

**DEFICITS IN EYE MOVEMENT  
CONTROL IN CHILDREN DIAGNOSED WITH  
22Q11.2 DELETION SYNDROME.**

by

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## Abstract

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**Background:** The 22q11.2 deletion syndrome (22q11.2 DS) causes a wide variety of symptoms, but the central nervous system (CNS) dysfunction is the one most likely to affect the day-to-day life of those affected by this genetic disorder. In addition to affecting the educational needs of children with 22q11.2 DS, the neurological deficits in childhood and adolescence could be related to future psychosis and schizophrenia, which can affect 30% of these patients. Thus, the development of screening tools for CNS dysfunction could help identify children who are most at risk for developing later psychosis, allowing them to receive additional care. As saccadic eye movement behaviours reflect the integrity of multiple brain structures, a battery of oculomotor tasks could help identify neurological deficits. This study sought to test the hypothesis that children with 22q11.2 DS would have deficits in oculomotor performance compared to typically developing children. **Methods:** A cohort of 16 children with 22q11.2 DS, and 32 age- and sex-matched controls completed prosaccade, antisaccade, delayed memory-guided sequential (DMS) and predictive eye movement tasks. **Results:** Compared to controls, children with 22q11.2 DS exhibited increased direction errors in the antisaccade task, increased timing errors in the DMS task, as well as decreased predictive and increased regular saccades in the predictive task. The group of children with 22q11.2 DS also exhibited an increase in saccade amplitude in the prosaccade, antisaccade and predictive tasks, increased error in saccade trajectory in the prosaccade, antisaccade and

DMS tasks and decreased saccade velocity in the predictive saccade tasks. **Conclusion:**

This study showed that performance in the eye movement tasks could be used to assess injury to the frontostriatal circuitry and cerebellum in children with 22q11.2 DS.

## **Co-Authorship**

The research described in this thesis was conducted by Sarah Kalwarowsky under the supervision of Dr. James Reynolds. The data was collected by Sarah Kalwarowsky with the assistance of Rebecca Titman and Stacey Kimmett and analyzed by Sarah Kalwarowsky. The first draft of this thesis was written by Sarah Kalwarowsky.

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## List of Abbreviations

22q11.2 DS	22q11.2 Deletion Syndrome
ADHD	Attention deficit/hyperactivity disorder
ANOVA	Analysis of variance
BG	Basal ganglia
BRIEF	Behavioral Rating Inventory of Executive Function
CANTAB®	Cambridge Neuropsychological Test Automated Battery
CC	Corpus callosum
CMS	Children's Memory Scale
CNS	Central nervous system
COMT	Catechol-o-methyltransferase
CV	Co-efficient of variation
dIPFC	Dorsolateral prefrontal cortex
DMS	Delayed memory-guided sequential
DNA	Deoxyribonucleic acid
DTI	Diffusion tensor imaging
EBN	Excitatory burst neurons
FASD	Fetal alcohol spectrum disorders
FDI	Freedom from Distractability Index
FEF	Frontal eye fields
FISH	Fluorescence <i>in situ</i> hybridization
fMRI	Functional magnetic resonance imaging
FP	Fixation point
IBN	Inhibitory burst neurons
IQ	Intelligence quotient
LIP	Lateral Intraparietal area
LLBN	Long-lead burst neurons
MRI	Magnetic resonance imaging
MT	Middle temporal visual area
OPN	Omnipause neurons
PCR	Polymerase Chain Reaction
PFC	Prefrontal cortex
PET	Positron emission tomography
PPC	Posterior parietal cortex
SCi	Intermediate layer of the superior colliculus
SEF	Supplementary eye fields
SRT	Saccadic reaction time
SWM	Spatial Working Memory task
T	Target
TEO	Temporo-occipital visual area
TMS	Transcranial magnetic stimulation
TMT-A & B	Trail Making Test, Part A & B
WIAT	Wechsler Individual Achievement Test
WISC	Wechsler Intelligence Scales for Children
WRAML	Wide Range Assessment of Memory Learning

## **Chapter 1**

### **Introduction**

Children with 22q11.2 Deletion Syndrome (22q11.2 DS) commonly present with cardiac abnormalities, facial malformations, palatal abnormalities, and central nervous system (CNS) dysfunction (Burn, 1999). The deletion affects approximately 1 in 3000 individuals, and it is the highest known genetic risk factor for the development of schizophrenia in adulthood (Kobrynski & Sullivan, 2007; Murphy *et al.*, 1999). The syndrome is the result of a 1.5-3 megabase heterozygous deletion on the long arm of chromosome 22 which encompasses approximately 30 genes (Scambler *et al.*, 1992). Children with 22q11.2 DS have been found to have borderline-low IQ as well as deficits in executive function (especially response inhibition and suppression) as well as deficits in attention and working memory (Goldmuntz, 2005; Campbell *et al.*, 2010). These deficits not only affect the daily life of children with the disorder, but are also likely related to the psychiatric disorders commonly found in this population, including schizophrenia, autism spectrum disorders, ADHD, anxiety disorders, depression, somatization, social withdrawal or obsessive-compulsive disorder (Fine *et al.*, 2005; Goldmuntz, 2005).

Currently, clinicians use standardized tests to assess CNS dysfunction, with emphasis on working memory, attention and executive function. These tests are often time consuming and can only be administered by qualified professionals. The

development of better screening tools could help identify children who are most in need of additional resources. Also, if a specific parameter of the tests could be correlated with future psychosis, these patients could be monitored more closely for future risk.

The measurement of eye movement control is a powerful tool for assessing sensory, motor and cognitive function, and has been in use to help assess brain function in clinical populations for many years. The control of eye movement is diffuse and is spread between cortical and subcortical structures. Therefore, eye movements are a simple way to quantify the integrity of large portion of neural circuitry. Many decades of human and animal studies have probed the role of each brain structure in the control of eye movements, allowing the correlation between oculomotor behaviour and certain brain regions. These eye movement tasks have never before been performed on children with 22q11.2 DS. The goal of this research project was to characterize the oculomotor behaviours in this population using eye movement paradigms that probed sensorimotor processing, executive function, procedural learning and working memory abilities.

## Chapter 2

### Literature Review

#### 2.1 22q11.2 Deletion Syndrome

22q11.2 Deletion Syndrome (22q11.2 DS) is a relatively common genetic disorder affecting around 1 in 3000 individuals (Kobrynski & Sullivan, 2007). Due to the variety and combinations of symptoms it produces, it has previously been called the DiGeorge syndrome, velocardiofacial syndrome, conotruncal anomaly face syndrome, Opitz G/BBB syndrome and Cayler cardiofacial syndrome (du Monctel *et al.*, 1996) before it was recognized that these syndromes had a common cause. 22q11.2 DS is the result of a heterozygous deletion on the long arm of chromosome 22 which spans 1.5-3 megabases and encompasses approximately 30 genes (Scambler *et al.*, 1992). The most frequent anomalies in individuals with 22q11.2 DS include cardiac malformations, hypocalcemia, mild conductive hearing loss, velopharyngeal insufficiency and cleft palate (Ryan *et al.*, 1997). The symptoms have been described by the mnemonic **CATCH-22**; **C**ardiac abnormality, **A**bnormal facies, **T**hymic aplasia, **C**left palate and **H**ypocalcemia (Burn, 1999). 22q11.2 DS also affects cortical development leading to behavioural, emotional and cognitive deficits. It is also the highest known genetic risk of schizophrenia, with approximately 30% of patients developing the disorder (Murphy *et al.*, 1999).

### **2.1.1 Overview of heritage:**

In the great majority (90-95%) of cases the 22q11.2 DS is a *de novo* deletion which occurs sporadically during spermatogenesis or oogenesis in one of the parents (Ryan *et al.*, 1997). This region of the chromosome has a structural feature which results in aberrant recombination during meiosis, leading to frequent deletions (which cause 22q11.2 DS) and duplications. However, in 5-10% of cases 22q11.2 DS is inherited from one of the parents. Because the deletion is autosomal dominant, if a parent has 22q11.2 DS there is a 50% chance of the parent passing on the deletion to their offspring (Steele *et al.*, 1972). Parents who have symptomatic 22q11.2 DS often have much milder symptoms than their offspring, with a lower frequency of congenital heart defect (Leana-Cox *et al.*, 1996).

### **2.1.2 Diagnosis:**

22q11.2 DS has such a variable phenotype that the only reliable method of diagnosis is to examine the region of DNA on chromosome 22 to determine if the deletion is present (Shprintzen, 2008). Patients found to have certain abnormalities have a high frequency of 22q11.2 DS and should be screened for the deletion. These disorders include; conotruncal cardiac anomalies, velopharyngeal insufficiency and neonatal hypocalcemia. The 22q11.2 deletion can be detected by using fluorescence *in situ* hybridization (FISH) analysis which uses probes for the deleted region (Liu *et al.*, 2010). FISH testing is very accurate, with fewer than 5% false negative tests, however, since the area deleted can vary in size, more advanced testing methods such as rapid polymerase

chain reaction (PCR) screening are being developed (Chen *et al.*, 2006; Tomita-Mitchell *et al.*, 2010). Prenatal screening is offered to pregnancies which are at high risk of 22q11.2 DS; if a parent has the syndrome, or if congenital heart malformations, or palate malformations are detected by ultrasound.

## **2.2 The cognitive-behavioural phenotype of 22q11.2 DS**

Since the discovery of 22q11.2 DS, the neuropathology underlying the syndrome has been extensively studied. Children with 22q11.2 DS face many challenges, not only because of the many health problems, but also because of the neurological deficits which are associated with the syndrome. Because the deletion can vary in size, there are a wide range of neurological deficits associated with 22q11.2 DS. In addition to the quantifiable neurological deficits, there are a host of psychiatric disorders that are commonly observed in children with 22q11.2 DS. These include autism spectrum disorders, ADHD, anxiety disorders, depression, social withdrawal or obsessive-compulsive disorder (Fine *et al.*, 2005; Goldmuntz, 2005).

In the earliest classifications of velo-cardio-facial syndrome, before the cause was known, intellectual impairment and learning disabilities were observed as a common symptom (Shprintzen *et al.*, 1981; Golding-Kushner *et al.*, 1985). In more modern studies in children with 22q11.2 DS cognitive deficits have been quantified as borderline to moderate (Gerdez *et al.*, 1999). Patients with 22q11.2 DS often have higher verbal IQ than performance IQ scores, meaning that the patients are better at processing and using verbal information than they are at perceptual processing and organization (Goldmuntz,

2005; Antshel *et al.*, 2008). These studies, which have quantified the deficits in 22q11.2 DS, show particular weaknesses in visual perception, visual memory, visual-spatial information processing and executive function (Bearden *et al.*, 2001; Simon *et al.*, 2002). Moss and colleagues (1999) determined that school-aged children with 22q11.2 DS show significantly weaker math scores than reading and spelling scores. This weakness in math is thought to be associated with the deficits in visual-spatial information processing. Neuroimaging studies have sought to identify the structural abnormalities which cause these behavioural deficits.

Between 30-40 genes are contained within the deleted region of chromosome 22, and since many of these genes are thought to be involved in early embryonic neuronal migration, children with 22q11.2 DS exhibit abnormal brain development (Table 2.1; Maynard *et al.*, 2003; McDonald-McGinn *et al.*, 2005). A meta-analysis of 22 magnetic resonance imaging (MRI) studies in patients with 22q11.2 DS revealed an overall reduction in brain volume, affecting both grey and white matter (Tan *et al.*, 2009). As a consequence of altered neuronal migration, people with 22q11.2 DS have frequent dysmorphology of many midline structures. Several studies, including the meta-analysis (Tan *et al.*, 2009), have found an increased volume of the corpus callosum in 22q11.2 DS patients (Shashi *et al.*, 2004; Antshel *et al.*, 2005). The septum pellucidum is a midline membrane which separates the two lateral ventricles. In fetal development there is a space, between the two layers of membrane, called the cavum septum pellucidum. This space usually closes during infancy, but children with 22q11.2 DS often show an

**Table 2.1 Genes within the chromosomal region affected by 22q11.2 DS**

<b>Gene</b>	<b>Gene Product</b>	<b>Gene</b>	<b>Gene Product</b>
<i>DGCR6</i>	DiGeorge syndrome critical region protein 6	<i>TBX1</i>	T-box transcription factor TBX1
<i>ProDH2</i>	Proline dehydrogenase 2	<i>GNB1L</i>	Guanine nucleotide-binding protein subunit beta-like protein 1
<i>DGCR5</i>	miscRNA	<i>COMT</i>	Catechol-O-methyltransferase
<i>LAN/ DGCR2/Idd</i>	Integral membrane protein DGCR2/Idd	<i>ARVCF</i>	Armadillo repeat protein deleted in velo-cardio-facial syndrome
<i>Stk22a</i>	Testis-specific serine/threonine-protein kinase 1	<i>T10</i>	Serine/Threonine-rich protein T10 in DGCR region
<i>Stk22b</i>	Testis-specific serine/threonine-protein kinase 2	<i>DGCR8</i>	Microprocessor complex subunit DGCR8
<i>HIRA</i>	Protein HIRA	<i>TRMT2A</i>	tRNA methyltransferase homolog A
<i>DGCR14</i>	Protein DGCR14	<i>RanBP1</i>	ran-specific GTPase-activating protein
<i>Gscl</i>	Goosecoid-like	<i>ZDHHC8</i>	Palmitoyltransferase ZDHHC8
<i>SLC25A1</i>	Tricarboxylate transport protein, mitochondrial	<i>RTN4R</i>	Reticulon-4 receptor
<i>CLTCL</i>	Clathrin, heavy polypeptide-like	<i>PRODH</i>	Proline dehydrogenase, mitochondrial
<i>NLVCF</i>	Unknown	<i>DGCR6L</i>	Protein DGCR6L
<i>Ufd1L</i>	Ubiquitin fusion degradation protein 1 homolog	<i>MED15</i>	Mediator of RNA polymerase II transcription subunit 15
<i>TMVCF</i>	Transmembrane protein deleted in VCFS	<i>CRKL</i>	Crk-like protein
<i>SEPT5</i>	Septin-5	<i>LZTR1</i>	Leucine-zipper-like transcriptional regulator 1
<i>GP1BB</i>	Platelet glycoprotein 1b beta chain	<i>ZNF74</i>	Zinc Finger Protein 74

Adapted from Maynard *et al.*, 2003 and McDonald-McGinn *et al.*, 2005

increased incidence of enlarged cavum septum pellucidum (Chow *et al.*, 1999; van Amelsvoort *et al.*, 2001). The cerebellar vermis and the pons of patients with 22q11.2 DS have also been shown to be reduced in volume (Eliez *et al.*, 2001; Bish *et al.*, 2006). Although white matter structures are generally more severely impacted than grey matter in subjects with 22q11.2 DS some cortical regions are still affected, especially the prefrontal cortex and hippocampi (Baker *et al.*, 2011). The meta-analysis also revealed that male subjects with 22q11.2 DS had larger decrements in parietal lobe volume than any other group (Tan *et al.*, 2009). Bearden and colleagues (2007 & 2009) performed studies of cortical thickness which revealed cortical thinning in the superior parietal cortex and right parieto-occipital cortex (involved in visuospatial processing), which was consistent with the meta-analysis. These studies also showed thinning of the inferior frontal gyrus (pars orbitalis; language development), ventromedial occipital-temporal cortex (visuospatial representation) and the anterior cingulate cortex (attentional control).

The cognitive profile in patients with 22q11.2 DS is very similar to the pattern of deficits seen in non-22q11.2 DS people with schizophrenia, perhaps explaining why the two disorders so often overlap (Campbell *et al.*, 2010). The widespread disruption of neural circuitry in 22q11.2 DS may explain why the deletion is the single greatest risk factor for schizophrenia. Patients with 22q11.2 DS also frequently suffer from other psychological problems, including depression and bipolar disorder (Murphy *et al.*, 1999).

### 2.2.1 Corpus callosum

One of the most consistent structural changes observed in the 22q11.2 population is abnormalities of the corpus callosum (CC). The CC is the fibre tract that connects the two cortical hemispheres and permits fast interhemispheric communication. This is especially important during complex tasks (Usiskin *et al.*, 1999; Gazzaniga, 2000). Occasionally, agenesis of the CC has been reported in subjects with 22q11.2 DS (Chow *et al.*, 1999).

However, more often, structural MRI studies have revealed that total CC area is significantly increased in patients with 22q11.2 and that all portions of the CC are greater in size except for the genu (Shashi *et al.*, 2004; Antshel *et al.*, 2005). Interestingly, ADHD and schizophrenia, both of which are prevalent in the 22q11.2 DS population, are associated with smaller CC volumes (Gothelf *et al.*, 2003; Murphy *et al.*, 1999). It was found that children with 22q11.2 DS and ADHD had smaller CC volumes than children with 22q11.2 DS alone (Antshel *et al.*, 2005). These investigators also reported that the size of the bending angle of the CC was smaller in children with 22q11.2 DS than the control children.

