Saccade inhibition in schizophrenia

Towards phenotyping based on brain function

Mathijs Raemaekers

Voor mijn ouders

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Inhibitie van saccades in schizofrenie

Op weg naar fenotypering op basis van hersenfunctie

(met een samenvatting in het Nederlands)

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Mathijs Antoine Helena Lambertus Louisa Raemaekers

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Promotor: Prof. Dr. R.S. Kahn

Co-promotor: Dr. N.F. Ramsey

Content

General introduction

1.1 Schizophrenia:

Schizophrenia refers to a heterogeneous group of psychotic disorders and is associated with a gross separation from reality and deterioration of mental and social life. Symptoms are classified in three dimensions including positive, negative and disorganized symptoms (Andreasen et al., 1994). The positive dimension refers to symptoms which are present in patients, but not in healthy individuals and include delusional thinking and hallucinations. Negative symptoms signify characteristics or abilities which are impaired or absent in patients relative to healthy individuals and include flattening of affect, impovered speech, and disturbance of volition. Disorganized symptoms include disorganizations or abnormalities in thinking, speech or behaviour.

Schizophrenia was formerly known by Kraeplin's term dementia praecox, and the term was used to describe individuals who exhibited symptoms that involved severe mental deterioration from an early age. Later, Eugen Bleuler coined the term "schizophrenia" to describe the splitting of mental associations, the disturbance that lies at the foundation of the disorder. Although the term schizophrenia dates from the 20th century, there have been accounts of mental states that resemble schizophrenia since 400 B.C. Today, schizophrenia has an incidence rate (number of new cases reported) of one per ten thousand people per year and a lifetime prevalence rate of one percent (McGuffin et al., 1995). The chance of being diagnosed with schizophrenia increases with the number of family members already diagnosed with schizophrenia. The age of onset ranges from late teens to mid-thirties, and there are gender differences: For men, the age of onset is early to mid-twenties. Women tend to have an age of onset in the late twenties and are more likely to exhibit more mood symptoms and have a better prognosis. Sixty to seventy percent of individuals diagnosed with the schizophrenia never marry, and about ten percent end their life in suicide (Caldwell and Gottesman, 1990).

Heritability:

There is now consensus that schizophrenia is in part a hereditary disorder. The disorder strongly tends to cluster within families and concordance rates are higher in monozygotic than in dizygotic twins (48% vs. 17%) (McGuffin et al., 1995) (Figure 1.1). Quantative analysis of suggests that approximately 80% or more of the variance in liability for schizophrenia is accounted for by genetic factors (Cardno and Gottesman, 2000). It is also understood that the disorder is not entirely genetically determined as the median concordance rate for schizophrenia observed among monozygotic

twin pairs is 46%, a feature that implicates non-genetic factors. So what is inherited is not the certainty of disease, but rather a predisposition or liability to develop the disorder (McGuffin et al., 1995).

The actual nature of the genetic factors for schizophrenia remains unknown. Although it is now generally accepted that schizophrenia is not caused by a single major gene, there is no reliable estimate of the number of genes that is involved. In addition, there is no model on how these genes interact with each other or with environmental risk factors in the development of schizophrenia. This troublesome situation may be partly attributed to obstacles that complicate efforts to identify genes for any complex disorder, such as unknown mode of inheritance, genetic heterogeneity, phenocopies, incomplete penetrance, and variable expressivity. Such a complex type of transmission can have a strong negative influence on the power of molecular genetic studies in their effort to detect genes and gene loci associated with the illness.

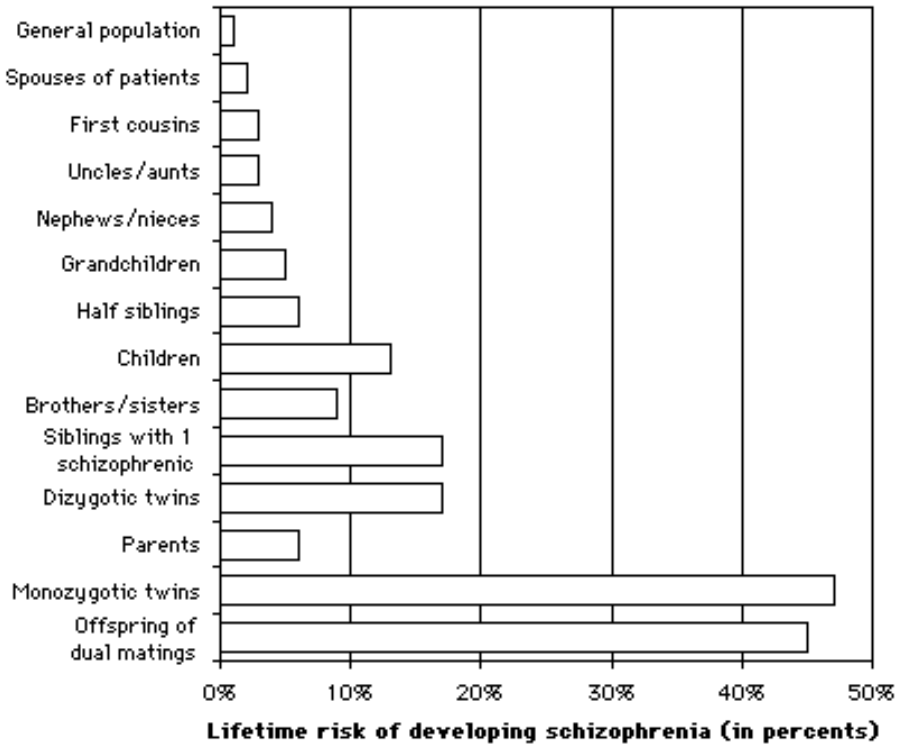

Figure 1.1. Lifetime risk on developing schizophrenia in percent (Gottesman 1991)

Identifying the genes, mode of transmission and responsible chromosomes has proved to be a difficult task for schizophrenia. The failure of most studies in obtaining clear replicated linkages have led to the scepticism that such approach would ever be successful. Fortunately, there are now signs of some real progress (Owen et al., 2004). Several linkages are beginning to emerge for a number of gene loci. Three of the best-supported regions are 6p24-22, 1q21-22 and 13q32-34. In these cases, single studies achieved genome-wide significance at $P<0.05$ and suggestive positive findings have also been reported in other samples. The other promising regions include 8p21-22, 6q21-25, 22q11-12, 5q21-q33, 10p15-p11 and 1q42. The study of chromosomal abnormalities in schizophrenia has also added to the evidence for susceptibility loci at 22q11 and 1q42. In addition, gene association studies have found some promising results for neuregulin, dysbindin, COMT, DISC1, RGS4, GRM3, and G72. Although these results have led to some optimism, many difficulties remain. Positive results have typically been obtained in very large sample sizes only, meaning that the effect sizes of individual loci and genes are very small. There is still need for alternative approaches

1.2 Endophenotypes:

Many linkage studies may have failed because the assumptions on which they are based, the existence of a single gene of major effect, complete penetrance, and no phenocopies, are incorrect. Although schizophrenia is normally regarded as a single diagnostic entity, the underlying genetic pattern may involve a constellation of susceptibility genes which can differ from patient to patient (Pulver et al., 2000). In addition, even when the necessary genotype for schizophrenia is present, it is not always expressed as clinical disease at the phenotypic level (the concordance rate in monozygotic twins is not 100%). Moreover, there could be a proportion of cases of schizophrenia who show the schizophrenia phenotype but who do not have the genotype. All these phenomena can potentially reduce the power of molecular genetic studies. However, these difficulties can at least partially be overcome by alternative phenotyping. In this perspective, endophenotypes are being defined and developed.

