects, we also explored the pattern of brain activation during performance of the task for a sequence-random contrast in a whole brain group-level voxel-wise analysis during early and late learning using a fixed-effects model to obtain trend level differences between the groups in regions that could be significant with the addition of more subjects. The results from the sequence-random contrast revealed a significant difference in the left putamen with the relatives displaying an increase in activation compared to controls on both days of learning. The same pattern emerged for trend level differences in the left and right caudate, right putamen, left globus pallidus, and in the bilateral anterior cingulate gyrus

Some of the results of this study (an overactivation of the basal ganglia by the relatives compared to controls) are actually in contradiction to the few studies that have been performed in schizophrenia patients and relatives thus far. Zedkova et al. (2006) and Reiss et al. (2006) examined schizophrenia patients taking atypical antipsychotic medication while completing the

SRT in the fMRI scanner. Behaviorally, both the patients and the controls performed similarly on the task. However, the patients did not activate the striatum, specifically the caudate, while completing the task compared to controls. In addition, the Zedkova et al. (2006) study also demonstrated that the patients did not show as much activation as compared to controls in frontal regions as well. Though both studies revealed more activation in certain brain regions of the patients that were greater than the controls in areas such as the superior temporal cortex, anterior cingulate cortex, globus pallidus, and dorsolateral prefrontal cortex, suggesting that the patients are utilizing compensatory mechanisms to successfully complete the task.

There has been only 1 published study examining the performance of relatives of ROS patients versus controls while completing the SRT in the scanner. Woodward et al. (2007) tested adult siblings and demonstrated comparable performance of the siblings to the controls on the task. And like schizophrenia patients, the siblings revealed less activation in the basal ganglia as well. In a recent study, we also added further support to that study by demonstrating less activation in the left caudate for adolescent siblings of schizophrenia patients compared to controls (Wagshal et al., unpublished).

However, the results of our current study go against both of these. One reason could be due to our low sample size, though our adolescent siblings and the Woodward et al. (2007) study also did not have a lot of subject in the experiment. Another reason for our contradictory results could be due to the fact that we had a mixed representation of adult relatives (5 childhood onset schizophrenia parents, 1 adult onset schizophrenia parent, and 2 adult onset schizophrenia siblings). The other studies might have found different results because they tested a homogenous population. Our adolescent siblings were comprised of siblings of childhood onset schizophrenia patients and Woodward et al. (2006) tested adult siblings of adult onset schizophrenia patients.

Ultimately, what we did find in our adult relatives population that was similar to the other studies were compensatory regions that demonstrated an increase in activation from baseline for the relatives. Therefore, future studies with a large number of participants and a homogeneous schizophrenia relatives population need to be carried out in order to further elucidating the role of the corticostriatal circuits and genetic liability for schizophrenia.

5.2.5 Figures

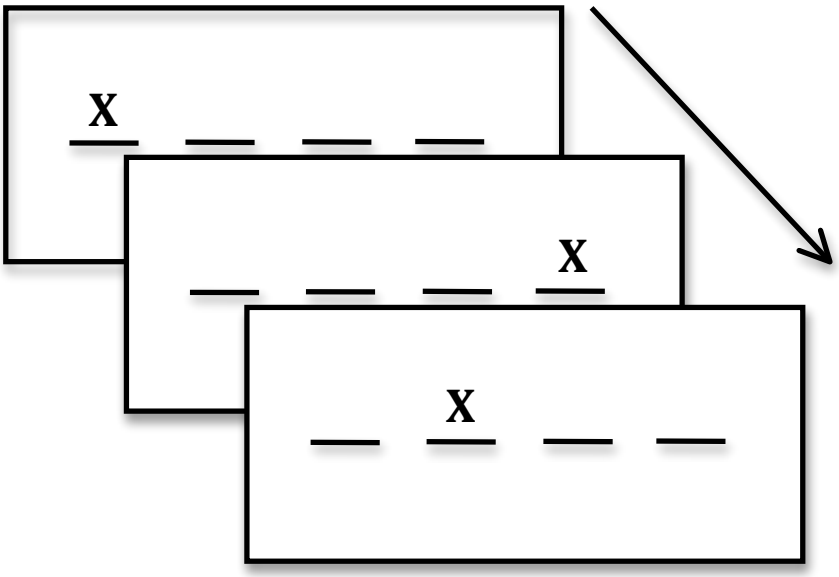

Figure 5.2.1. Serial Reaction Time Task. The SRT involves a visual target appearing in one of 4 screen locations. Participants have to respond as quickly and accurately as possible to the target by pressing one of four spatially compatible buttons. On some sets of trials there is a sequential order of the target (however, the participants do not know this) while on others there is a pseudorandom order of the target.