Antshel and colleagues (2005) noted that the volume of the CC was correlated with behavioural difficulties. They speculated that the increased size of CC in subjects with 22q11.2 DS is due to disrupted neural pruning throughout development which would cause deficits in executive functions. This hypothesis is supported by Baker and colleagues (2011) who determined that adolescents with 22q11.2 DS showed more

extensive CC enlargements when compared to higher IQ controls than IQ-matched controls. Diffusion tensor imaging (DTI) studies, which allow the exploration of microstructural integrity in white matter tracts, have also found abnormalities in regions of the CC (Barnea-Goraly *et al.*, 2003). These abnormalities reflect alterations in density, coherence and myelination of the CC in the 22q11.2 DS group. Together, these studies suggest that the enlarged CC in subjects with 22q11.2 DS is due to disruption in neural pruning which could explain some deficits in overall IQ as well as executive functions.

### **2.2.2 Cerebellum**

Abnormalities in the cerebellum are consistently reported in children with 22q11.2 DS (Eliez *et al.*, 2001; Bish *et al.*, 2006; Baker *et al.*, 2011). The cerebellum coordinates and modulates actions based on inputs from all four cortical lobes, the spinal cord, the brain stem and the thalamus (Devinsky & D'Esposito, 2004). The cerebellum is normally associated with motor coordination, however, anatomical and physiological evidence also suggests that the structure plays a role in cognitive and emotional functions as well as attention (Devinsky & D'Esposito, 2004; Bish *et al.*, 2006). The cerebellum has a well-documented role in several types of eye movements. The vestibulocerebellum, part of the vestibular pathway, is involved in the control of smooth pursuit, eye-head tracking and the control of the vestibulo-ocular reflex (Zee *et al.*, 1981). The ocular motor vermis and caudate fastigial nucleus modulate saccades. These structures modulate saccade amplitude and monitor saccade accuracy and plan corrective saccades, if necessary (Robinson *et al.*, 2002; Colnaghi *et al.*, 2010).

MRI studies in children with 22q11.2 DS have revealed that there are reductions in both the grey matter of the superior cerebellar vermis and the white matter of the cerebellar peduncles when compared to IQ-matched controls (Eliez *et al.*, 2001; Baker *et al.*, 2011). Eliez and colleagues (2001) found a reduction in cerebellar lobules VI-VII, however, when Baker and colleagues (2011) performed a similar study with IQ-matched controls, they did not see this reduction. All of these results have been controlled for overall brain size. These reductions in cerebellar volume may contribute to some of the cognitive impairments in 22q11.2 DS including impairments in attentional orienting and social communication as well as control over saccade metrics.

### **2.2.3 Cerebral Cortex**

MRI volumetric studies have revealed that total cerebral cortical volume is reduced by 8.5-11% in children with 22q11.2 DS when compared to typically developing children. However, certain regions of the cortex were more severely affected than others; in children with 22q11.2 DS this loss of volume follows a rostro-caudal gradient: frontal<temporal<cerebellar<occipital (Eliez *et al.*, 2000; Kates *et al.*,2001).

Children with 22q11.2 DS often exhibit deficits in behavioural and executive control, which is indicative of frontal lobe dysfunction. Despite the frontal lobe being relatively preserved, a meta-analysis of brain volume revealed that the right frontal lobe in children with 22q11.2 DS was decreased in volume compared to controls, with the reduction mainly occurring in the frontal grey matter (Tan *et al.*, 2011). In addition to the slight decrease in frontal lobe volume, children, adolescents and young adults with

22q11.2 DS have been found to have other indications of dysfunction in the orbito-frontal region; specifically decreased cortical folding and decreased cortical thickness when compared to controls (Schaer *et al.*, 2006; Bearden *et al.*, 2007). Children with 22q11.2 DS, despite having relative strengths in verbal memory, very frequently suffer from early language delays and exhibit deficits in language comprehension (Gerdes *et al.*, 1999). The orbito-frontal region is an important structure for language development, and thinning in this region may explain some of the early language delays and deficits in executive function seen in 22q11.2 DS (Chen *et al.*, 1996).

The parietal lobe has multiple functions, and in particular serves a central role in sensory-motor integration, visual attention and perception (Devinsky & D'Esposito, 2004). The reduction in volume of the parietal lobe may be the anatomical reason underlying the difficulties in visuo-spatial processing and difficulties with math that are often seen in the 22q11.2 DS (Gothelf *et al.*, 2008).

Patients with 22q11.2 also show decreased volume in the temporal lobe. A decreased volume in the temporal lobe is also a common anatomical change in schizophrenia, which develops in 30% of patients with 22q11.2 DS over their lifetime (Bassett & Chow, 2008; Murphy *et al.*, 1999). It is for this reason that linkage studies have suggested an association between 22q11.2 DS and schizophrenia (Williams *et al.*, 2003). The temporal lobe volume reduction may be associated with the relative risk of developing schizophrenia (Tan *et al.*, 2009).

The occipital lobe generally has the greatest deficit in grey matter in subjects with 22q11.2 DS and shows the most cortical thinning (Bearden *et al.*, 2007). The thinning of the occipital cortex, which is responsible for processing visual information and is critical for directing spatial attention, may help explain some of the deficits in visuo-spatial processing which are common in patients with 22q11.2 DS.

### **2.2.3 Hippocampus**

The hippocampus is another structure commonly affected in children with 22q11.2 DS. Studies have shown that left hippocampal volume is significantly reduced in children with 22q11.2 DS and that this correlates closely with IQ (DeBoer *et al.*, 2007). Baker and colleagues (2011) determined that the hippocampal volume was lower than high-IQ controls, but not for IQ-matched controls. This suggests that a reduced hippocampal volume is indicative of a learning disability, regardless of cause.

By combining the findings from imaging and behavioural studies, a pattern of brain injury associated with 22q11.2 DS is emerging. This pattern provides new insight into the neuropathology behind the 22q11.2 DS cognitive-behavioural phenotype, perhaps making links between genes and neurological development.

### **2.2.4 Catechol-O-methyltransferase**

Due to the large number (30-40) of genes implicated in the 22q11.2 deletion, it is difficult to pinpoint which and how many of them are responsible for the neurological deficits. A mouse model of 22q11.2 DS showed that diminishing doses of the affected

genes compromise the neurogenesis and the differentiation of cells in the developing cerebral cortex (Meechan *et al.*, 2009). The gene for catechol-O-methyltransferase (COMT), which catalyses the degradation of the catecholamine neurotransmitters including dopamine, epinephrine and norepinephrine, has been deemed a likely candidate for some of the neurobehavioural effects. Because patients with 22q11.2 DS are hemizygous for the COMT gene, several studies have attempted to quantify the relationship between the COMT genotype and the cognitive deficits exhibited by the patients. While some studies have found no association between the COMT gene polymorphism and the cognitive deficits (Campbell *et al.*, 2010; Glaser *et al.*, 2006), other studies have found evidence for such an association (Shashi *et al.*, 2006; Bearden *et al.*, 2004). This could be due to differences in the outcomes that were measured in these studies, but more likely points to a complex relationship between this gene and the outcomes, with environmental factors playing a larger role than previously anticipated (Vorstman *et al.*, 2009).

### **2.3 Assessing CNS dysfunction in 22q11.2 DS**

Dysfunction of the CNS in children with 22q11.2 DS has been assessed using numerous behavioural checklists and tests of executive function. Checklists have been used to quantify behavioural dysfunction in children with 22q11.2 DS; these include the *Behavioral Rating Inventory of Executive Function* (BRIEF) which can be administered to the parents and teachers of the subjects (Gioia *et al.*, 2000; Antshel *et al.*, 2005). These studies found that more behavioural difficulties were reported in children with 22q11.2

than their control counterparts. Intellectual ability in children with 22q11.2 DS has been evaluated using the *Wechsler Intelligence Scales for Children* (WISC) for school-aged children (Wechsler, 1992; Campbell *et al.*, 2009; Baker *et al.*, 2011). This test examines verbal comprehension, perceptual organisation, freedom from distractibility and processing speed. Studies employing this test have determined that children with 22q11.2 DS have lower IQ than their control counterparts, with performance IQ being more severely affected than verbal IQ. Intellectual functioning has also been assessed using the *Wechsler Individual Achievement Test* (WIAT) which measures the academic achievement of children as young as four (Wechsler, 2001; Antshel *et al.*, 2005). These checklists and rating scales are useful for assessing general life outcomes, including academic, social and emotional problems; however, domain-specific tests of neuropsychological function are required to define the cognitive profile of an individual (Antshel *et al.*, 2008). In order to assess the cognitive deficits specific to children with 22q11.2 DS, tests of attention and information processing, executive function, memory and learning are employed.

### **2.3.1 Tests of attention and information processing**

Attention is defined as the process of selectively concentrating on one aspect of the environment, while ignoring other stimuli. Attentional deficits have been examined in children with 22q11.2 DS, because they are a common co-morbidity of the disorder (Goldmuntz, 2005). The *Trail-Making Test A* requires brief, focused, attention while the children connect dots labelled with ascending numbers as quickly as possible (Reitan &

Wolfson, 1992). Performance on the *Freedom from Distractability Index* from the WISC is measured using the Arithmetic (timed arithmetic question), Digit Span (recalling a sequence of numbers), Coding (time-limited marking of shapes with different lines) and Symbol Search (must identify if target symbols appear in a row) subtests and is related to ADHD (Dickerson Mayes *et al.*, 1998). Woodin and colleagues (2001) administered the *Freedom from Distractability Index* and *Trail-Making Test A* to find that, during the *Trail-Making Test A* the 22q11.2 DS group performed similarly to controls, whereas deficits in the *Freedom from Distractability Index* were observed within the 22q11.2 DS population. Campbell and colleagues (2010) used a task from the *Maudsley Attention and Response Suppression Battery* to examine other elements of executive function (Rubia *et al.*, 1999). They used a Stroop task, where subjects must identify the required piece of information during both congruous and incongruous trials, to determine if the patients focus on important information only. During this task, children with 22q11.2 DS performed no differently to their control counterparts. General tests evaluating attention have been administered to children with 22q11.2 DS, but more specific tasks, probing the specific circumstances under which the children have attentional difficulties have yet to be studied in this population.

### **2.3.2 Tests of executive function**

Executive function is defined as the set of cognitive abilities that control and regulate other behaviours; these include the ability to initiate and stop actions, to change behaviour and to plan future behaviour. Children with 22q11.2 DS exhibit structural

changes in the frontal lobe, which is responsible for executive function (Tan *et al.*, 2011). Executive functions are important for academic success as well as other life skills, including performing tasks, making plans, changing plans and controlling impulses. The *Trail Making Test B* can be used to measure how well subjects can shift their attention, because they are required to draw a line through alternating numbers and letters in numeric and alphabetical order. In children with 22q11.2 DS, Woodin and colleagues (2001) found deficits in the *Trail Making Test B*, suggesting that children in the clinical group are worse at shifting their attention than their control counterparts.

Campbell and colleagues (2010) used two tasks from the computerized *Cambridge Neuropsychological Testing Automated Battery* (CANTAB) to assess executive function in 22q11.2 DS children. Firstly, they used the Set Shifting Task (Downes *et al.*, 1989), where the patients are required to discriminate stimuli by trial and error, as a measure of attentional set shifting. Consistent with the study by Woodin and colleagues (2001), subjects with 22q11.2 DS made more errors in this task. Secondly, they used the Stockings of Cambridge (Owen *et al.*, 1990), where patients must perform problem-solving tasks as a measure of planning ability. Children with 22q11.2 DS exhibited reduced planning ability in this task as well. Campbell and colleagues (2010) also used a Go-NoGo task from the *Maudsley Attention and Response Suppression Battery*. During the Go-NoGo task, subjects must execute or inhibit a motor response depending on which stimulus appears on the screen to measure the inhibition of motor responses. Children with 22q11.2 DS made more premature responses in the Go-NoGo

task than their control counterparts, indicating a deficit in response inhibition. Using all these tests of executive function, a cognitive profile of children with 22q11.2 DS has been outlined.

### **2.3.3 Tests of learning and memory**

Memory is described as the ability to recall previously presented information whereas learning is a modification of behaviour based on past events. The hippocampus is central to the formation of memory, and several studies have shown that children with 22q11.2 DS have smaller hippocampi than control counterparts (DeBoer *et al.*, 2007; Debanné *et al.*, 2006). The *Wide Range Assessment of Memory Learning* (WRAML) is a standardized test that measures both immediate and delayed memory in three subtests: 1) Verbal, 2) Visual and 3) Attention-Concentration. Children with 22q11.2 DS showed average performance on rote verbal learning and memory, but deficits in visual-spatial memory and, more complex, delayed story memory on the WRAML test (Woodin *et al.*, 2001). The *Children's Memory Scale* (CMS) measures immediate and delayed memory in the following categories; generalized learning and memory, as well as auditory/verbal and visual-spatial memory (Cohen, 1997). On this test, children with 22q11.2 DS performed in the low average-borderline range in all categories, with an additional deficit in delayed visual memory (Campbell *et al.*, 2010). CANTAB has a *Spatial Working Memory* (SWM) task where patients must search through boxes for blue tokens, remembering where they have looked before (Owen *et al.*, 1990). Campbell and colleagues (2010) found that children with 22q11.2 DS also exhibited deficits in the SWM task. These tasks

have revealed a consistent profile of children with 22q11.2 DS, but the performance on these tasks has yet to be correlated with volumes of neurological structures, such as the hippocampus.

These traditional neuropsychological tests, while effective, are limited because of the amount of time required to complete them and the need for specially trained professionals to administer them. If there were different tasks that could also objectively assess cognitive function in children with 22q11.2 DS using tools that are both mobile and easy to administer, they would have significant advantages when compared to these neuropsychological tests.

#### **2.4 Saccadic eye movements as measures of cognitive function**

Saccades are rapid, ballistic eye movements that permit the fixation of objects of interest onto the fovea, the region of the retina with the highest visual acuity (Leigh & Zee, 1983). Because we have a limited cognitive capacity with which to process visual information, we must integrate the sensory information, narrowing down the number of motor responses, or saccades, needed to gather information from our environment (Wurtz & Mohler, 1974). In an experimental setting, saccadic eye movement tasks have several advantages which make them convenient for probing brain function. Firstly, sensory input can be highly controlled by designing the experimental setting specifically to examine different aspects of sensory-motor integration (see for review: Gooding & Basso, 2008). Secondly, the saccade parameters can be precisely measured using remote monitoring equipment. This equipment is designed to produce little to no discomfort to

the participant, making it an ideal tool for assessing brain function in children. Thirdly, mobile eye tracking equipment is available, which allows testing in remote communities where there may not be other types of testing equipment. Finally, due to the significant overlap in the brain structures affected by 22q11.2 DS and those involved in eye movement control, saccadic eye movement tasks should represent a suitable tool for providing insights into the CNS dysfunction associated with 22q11.2 DS.