Endophenotypes are measurable components unseen by the unaided eye along the pathway between disease and distal genotype. They have emerged as an important concept in the study of complex neuropsychiatric diseases as they represent simpler clues to genetic underpinnings than the disease syndrome itself. The approach assumes that psychiatric diagnoses can be decomposed or deconstructed, which can result in more straightforward-and

successful-genetic analysis. In addition, if successful linkage to an endophenotype is achieved, the results will provide more insight in how a certain genetic defect may eventually result in psychotic illness. However, endophenotypes for psychiatric disorders must meet certain criteria, including (Gottesman and Gould, 2003):
-The endophenotype is associated with the illness.
-The endophenotype is heritable.
-The endophenotype can be detected in remitted patients who do not suffer from active illness (state-independence).
-The endophenotype and the illness co-segregate in affected families.
-The endophenotype found in ill family members is found in unaffected family members at a higher rate than in the general population.
- The endophenotype must have an association with a candidate gene or gene region.
Numerous candidate endophenotypes have already been proposed for schizophrenia across various domains. They include cognitive phenotypes (like impairments in working memory, executive functions, sustained attention, verbal memory, and episodic memory), electrophysiological phenotypes (like the abnormalities in the P50 sensory gating, the PPI prepulse inhibition, and hemispheric EEG coherence) and further biochemical, endocrinological, and neuroanatomical phenotypes (Gottesman and Gould, 2003). All of these candidate endophenotypes meet one or more of the criteria. None of the endophenotypes has consistently met all the criteria.

Abnormalities in smooth pursuit eye movements in schizophrenia:
Abnormalities in eye movements are high on the list of candidate endophenotypes for schizophrenia. As early as 1908, Diefendorf and Dodge observed a deficit in eye movements in patients with ‘dementia praecox’. Patients were impaired in accurately tracking a moving pendulum with their eyes. The human eye can move in a rapid ballistic motion between two positions, which is called a saccadic eye movement. The eye can also move slowly and smoothly when tracking a moving object, which is called smooth pursuit eye movement. Schizophrenic patients find it difficult to maintain smooth pursuit eye movements, and make more saccadic eye movements interrupting the smooth pursuit. The relationship between schizophrenia and the smooth pursuit eye movement disorder was rediscovered by Holzman (Holzman et al., 1973). One year later, Holzman found that the same abnormality was also present in non-schizophrenic healthy relatives of patients (Holzman et al., 1974). Smooth pursuit eye movements were disordered in about 70% of schizophrenics, about 45% of their first-degree

relatives, and about 5% in the general population. This suggested that abnormal smooth pursuit may be an indicator of (genetic) risk for schizophrenia. There is now increasing evidence that this abnormality may be related to the susceptibility locus 6p21 in schizophrenia (Arolt et al., 1996,Matthysse et al., 2004).

The antisaccade task:
The finding of the smooth pursuit deficit has led to the investigation of other eye movement abnormalities in schizophrenia. Especially eye movement paradigms that are sensitive to impairments in executive functioning have generated promising results. One of these paradigms is the antisaccade task. The antisaccade task is the most commonly used paradigm to measure the ability to inhibit saccadic eye movements and was first introduced by Hallett (Hallett, 1978b) (Figure 1.2). During an antisaccade task, subjects must inhibit an eye movement towards a novel stimulus, suddenly appearing to the left or right of a central fixation cross, and instead make an eye movement in the opposite direction. The counterpart of the antisaccade is the prosaccade. During the prosaccade task, subjects must make a saccadic eye movement towards the stimulus. The saccadic onset latencies of antisaccades are typically longer than for prosaccades. In addition, subjects make more errors during antisaccades (initial saccade in the wrong direction).

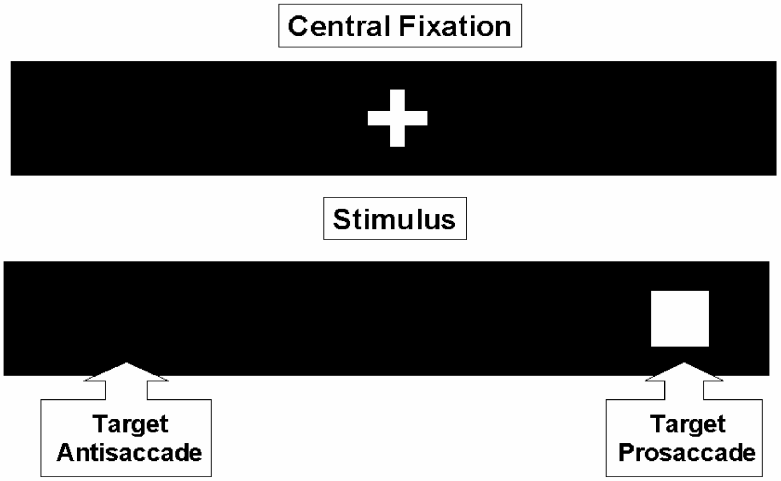

Figure 1.2. Before the onset of the stimulus the subjects remain fixated on the central cross. A stimulus appears randomly on the left or right. Depending on previously given instructions, the subject makes a saccade towards the stimulus (prosaccade) or towards the mirror location of the stimulus (antisaccade).

Many variations can be built into the paradigm to influence the performance. Pro-cues can be presented that give information regarding the direction of the subsequent saccade, with different lead times for the cues. Voluntary attention can be brought to a particular location in the visual field before the presentation of the stimulus. However, the most common variation regards the moment at which the fixation cross is extinguished. In the 'gap' paradigm the fixation cross is extinguished before the onset of the stimulus, in the 'step' paradigm it is extinguished simultaneously with the stimulus, in the 'overlap' paradigm it is extinguished after the onset of the stimulus. The 'gap' paradigm, with a duration of 200 ms between the stimulus and disappearance of the fixation cross, has been associated with the fastest reaction times, but also the highest error rates (Fischer et al., 1997b).