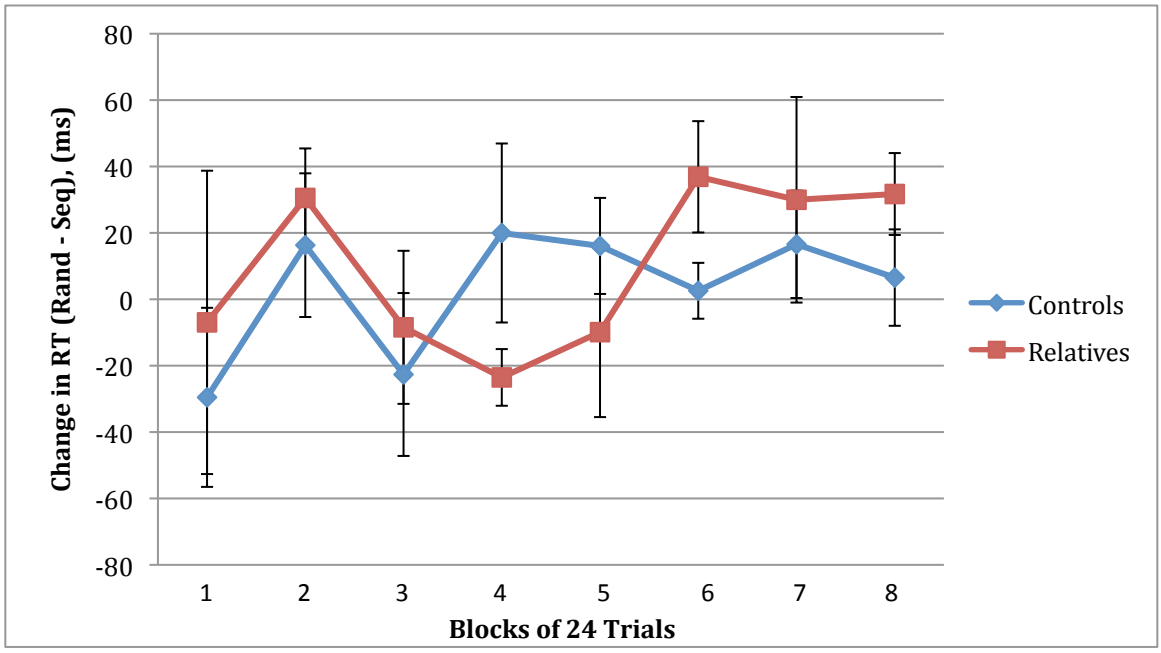

Figure 5.2.2. SRT Reaction Time- Early Training. Error bars represent the standard error of the mean.

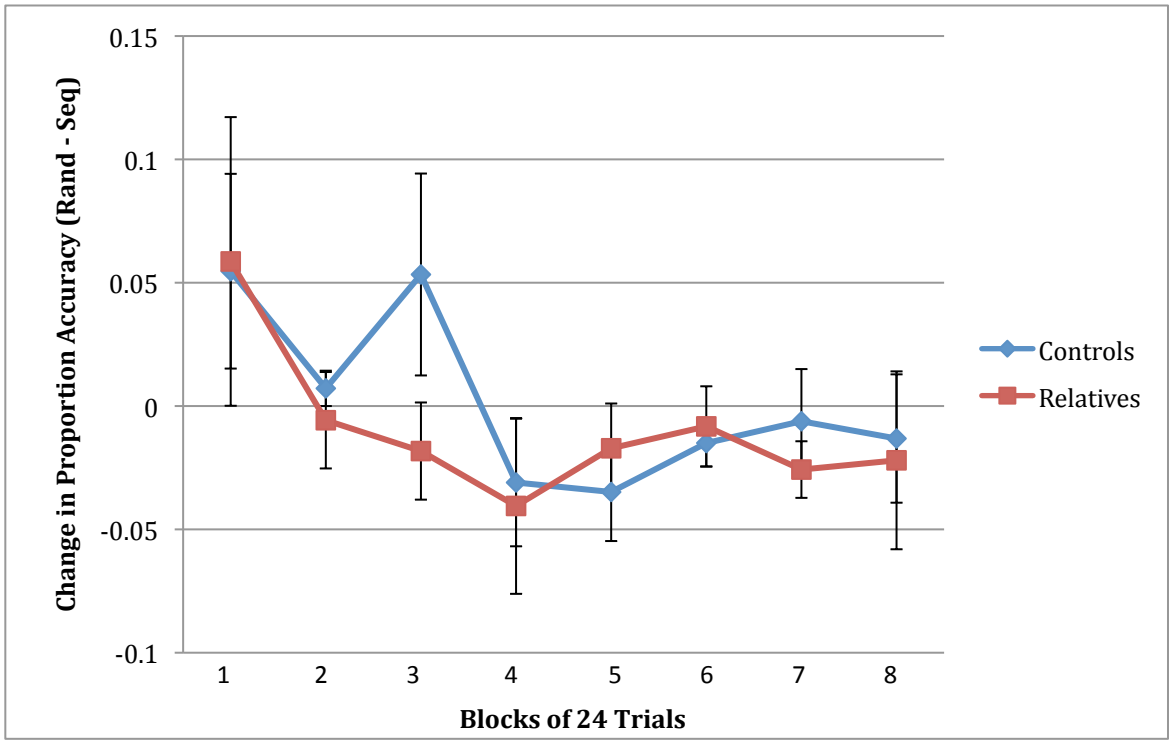

Figure 5.2.3. SRT Accuracy- Early Training. Error bars represent the standard error of the mean.