#### **2.4.1 Neurocircuitry of saccadic eye movements**

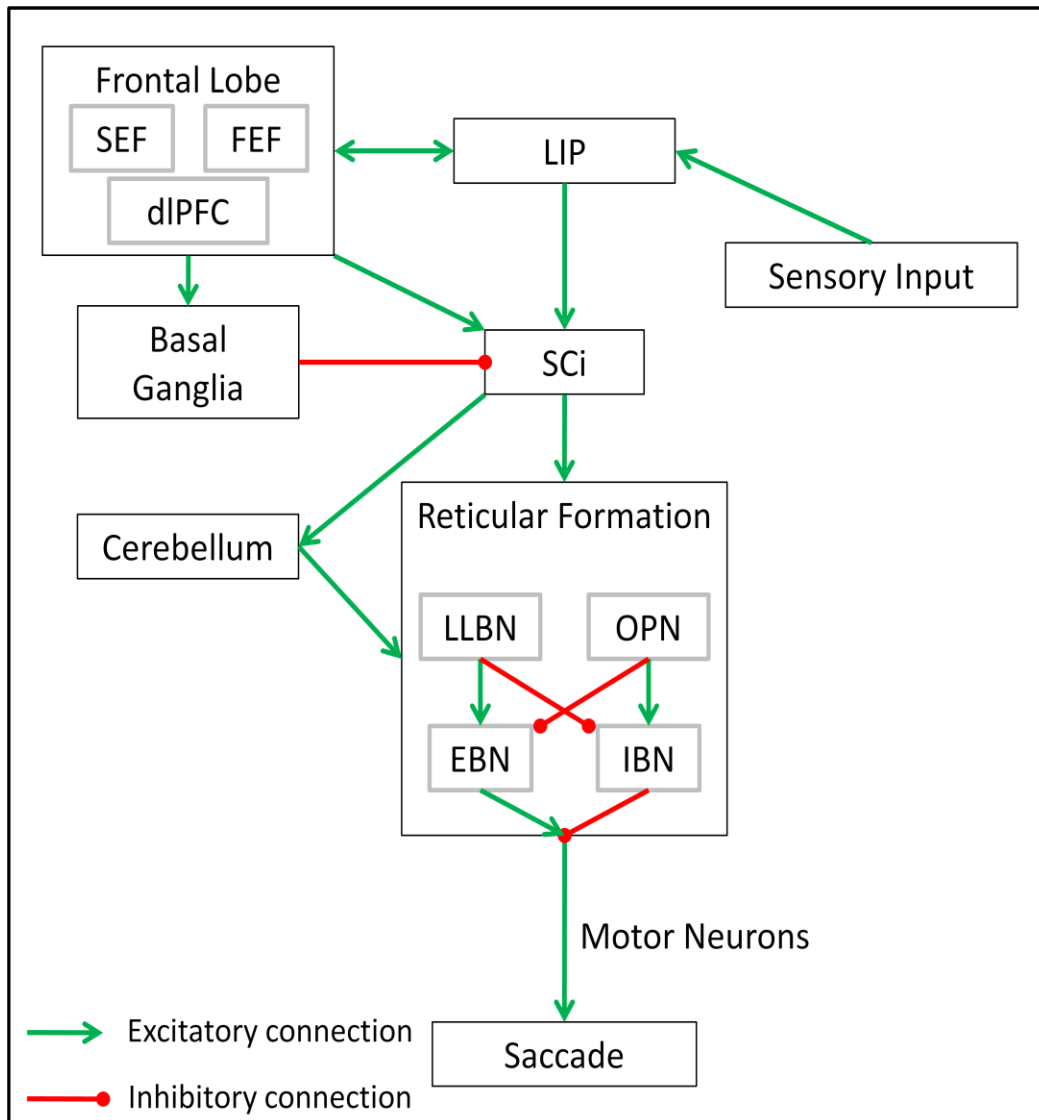
Saccadic eye movements are useful for assessing brain function for several reasons. They are easy to administer, their neurophysiology has been well characterized and they can be modified to probe specific brain areas. Eye movement control is spread through many brain structures, spanning both cortical and subcortical regions of the CNS, allowing overall brain function to be measured. Structures involved in oculomotor control include; the parietal and frontal cortices, the basal ganglia, the thalamus, the superior colliculus, the cerebellum, and the brainstem reticular formation (Leigh & Zee, 1999). Each of these structures have specific roles in the control of eye movement behaviour, which, historically, have been elucidated from lesion studies and electrophysiology in animals, and have been recently confirmed using functional MRI (fMRI) and transcranial magnetic stimulation (TMS) experiments which can be performed on humans (Sweeney *et al.*, 2007). Eye movement paradigms can be tailored to assess specific domains of cognitive function and provide insight into the underlying neuropathology associated

with different clinical populations. They have been used to clarify the pathophysiology of autism, ADHD and Tourette's syndrome among others (Sweeney *et al.*, 2004).

Eye movements are generated by motor neurons which originate in the reticular formation and directly innervate the ocular muscles. See Fig. 2.1 for a diagram of the saccade circuitry explained below, which has been adapted from Munoz & Everling (2004). These motor neurons discharge a burst of action potentials to elicit saccades and maintain tonic discharge to hold the eyes in place during an eccentric fixation (Sparks, 2002). These motor neurons are under the control of excitatory and inhibitory burst neurons, (EBN and IBN), also within the reticular formation, which elicit bursts during saccades to produce a desired movement (Okhi *et al.*, 1988; Scudder *et al.*, 1988). The EBN and IBN are, in turn, influenced by excitatory long-lead burst neurons (LLBN) and the inhibitory omnipause neurons (OPN) also found within the reticular formation. For a saccade to be initiated, the LLBNs must produce a high frequency burst activity, while the OPNs stop inhibiting the saccade.

The control of these premotor areas is governed by the intermediate layers of the superior colliculus (SCi), which contains a topographic map of the surroundings in retinal coordinates. It is thought that, via inputs from the parietal and frontal cortices, the basal ganglia (BG) and the cerebellum, the SCi forms a salience map of the subject's surroundings which can be used to select the next saccadic eye movement (Schall, 1995).

The posterior parietal cortex (PPC), which receives inputs from the visual, auditory and somatosensory system, is important for the planning of visually-guided



**Figure 2.1 The Neural Circuitry Outlining Voluntary Saccade Control**

Adapted from Munoz & Everling, (2004).

LIP - Lateral intraparietal area, SEF - Supplementary eye fields, FEF - Frontal eye fields, dIPFC - Dorsolateral prefrontal cortex, SCi - Intermediate layer of the superior colliculus, LLBN - Long-lead burst neurons, OPN - Omnipause neurons, EBN - Excitatory burst neurons, IBN - Inhibitory burst neurons

saccades. Within the PPC is the lateral intraparietal area (LIP) which also contains a retinotopic saliency map, plays a role in sensorimotor integration and projects to the SCi (Bisley & Goldberg, 2003). Within the frontal lobe, the frontal eye fields (FEF) contribute to transforming visual signals into saccade commands, because they have both sensory and motor connections (Schall, 1997). Because the FEF are reciprocally connected with many other cortical regions, including the LIP, the middle temporal visual area (MT), the prefrontal cortex (PFC) and the temporo-occipital visual area (TEO), the FEF can modulate incoming sensory information and pass it on to downstream neurons in the SCi, the BG, the cerebellum and the reticular formation (Schall, 2002). The supplementary eye fields (SEF) and the dorsolateral prefrontal cortex (dlPFC), also within the frontal lobe, have been shown to have roles in working memory and decision-making. Both of these areas provide input to saliency maps by connecting to the SCi directly, and indirectly via the FEF. Additional contributions to the control of saccades come from both the cerebellum and the BG. The cerebellum plays a role in monitoring saccade accuracy, by steering and stopping saccades as they are ongoing. The cerebellum also plays a role in planning corrective saccades if necessary. The cerebellum exerts an influence on saccadic accuracy by innervating the EBN and IBN (Robinson *et al.*, 2002; Colnaghi *et al.*, 2010). The BG also influences the saccadic eye movement system via projections to the SCi. The BG helps to determine which saccades would be useful because it receives inputs strongly modulated by working memory, expectation of reward and attention (Hikosaka *et al.*, 2000). Because such a wide range of brain structures are

involved in oculomotor control, a brain injury which affects any of the structures can result in deficits in the performance of saccadic eye movement paradigms. These deficits can easily be quantified to better understand the neuropathology that is occurring.

#### **2.4.2 Saccadic eye movement tasks**

As addressed above, many brain regions, namely the cerebral cortex, hippocampus and cerebellum, are affected in children with 22q11.2 DS. The overlap of brain regions affected by the deletion and those involved in eye movement control suggest that saccadic eye movement behaviours may provide insight into the CNS dysfunction observed in children with 22q11.2 DS. Although eye movement paradigms have not yet been used to study children with 22q11.2 DS, they have been used to clarify the neuropathology of autism, ADHD, Tourette's syndrome and Fetal Alcohol Spectrum Disorders (FASD) (Sweeney *et al.*, 2004; Green *et al.*, 2007). In the current study, subjects performed four eye movement tasks that assessed the ability to generate different saccade types; prosaccades, antisaccades, delayed memory-guided saccades and predictive saccades.

Prosaccades are visually-guided saccades which are directed to a peripheral target. Because prosaccades are a natural behaviour, we can measure the saccadic reaction time (SRT) and the accuracy of the endpoint of the saccade which is a quantification of simple sensory-motor processing. The antisaccade task requires subjects to look away from a peripheral target. This unusual behavior requires two processes: 1) the suppression of the automatic prosaccade towards the target and 2) the generation of

an internally-driven antisaccade away from the target (Fukushima et al., 1988). Because this task places an increased cognitive demand on the subject, regions of the frontal cortex and basal ganglia are recruited to exert top-down control (Munoz & Everling, 2004). Pro- and antisaccade tasks have not previously been used to study behaviour in subjects with 22q11.2 DS. However, because these individuals have widespread neurological involvement including deficits in executive function, it seems reasonable to suggest that subjects with the deletion would make more errors and require increased processing time than control subjects.

Because spatial working memory and cognitive inhibition abilities are areas of specific weakness in 22q11.2 DS, a delayed memory-guided sequential (DMS) saccade task was also used. Subjects were required to generate two memory-guided saccades to locations where peripheral targets had previously appeared. Studies have found that the fronto-parietal network is chiefly responsible for the response inhibition, working memory, and generation of saccade sequences which are required to successfully perform this task (Corbetta & Shulman, 2002). Although children with 22q11.2 have not been tested on a memory-guided saccade task, children with attention deficit/hyperactivity disorder (ADHD), and adults with Huntington's disease and Parkinson's disease demonstrated deficits in response inhibition with the frequent initiation of early responses (Chan *et al.*, 2005; Mostofsky *et al.*, 2001; Peltsch *et al.*, 2008). Because children with 22q11.2 DS have shown deficits in spatial working memory and executive function, I hypothesized that they would show deficits in this task as well.

The way in which procedural learning was tested in this population was to administer a predictive task, in which subjects follow a target alternating between two known locations in the right or left hemi-field. To perform well in this task, subjects must adjust their motor responses to the predictable movement of stimuli with practice, thus reducing SRTs as the trials progress (Smit & Van Gisbergen, 1989). The FEF have a key role in predictive saccade generation; lesions in this area are known to impair predictive saccades and both positron emission tomography (PET) studies have revealed increased activity in these regions as predictive behaviours become apparent (Rivaud *et al.*, 1994; O'Driscoll *et al.*, 2000). Lesions of the cerebellum, where smooth pursuit movements are controlled, also have a detrimental effect on predictive saccades (Isotalo *et al.*, 1995). Patients with BG degeneration, including Parkinson's disease demonstrate difficulty generating predictive saccades, also implicating the basal ganglia in this process (Bronstein & Kennard, 1985; O'Driscoll *et al.*, 2000).

Because these four eye movement tasks provide objective and sensitive measures of sensory-motor processing, response inhibition, working memory, and procedural learning abilities, they provide an ideal opportunity to quantify the oculomotor behaviours that reflect both the automatic and higher-order cognitive abilities that are often deficient in the population with 22q11.2 DS.

## **2.5 Research rationale, hypotheses and objectives**

The neural circuitry involved in the control of eye movements overlaps with several brain regions that are significantly affected by 22q11.2 DS. These include many

parts of the cerebral cortex, the hippocampus, and the cerebellum. Previous studies using neuropsychological tests have documented deficits in executive function, spatial working memory, and cognitive flexibility, specifically in areas of sensory-motor processing and response inhibition in children with 22q11.2 DS. The goal of this research project was to characterize oculomotor behaviours in a previously untested population using eye movement paradigms that probed sensorimotor processing and executive function, procedural learning and working memory abilities.

Objective:

- To assess cognitive flexibility, spatial working memory, and motor learning in children with 22q11.2 DS using a remote eye tracking system.

Hypothesis:

Using a remote eye tracking system, children with 22q11.2 DS will:

- Demonstrate deficits in the prosaccade and antisaccade tasks, on measures of saccadic reaction time and increased direction errors.
- Show deficits in spatial working memory and response inhibition in the DMS task on measures of accuracy and errors.
- Exhibit deficits in motor learning in the predictive task as measured by deficient adjustment of reaction time with repetition.

## Chapter 3

### Materials and Methods

#### 3.1 Oculomotor control in children with 22q11.2 DS

All experimental procedures were reviewed and approved by the Research Ethics Boards at Queen's University and the Children's Hospital of Eastern Ontario. Typically developing subjects were recruited from the Kingston area and 22q11.2 DS patients were recruited from the patient population at the Children's Hospital of Eastern Ontario (CHEO) in Ottawa, Ontario. Prior to data collection, participants and parents/guardians were introduced to the experimental procedures and completed consent and personal information forms. Control subjects were excluded if they had any neurological, psychiatric or visual disorders, other than corrective lenses. As many children with 22q11.2 DS are prescribed a variety of pharmacological agents, medication history was collected. Participants were not asked to withhold any medications typically taken before the testing session as their effect on eye movement performance in other clinical populations has been unclear (Green et al., 2009a). Other medical history, including major surgical procedures and hospitalizations, was taken from the medical records of the participants. Children in the 22q11.2 DS group included both those in whom deletion was spontaneous, and those who had inherited the deletion from one of the parents. Subjects were tested at Hotel Dieu Hospital and at the CHEO. They received snacks (juice and granola bars) during the sessions and were allowed breaks when necessary. Participants received a \$10 gift card for the 1-hour session.

### **3.2 Saccadic eye movement recordings**

During the eye movement testing sessions, participants were seated comfortably in a dark, quiet room on a stable chair. A small target sticker was placed on their forehead as part of the remote eye tracking system. Eye movement recordings were obtained using the Eyelink 1000 (SR Research, Mississauga, ON) which was positioned in front of participants so that the 17" LCD monitor and mounted infrared camera were at a distance of 58 cm, as measured by the system, from the participant's left eye. The position of the left pupil was digitized in both the vertical and horizontal axes at a sampling rate of 500 Hz. Subjects performed four saccadic eye movement tasks: prosaccade, antisaccade, delayed memory-guided sequential and predictive. In both the pro- and antisaccade tasks, a central fixation point (FP) was illuminated for a randomized interval between 800 and 1200 ms to begin each trial. After a delay of 200 ms following the disappearance of the FP, a peripheral target (T) appeared at 10° to the left or right of the central FP. Participants were given a 1000 ms time-frame to initiate and complete a saccade. In the prosaccade task, participants were instructed to look towards the T as soon as it appeared (Fig. 3.1A); while in the antisaccade task, participants were instructed to look away from the T and towards the opposite side of the screen (Fig. 3.1B). One block of 60 trials was collected for the prosaccade task and either one or two blocks of 60 trials each of antisaccades were obtained.

For both tasks, saccadic reaction time (SRT) was defined as the time from the appearance of the peripheral T to the initiation of the first saccade that exceeded 30°/s. Intrasubject variability was assessed by calculating the coefficient of variation (CV) for

each individual subject (using the standard deviation of the SRT divided by the mean). Saccades initiated at 90-140 ms after T appearance were defined as express saccades, the shortest latency visually-guided saccades (Fischer et al., 1993). Saccades generated less than 90 ms after the appearance of T were classified as anticipatory saccades. Direction errors were defined as saccades initiated in the wrong direction and with a velocity exceeding  $30^{\circ}/s$  with respect to the instruction (i.e., away from the T in the prosaccade task; towards the T in the antisaccade task). Additionally, the error of the saccade trajectory was measured (in degrees) as the angle between a direct path to the target and the trajectory of the first saccade in the correct direction. A trial was marked as containing step saccades if it took multiple saccades to reach the target (Fig. 3.1A).

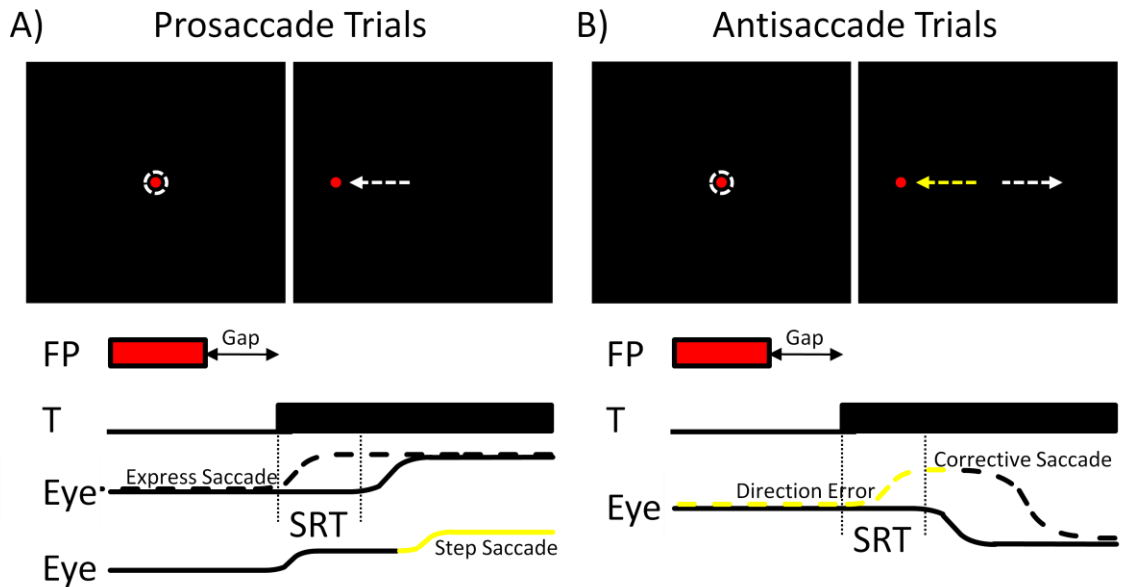
In the DMS task, subjects were instructed to look at a central FP (time length of FP = 200-1000 ms, randomly distributed) while peripheral targets appeared. The screen was divided into four quadrants in which the peripheral T could appear. Each quadrant consisted of 9 potential T locations in a 3 by 3 grid centered at a  $10^{\circ}$  visual angle from the FP. Two targets were illuminated in succession for 100 ms each within two of the four quadrants of the screen. A delay period of 0, 600, 1200, or 1800 ms between the disappearance of the second peripheral target and the disappearance of the FP was used (Fig.3.2). The participants were instructed to remember the order and spatial location of the peripheral targets, and to make two saccades as accurately as possible to these locations in the same sequence after the disappearance of the FP. One or two blocks of 72 trials were completed in this task. Outcome measures for the DMS task included SRT of

both the first and second saccade, defined as the time from the disappearance of the FP and the initiation of a saccade that exceeded 30°/s. Trials were assigned as either correct, timing errors (subject initiated the saccade sequence before the go signal), or sequence errors (subject made saccades to the peripheral T locations in the incorrect order). Trials could also be combined errors where both sequence and timing errors occurred.