Antisaccades and schizophrenia:
Antisaccade performance in schizophrenia was first investigated by Fukushima (Fukushima et al., 1988). In his experiment, patients and control subjects performed three tasks, that is, prosaccades, antisaccades and no saccades (remain fixated when the peripheral stimulus is presented). Patients had increased saccadic onset latencies for antisaccades, but not for prosaccades. In addition, patients made more errors during the antisaccade and the no-saccade task, but not during the prosaccade task. This finding has been replicated numerous times in the following years, and is now a well established phenomenon (see Brownstein for a review)(Brownstein et al., 2003). The deficit is unlikely to be a result of the use of antipsychotic medication, and is also present in patients without a current psychotic episode (Curtis et al., 2001,Green and King, 1998). The test retest reliability of the antisaccade task performance is high for control subjects(Ettinger et al., 2003), but also for schizophrenic patients and their relatives (Calkins et al., 2003b). These data are all supportive for a trait-like characteristic. The antisaccade abnormality could thereby be a possible candidate endophenotype for schizophrenia.

It has been argued that the smooth pursuit and saccadic inhibition abnormality are part of a same underlying deficit. Increased saccadic intrusion during smooth pursuit could theoretically be explained by an inability to suppress anticipatory saccades ahead of the moving target. However, no relationship has been detected between the saccadic inhibition and the smooth pursuit deficit (Hutton et al., 2004,Radant et al., 1997). This suggests that the underlying neural or genetic mechanisms for the antisaccade abnormality are fundamentally different.

Neural correlates of abnormal antisaccade performance:
Although various imaging studies have investigated brain activation during antisaccades in patients, the results have been inconsistent (Nakashima et al., 1994,Crawford et al., 1996,McDowell et al., 2002). The notion that the Dorsolateral Prefrontal Cortex (DLPFC) is involved is largely based on reports of hypofrontality in schizophrenia (Weinberger and Berman, 1988) in combination with the finding of impaired antisaccade performance in patients with lesions affecting the DLPFC (Pierrot-Deseilligny et al., 1991a). However, the underlying mechanisms for antisaccades are obviously far more complex. Impaired antisaccade performance has been related to a broad range of neural and psychiatric illnesses including bipolar disorder (Katsanis et al., 1997), obsessive compulsive disorder (Rosenberg et al., 1997b), Tourette syndrome (Dursun et al., 2000), Alzheimer's disease (Shafiq-Antonacci et al., 2003), Huntington's disease (Dursun et al., 2000) , Parkinson's disease (Armstrong et al., 2002), adult Attention-Deficit Hyperactivity Disorder (Feifel et al., 2004) and HIV infection (Johnston et al., 1996). These data suggest that antisaccade performance can be affected by many different factors, which indicates the involvement of a widely distributed network. Data from tracer and cell recording studies in monkeys in combination with neuropsychological and imaging studies in humans are beginning to unravel this network. At the heart of it, is the oculomotor system.

1.3 The oculomotor system:

Although saccades are motor acts, it is not the motor system that contributes to the generation of saccadic eye movements. The functional segregation between the motor and the oculomotor system exists as a result of the fundamentally different requirement for these two systems. Whereas limb movements are relevant to environmental manipulation, eye movements are involved in orienting. In order to handle objects, movements must be programmed in allocentric co-ordinates, in other words a representational system using body and limb position in respect to the environment. Orienting behaviour on the other hand is based on egocentric representation, as eye movements must be defined by visual angles in relation to current eye or head position.

The oculomotor system has a hierarchical organisation, with the cortical structures at the top, and downwards the basal ganglia and the midbrain structures. The basal ganglia project back to the cortex through the thalamus, thereby creating a loop (Alexander et al., 1986) (Figure 1.3). The basal

ganglia also connect to the superior colliculus in the midbrain, thereby providing an escape pathway from the loop. The superior colliculus is the last gateway in the network before the saccade generator in the brainstem.

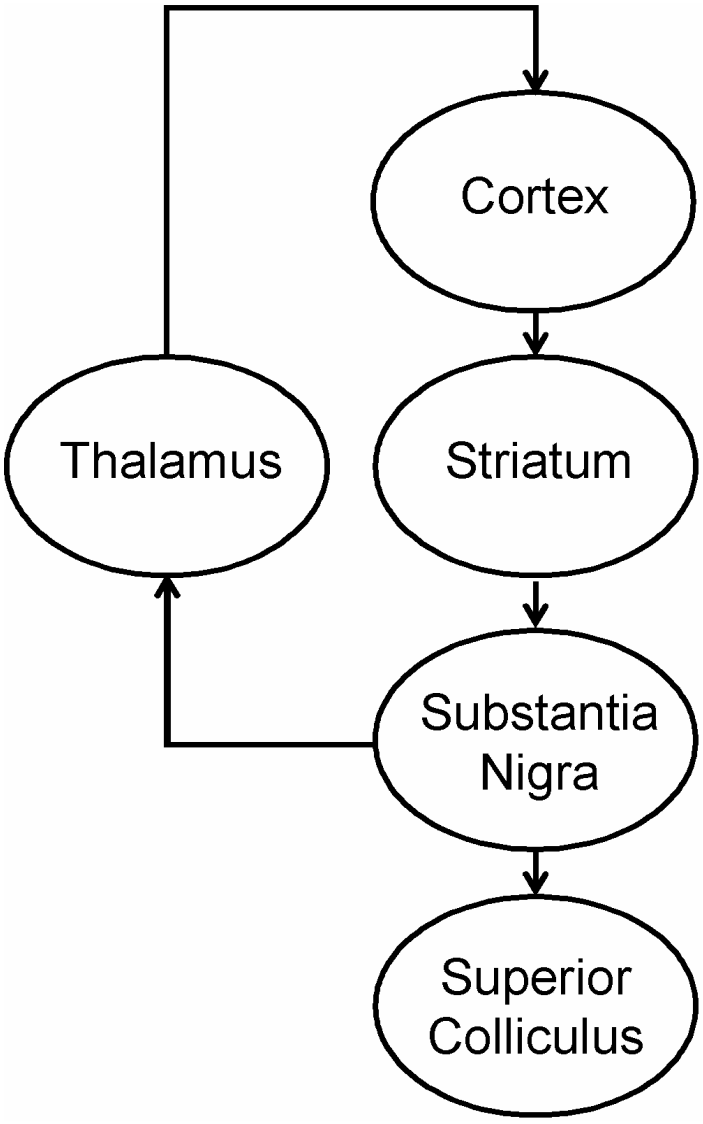

Figure 1.3. Schematic of the basal ganglia-thalamocortical circuit of the oculomotor system (Alexander 1986)

The brainstem:
A saccade is produced by discharge of the Saccade Related Burst Neurons (SRBN) in the pontine and the mesencephalic reticular formations (Figure 4). The SRBNs activate an integrator circuit, which generates a tonic excitatory input to the motor neurons, and also project to the motor neurons directly. The combination of tonic and burst input to the motor neurons produces the saccade (Frens and Van Opstal, 1998). The SRBNs are under tonic inhibitory control of the OmniPause Neurons (OPN) located in the midline of the reticular formation. These OPN neurons pause before saccade execution, thereby temporally releasing the SRBN's from inhibition and open the gate for saccade execution (Everling et al., 1998a).