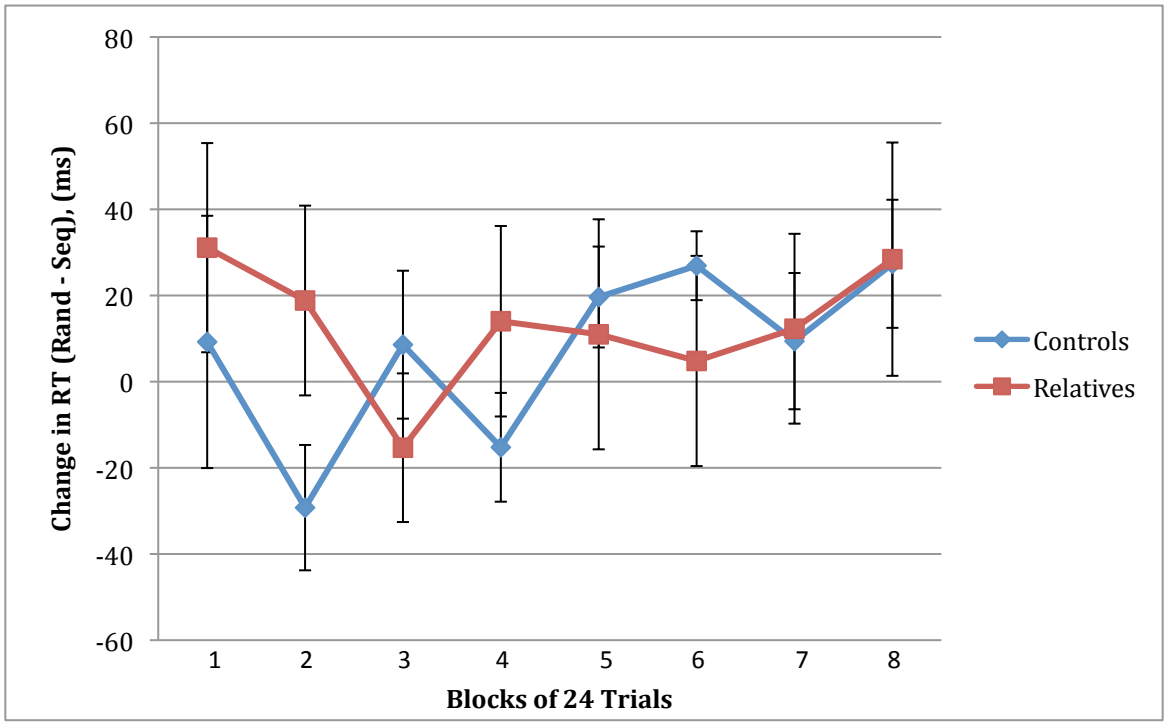

Figure 5.2.4. SRT Reaction Time Extended Training. Error bars represent the standard error of the mean.

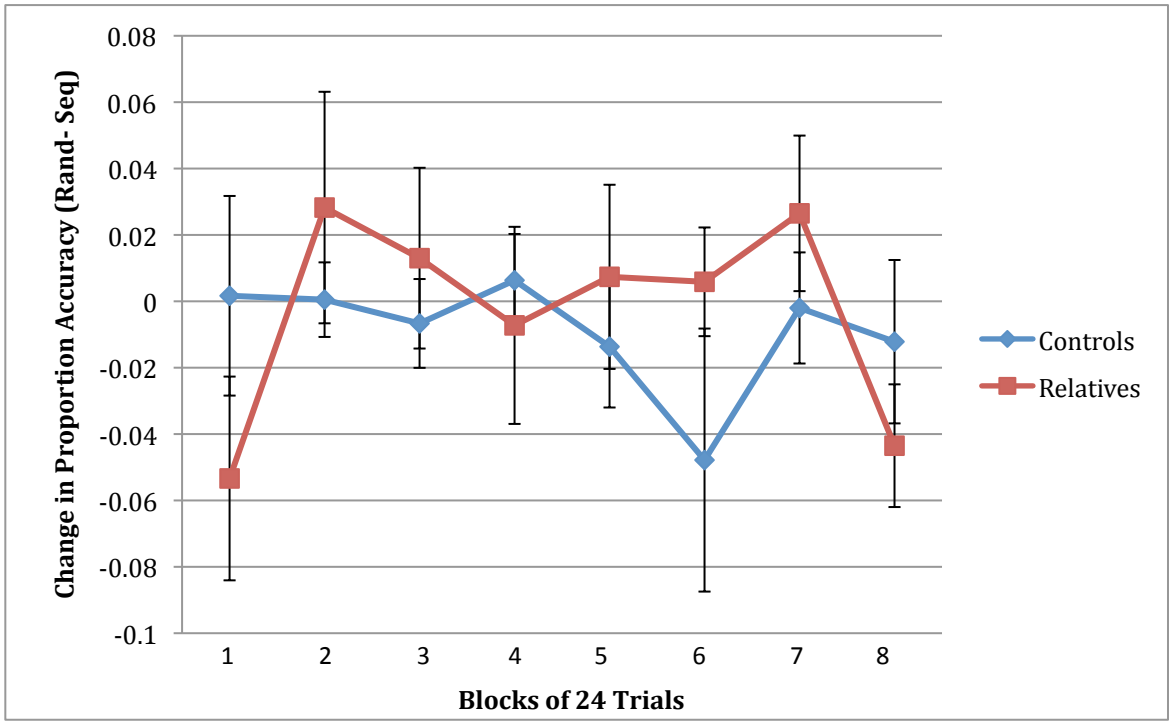

Figure 5.2.5. SRT Accuracy Extended Training. Error bars represent the standard error of the mean.