Additionally, saccades were assessed for accuracy, measured in degrees from the closest fixation point to the actual peripheral target location.

In the predictive saccade task, a central FP (illuminated for a random interval between 1000 and 1500 ms) appeared, after which 12 peripheral T alternated between two fixed locations 10° to the right and left of the FP (Fig. 3.3). Targets alternated at either specific inter-stimulus intervals (ISIs; blocked trials) or pseudo-randomly (interleaved trials). The blocked condition of the task was comprised of 15 trials at each ISI of 750 ms and 1000 ms (total 30 trials). The interleaved condition comprised 15 trials with stimuli alternating randomly at one of the following time intervals: 500, 750, 1000, 1250, or 1500 ms.

Participants were instructed to move their eyes in time with the dots without missing any of the 12 stimuli. Outcome measures for the predictive task included the SRT of saccades made to each of the twelve stimuli. SRTs from each stimulus were collapsed and further subdivided into four different saccade categories: anticipatory (saccades made more than 300ms before the appearance of the stimulus) predictive (saccades made between 300ms before and 100ms after the appearance of the stimulus -



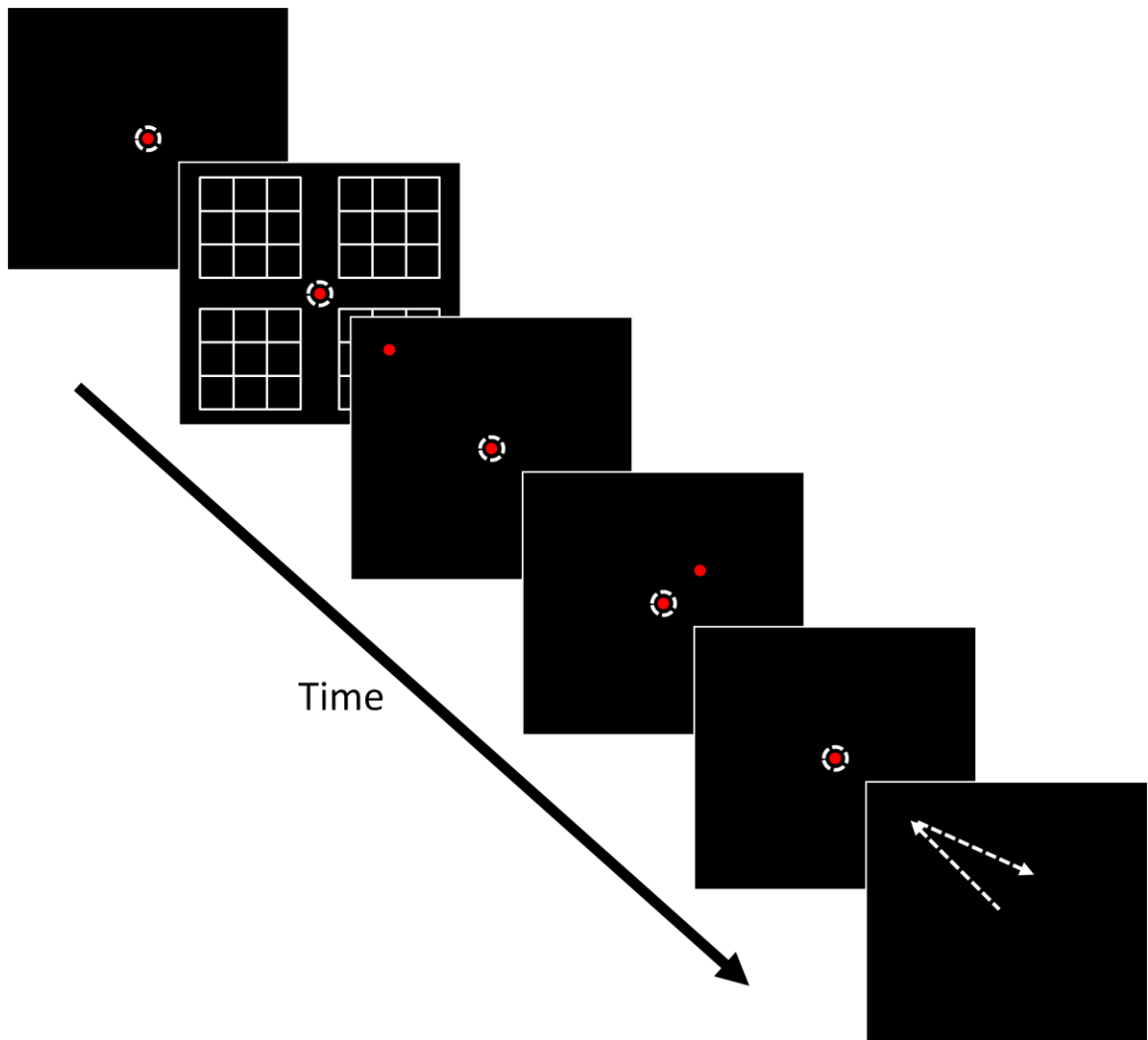
**Figure 3.1 Prosaccade and antisaccade task paradigms.**

(A) Prosaccade: subjects look from the fixation point (FP) to the peripheral target (T).

Express saccades, as well as regular latency saccades, are often generated. (B)

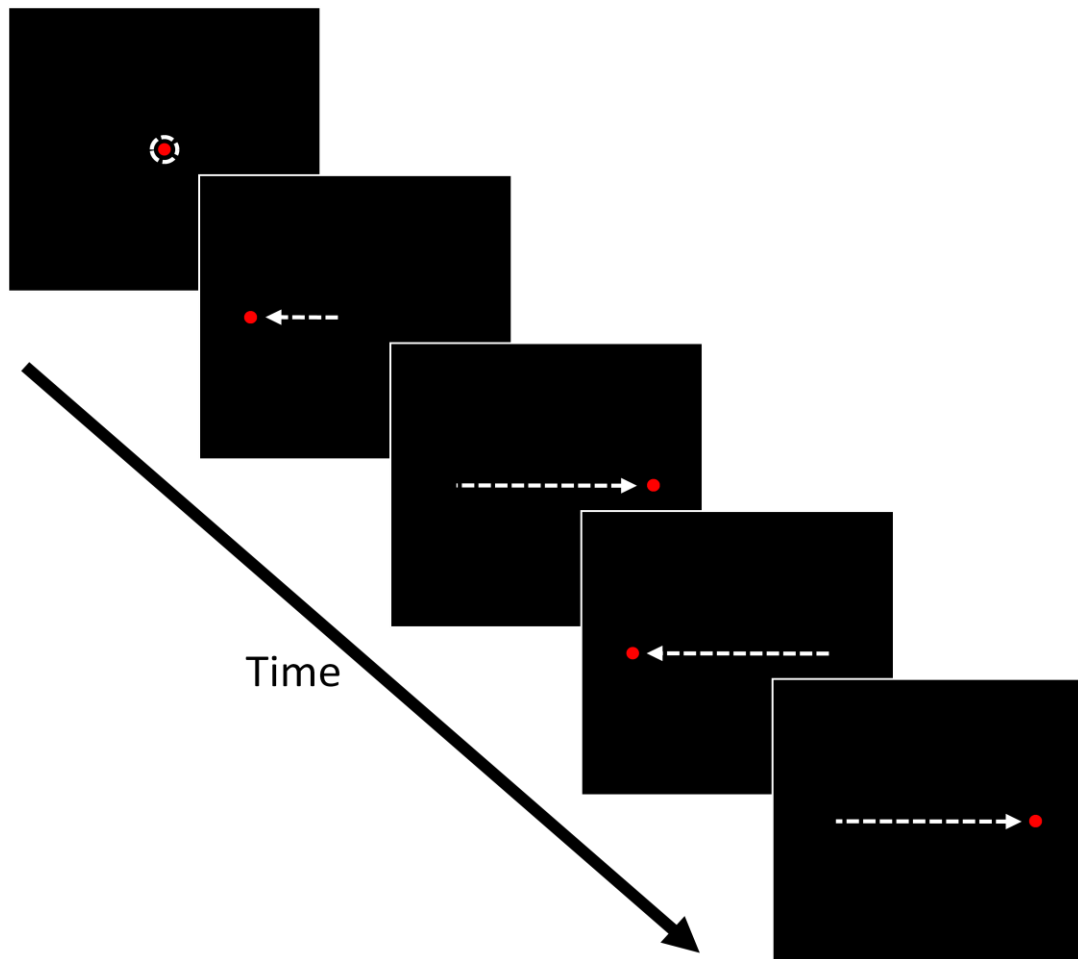
Antisaccade: subjects look from the FP to the opposite side of the screen to the T.

Direction errors are generated when subjects look towards the T; however they are often altered by a corrective saccade.



**Figure 3.2 Delayed memory-guided sequential saccade paradigm.**

The subject begins at a central fixation. Each quadrant of the screen is divided into 9 possible peripheral target locations in which two peripheral targets flash sequentially, each for 100 ms. After the disappearance of the central FP, the subject must initiate two saccades to the remembered target locations in the same sequence in which they flashed.



**Figure 3.3 Predictive saccade task paradigm.**

The subject begins at a central fixation point. The T then alternates between two fixed locations  $10^\circ$  from centre, for a total of 12 peripheral stimuli. Blocked trials consist of regular ISIs (either 750 ms or 1000 ms), while interleaved trials incorporate pseudo-randomized timing.

therefore not visually guided), express (100-150 ms after the appearance of the stimulus), and regular (>150 ms after the appearance of the stimulus). The distribution of saccade type was quantified for each individual, which allowed the analysis of velocity and amplitude by saccade type.

### **3.3 Statistical analysis of eye movement data**

Differences between groups were analyzed using unpaired *t*-tests if the values passed the D'Agostino & Pearson omnibus normality test. If not, a Wilcoxon signed rank test was used. Effect sizes were also calculated for the dependent variables using Cohen's *d* scores. An effect size below 0.2 was considered small, between 0.2 and 0.8 was considered medium, and an effect size above 0.8 was considered large (Cohen, 1988). Specifically in the DMS task, two-way repeated measures analysis of variance (ANOVA) was used to examine the effect of delay on outcome measures, with the two independent variables being group (control versus 22q11.2 DS) and delay (0-1800 ms). In the predictive task, two-way repeated measures ANOVA was used to examine the effect of stimulus and group on SRT and to examine the occurrence of predictive saccades between groups and ISI. Additionally, Pearson correlations were used in this task to determine the relationship between the generation of predictive saccades and regular saccades for each individual. Finally, amplitudes and velocities were analyzed across saccade category using two-way ANOVA with Bonferroni post-hoc tests.

### **3.4 Saccadic eye movement tasks**

All children participating in the study were tested using the eye movement task paradigms described in section 3.1.1. Due to the challenging nature of the DMS task, as well as the decreased ability to sustain attention in the clinical population, not all participants were able to complete all four paradigms. Of the 16 22q11.2 DS children involved in the study, 16 performed the pro- and antisaccade tasks, 15 performed the predictive task, and 9 performed the DMS task during the testing session. Of the 32 control children participating in the study, 32 performed the pro- and antisaccade tasks, 26 performed the predictive task, and 28 performed the DMS task during the testing session. Some of the control children performed a different version of the predictive task than that described above, and were therefore excluded from the analysis.

## Chapter 4

### Results

#### 4.1 Demographic Information

Demographic information was collected for both the control and the test groups (Table 4.1; 22q11.2 DS n=16, age=11.1±1.8; Control n=32, age=11.0±0.6). The 22q11.2 DS subjects were all diagnosed by FISH prior to being recruited (age diagnosed = 4.0±2.0 years). As many children with 22q11.2 DS are prescribed a variety of pharmacological agents, medication history was collected. Of the 16 children with 22q11.2 DS, 3 were regularly taking stimulant medications (i.e. Ritalin®, Concerta®), while 5 were taking other medications (i.e. antipsychotics, anticonvulsants, antidepressants). It was also determined that 3 of the patients inherited their deletion from one of their parents while 12 had a spontaneous deletion. One patient did not have their biological parents tested for the deletion. Information on comorbidities prevalent in the 22q11.2 DS group was also collected, with abnormal facies, cardiac abnormalities and palate malformations affecting over 50% of the patient group (Table 4.1). There was a significant difference in the parent/caregiver level of education (Table 4.1; 22q11.2 DS years education=14.5±0.6; Control years education=17.4±0.4,  $p<0.0001$ ), which was collected to indicate the socio-economic status of the two experimental groups.

## **4.2 Saccadic eye movement tasks**

### **4.2.1 Prosaccade task**

Correct trials were defined as trials where the initial saccade was made in the direction of the target. In the prosaccade task, children in 22q11.2 DS group did not differ from age- and sex-matched controls in the number of correct trials (Fig. 4.2A;  $p=0.77$ ) measures of SRT (Fig.4.1A & 4.2C;  $p=0.27$ ), CV of SRT ( $p=0.26$ ), or direction errors (Fig. 4.1A;  $p=0.46$ ). Differences were not observed in additional measures of saccade metrics (Table 4.2; express saccades, velocity) or performance (Table 4.2; number correct trials, percent anticipatory errors). The amplitude of the saccade was defined as the angle of rotation of the eye toward the target. The error of saccade trajectory was measured as the difference in angle between the initial saccade made by the subjects and the optimal path to the target. Children in the 22q11.2 DS group exhibited an increase in the amplitude of saccades to the peripheral target (Fig. 4.2G;  $p=0.004$ ) and greater error of saccade trajectory (Fig. 4.2E;  $p=0.002$ ) compared to controls. Effect size analysis using the Cohen's  $d$  value, revealed a very large effect size of saccade trajectory (Table 4.3;  $d=1.35$  (effect size  $r = 0.53$ )).

### **4.2.2 Antisaccade task**

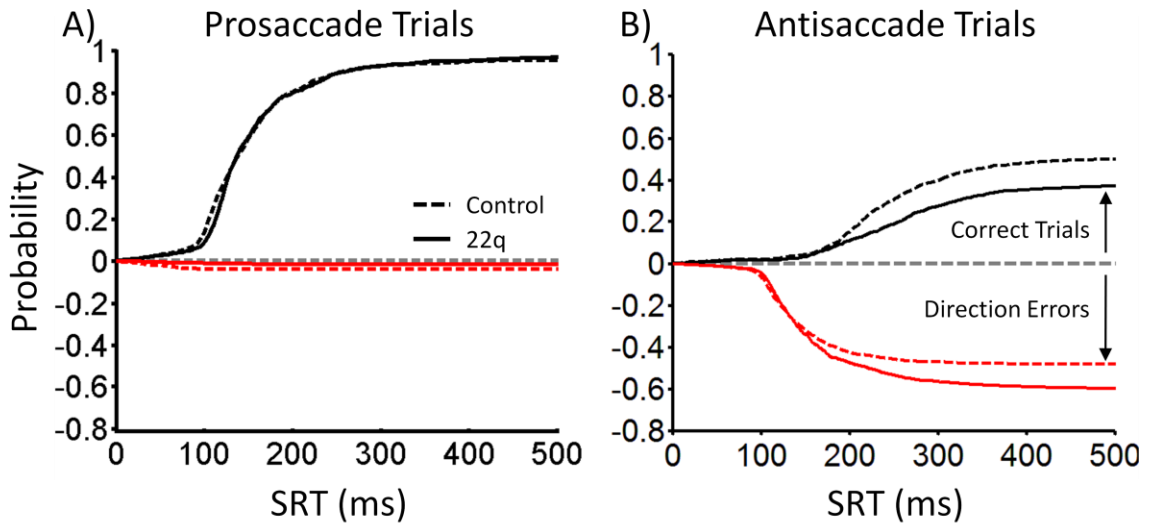
In the antisaccade task, children in the 22q11.2 DS group completed the same number of correct trials as controls (Table 4.2;  $p=0.58$ ). However, in comparison to controls, children in the 22q11.2 DS group had increased SRT (Fig. 4.2D;  $p=0.04$ ), greater magnitude of errors in saccade trajectory (Fig. 4.2F;  $p<0.001$ ), and increased

saccade amplitude (Fig. 4.2H;  $p < 0.05$ ) and saccade velocity (Table 4.2;  $p = 0.02$ ). No differences were observed in measures of anticipatory errors or express saccades (Table 4.2). As direction errors in the antisaccade task are highly age-dependent (Fischer *et al.*, 1997) performance in this task was age-corrected by calculating the residuals from the linear regression line obtained for control subjects (Fig. 4.3A). This analysis revealed that, independent of age, the 22q11.2 DS group had significantly more direction errors than the control group (Fig 4.3B;  $p = 0.02$ ).