The superior colliculus:
The superior colliculus (SC) contains an oculomotor map with two dimensional target vectors. This means that the locations of stimuli or spatial targets are programmed in angle and distance from current central fovea. Stimulation of eye movement related neurons in the SC causes a saccade with a fixed angle and amplitude. Firing of saccade related neurons within the collicular map activates the SRBN's, and simultaneously inhibits the OPN's (Pare and Guitton, 1994) (Figure 1.4), thereby unlocking the SRBN's. The SRBN's can then trigger a saccade through the integrator circuit.

The SC can operate as an independent saccade initiator without cortical intervention. There is evidence for axons from neurons of the optic that branch off to the oculomotor map in the SC before reaching the occipital lobe. Through these connections, a visual stimulus can potentially induce a saccadic eye movement without conscious awareness or cortical intervention. This loop in itself forms an extremely short pathway for the generation of reflexive prosaccades.

Apart from the neurons of the collicular map, there is a sub-population of neurons situated in the rostral pole of the SC with a different function. Neurons in the rostral pole tonically inhibit the collicular map and innervate the OPN's during fixation. Like the OPN's, the neurons in the rostral pole show saccade related discharge pauses (Munoz and Wurtz, 1993). Muscimol (GABA agonist) injections in the rostral pole of the SC in behaving monkeys, suppresses rostral pole activity, and reduces onset latencies of saccades to visual targets. In addition, these monkeys have difficulty holding gaze in the presence of visual distracters (Munoz and Wurtz, 1993). The SC is also likely to play a role in saccade suppression in human subjects. A patient with a unilateral lesion affecting the superior colliculus had a

lateralized difficulty inhibiting reflexive eye movements during antisaccades (Pierrot-Deseilligny et al., 1991b).

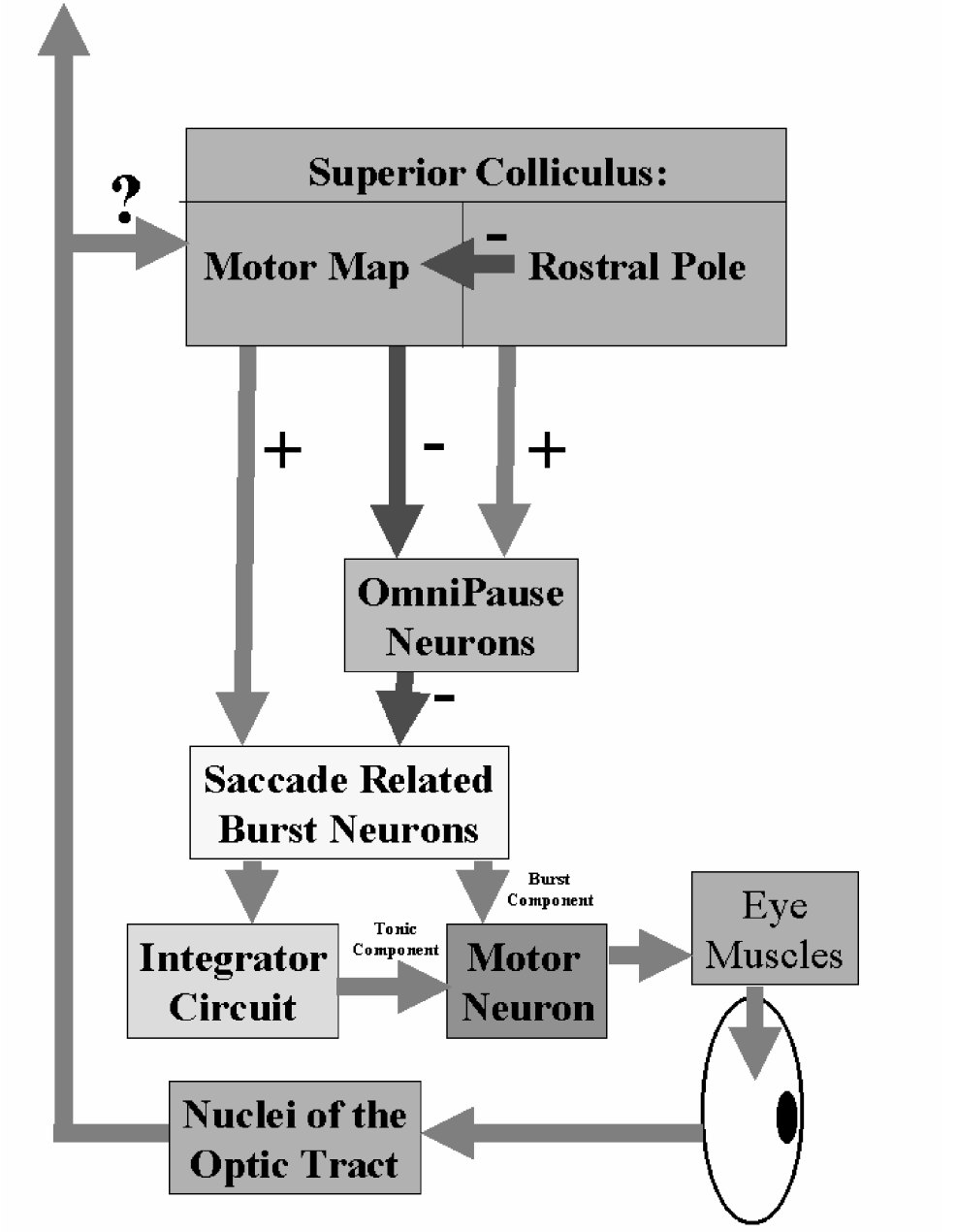

Figure 1.4. Schematic of the midbrain and brainstem machinery involved in the generation and suppression of saccadic eye movement.

The cortical structures of the oculomotor system:
The SC is an evolutionary very old structure and is unlikely to be capable by itself to decide when to look, and when not to look. During antisaccades, SC activation must therefore be under control of cortical structures. The cortical part of the oculomotor system encompasses a number of different eye fields. Neuronal activity in these eye fields corresponds to orienting behaviour. Although there is substantial overlap in function, each eye field has its specialisation. The most important cortical eye fields are the Parietal Eye Fields (PEF), the Frontal Eye Fields (FEF), the Supplementary Eye Fields (SEF) and an eye field in the Dorsolateral Prefrontal Cortex (DLPFC) (Figure 1.5).