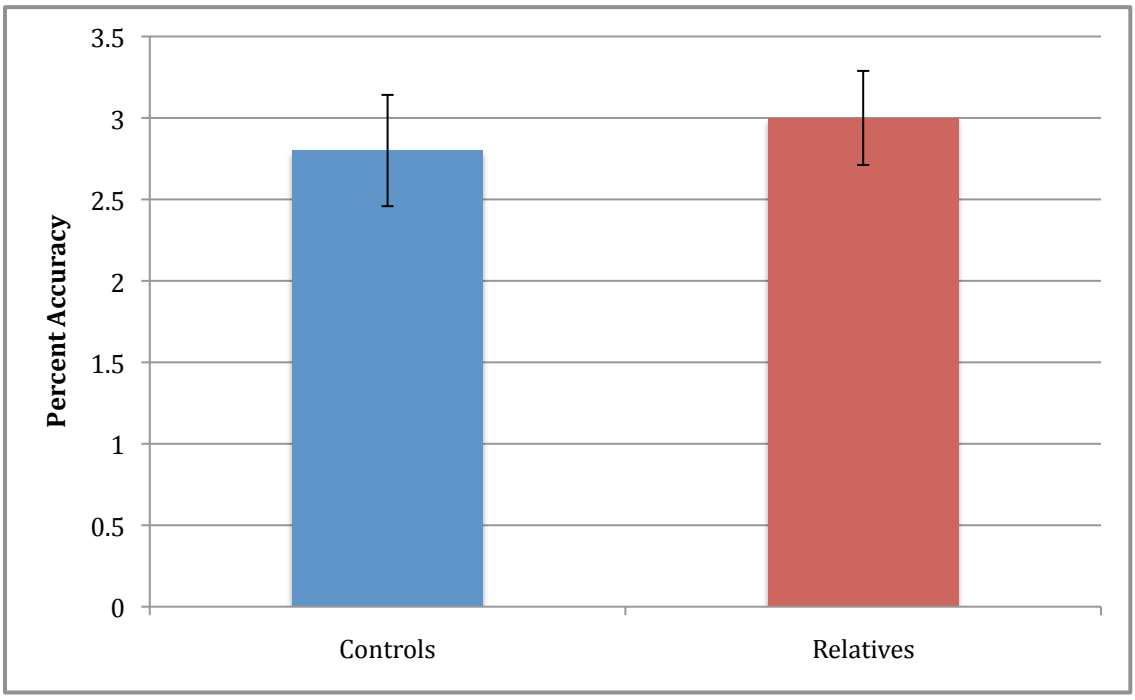

Figure 6. SRT Declarative Knowledge Questionnaire. Error bars represent the standard error of the mean.

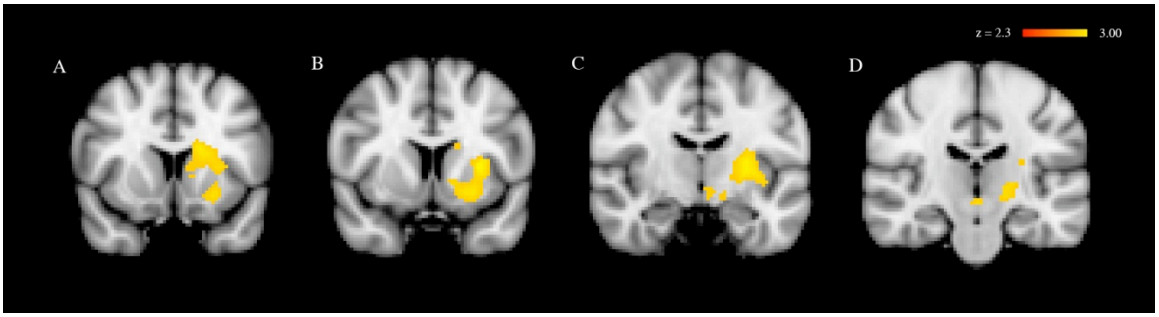

Figure 5.2.7. fMRI Images during Extended Training on the SRT (Mixed Effects). Extended Training (after 3312 additional trials) in the serial reaction time task. Images are from the voxel-wise group-level analysis ($z > 2.3$, cluster-corrected thresholded at $p = 0.05$) for sequence-random. A = left caudate, B = left putamen, C = left globus pallidus, and D = left thalamus for relatives > baseline.

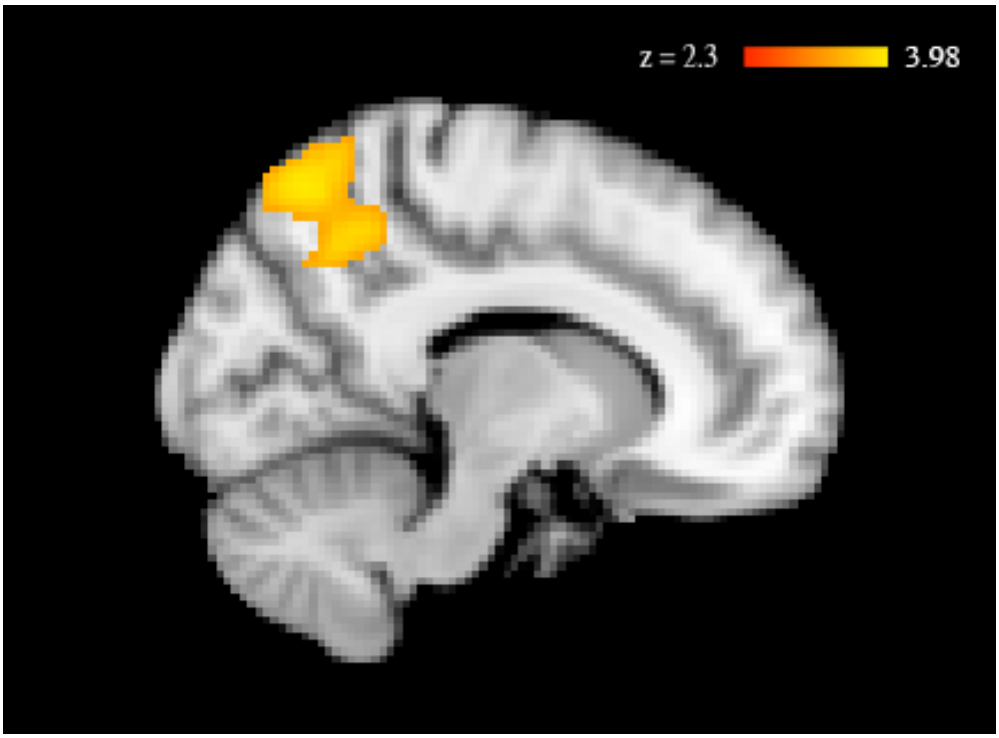

Figure 5.2.8. fMRI Images during Early Training on the SRT (Fixed Effects). Early Training (first 192 trials) in the serial reaction time task. Images are from the voxel-wise group-level analysis ($z > 2.3$, cluster-corrected thresholded at $p = 0.01$) for sequence-random in relatives > controls for a fixed-effects analysis. This region depicts the precuneus cortex.