**Table 4.1 Demographic information for control and 22q11.2 DS groups**

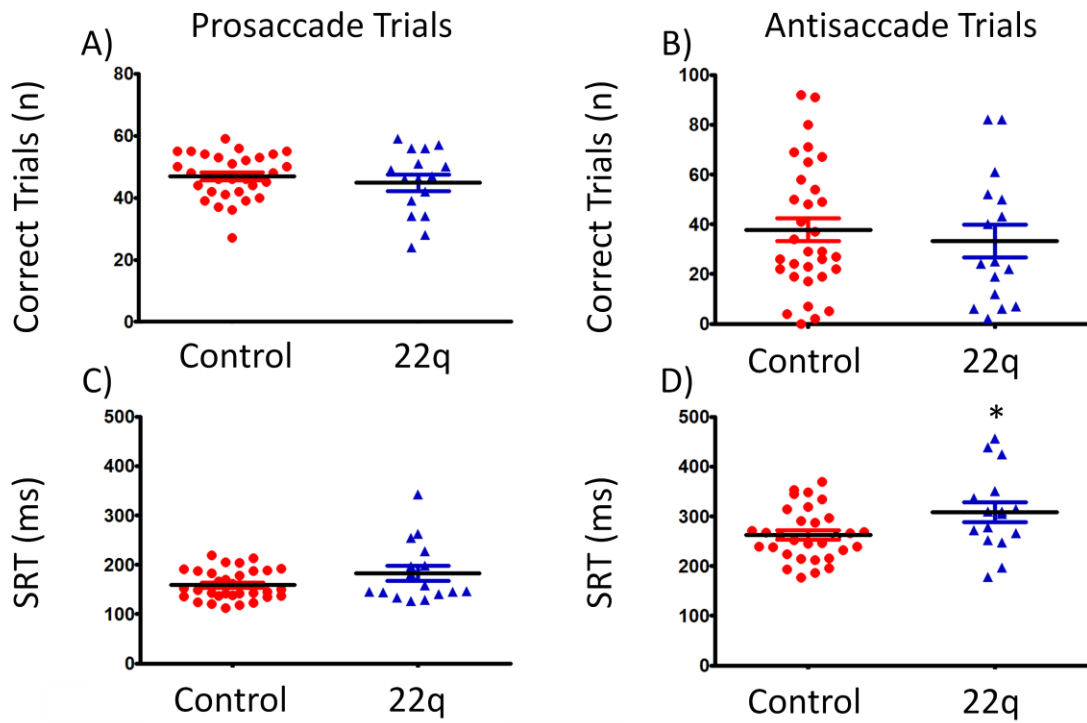
<b>Category</b>	<b>Control (32)</b>	<b>22q (16)</b>
Age $\pm$ SEM (years)	11.0 $\pm$ 0.6	11.1 $\pm$ 1.8
Male : female	18:14	9:7
Parent mean years education $\pm$ SD	17.4 $\pm$ 0.4	14.5 $\pm$ 0.6*
Living with biological parents, <i>n</i> (%)	31 (97)	15 (94)
Parents employed, <i>n</i> (%)	61 (95)	30 (94)
<b>Diagnosis</b>		<b>n (%)</b>
By FISH:		16 (100)
Hereditary		3 (19)
<i>de novo</i> deletion		12 (75)
Unknown		1 (6)
<b>Medication</b>		<b>n (%)</b>
Stimulants		3(19)
Other		5(31)
Unknown		1(6)
<b>Co-morbidities</b>		<b>n (%)</b>
Cardiac Abnormalities		11 (69)
Abnormal Facies		13 (81)
Thymic Aplasia		2 (13)
Palate Malformations		9 (56)
Hypocalcemia		3 (19)
Hearing Impairment		4 (25)
ADHD		4 (25)
Seizure disorder		1 (6)

*Other* drugs include antipsychotics, antidepressants, and anticonvulsants. Medication information was unavailable for one participant. None of the children in the control group were on medications or reported co-morbidities. In the chart, ‘parent’ refers to either the parent or care-giver of the child.



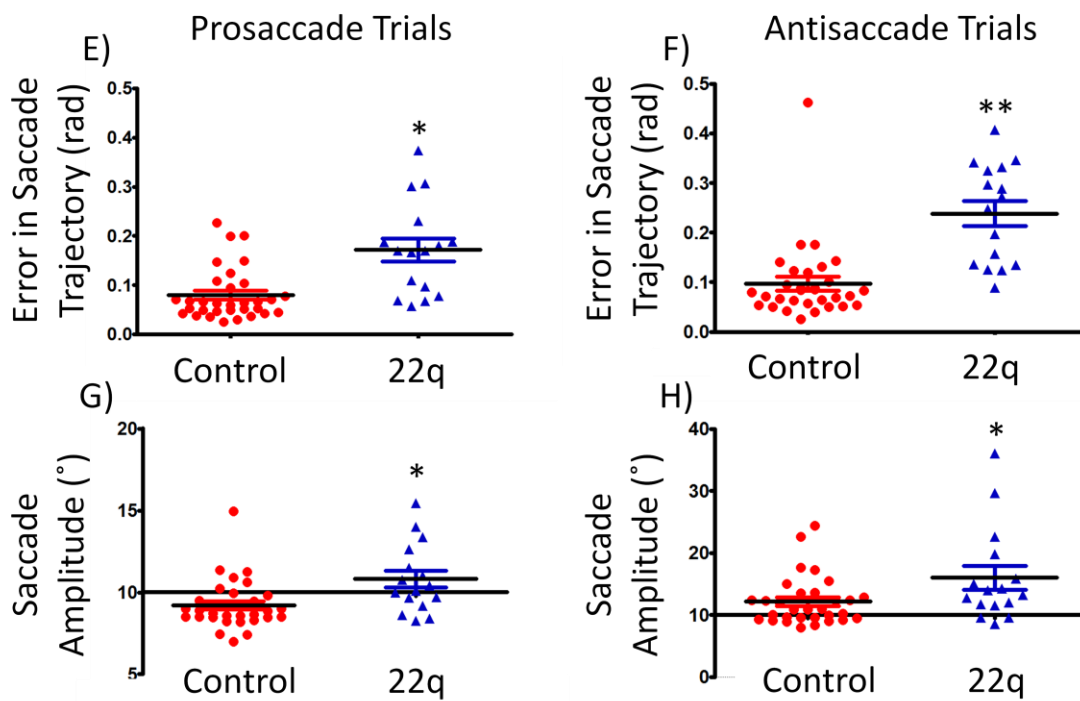
**Figure 4.1 Prosaccade and antisaccade task cumulative frequencies.**

Cumulative distribution of saccadic reaction times (SRTs) for correct trials (positive values) and direction errors (negative values) for the prosaccade (A) and the antisaccade (B) tasks comparing control (dashed lines) and 22q11.2 DS (solid lines) children.



**Figure 4.2 Prosaccade and antisaccade task parameters.**

Comparisons between 22q and control groups in the prosaccade and antisaccade tasks included (A)&(B) number correct trials, (C)&(D) SRT \* $p < .05$ , \*\* $p < .0001$

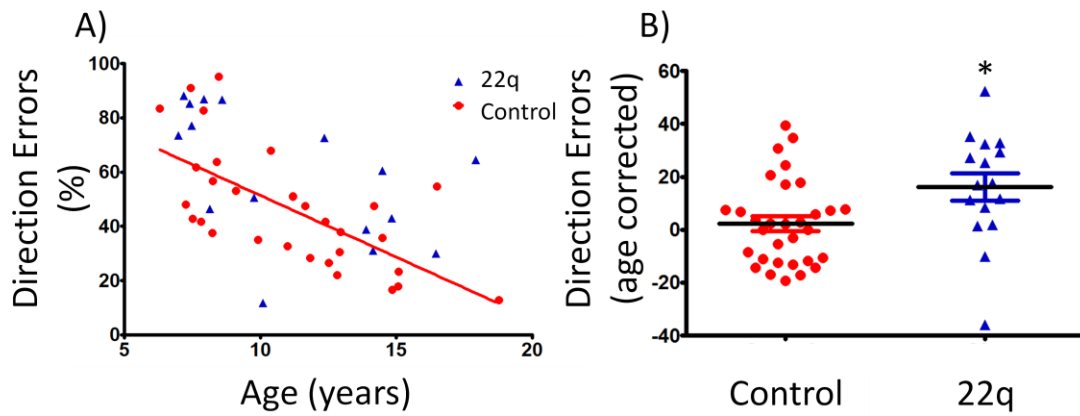


**Figure 4.3 Prosaccade and antisaccade task parameters.**

Comparisons between 22q and control groups in the prosaccade and antisaccade tasks included (E)&(F) the error in saccade trajectory, (G)&(H) Saccade Amplitude \* $p < .05$ , \*\* $p < .0001$

**Table 4.2 Saccade parameters for control and 22q11.2 DS groups**

<b>Task</b>	<b>Parameter</b>	<b>Control (32) Mean±SEM</b>	<b>22q (16) Mean±SEM</b>	<b><i>p</i>-value</b>
<b>Prosaccade</b>	Anticipatory Errors (%)	12.86±1.56	8.37±1.62	0.08
	Express Saccades (%)	50.05±4.05	47.15±6.83	0.70
	Saccade Velocity (°/s)	409.0±46.26	406.9±38.84	0.49
	Correct Trials (n)	46.91±1.25	44.88±2.65	0.43
<b>Antisaccade</b>	Anticipatory Errors (%)	7.61±1.44	6.52±1.77	0.65
	Express Saccades (%)	4.02±1.26	3.08±1.26	0.63
	Saccade Velocity (°/s)	524.8±80.78	1122±291.6	0.58
	Correct Trials (n)	37.72±4.58	33.31±6.58	0.02
<b>DMS (T1)</b>	CV of SRT	48.82±1.85	33.30±6.20	0.04
	Saccade Accuracy (°)	1.83±0.15	1.93±0.17	0.74
<b>DMS (T2)</b>	CV of SRT	31.17±1.48	34.16±5.61	0.62
	Saccade Accuracy (°)	2.18±0.22	2.10±0.17	0.77



**Figure 4.4 Antisaccade direction errors**

Direction errors for the antisaccade task were analyzed as a function of (A) age. Then the group comparison was performed after (B) correcting for age.  $*p < .05$

**Table 4.3 Effect sizes of eye movement task parameters.**

<b>Task</b>	<b>Parameter</b>	<b>Cohen's <i>d</i></b>	<b>Effect size <i>r</i></b>	<b>Effect size classification</b>
<b>Prosaccade</b>	Error of saccade trajectory	1.35	0.53	Large
	Saccade Amplitude	0.95	0.41	Large
<b>Antisaccade</b>	Error of saccade trajectory	1.66	0.62	Large
	Saccade Amplitude	0.72	0.32	Medium
	SRT	0.73	0.33	Medium
	Saccade Velocity	0.79	0.35	Medium
	Direction errors (age corrected)	0.79	0.35	Medium
<b>DMS</b>	% Step Saccades	1.12	0.43	Large
	Path Length	2.13	0.67	Large
	Timing Errors	1.01	0.40	Large
	Sequence Errors	0.01	0.004	ns
	Timing and sequence errors	1.43	0.52	Large
<b>Predictive</b>	750 ms – regular saccades	0.62	0.29	Medium
	1000 ms – regular saccades	1.32	0.54	Large
	Interleaved - predictive saccades	0.25	0.12	Medium
	Interleaved – regular saccades	-1.85	0.67	Large

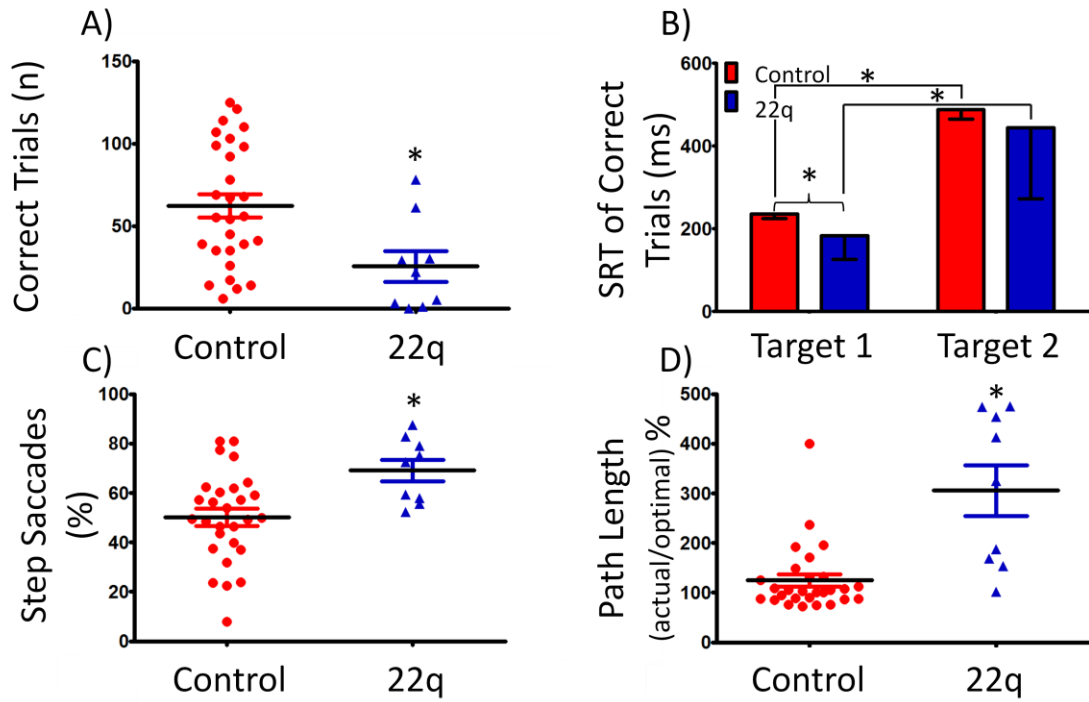
### 4.2.3 Delayed memory-guided sequential task

The percent of trials completed correctly in this task was analyzed using a two-way repeated measures ANOVA, with the dependent variables being group and delay. This analysis revealed an effect of group ( $F(1,134)=22.22, p<0.0001$ ) but no effect of delay ( $F(3,134)=2.27, p=0.08$ ) on the percent of correct trials. There was no interaction between the two variables ( $F(3,134)=0.44, p=0.73$ ). When evaluating correct trials exclusively, a Mann-Whitney U-test revealed that the control group had a higher SRT toward the first target than the 22q subjects (Fig. 4.4B;  $p=0.008$ ). The SRT toward the second target was not significantly different between the two groups (Fig. 4.4B;  $p=0.09$ ). Additionally, the CV of SRT to the first target was significantly higher for the control group than it was for the 22q group (Table 4.2;  $p=0.04$ ), but there was no group difference for the CV of SRT to the second target (Table 4.2;  $p=0.62$ ). Groups did not differ in the accuracy of correct saccades directed to either the first (Table 4.2;  $p=0.74$ ) or second (Table 4.2;  $p=0.77$ ) target. The 22q11.2 DS patients performed significantly fewer correct trials than the control group (Fig. 4.4A;  $p=0.008$ ). The patients also made step saccades in a significantly higher percent of trials than their control counterparts (Fig. 4.4C;  $p=0.007$ ). Also, the 22q11.2 DS patients were significantly less efficient during the DMS task, with a much greater path length (Fig. 4.4D;  $p<0.0007$ ).