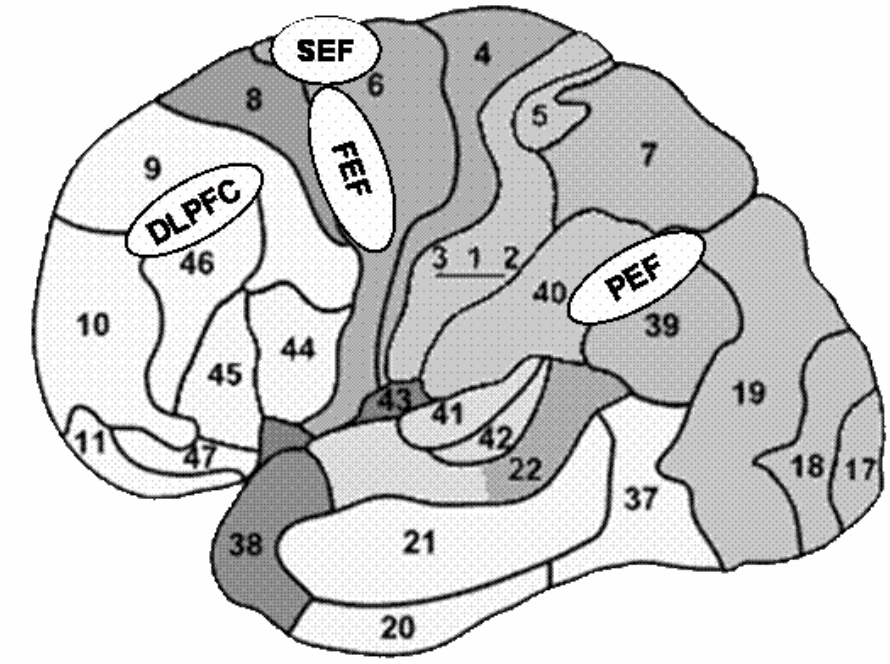

Figure 1.5. Approximate location of the four most important eye fields superimposed on the Brodmann area map.

Parietal Eye Fields (PEF):
The PEF are located on the lower bank of the intraparietal sulcus (LIP), an area that corresponds to Brodmann area 39 and 40 in human subjects (Muri et al., 1996). The PEF receive visual information from striate and extrastriate regions. This visual input mostly originates from the termination zones of the magnocellular system. The receptive fields in the PEF encode locations of external stimuli or events in retinotopic coordinates (relative to

current central fovea). The stimulus locations are dynamically remapped following eye movements, suggesting an important role for the PEF in visuomotor integration (Colby et al., 1995). Retinotopic encoding of external stimuli is relevant to gaze shifts and matches neuronal organisation in the other cortical eye fields and the SC. Apart from being visually responsive, neurons in the PEF show additional activation related to the execution of saccadic eye movements. Efferents from the PEF to the SC can trigger a saccade before the identity of a stimulus has been determined, thereby forming another quick pathway for reflexive saccades (Pare and Wurtz, 1997). Visuo-motor integration of external stimuli is relevant to both prosaccades and antisaccades. In prosaccades it provides the saccadic target location, in antisaccades it provides the information by which the correct response can be calculated.

Frontal Eye Fields (FEF):
The FEF in humans is located on the precentral gyrus, at the intersection between the precentral and superior frontal fissure (Paus, 1996). The FEF is, like the PEF, retinotopically organised. Microstimulation of the FEF causes fixed vector saccades, where angle and amplitude of the saccade are dependent on the site of stimulation (Segraves and Park, 1993). The FEF receives afferents from the PEF and the Inferotemporal Cortex (ITC). Whereas the projections from the ITC terminate in the lateral FEF, the PEF projects mainly to the medial and central FEF. The input from the ITC to the lateral FEF corresponds to information transfer from the 'what' trajectory. Input from the PEF corresponds to information transfer from the 'where' trajectory. Whereas the lateral FEF is involved in small amplitude saccades, which would typically be associated with scanning objects, the central and medial FEF encode large amplitude saccades in addition to neck and torso movements, which would be associated with orienting and localization (Schall et al., 1995). In addition, the FEF receives strong visual input directly from V2, V3, V4, and V5 holding the same segregation. Peripheral vision neurons with a large receptive field project to the medial and central FEF, while central vision neurons with a small receptive field project to the lateral FEF(Bullier et al., 1996). The strong visual innervations, in addition to the convergence of the temporal 'what' and medial 'where' trajectory, suggest that the FEF is involved in purposeful selection of targets from the visual environment. Neural substrates of target selection through processes of inhibition and excitation have been found in the FEF with cell recordings in monkeys (Thompson et al., 1996).

In addition to the saccade related neurons, the FEF contain neurons which fire tonically during fixation, and pause before the onset of a saccade. Fixation neurons are under inhibitory control of fixation disengagement neurons. Fixation disengagement neurons discharge at the offset of a fixation stimulus during a visually guided gap task, thereby triggering a pause in the tonic activation of fixation neurons (Dias and Bruce, 1994). Cell recordings detected higher activation in fixation neurons during a 'race model' experiment, when the upcoming saccade had to be withheld, compared to when it had to be executed (Hanes et al., 1998). Hence, fixation and saccadic inhibition may involve similar processes at a neuronal level. Fixation neurons in the FEF can inhibit saccades by connections to the rostral pole of the SC or to the OPN neurons (Figure 1.4), but probably also by indirect connections through the basal ganglia (Berthoz, 1996).

Supplementary Eye Field (SEF):
The SEF, or dorsomedial eye fields, have been located along the interhemispheric fissure, extending minimally into the dorsal cortical surface, as detected with fMRI in humans (Luna et al., 1998). Although electrocortical stimulation of both the SEF and the FEF elicits saccades, the stimulation threshold for the SEF is higher, suggesting a lower density of saccade encoding neurons (Tehovnik and Sommer, 1997). Furthermore, the type of saccade encoding differs from the FEF. Whereas stimulation of saccade related neurons in the FEF causes saccades with a fixed vector, stimulation of SEF neurons induce a saccade to a fixed location in relation to the position of the head (craniotopic) (Tehovnik et al., 1998).Whereas remapping in the FEF occurs after eye movements, remapping in the SEF is more related to head movements and corresponding vestibular input. SEF lesions impair orientation after body or head transformations, but not after saccadic eye movements (Israel et al., 1995). The FEF and the SEF both receive projections from the PEF. However, the SEF does not receive direct afferents from visual areas. SEF generated saccades therefore seem to be more related to an internal context, more self-initiated, and less related to the environment. Continuous stimulation of neurons in the craniotopic map inhibits saccades towards visual distractors (Tehovnik and Lee, 1993).

Cell recordings in the SEF in monkeys performing prosaccades and antisaccades have demonstrated increased firing in neurons encoding the correct target location during antisaccades. The balance between the activity of visuo-motor neurons and of the neurons encoding the correct target location was predictive of trial performance (Schlag-Rey et al., 1997) EEG recordings have found evidence for similar processes in humans.

Antisaccades are preceded by a stronger negativity over the dorsomedial frontal cortex than prosaccades (Everling et al., 1997). Correct antisaccades are preceded by a stronger negativity than incorrect antisaccades (Everling et al., 1998b).