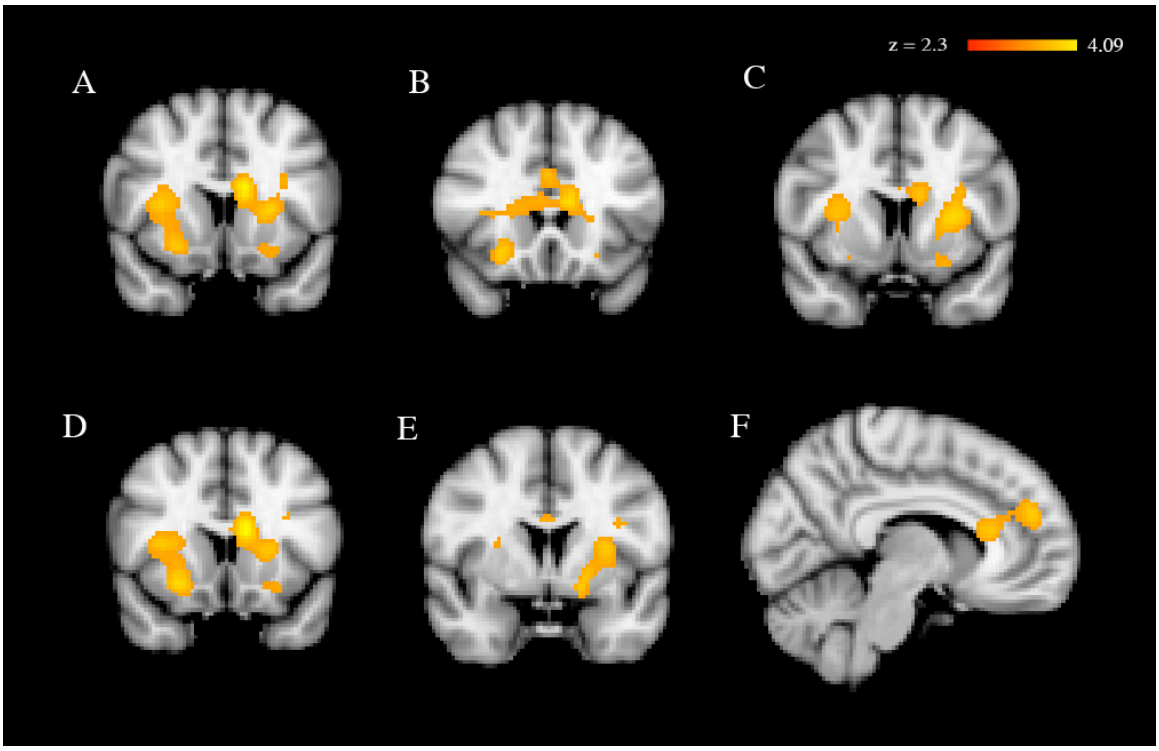

Figure 5.2.9. fMRI Images during Extended Training on the SRT (Fixed Effects). Extended Training (first 192 trials) in the serial reaction time task. Images are from the voxel-wise group-level analysis ($z > 2.3$, cluster-corrected thresholded at $p = 0.01$) for sequence-random in relatives-controls for a fixed-effects analysis. A = left caudate, B = right caudate, C = left putamen, D = right putamen, E = left globus pallidus, and F = bilateral anterior cingulate, for relatives > controls.