The types of errors made during performance of the DMS task were analyzed. 22q11.2 DS patients did not differ from the control group in the percentage of sequence errors (Fig. 4.5A). However, the 22q11.2 DS group made more timing (Fig. 4.5C) errors in the DMS task. The types of errors were also analyzed using a two-way repeated

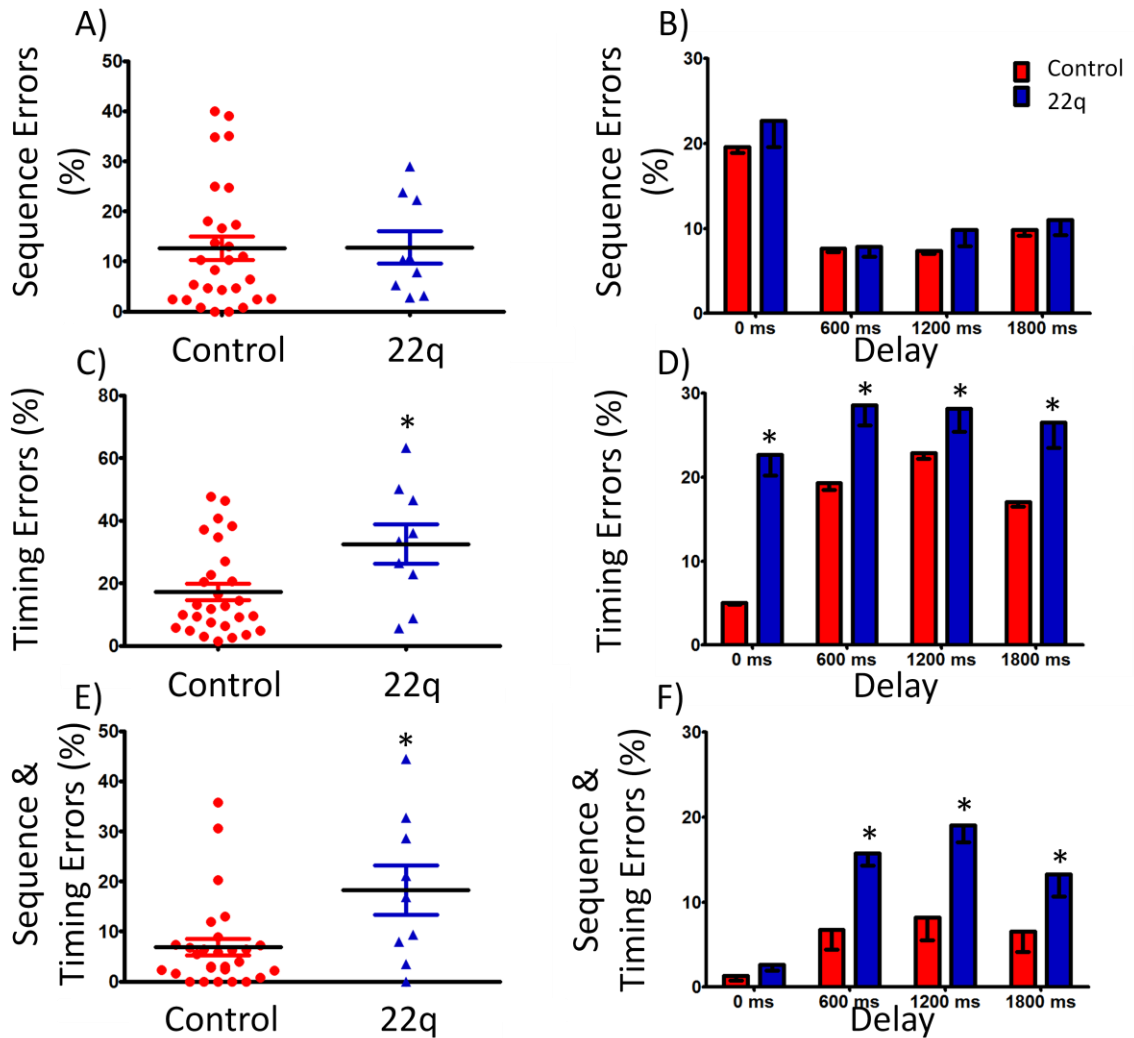
measures ANOVA, with the dependent variables being group and delay. Sequence error analysis revealed no effect of group (Fig 4.5 B;  $F(1,133)=0.0003$ ,  $p=0.99$ ) nor an interaction between the variables ( $F(3,133)=0.047$ ,  $p=0.99$ ). However, the ANOVA did reveal an overall effect of delay on the percentage of sequence errors ( $F(3,133)=6.32$ ,  $p=0.0005$ ). Conversely, timing error analysis revealed a significant effect of group ( $F(1,134)=13.05$ ,  $p=0.0004$ ) but not delay (Fig. 4.5D;  $F(3,134)=1.04$ ,  $p=0.37$ ). There was also no interaction between the two variables ( $F(3,134)=1.55$ ,  $p=0.20$ ). The trials where both sequence and timing errors were made were also analysed. There was a significant effect of delay ( $F(3,134)=6.57$ ,  $p=0.0004$ ) and group (Fig. 4.5F;  $F(1,134)=18.35$ ,  $p<0.0001$ ), but no interaction between the variables ( $F(3,134)=1.55$ ,  $p=0.21$ ).

When considering the direction of saccades elicited in trials with timing errors, children in the 22q11.2 DS group made more first saccades toward the second target, although this effect was not significant (Fig. 4.6;  $p=0.08$ ). Effect size analyses revealed large effect sizes in number of timing errors (Table 4.1;  $d=1.01$ ), sequence and timing errors (Table 4.1;  $d=1.43$ ), percent of trials containing step saccades (Table 4.1;  $d=1.12$ ) and path length (Table 4.1;  $d=2.13$ ).



**Figure 4.5 Saccade parameters for the DMS task**

(A) The number of correct trials and (B) the corresponding SRT for each target in these trials. (C) The percent of trials containing step saccades. (D) The path length for each subject as a measure of planning efficiency. \* $p < .05$



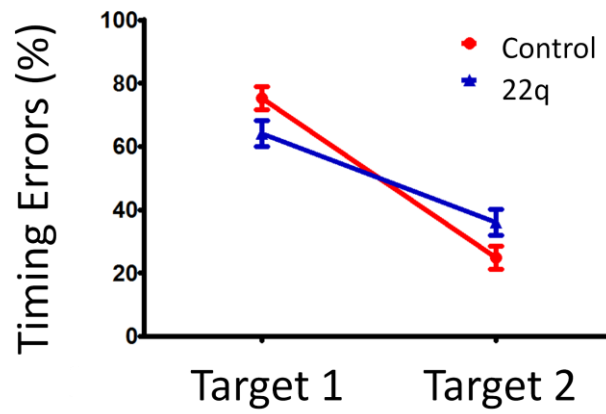
**Figure 4.6 Sequence and timing errors of the DMS task.**

(A) The percent sequence errors in the DMS task for all trials and (B) grouped by delay.

(C) The percent timing errors for all trials and (D) grouped by delay. (E) The percent

sequence and timing errors in the DMS task for all the trials and (F) grouped by delay.

\* $p < .05$



**Figure 4.7 Timing errors of the DMS task.**

Timing errors were classified by the direction of the first saccade.

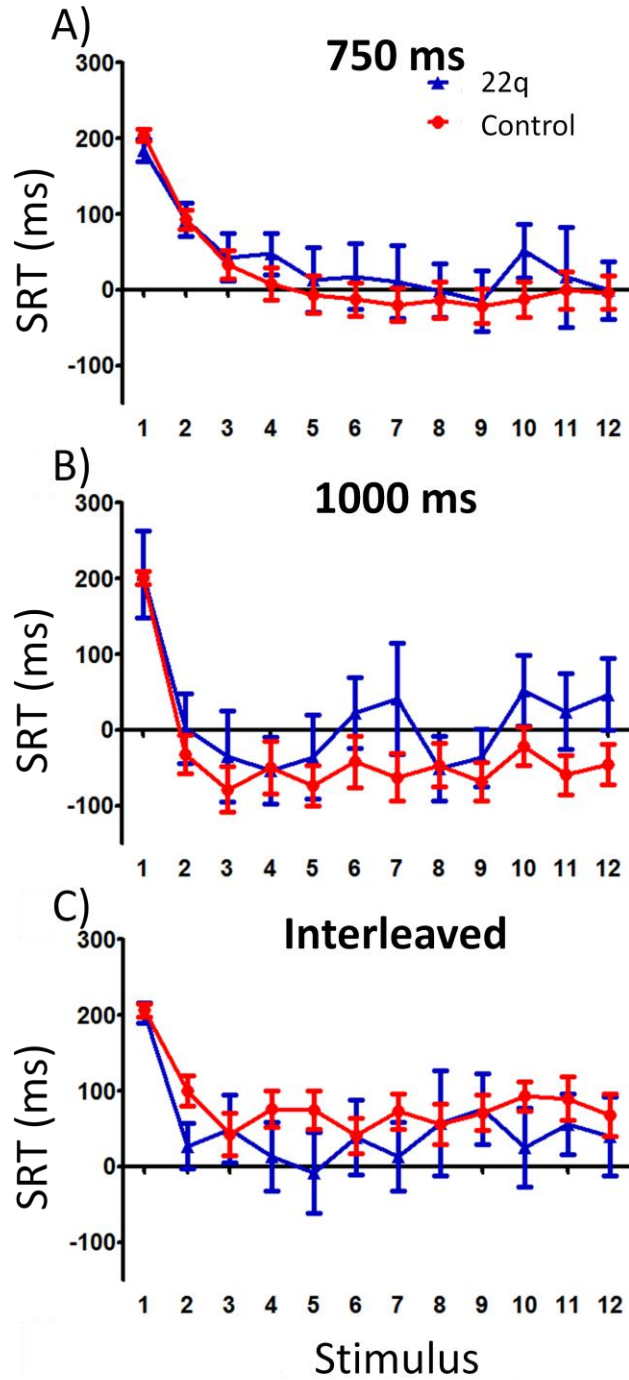
#### 4.2.4 Predictive saccade task

In the predictive saccade task, two-way repeated measures ANOVA, with group and stimulus number as dependent variables, revealed that SRTs did not differ between groups in the blocked trials with ISI of 750 ms (Fig. 4.7A;  $F(1,456)=2.31, p=0.13$ ). However, the stimulus number had a significant effect, showing that both groups learn to predict the stimuli over time ( $F(11,456)=8.63, p<0.0001$ ). There was no interaction between the dependent variables ( $F(11,456)=0.28, p=0.99$ ). With ISI of 1000 ms, however, children in the 22q11.2 DS group had significantly longer SRTs than the control group (Fig. 4.7B;  $F(1,456)=9.30, p=0.002$ ). There was also an effect of stimulus number, again indicating that both groups modify their behaviour over time ( $F(11,456)=7.15, p<0.0001$ ). Again, there was no interaction between patient group and stimulus in the trials with an ISI of 1000 ms ( $F(11,456)=0.49, p=0.91$ ). For the interleaved trials, analysis revealed that the 22q11.2 DS subjects had significantly shorter SRTs than the control group (Fig. 4.7C;  $F(1,468)=5.90, p=0.02$ ). The stimulus number continued to have a significant effect on SRT ( $F(11,468)=3.69, p<0.0001$ ). There was still no interaction between the two dependent variables ( $F(11,468)=0.52, p=0.89$ ). Histograms for both the control and 22q11.2 DS groups revealed a bimodal distribution of SRTs, in both of the blocked trial conditions (see Fig. 4.8 for an example with ISI 750 ms). Based on the distribution of SRTs, saccades were categorized into four groups: anticipatory ( $SRT < -300$  ms), predictive ( $-300 \text{ ms} < SRT \leq 100$  ms), express ( $100 \text{ ms} < SRT \leq 150$  ms), or regular ( $SRT > 150$  ms). Analysis of the percent of saccades in each of these categories revealed no significant difference between the control and 22q11.2 DS

groups. Two-way repeated measures ANOVA revealed an effect of ISI ( $F(2,78)=64.55$ ,  $p<0.0001$ ) on the generation of predictive saccades, along with an interaction between ISI and the subject group (Fig. 4.10A;  $F(2,78)=3.67$ ,  $p=0.03$ ). For both the blocked groups of trials, the control group made a higher percentage of predictive saccades than the 22q11.2 DS group, however, in the interleaved trials, the 22q11.2 DS subjects appeared to make predictive saccades to the unpredictable targets. In the interleaved trials, both groups generated similar proportions of predictive saccades. The two-way repeated measures ANOVA also showed a significant effect of ISI (Fig. 4.10B;  $F(2,78)=30.06$ ,  $p<0.0001$ ) on the generation of regular saccades as well an interaction between the subject group and ISI which approached significance ( $F(2,78)=2.93$ ,  $p=0.06$ ). For both the blocked groups of trials, the group of children with 22q11.2 DS made more regular saccades than the control group. With the interleaved trials, however, this effect appeared to be reversed, with the 22q11.2 DS group making fewer regular saccades. Additionally, a negative correlation was found between the percent of regular saccades and the percent of predictive saccades for both 22q11.2 DS (Fig. 4.11A;  $r=-0.87$ ,  $p<0.0001$ ) subjects and controls (Fig. 4.11A;  $r=-0.93$ ,  $p<0.0001$ ) in the 750 ms ISI. There was a significant negative correlation for the 22q11.2 DS subjects (Fig. 4.11B;  $r=-0.64$ ,  $p<0.0001$ ), and for the control subjects (Fig. 4.11B;  $r=-0.39$ ,  $p<0.01$ ) during the 1000 ms ISI trials. During the interleaved trials, there was a negative correlation between the regular and predictive saccades for both the control group (Fig. 4.11C;  $r=-0.70$ ,  $p<0.0001$ ) and the 22q11.2 DS group (Fig. 4.11C;  $r=-0.73$ ,  $p<0.002$ ).

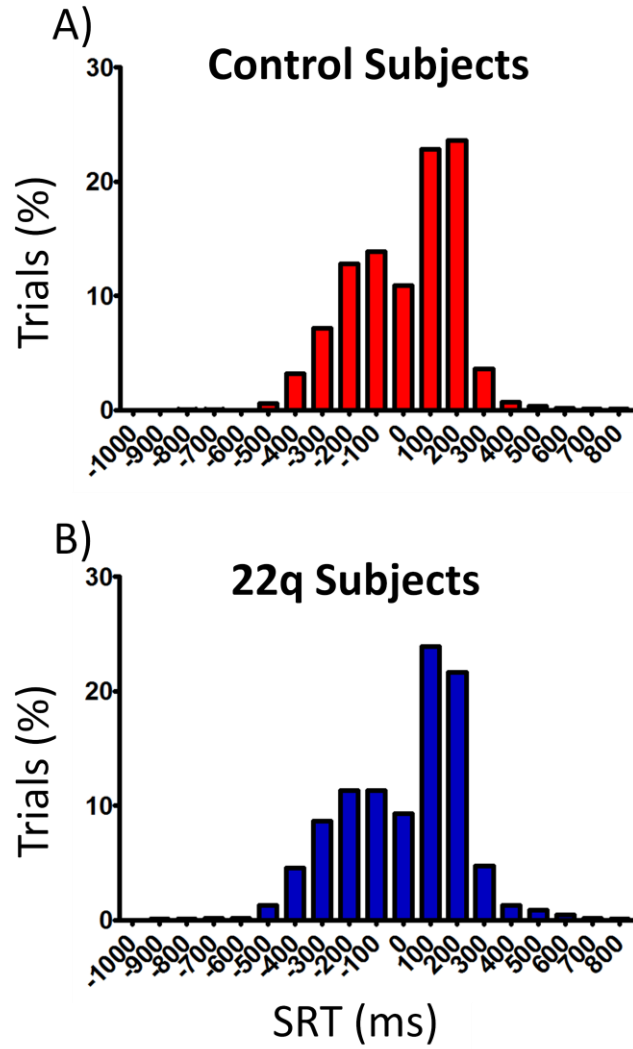
In addition, cumulative percent distributions of amplitudes for all saccades elicited by each group were separated by saccade type. In the 750 ms ISI trials, a two-way ANOVA revealed an effect of group on amplitude (Fig. 4.12A;  $F(1,148)=6.66$ ;  $p=0.01$ ), which was driven by overshooting saccades from the 22q11.2 DS group. In the 1000 ms ISI trials, neither the group nor the type of saccade had an effect on saccade amplitude (Fig. 4.12B). In the interleaved trials, there was a significant effect of group (Fig. 4.12C;  $F(1,151)=10.68$ ,  $p<0.001$ ).

Lastly, cumulative percent distributions of velocity were considered within each saccade category. To control for the effect of amplitude on peak velocity, only saccades with amplitudes within the 18-21° range were included in the analysis. Two-way ANOVA with group and saccade type as the independent variables revealed an effect of saccade type on peak velocities in the 750 ms ISI condition (Fig. 4.13A;  $F(3,1712)=13.74$ ,  $p<0.0001$ ), the 1000 ms condition (Fig. 4.13B;  $F(3,1693)=3.08$ ,  $p=0.03$ ) and the interleaved condition (Fig. 4.13C;  $F(3,1985)=3.98$ ,  $p=0.008$ ). The two-way ANOVA revealed that the group of children with 22q11.2 DS had significantly lower saccade velocity than the control group in the 750 ms ISI condition (Fig. 4.13A;  $F(1,1712)=72.35$ ,  $p<0.0001$ ), the 1000 ms condition (Fig. 4.13B;  $F(1,1693)=69.50$ ,  $p<0.0001$ ) and the interleaved condition (Fig. 4.13C;  $F(1,1985)=58.97$ ,  $p<0.0001$ ). The control and 22q11.2 DS groups have the same saccade velocity for anticipatory saccades. For predictive, express and regular saccades, the children with 22q11.2 DS had lower saccade velocities than their control counterparts.