The Dorsolateral Prefrontal Cortex (DLPFC):
The DLPFC is integrated in the oculomotor system as well. More specifically, Brodmann Area 46, corresponding to the region surrounding the principal sulcus in rhesus monkeys, has been implicated. The DLPFC is most notably involved in working memory (Funahashi et al., 1997). Visuo-spatial working memory is relevant to the upkeep of remembered targets during memory guided saccades such as the oculomotor delayed response task. There is evidence for a memory eye field in the DLPFC. Unilateral lesions of the principal sulcus in monkey's affect memory guided saccades when the target is contralateral to the side of the lesion (Israel et al., 1995). In addition, dopamine antagonist injections in the DLPFC impair performance on an oculomotor delayed response task on targets contralateral to the injection side (Sawaguchi and Goldman-Rakic, 1994).

Involvement of the DLPFC in a delayed antisaccade task has been investigated in monkeys with cell recordings around the principal sulcus . During the delay, the response in some neurons was characterised by a transient increase during the delay, representing a shift in the hemifield (Funahashi et al., 1993). Antisaccade abnormalities in humans have been found in patients with infarcts affecting the dorsal prefrontal cortex (Pierrot-Deseilligny et al., 1991a). However, 100% distractibility on the antisaccade task has been found after a lesion affecting the right VLPFC (Walker et al., 1998). In parallel with involvement of the prefrontal cortex, saccadic inhibition during antisaccades is dependent on working memory (Roberts et al., 1994). Simultaneous performance of a mental arithmetic task increases onset latencies and number of errors for antisaccades, but not for prosaccades.

The basal ganglia:
Output from the cortical oculomotor areas is transferred downwards through the basal ganglia. The striatum, which consists of the putamen and caudate nucleus, is the main input site of the basal ganglia. Efferents from the cortical areas of the oculomotor system selectively terminate in the body of the caudate nucleus and medial putamen (Alexander et al., 1986) (Figure 1.6).

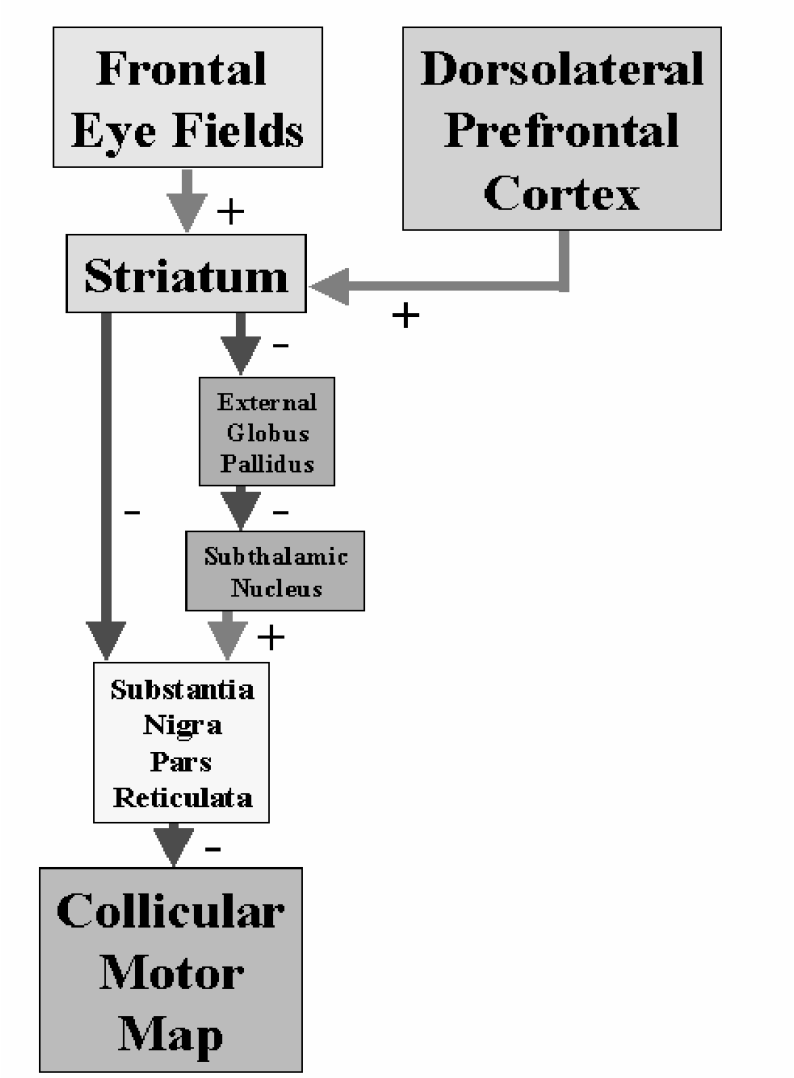

Figure 1.6. Schematic of the inhibitory/excitatory cascade through the sub cortical portion of the oculomotor system

From the striatum on, the information is further passed through to the substantia nigra pars reticulata (SNr), while maintaining segregation with regard to saccade type and vector (Berthoz, 1996). SNr neurons tonically inhibit the oculomotor map in the SC (Hikosaka and Wurtz, 1983). Striato-nigral connections can be either direct or indirect. The direct connection selectively inhibits the SNR, thereby releasing the SC from tonic inhibition and opening the gate for saccade execution. The indirect connection passes through the external globus pallidus and the subthalamic nucleus. Aside

from the inhibitory control directly from the striatum, the SNr receives excitatory projections from the subthalamic nucleus. The subthalamic nucleus is under tonic inhibitory control of the external globus pallidus. Fixation neurons of the FEF cause a suppression of the GPe through the striatum, thereby releasing the STN from inhibition causing further excitation of the SNr. By this route inhibitory output of the striatum is reversed, thereby contributing to suppression of the SC and maintaining fixation (Berthoz, 1996).

Impairments of the basal ganglia affect more complex types of saccades. Inhibiting dopamine function in the caudate nucleus by MPTP injections in rhesus monkeys causes a reduction in the number of spontaneous saccades (Kato et al., 1995), and affects accuracy and metrics of memory guided saccades (Kori et al., 1995). In addition, degenerative diseases of the basal ganglia like Parkinson's and Huntington's disease impair antisaccade performance in humans (Dursun et al., 2000,Armstrong et al., 2002).

Summary:
There is evidence for neural correlates of saccadic inhibition in several nodes in a widespread network of brain areas involved in oculomotor functioning. Implicated regions probably include the FEF, the SEF, the DLPFC, parts of the basal ganglia, and the SC. In addition, Paus et al. (Paus et al., 1993) found differential task related foci in the anterior cingulate in rhesus monkeys during higher order motor control when behavioural output was motor, oculomotor or verbal. Such a cingulate eye field may be involved in saccadic inhibition as well, as an impairment in saccadic inhibition during antisaccades was found in two patients after brain infarcts affecting the anterior cingulate (Gaymard et al., 1998). Finally, evidence has been found for implication of the insula as well in suppressing saccadic eye movements (Petit et al., 1993).