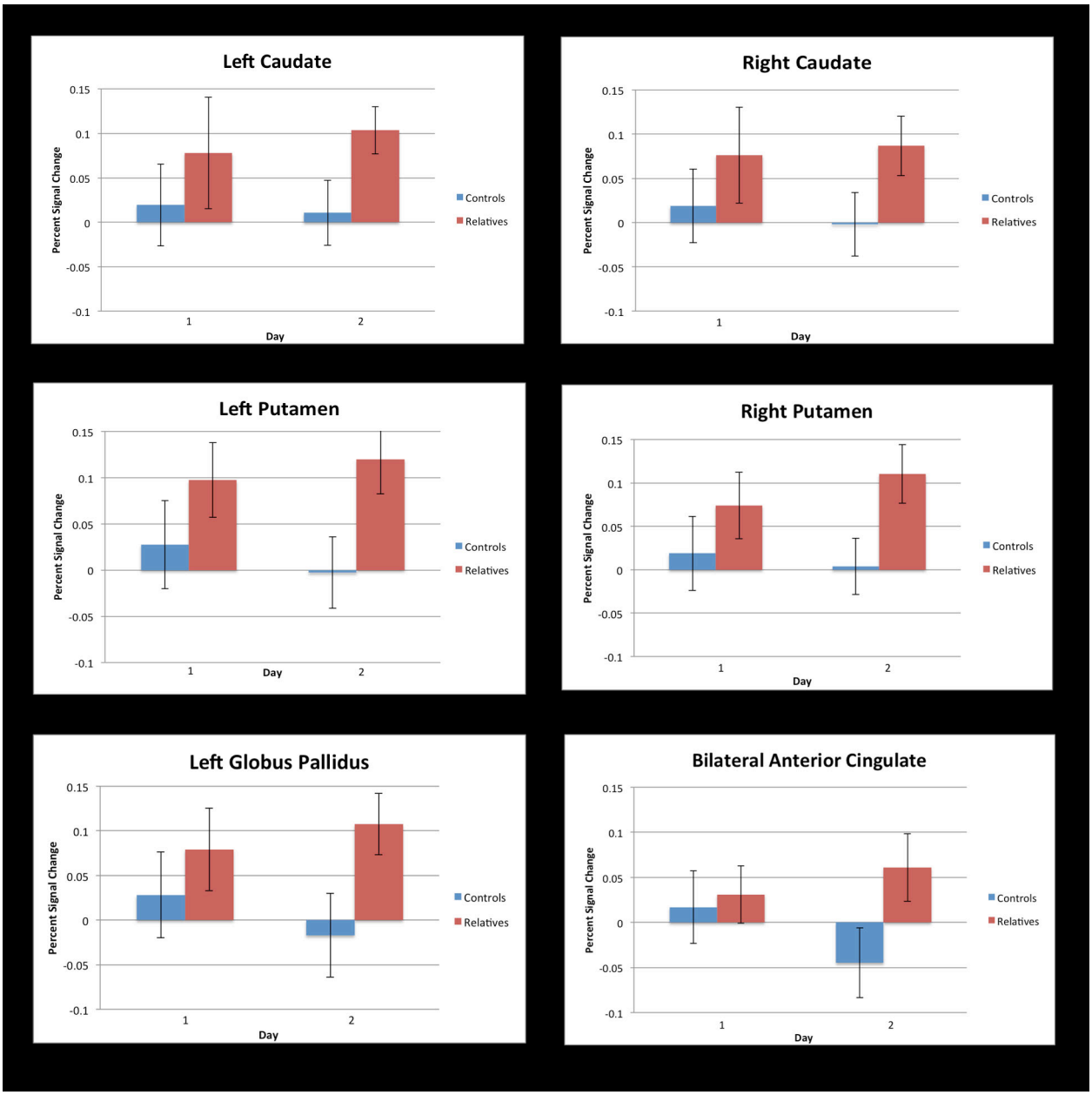

Figure 5.2.10. ROI graphs of the SRT. Percent signal change during the task for sequence versus random within the left and right caudate, left and right putamen, left globus pallidus, and bilateral anterior cingulate gyrus. Error bars represent the standard error of the mean.

5.2.6 Tables

Variable	Controls (n = 6) M	SD	Relatives (n = 8) M	SD
Age	41.33	4.03	42.25	3.96
Gender*	1/5		2/6	
Education	15.00	2.10	12.88	4.61
Vocabulary**	53.83	13.43	45.33	6.51
Blocks	63.00	5.22	43.88	10.18

Table 5.2.1. Demographic Table.

*Men/Women, ** WASI Vocabulary subtest (missing 5 relatives)

Region	Coordinate (mm) X	Y	Z	Max Z-Value
Day 2 for relatives > baseline for sequence > random				
Left Caudate	53	69	45	2.780
Left Putamen	59	67	39	2.300
Left Globus Pallidus	57	58	37	2.600
Left Thalamus	53	53	36	2.531

Table 5.2.2. Voxel-wise Region Coordinates (Mixed Effects). Brain regions that showed significant activation at the group-level analysis for sequence-random. Z-statistical maps were whole-brain cluster corrected at $Z > 2.3$, $p = 0.05$. There were no significant regions for Day 1 controls > relatives, relatives > controls, controls > baseline, or relatives > baseline and Day 2 controls > relatives, relatives > controls, or controls > baseline.

Region	Coordinate (mm)			Max Z-Value
	X	Y	Z	
Day 1 for relatives > controls for sequence > random				
Bilateral Precuneus Parietal Cortex	39	35	58	3.195
Day 2 for relatives > controls for sequence > random				
Left Caudate	51	69	44	3.559
Right Caudate	36	73	42	2.618
Left Putamen	58	67	40	3.390
Right Putamen	34	70	31	3.285
Left Globus Pallidus	54	64	33	3.000
Bilateral Anterior Cingulate Gyrus	41	81	47	2.350

Table 5.2.3. Voxel-wise Region Coordinates (Fixed Effects). Brain regions that showed significant activation at the group-level analysis for sequence-baseline. Z-statistical maps were whole-brain cluster corrected at $Z > 2.3$, $p = 0.05$. There were no significant regions for Day 1 relatives > controls and Day 2 relatives > controls.

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CHAPTER 6. Conclusion

Though skill learning has been studied extensively in schizophrenia patients, these series of studies are the first to examine adolescent childhood onset schizophrenia (COS) patients on skill learning and the first or one of the first to employ functional magnetic resonance imaging (fMRI) in adolescent and adult relatives of schizophrenia patients on skill learning. Tasks of cognitive skill learning, the improvement of performance in a task that is not motor or perceptual in nature, and motor skill learning, a change in motor performance, were utilized to measure cortico-striatal function in individuals at genetic risk for schizophrenia.

Individuals with schizophrenia possess deficits in several domains, such as in working memory, attention, executive control, language, and declarative memory (Asarnow, 1999; Gold & Harvey, 1993; Kuperberg & Heckers, 2000; Riley et al., 2000). A subset of these deficits is also present in relatives of schizophrenia patients as well (Asarnow, Steffy, MacCrimmon, & Cleghorn, 1977; Sitskoorn et al., 2004; Szöke et al., 2005; Snitz, Macdonald, & Carter, 2006; Vink et al., 2006). Skill learning is an area that has been much less examined in the schizophrenia relatives literature and was the focus of this dissertation.

For cognitive skill learning, our results on the weather prediction task (WPT) demonstrated differences in behavior and the pattern of brain activation for adolescent siblings vs. controls as well as adult relatives vs. controls. The experiment with adolescent siblings and controls revealed group differences early in learning (the first 50 trials) with the controls showing clear learning while the adolescent siblings do not. Even after extended training in which another 800 trials of the task were completed, the siblings never caught up to the level of performance of controls and the COS siblings reached a lower level of asymptotic performance than controls.

We also wanted to determine whether there was a difference in the pattern of brain activation between COS siblings and controls. Given that our behavioral data indicated behavioral differences between groups early in training and during extended training with asymptotic performance, we scanned both early and late in learning to assess differences in brain activation during these two time points. We first wanted to find areas that were significantly different in activity between siblings and controls during both days of learning when they completed the task as compared to baseline. Our voxel-wise whole brain group level analysis revealed that for early learning, the right putamen, right hippocampus, bilateral anterior cingulate, bilateral posterior cingulate showed greater activation for siblings > controls. Voxel-wise analysis for later learning revealed that the bilateral anterior and posterior cingulate, right and left putamen, left caudate, and right hippocampus were significantly more active for siblings > controls.