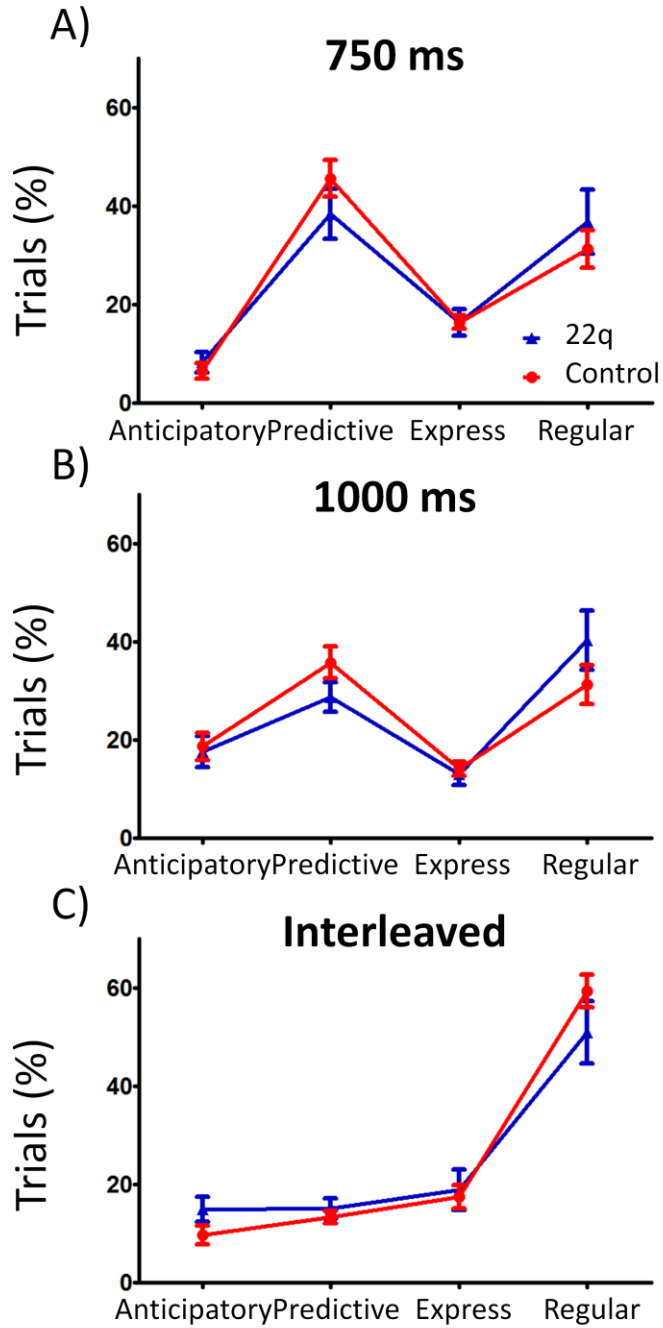
**Figure 4.8 Predictive task SRT.**

Saccadic reaction time to each peripheral stimulus for both the control (red) and 22q (blue) groups in the blocked trials (A, B) and the interleaved trials (C).



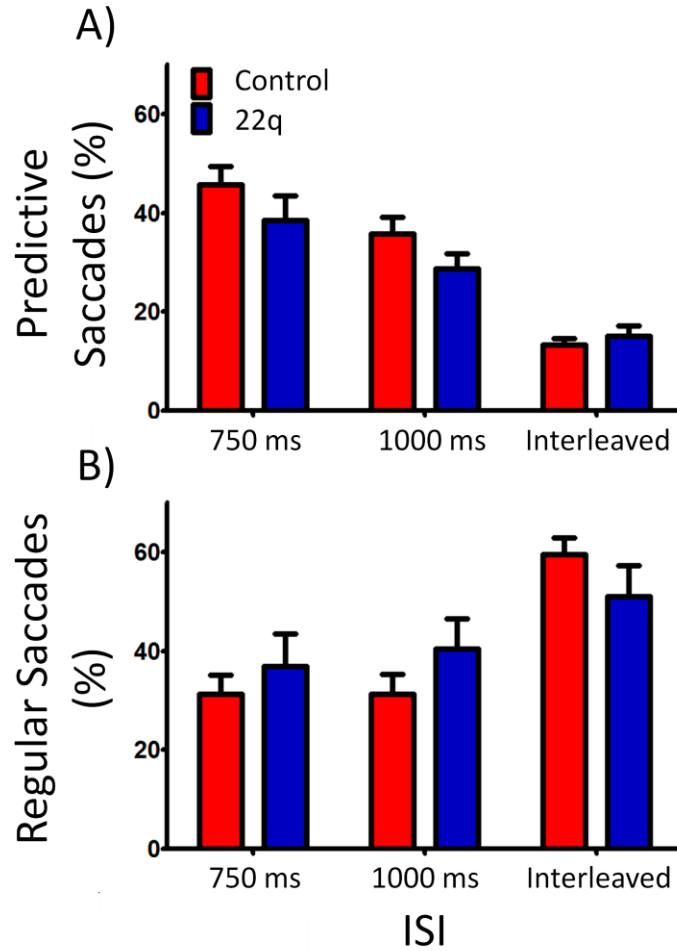
**Figure 4.9 Predictive task SRT distribution.**

Distributions of SRTs for the 750 ms ISI condition for both control (A) and 22q (B) groups.



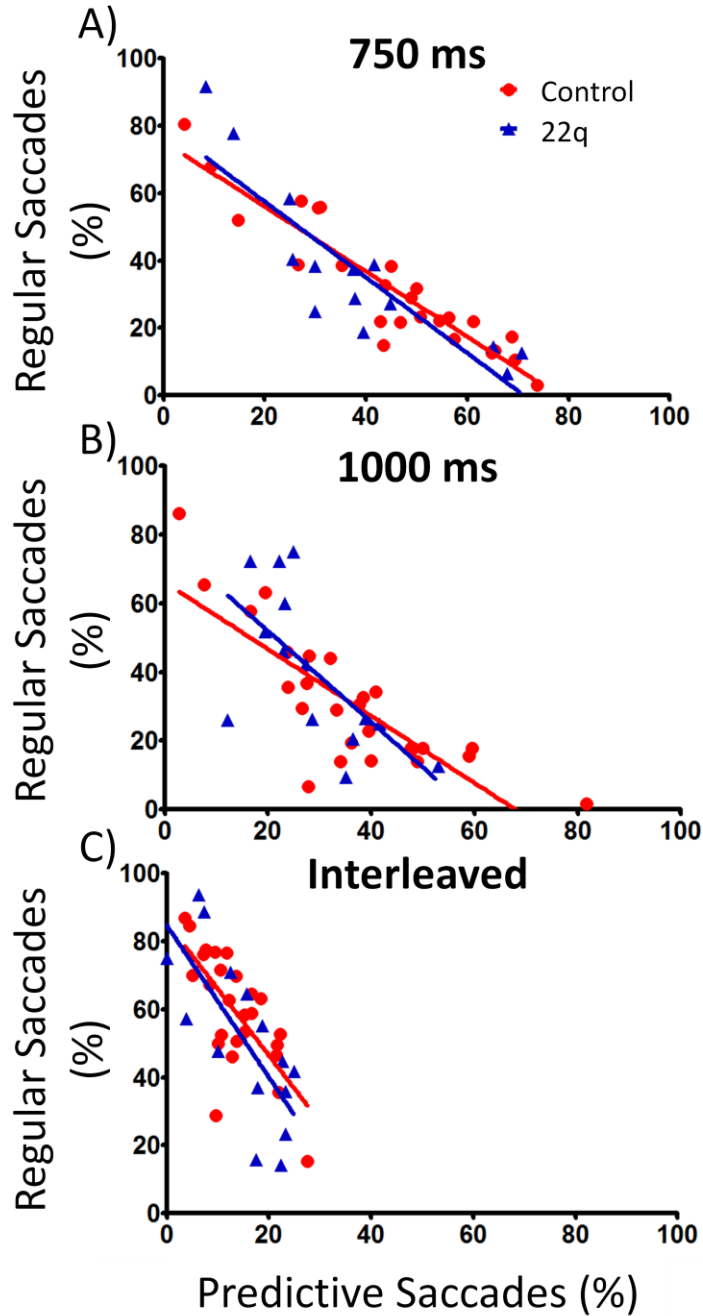
**Figure 4.10 Distribution of saccade type in the predictive task.**

The distribution of saccade type for the control (red) and 22q (blue) groups in the blocked (A,B) and the interleaved (C) trials.



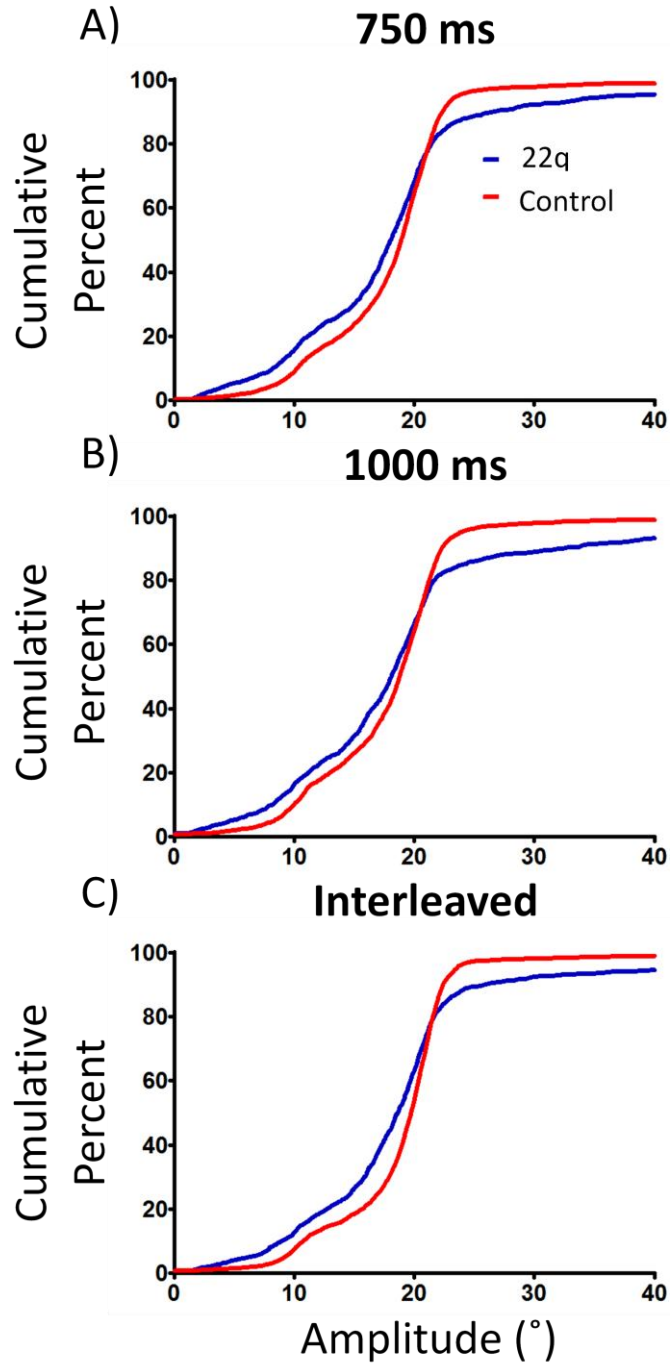
**Figure 4.11 Predictive and regular saccades in the predictive task.**

The percent of trials in which (A) predictive saccades (SRT between -300ms and 100ms) and (B) regular saccades (SRT >150ms) were generated in each inter-stimulus interval for both the control (red) and 22q (blue) groups.



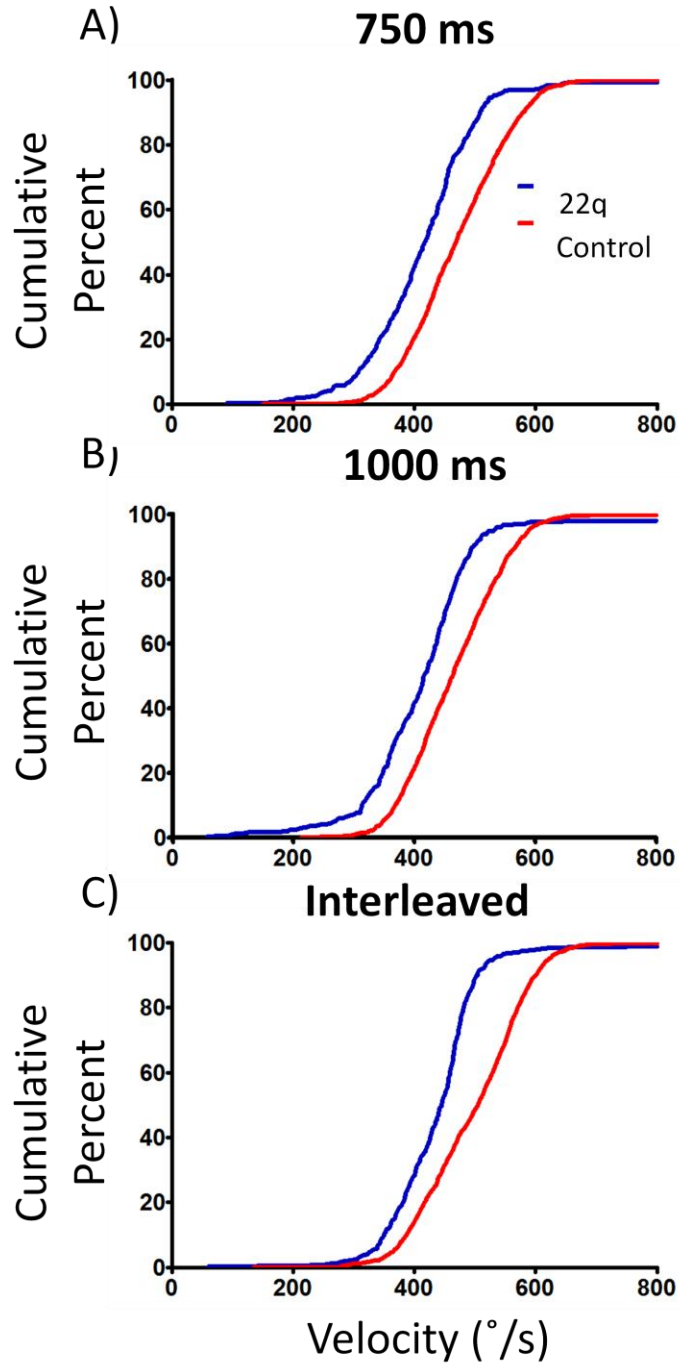
**Figure 4.12 Regular saccade and predictive saccade correlations.**

The percent of trials in which control and 22q children generate regular saccades (SRT >150 ms) is correlated with the percent of trials in which they generate predictive saccades (SRT between -300ms and 100ms) for ISI: (A) 750 ms and (B) 1000 ms and (C) Interleaved trials.



**Figure 4.13 Cumulative percent distributions of saccade amplitude.**

Amplitudes (in degrees) of all saccades for the (A) 750 ms ISI, (B) 1000 ms ISI, and (C) interleaved trials.



**Figure 4.14 Cumulative percent distributions of saccade velocity.**

Velocity (in °/s) of all saccades for the (A) 750 ms ISI, (B) 1000 ms ISI, and (C) interleaved trials.

## **Chapter 5**

### **Discussion**

#### **5.1 Saccadic eye movement tasks**

The current study used a battery of eye movement tasks to assess CNS dysfunction in children with 22q11.2 DS. We used eye movement paradigms which were specifically designed to probe commonly reported deficits in this population. These included the prosaccade, antisaccade, delayed-memory guided and predictive saccade tasks. These tasks assessed aspects of sensorimotor function, cognitive flexibility, working memory, and procedural learning. Children with 22q11.2 DS did not exhibit differences from controls in SRT or direction errors in the prosaccade task. However, the children with 22q11.2 DS did show some deficits in the antisaccade and the spatial working memory tasks, and striking deficits in response inhibition in the DMS task. Children with 22q11.2 DS did not show deficits in motor learning in the predictive task, but they also exhibited deficits in response inhibition in this task. In addition to the hypothesized results, the saccade metrics in all four of the tasks suggested impairment in cerebellar function. From these results, it appears that this battery of saccadic eye movement tasks may be used to assess the integrity of frontostriatal structures and circuitry, and cerebellar function in the 22q11.2 DS population.

## **5.2 Visually-guided saccade circuitry**

Because the prosaccade task tests the automatic saccadic responses to visual stimuli, it is an ideal task to test the basic, visually-guided saccade circuitry. Because the children with 22q11.2 DS did not show any differences in response accuracy, SRT or direction errors, it indicates that the visually-guided saccade circuitry is relatively intact. As summarized in Figure 2.1 (adapted from Munoz & Everling, 2004), the generation of visually-guided saccades is mediated by the SCi, with inputs from the parietal and occipital visual cortices. Because the main parameters of the prosaccade task were not significantly different between children with 22q11.2 DS and their age- and sex-matched controls, this is a strong indication that the occipital and parietal cortices as well as the collicular control of visually-guided saccades is unaffected by the chromosomal deletion.