The involvement of such a widespread neural network could have important implications for the relationship between antisaccade performance, and the underlying neural mechanisms. On one hand, the neural system subserving antisaccades could be impaired on many different levels. In that case, the antisaccade deficit will not be very specific for any kind of neurofunctional deficit. As support for this, many different brain lesions, psychiatric abnormalities and neural diseases, lead to impaired antisaccades. Hence, the neural deficit underlying an antisaccade impairment could be very heterogeneous, even within schizophrenia. Heterogeneity in the underlying neural mechanism would also implicate heterogeneity of the genetic mechanisms underlying the antisaccade impairment. Thereby the

relationship between the antisaccade deficit, and underlying genes will be reduced.

Alternatively, the involvement of such a complex network could have an effect that is more or less opposite. Instead of antisaccade performance being vulnerable to many kinds of factors affecting the central nervous system, the system could also have a substantial functional redundancy, due to overlap in functionality between the different eye fields, parallel connectivity, and inhibitory mechanism on many different levels (Berthoz, 1996). In that case, an abnormality on the level of brain function could be overcome by backup or compensatory mechanisms, and would not become apparent at the behavioural level. However, the genetic abnormality causing the brain function abnormality will not become apparent at the behavioural level either. The penetrance of these genes will thus be reduced when measuring antisaccade performance.

In any event, the complexity of the neural network underlying antisaccades will have a negative impact on the relationship between genes and the behavioural measure of saccadic inhibition. To counteract this problem, measurements of antisaccades would preferably be done not only at the behavioural level, but also at the neurofunctional level. With functional magnetic resonance imaging (fMRI), we now have the possibility to do so. In this thesis, event related BOLD-fMRI is used to investigate the neural mechanisms underlying of antisaccade performance.

1.4 BOLD-fMRI

In order to view differences in brain function between task conditions and subjects, an MRI scanner must be able to detect some physical difference in the brain. Deoxyhemoglobin (rHb) (the unoxygenated form of hemoglobin in our blood) has paramagnetic properties that can be imaged using MRI. When neural activity is locally increased, e.g. when a subject makes a saccadic eye movement, there is a reduction of rHb due to an increase of regional cerebral blood flow (this flow brings in more oxygen, which reduces the amount of rHb) (Logothetis et al., 2001). This reduction of rHb leads to a smaller local magnetic field inhomogeneity which results in an increase in MRI signal. Caution should however always be taken when interpreting fMRI results. The relationship between the blood oxygenation level dependent (BOLD) signal change and haemodynamics are not well understood and are nonlinear(Rees et al., 1997). The initial hypo-oxygenation response to neural activation is highly localized, but then is followed by several seconds of hyperoxygenation, which is more widely dispersed up to 5mm from the localized activity area (Zarahn, 2001). This

may mean that fMRI is limited in spatial resolution by these physiological changes around the stimulated area. Regardless of these limiting effects, BOLD fMRI still has good spatial resolution for a non-invasive tool. BOLD fMRI has become the most widely used fMRI technique by far and continues to yield new information about brain function and the relationship between the mind and the body.

Event related fMRI:
Event Related paradigms differ from the more widely used blocked paradigms in that individual trial events are measured, rather than a temporally integrated signal. This allows for more sophisticated fMRI designs, e.g. the mixing of event types. The separate signal contributions of the events can be estimated and subsequently be compared directly. Several features of fMRI data have proven to be critical in allowing event-related procedures to be developed. First, due to technological advances, fMRI data can now be acquired at improved speed. This allows for an increase in the sampling frequency at which functional brain volumes can be acquired. The speed is now sufficient to measure the short fluctuations in the BOLD signal which typically occur in an event related fMRI design. Second, even very brief periods of neural activity give rise to measurable signal changes, despite the delayed and prolonged nature of the time course of the haemodynamic response. This means that event related fMRI can also be employed for measuring saccade related brain activations, which last at best a few hundred milliseconds.

Event related designs have profound advantage over blocked designs in that they allow for mixing of events. This mixing can be induced by the researcher either by presenting different stimuli or by requesting different responses from the subjects. Mixing can also be induced post hoc, by classifying different responses of the subjects, e.g. correct and incorrect. The disadvantage of event related fMRI is that in order to be able to temporally separate responses, and allow the condition of linearity to still apply, there must be an interval between events of at least a few seconds. This limits the number of trials which can be presented within a given time interval. Fewer trials means a reduced statistical power.

1.5 Outline of the thesis:

The main aim of this thesis is to assess the potential of brain activation maps, obtained with the antisaccade paradigm, for use as an endophenotype for schizophrenia. Five studies are undertaken to develop a suitable fMRI

design, and test properties of activity maps for potential use as endophenotypes for genetic research.

The first step in the process is the development of an fMRI design that is capable of picking up all the relevant activations that are associated with antisaccades. The first study (chapter 2) addresses the necessity of using event related fMRI for detecting saccade related brain activations in the antisaccade paradigm. Although blocked designs are statistically more powerful, their inability to discern activations of the separate events within the task may make them inadequate for the job.

The second study (chapter 3) addresses the first criterion for an endophenotype, the association with the illness. Although the presence of an antisaccade deficit in schizophrenia is well established, it is not yet known if and which specific brain areas are involved in the deficit. FMRI is used to investigate differences between patients and control subjects in brain activation associated with saccadic inhibition.

The third study (chapter 4) will assess whether the abnormalities in brain activation that are found in schizophrenia patients, can also be detected in their unaffected family members. As a good endophenotype is more closely linked to underlying genes than the diagnostic criterion, the increased genetic risk in unaffected family members should be reflected in an increased incidence of the abnormal brain activation endophenotype.

Although endophenotypes should be stable traits as they are strongly genetically determined, not all of them will remain unchanged over the entire lifespan due to environmental influences and interactions with genetic determinants. If individual subjects are to be classified based on their brain activation maps, normal or abnormal will greatly depend on their particular age. To be able to correct for age induced changes in activation, age effects on brain activation during the antisaccade paradigm will be assessed (chapter 5).

The use of brain activation maps as endophenotype in a linkage analysis involves the classification of individual data. Misclassification can reduce the chance of finding linkage between a trait and DNA locus dramatically. Hence, the reproducibility of the individual brain activation maps is of the utmost importance. The fifth study (chapter 6) will therefore investigate the test-retest reliability of brain activation maps that are associated with the antisaccade paradigm.

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Brain activation related to retrosaccades in saccade experiments.

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Abstract:
In saccade experiments, each trial (e.g. prosaccade/antisaccade) is by definition followed by a saccade which returns the gaze back to the center (retrosaccade). This event can complicate brain-imaging results when using a simple block design. We used an event related fMRI design involving prosaccades and antisaccades (testsaccades) to examine brain activation associated with retrosaccades. Testsaccades activated visual and oculomotor related brain areas. During retrosaccades, these areas were active at a lower level than during testsaccades. In the Supplementary Eye Fields, the Insula, and Striatum the retrosaccades gave rise to negative BOLD-responses. In the striatum these negative responses were equal in size to the positive responses of the testsaccades. This could mask brain activity of testsaccades when not taken into account.