Next we wanted to determine exactly how these areas change during learning and the differences between the groups. So we conducted an independent anatomical region of interest (ROI) analysis on the regions that passed threshold at the group level voxel-wise analysis. The ROI analysis revealed that the COS siblings demonstrated increased activation early in training and decreased activation after extensive training in striatal and medial frontal regions, while the controls maintained or marginally increased their level of activation in these regions across days of training. In the posterior cingulate cortex, a different pattern emerged, with controls showing a marked deactivation in the region early in learning, that was not present on the second day, while in the COS siblings, posterior cingulate activation remained near baseline levels throughout training.

Decreased activation in the striatum in the siblings may reflect a lack of effective utilization of neostriatal circuits in the siblings of COS patients, which may contribute to their poorer level of asymptotic performance on the WPT. For the anterior cingulate the controls maintained their level of activation across training, while the siblings demonstrated an increase in activation compared to baseline early in training and a sharp decrease in activation later in training. This is similar to findings in patients with schizophrenia and adult relatives of these patients, who have been shown to exhibit decreased anterior cingulate cortex activation in sustained attention tasks (Diwadkar et al., 2011; Sepede et al., 2010; Filbey et al., 2008; Liddle et al., 2006; Neuhaus et al., 2007). Therefore, our results are consistent with the idea that alterations in the executive control functions of the anterior cingulate cortex may be a hallmark of genetic liability for schizophrenenia.

In the next study, we wanted to know whether the adult relatives would replicate the behavioral and neural pattern seen with adolescent siblings of COS patients. There were several possibilities that could account for the group differences evident in the adolescents: 1) the group differences seen in the adolescents would be larger because adult subjects may be able to utilize compensatory strategies or 2) the group differences we observed in the adolescents may also reflect different developmental trajectories in individuals with genetic risk for schizophrenia that are attenuated in adulthood. In order to test these theories, we examined adult relatives and controls in the WPT.

The adult relatives of patients with schizophrenia did not display any impairment on the WPT compared to controls either during early or late training on the single-task trials. In contrast, dual task probe trials inserted during the second day of training appeared to differentially affect the groups. These results suggest that during learning on the first session of

the second day of training both groups were not performing the task in an automated manner. However, by the second dual task probe block, the controls showed no cost of the tone counting task on WPT performance, while the relatives' performance continued to suffer under dual task conditions at this point.

The effect of the addition of a secondary task decreases declarative memory retrieval by occupying working memory, and results in relying on controlled rather than automatic processing (Foerde, Poldrack, Knowlton, 2006; Logan, 1978; Craik et al., 1996). Our results demonstrate that while the two groups reach similar behavioral endpoints, the relatives of patients with schizophrenia have more difficulty learning the task automatically and continue to rely on controlled processing even after extensive practice. There is support in the literature that patients with schizophrenia have diminished processing resources compared to controls, and thus their ability to automate skill is also more impaired (Braff, 1985; Holzman, 1987; Nuechterlein, 1991). Our data indicate that adult relatives of patients may be similarly affected by a concurrent task.

The results of the adult relatives study and previous work (Wagshal et al., under review; Weickert et al., 2010) support the idea that deficits in the WPT are characteristic of the genetic liability for schizophrenia. However, our results suggest that the adult relatives of patients with schizophrenia may be able to engage compensatory strategies to achieve normal levels of performance. It may be that the adolescent siblings of COS patients tested in Wagshal et al. (under review) were unable to utilize such a strategy. In order to gain a better understanding of how the adult relatives were successfully learning the WPT and if compensatory regions were engage to contributed to their normal performance of the task, the next study examined the adult relatives and controls under fMRI conditions.

The results of the study with adult relatives and controls that completed the WPT in the scanner indicate that behaviorally, the adult relatives performed comparable to controls on the WPT, despite the fact that they did not achieve automaticity of the task and they displayed different patterns of neural activation during learning of the task. The fMRI data reveal that the adult relatives are at baseline during early learning and then have a sharp decrease in activation in the striatum, while the controls maintain their same level of activation near baseline across learning. For the bilateral anterior cingulate the relatives to have a slight decrease in activation on the first day and then a sharp decrease in activation during the second day. These results are paralleled but to an even greater extent in the left hippocampus for the relatives.

The significant difference in the brain activation pattern seen across both groups in the striatum signifies a lack of utilization in the relatives during later learning while controls recruited this region to help themselves complete the task. Though the adult relatives were successful in learning the WPT, this pattern of brain activation in the striatum is similar to the previous study with adolescent siblings of COS patients who displayed impaired learning of the WPT compared to controls (Wagshal et al., In Preparation). The difference in the pattern of brain activation in the striatum signifies that the relatives are using another region to successfully learn the WPT compared to controls. Our hypothesis was that the relatives would engage the hippocampus and would rely on controlled processing to aid in their successful learning of the task. Though inconclusive due to the extremely low power and lack of subjects, the results from our pilot data do not support this theory. Our preliminary data demonstrate that the hippocampus is not engaged at all during either early or late learning. However, a limitation of the study, besides the low number of subjects, was that the baseline was very low level. A future study should implement a higher level baseline in which the subjects are more engaged so that mind

wandering (and thus an increase in hippocampus activation during the baseline) does not occur and so hippocampus activity can be measured more accurately.

We have provided evidence of an impairment in cognitive skill learning in adolescents as well as a dual task effect in adult relatives. We have also demonstrated different patterns of brain activation in the schizophrenia relatives compared to controls with an underactivation of the striatum in both adolescents and adults. The next series of experiments were to determine if there was also evidence of corticostriatal dysfunction in relatives of schizophrenia patients on the serial reaction time (SRT) task, a measure of motor skill learning.