## **5.3 Frontostriatal structures and saccade control**

Once it was determined that the circuitry controlling visually-guided saccades was largely intact, this study examined higher cognitive control of saccadic eye movements using the antisaccade, DMS and predictive saccade tasks. This higher cognitive control of visually-guided saccades and internally driven saccades has previously been shown to be regulated by the prefrontal cortex and the BG (Hikosaka *et al.*, 2000; Schall, 2002). Children with 22q11.2 DS exhibit deficits in the antisaccade, DMS and predictive saccade tasks which suggests that they have dysfunction in one or both of these brain regions.

The generation of an antisaccade requires that the subjects do two things; 1) suppress the automatic, visually-guided saccade and 2) generate an internally-guided saccade away from the stimulus (Munoz & Everling, 2004). Several brain structures are involved in driving these behaviours. Activity in the contralateral FEF and SCi increases in response to the appearance of the visual stimulus. If this activity reaches a saccadic threshold, a visually-guided prosaccade will be generated, which in this task is a direction error. In order for the ipsilateral brain regions to initiate an internally-guided antisaccade, this contralateral activity must be suppressed. It is thought that the inhibition of the presaccadic activity in the FEF and SCi is mediated by the executive control of the dlPFC and/or the substantia nigra pars reticulata of the BG. The FEF, SEF and dlPFC all project to the SCi, either directly or indirectly through the BG, and allow for the executive control over the saccadic premotor circuitry (Connolly *et al.*, 2002; Pierrot-Deseilligny *et al.*, 2004). While the contralateral activity is being suppressed, the ipsilateral frontal cortex and SCi are critical for the generation of the voluntary antisaccade (Hikosaka & Wurtz, 1985; Munoz & Everling, 2004). Therefore, deficits in the executive control over the FEF and SCi activity by the prefrontal cortex or BG can result in greater SRT and increased direction errors in the antisaccade task.

In this study, children with 22q11.2 DS had significantly higher SRT and made more direction errors than their age- and sex-matched controls. Higher SRT indicates that these children spend a longer time deciding which action to take and creating an internally driven saccade, whereas the increased direction errors indicate that these

children are unsuccessful at suppressing the instinctive responses. These deficits are attributed to poor executive control over saccade generation which have been recorded in clinical populations with impairments in frontostriatal circuitry including FASD, ADHD, Huntington's disease and Parkinson's disease (Green *et al.*, 2007; Munoz *et al.*, 2003; Peltsch *et al.*, 2008; Chan *et al.*, 2005).

The DMS task is similar to the antisaccade task because it also assesses the executive control of internally-guided saccades. This task differs from the antisaccade task because subjects must use previously presented sensory information to plan and initiate a motor response. In this task subjects must do three things; 1) they must suppress their instinctive response to look at the stimuli until they are given the go signal, 2) they must remember the location and order in which the targets appeared and 3) they must generate voluntary saccades toward the remembered locations of the stimuli. Because of the complexity of this task, it requires the integration of multiple domains of cognitive function. Subjects suppress their responses and use spatial working memory to generate the appropriate saccade sequences. In the present study, children with 22q11.2 DS completed fewer correct trials, indicating an inability to successfully utilize these multiple domains.

The children with 22q11.2 DS showed increased timing errors in the DMS task, reflecting deficits in the ability to suppress saccadic responses until the go signal. Previous studies have revealed increased timing errors in populations with ADHD, Huntington's disease and Parkinson's disease, again implicating the frontostriatal

circuitry in the successful suppression of responses during the delay period (Mostofsky *et al.*, 2001; Peltsch *et al.*, 2008; Chan *et al.*, 2005). Along with the timing errors which were directed at the first target, children with 22q11.2 DS made significantly more timing errors to the second target than their control counterparts. This indicates that children with 22q11.2 DS are unable to suppress their responses while they are planning their saccades.

Interestingly, children with 22q11.2 DS did not make significantly more sequence errors than their control counterparts, nor did they exhibit deficits in saccade accuracy. This is unusual because a number of other studies have reported that children with 22q11.2 DS exhibit deficits in spatial working memory (Owen *et al.*, 1990; Cohen, 1997; Campbell *et al.*, 2010). Although their saccades were equally accurate, children with 22q11.2 DS had significantly fewer viable trials than their control counterparts. In addition to this, children with 22q11.2 DS took significantly less efficient saccade paths, and made many more step saccades while getting to the targets. The SEF and the dlPFC may be involved in the execution of memory-guided saccade sequences. The SEF have a role in the generation of complex oculomotor sequences (Pierrot-Deseilligny *et al.*, 2004). A TMS study in which stimulation was applied to the SEF resulted in the disruption of saccade sequence order (Tobler & Muri, 2002). However, children with 22q11.2 DS did not make more sequence errors than the control group. Not only is the dlPFC implicated in response inhibition, but also in short-term spatial memory (Pierrot-Deseilligny *et al.*, 2003). In humans with lesions to the dlPFC, increased variability is

observed in the accuracy of memory-guided saccades, suggesting its role in the encoding of spatial information (Israël *et al.*, 1995). Although injury to the dlPFC could explain some of the problems with response suppression in the DMS task, children with 22q11.2 DS did not have deficits in saccade accuracy in this task. This pattern of deficits is more likely due to cerebellar dysfunction, which will be discussed in Chapter 5.4.

Predictive saccade tasks have been used to assess procedural learning abilities for nearly 50 years (Stark *et al.*, 1962). Procedural learning abilities are associated with cerebellar and BG function, but are also associated with working memory as both spatial and temporal information must be encoded to generate internally-guided saccades (Gagnon *et al.*, 2002). Because of the working memory association, the FEF and dlPFC have both been associated with the generation of internally-guided predictive saccades. It has been shown that lesions in the FEF, BG and cerebellum affect predictive saccades more than reflexive saccades, implicating these regions in this task (Bronstein & Kennard, 1985; Tian *et al.*, 1991; Rivaud *et al.*, 1994; Isotalo *et al.*, 1995). Results from the blocked trials of this task suggest that children with 22q11.2 DS are capable of procedural learning, as internally-guided saccades are evident in the decrease of SRTs after several stimuli. The interleaved trial performance however, demonstrates that this “predictive” behaviour is also observed in an unpredictable condition. It is therefore possible that the children with 22q11.2 DS are not necessarily eliciting predictive saccades, but are exhibiting inappropriate anticipatory responses. This hypothesis is reinforced if one examines Fig. 4.9C, where the children with 22q11.2 DS appear to be

making fewer regular saccades and more anticipatory saccades than their control counterparts. This may be attributed to deficits in temporal working memory or inhibitory control. In order to correctly generate predictive saccades, individuals must utilize both spatial and temporal information to modify the motor response. This is supported by the observation that the group of control children generated more predictive saccades than any other type in the blocked trials, while this effect disappeared in the interleaved trials. Because the children with 22q11.2 DS performed significantly fewer predictive saccades in the blocked trials than their control counterparts, that is indicative of deficits in temporal working memory. Additionally, the group of children with 22q11.2 DS showed increased amplitude of saccades in the predictive task, indicating that they are overshooting the targets. As these saccades are generated by an internal representation of target location, they are essentially a memory-guided saccade. Therefore the overshooting shown by the children with 22q11.2 DS suggests a deficit in spatial working memory. Because the children with 22q11.2 DS performed fewer predictive saccades than the control group and had a tendency to overshoot the targets with their saccades, they exhibit a deficit in both spatial and temporal working memory.

The results from the interleaved trials suggest that the group of children with 22q11.2 DS have deficits in inhibitory control. In this condition, the children in the control group were able to identify the unpredictable nature of stimulus timing, and therefore continued to generate visually-guided saccades as opposed to switching to internally-guided saccades. In contrast, the children with 22q11.2 DS generated

internally-guided anticipatory saccades in this task after the first few stimuli. Similar to the anticipatory saccades observed in the antisaccade task, this behaviour may be due to an inability to suppress an internally-driven motor response or to maintain fixation.

Taken together, the results from the antisaccade, DMS, and predictive saccade tasks all suggest impairments in the frontostriatal circuitry and the associated oculomotor areas. These impairments are consistent with the behavioural studies that demonstrate deficits in response inhibition/suppression and working memory in children with 22q11.2 DS.

#### **5.4 Cerebellar Function**

While the frontostriatal circuitry is largely responsible for the initiation and executive control of saccades, the cerebellum is also involved in the regulation of saccade metrics. The cerebellar pathway of eye movement control performs three key functions: 1) adding to the directional drive of the saccade (contributing to the first angle) 2) monitoring the progress of the saccade toward the target (path length), and 3) inhibiting saccadic drive to the motor neurons, ending the saccade (contributing to saccade amplitude). The cerebellum receives oculomotor input from the pontine LLBN, which project mainly to lobules VIc and VII in the posterior portion of the cerebellar vermis (Quaia *et al.*, 1999). The Purkinje cells in the vermis then project to the oculomotor region of the fastigial nucleus which then innervates the EBN, IBN and OPN of the brainstem oculomotor circuitry (Noda *et al* 1990; Scudder *et al* 2000).

In the pro- and antisaccade tasks, the children with 22q11.2 DS made significantly longer saccades and were less efficient in their saccade trajectory than control children. These findings were more robust in the antisaccade task, likely due to the lack of a visual stimulus to guide the saccadic response. As cerebellar regions involved in oculomotor control receive inputs from the LLBN and send projections back to the brainstem premotor circuitry, it has been proposed that the cerebellum receives an efference copy of each saccade generated which is subsequently used to tailor the fastigial output in order to keep saccades on the right trajectory and terminate them on target (Quaia *et al.*, 1999). Therefore, the increase in the error of saccade trajectory and saccade amplitude observed in both the pro- and antisaccade tasks may be an indicator of cerebellar dysfunction in children with 22q11.2 DS.

Quantifying the cerebellar dysfunction in the DMS task was more difficult because the task is more complex. The vast increase in step saccades and path length is likely to be due to dysfunction of the cerebellar control over eye movements. Both animal and human studies of cerebellar lesions observe dysmetric visually-guided saccades with greater variability in both amplitude and velocity (Robinson & Fuchs, 2001). So the inefficient, wandering pathways to the targets could be due to impaired feedback regulation of the motor movement and/or an inability to stop the saccades properly.

The predictive saccade task also indicated cerebellar dysfunction in children with 22q11.2 DS. In the predictive saccade task, like in the anti- and prosaccade tasks, children with 22q11.2 DS made significantly longer saccades than their control

counterparts, signaling the inability of the cerebellum to stop the saccades. As well as increased saccade amplitude, the peak velocity of visually-guided saccades was diminished in the 22q11.2 DS group when compared to controls in both the blocked and interleaved conditions. This finding is indicative of cerebellar injury. In 2000, Robinson showed that lesions in the interpositus nucleus consistently reduced the peak velocity of saccades in monkeys. Together, findings from the pro- and antisaccade, DMS and the predictive saccade task, suggest that these eye movement paradigms may be sensitive to cerebellar dysfunction in children with 22q11.2 DS.

These findings are consistent with reports that children with 22q11.2 DS commonly have dysmorphologies of the cerebellum. Volumetric studies have shown that cerebellar structures, especially the vermis and other midline structures are reduced in volume in 22q11.2 DS (Bish *et al.*, 2006; Gothelf *et al.*, 2008) even when compared to IQ-matched controls (Baker *et al.*, 2011). These volumetric reductions overlap with the structures involved in cerebellar oculomotor control, which include the posterior portion of the cerebellar vermis and the fastigial nucleus, which is the most midline of the cerebellar nuclei (Quaia *et al.*, 1999). In addition to this, an fMRI study in people with autism spectrum disorders found a consistent lack of activation in the posterior vermal regions during tasks requiring spatial attention (Haist *et al.*, 2005). This all builds a strong case that the eye movement tasks in this study could be used to assess the damage to the cerebellum in patients with 22q11.2 DS.

## 5.5 Comparisons to previous studies in 22q11.2 DS

Because no previous eye movement studies have been performed in individuals with 22q11.2 DS, this study has provided a new method of examining the neurological deficits seen in this population. Knowing the anatomical structures involved in these eye movement behaviours also allows for correlations to be drawn between the behavioural deficits seen in the eye movement study and known volumetric changes in the patient population. Previous studies using traditional methods of assessing cognitive deficits have aligned well with the results we found in this study.

Using the antisaccade, DMS and predictive tasks, deficits were found in executive function, notably response inhibition. Similar deficits in executive function have been found in traditional behavioural tests (Bearden *et al.*, 2001). The DMS task revealed deficits in spatial working memory, which aligns with volumetric studies showing thinning of the occipital-temporal cortex and many cognitive tests (Simon *et al.*, 2002).

A unique behavioural finding of this study was that children with 22q11.2 DS may express severe deficits in the cerebellar control of eye movements. This corresponds with volumetric studies which have shown reduced volumes of cerebellar midline structures, but which have previously only been associated with reduced IQ, not more specific behavioural deficits (Baker *et al.*, 2011).

## **5.6 Clinical Relevance**

The present study suggests that a battery of eye movement tasks may be implemented to assess both frontostriatal and cerebellar dysfunction in children with 22q11.2 DS. Although children with severe symptoms are usually identified in early childhood, usually due to cardiac and palate malformations, children with subtle symptoms may be more difficult to identify. Even if patients with 22q11.2 DS don't have life-threatening symptoms, it is important that they are identified early because of the risk of psychosis in adulthood as well as the risk of more severe symptoms in potential offspring. Thus, the development of screening tools for CNS dysfunction in 22q11.2 DS could allow for the identification of individuals requiring genetic testing. An effective screening tool must be sensitive and specific to the neurobehavioural phenotype of 22q11.2 DS. Therefore, the comparison of oculomotor behaviours in 22q11.2 DS to other disorders with similar clinical presentations must be considered. Another clinical use of these eye movement tasks could be to correlate eye movement behaviour with future risk of psychosis, allowing the close monitoring of those patients who are most at risk.

## **5.7 Limitations**

In this initial study of novel eye movement tasks in children with 22q11.2 DS, there are several methodological considerations that must also be accounted for. Firstly, this study only tested 16 children with the deletion and these children were spread over a large age range during which a lot of development takes place. Narrowing the age range would allow a more in depth look at each stage of development, while increasing the

number of participants would allow us to better quantify the range of deficits in the eye movement tasks in the children with 22q11.2 DS.

Secondly, in this study, the children performed four tasks in one session. Although they were given breaks, the length of the testing session was bound to have detrimental effects on performance due to diminished attention. It would therefore be advantageous to administer these tasks in random order to control for the effects of fatigue on performance. Furthermore, the sustained attention required for the DMS task was extremely difficult for these children, in particular at the lower age range. Since only some of the participants were able to complete this task, the results may reflect the higher functioning end of the spectrum. This also limits the use of the DMS task in a clinical setting, as children must be able to perform the task sufficiently to obtain any indications of CNS dysfunction.

Lastly, it was noted in the evaluation of the parent's years of education that the control group had more highly educated parents than the children with 22q11.2 DS. We also did not find IQ-matched control children. It would be advantageous to control for socio-economic status and IQ to ensure that these are not confounding factors in future studies.

## **5.8 Future directions**

The use of these eye movement tasks in a larger population of children with 22q11.2 DS would allow for a greater exploration of the range of behavioural deficits in

the disorder. Examining more children at each age could give us a better idea of how the deficits in these children change as the children develop. The incorporation of MRI and DTI imaging techniques would provide further information regarding the neuropathology underlying these behavioural deficits and could allow correlations to be made between behaviour and anatomy. These eye movement tasks could be used to assess the effectiveness of behavioural interventions in the population of children with 22q11.2 DS. Lastly, a longitudinal study could correlate eye movement behaviour in childhood or adolescence with future psychosis.

## Chapter 6

### Summary and Conclusions

This thesis tested the hypothesis that children with 22q11.2 DS would demonstrate deficits in multiple saccade paradigms, including prosaccade, antisaccade, delayed memory-guided sequential and predictive saccades. These tasks were selected in order to probe aspects of cognitive flexibility, spatial working memory, and motor learning.

Compared to age- and sex-matched typically developing children, the 22q11.2 DS group demonstrated deficits in response inhibition and spatial working memory in the antisaccade, DMS and predictive tasks, suggesting injury to the frontostriatal circuitry and its associated structures. Additionally, results from the prosaccade, antisaccade, DMS and predictive tasks suggested that saccade amplitude, velocity and trajectory were indicative of cerebellar dysfunction in this population. Together, this battery of eye movement tasks may allow the assessment of frontostriatal and cerebellar function; regions frequently atypical in 22q11.2 DS.

Future studies should employ the use of imaging data in order to further identify the neural correlates of the behavioural deficits observed in the eye movement tasks. In addition, large scale studies would allow the exploration of these findings in a sampling better reflecting the general population and a longitudinal study may be able to correlate eye movement behaviour with future psychotic events.

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