There are mixed results in the patient literature concerning specific types of motor skill learning. Studies using the Pursuit Rotor and Mirror Tracing tasks have demonstrated that patients with schizophrenia show similar learning comparable to controls (Huston & Shakow, 1949; Goldberg et al., 1993; Granholm, Bartzokis, Asarnow, & Marder, 1993; Clare, McKenna, Mortimer & Baddeley, 1993; Kern, Green, & Wallace, 1997; Weickert et al., 2002; Scherer, Stip, Paquet & Bedard, 2003; Bedard et al., 2000). However, on another measure of motor skill learning, the Serial Reaction Time (SRT) task, there is less agreement in the literature of whether or not patients possess a deficit. Ultimately, it seems that the type of medication plays a large role in SRT impairment due to alterations in striatal D2 receptors (Paquet et al., 2004; Kumari et al., 2002). In order to avoid a confound of medication, we examined relatives of schizophrenia patients.

Our behavioral study with adolescent COS siblings and controls revealed comparable performance with each group demonstrating sequence specific learning. Next, we wanted to determine whether there was a difference in the pattern of brain activation between adolescent siblings vs. controls. Our behavioral data indicated no behavioral differences between groups

early in training and during extended training, though the results of past studies with adult schizophrenia patients and siblings indicated neural differences between the groups (Zedkova et al., 2006; Reiss et al., 2006; Purdon et al., 2011; Woodward et al., 2007). Therefore, we scanned both early and late in learning to assess differences in brain activation during these two time points. The fMRI results in the whole brain group level analysis indicated no differences in the pattern of brain activation for sequence specific learning for siblings compared to controls or vice versa. Therefore we conducted a sequence-baseline contrast. Regions in the basal ganglia, hippocampus, thalamus, SMA, supramarginal gyrus, precuneus parietal cortex, superior frontal gyrus, and middle frontal gyrus passed threshold for both the controls and siblings. However, ROI analyses were only conducted in regions that were different among controls and siblings, which were in the left and right caudate, left and right putamen, left globus pallidus, and left and right thalamus that were significantly more active for controls for sequence > baseline. We also wanted to compare later learning on the second day and the left hippocampus, left caudate, left putamen, left globus pallidus, and left thalamus were significantly more active for controls for sequence > baseline.

We wanted to determine exactly how these areas change in the pattern of brain activation during early and late learning on day 1 and 2 and if there were differences between both the groups. An independent ROI analysis were conducted on the regions that passed threshold at the voxel-wise analysis. The COS siblings showed increased activity early in training and decreased activity after extensive training, while the controls maintained their increased level of activation throughout both days of training. However, due to a lack of power caused by a small sample size in the siblings (N = 8), we were not able to demonstrate more brain regions that were significantly different from controls. Nevertheless, the pattern of activation during early and late

learning looks very promising in these regions and the addition of more siblings could potentially add more power to find a significant difference in these regions between the groups.

In the next study we wanted to know whether the adult relatives would replicate the behavioral pattern seen with adolescent siblings of COS patients and that found in the literature. The relatives and controls performed comparably on the SRT, which mirrored past studies. Next we wanted to determine if there were differences in the pattern of brain activation between the adult relatives and controls. For sequence specific learning, there were no regions passed threshold in the voxel-wise analysis for early learning. However, there were 4 regions that passed threshold for late learning on the second day for sequence-random. These regions included the left caudate, left putamen, left globus pallidus, and left thalamus that were significantly more active for the relatives compared to baseline. Next, we also we wanted to determine how these areas change in the pattern of brain activation during early and late learning on day 1 and 2 and if there were differences between both the groups. So we conducted an independent anatomical ROI analysis; unfortunately no regions were significant or at trend levels. However the voxel-wise whole brain analysis demonstrated significant differences in later learning with an increase in these regions for the relatives but not for controls when compared to baseline.

Due to a lack of subjects, we also explored the pattern of brain activation during performance of the task for a sequence-random contrast in a whole brain group-level voxel-wise analysis during early and late learning using a fixed-effects model to obtain trend level differences between the groups in regions that could be significant with the addition of more subjects. The results from the sequence-random contrast revealed a significant difference in the left putamen with the relatives displaying an increase in activation compared to controls on both

days of learning. The same pattern emerged for trend level differences in the left and right caudate, right putamen, left globus pallidus, and in the bilateral anterior cingulate gyrus.

However, these results do not support the literature, which would predict an underactivation in the basal ganglia for the relatives. There are several possible reasons for these results. First, we had very low sample size (8 relatives and 6 controls), though no study that has been conducted so far has examined an extensive number of subjects. A second reason could be due to our mixture of relatives (5 childhood onset schizophrenia parents, 1 adult onset schizophrenia parent, and 2 adult onset schizophrenia siblings). The other studies might have found different results because they tested a homogenous population. The adolescent study comprised of siblings of childhood onset schizophrenia patients and Woodward et al. (2007) tested adult siblings of adult onset schizophrenia patients. However, what we did find in our adult relatives population that was similar to the other studies were compensatory regions that demonstrated an increase in activation from baseline for the relatives, specifically in the hippocampus and thalamus.

Ultimately, these series of experiments of cognitive and motor skill learning have demonstrated impaired cortico-striatal function in adolescent and adult relatives of schizophrenia patients. Future studies with a large number of participants and a homogeneous schizophrenia adult relative population need to be carried out in order to further elucidating the role of the corticostriatal circuits and genetic liability for schizophrenia. Knowing which strategies and brain regions that schizophrenia patients and their relatives employ can give insights into whether corticostriatal dysfunction is an endophenotype of schizophrenia, independent of the effects of medication and the illness. Thus, understanding the behavioral and neural mechanisms

of implicit learning is an important step that will help target future treatments to develop successful strategies to help overcome or prevent impairments due to the disease.

6.1 References

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