2.1 Introduction:

The antisaccade paradigm is a well established task in clinical and experimental brain research (Hallett, 1978a). The antisaccade task measures a subject's ability to suppress an eye movement towards a new peripheral stimulus and instead make a voluntary saccade in the opposite direction. The reference task typically involves prosaccades, where subjects must make the saccade towards the peripheral stimulus. Many functional magnetic resonance imaging (fMRI) studies have examined the neural network involved in antisaccades and prosaccades (Connolly et al., 2000,Connolly et al., 2002,Cornelissen et al., 2002,Curtis and D'Esposito, 2003,Desouza et al., 2003,Kimmig et al., 2001,Matsuda et al., 2004,Muri et al., 1998).

Although generation of antisaccades is a conceptually simple act, the number of neuronal processes involved is considerable. Not only does the task induce activation directly related to the prosaccade or antisaccade, but recent experiments also demonstrate activation related to preparatory set prior to the saccadic event (Curtis and D'Esposito, 2003,Desouza et al., 2003). In addition, the prosaccade and antisaccade trials are always followed by a saccade back to center, in preparation for the next trial. Contributions of retrosaccade activity has not been studied with fMRI, so it is not known whether they are significant. In spite of this, ignoring retrosaccades in the analyses of imaging data, may have given cause to some unexpected results.

We obtained unexpected results when we attempted to improve our fMRI design for antisaccades and prosaccades. In a previous experiment, when using a sparse event related fMRI study, we found that during antisaccades compared to prosaccades there was increased activation in the striatum, the frontal eye fields (FEF), and the supplementary eye fields (SEF) (Raemaekers et al., 2002a). With the objective of shortening scantime and increasing sensitivity, we piloted a block-design with shortened intertrial intervals. This design yielded the expected activity in the FEF and the SEF. However, it failed to replicate striatal activity during antisaccades and for the contrast between antisaccades to prosaccades. These differences in results could only originate from differences in design properties. Most importantly, the event related design can separately model the antisaccades and prosaccades (testsaccades), and the saccades which return the view to central gaze (retrosaccades), whereas the block-design cannot dissociate these two responses. In a block-design the response associated with the retrosaccade could therefore effectively mask or confound the event of interest (e.g. the prosaccade or antisaccade).

This experiment addresses prosaccades and antisaccades within a sparse event related fMRI design. The use of event related fMRI allows us to separately establish brain activation related to prosaccades and antisaccades on one hand, and activation related to retrosaccades on the other. An opposite or negative response related to retrosaccades in the striatum and other regions could potentially mask other neural events within a trial when the interstimulus interval is short, and could thereby explain the findings of our blocked design. The event related design on the other hand would adequately detect all neural events within the striatum during the task paradigm.

2.2 Methods:

Subjects:
38 subjects participated in the experiment. All were right handed according to the Edinburgh Handedness inventory (Oldfield, 1971b)(mean, 0.84; SD, 0.18). All subjects gave informed consent for participation (approved by the Human Ethics Committee of the University Medical Center Utrecht).

Scanning protocol:
All images were obtained with a Philips ACS-NT 1.5T scanner (Philips Medical Systems, Best, the Netherlands) with fast gradients (PT6000). The head was held in place with a strap and with padding. For functional scans, a navigated 3D-PRESTO pulse sequence (Ramsey et al., 1998a) was used with following parameters: TE 37 ms, TR 24 ms, flip angle 9.5 degrees, matrix 48*64*24, FOV 192*256*96 mm, voxel size 4 mm isotropic, scan duration 1.49 s per 24-slice volume. A T1 weighted structural image was acquired at the end of the experiment.

Task design:
The fMRI design used a PC, a rear projection screen and a video-projector system for presentation. All stimuli were projected in white on a dark background unless notified differently. All events were time-locked to the fMRI scans. The first design consisted of two tasks, i.e. prosaccade and antisaccade, which had identical stimuli. Each new trial started with the disappearance of a fixation cross (0.9 ° visual angle) at central view. After a 200 ms gap period, a square (0.9° visual angle) was presented semi-random 8.7° to the left or right of central fixation. The square was extinguished after 3240 ms, simultaneously with the reappearance of the fixation cross at central view. A new stimulus was triggered by the scanner every ninth scan,

thereby generating a fixed stimulus interval of 13.4 s giving stimulus related blood oxygenation level dependent (BOLD) signal time to return to baseline (Bandettini and Cox, 2000). The two events, e.g. the testsaccade and the retrosaccade, were placed in time in this way, in order to keep the multiple correlations (R^2) to a minimum when regressing all other factors in the design matrix on the factor of interest (i.e. the testsaccade or the retrosaccade factors). This ascertains that the parameter for the factor if interest can be estimated with the highest possible reliability. The variance inflation factor ($VIF=1/(1-R^2)$) for the testsaccade factors is displayed in figure 2.1 for different time intervals between the testsaccade and retrosaccade (the graph is approximately equal for the retrosaccades).

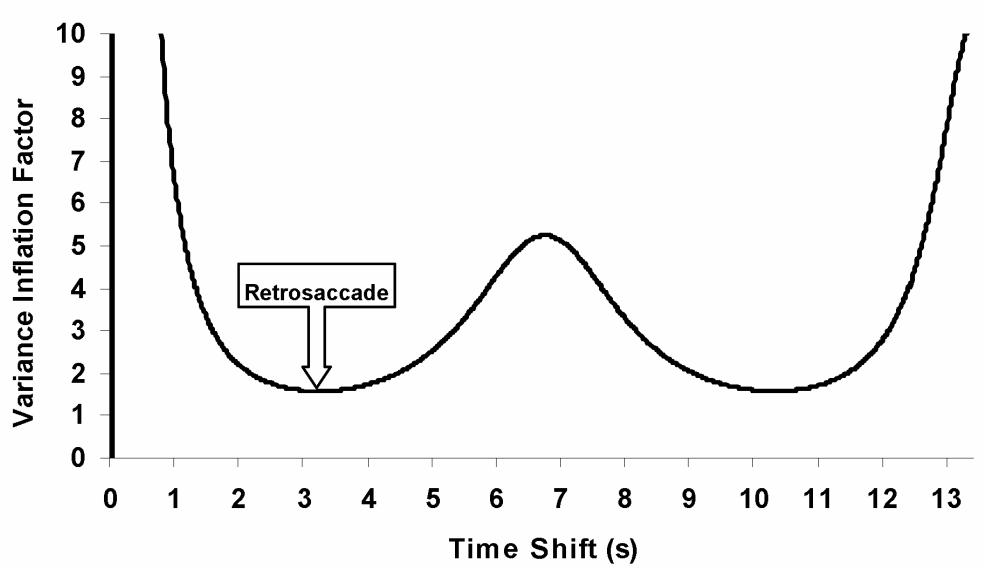

Figure 2.1. Variance inflation factor for the PRO/ANT factors over latency for the retrosaccade. The retrosaccade was placed at the first minimum, 3240 ms after the pro/anti-saccade. Thus, the subjects returned their gaze to the reappearing central fixation cross 3240 ms after the onset of the peripheral stimulus.

Instructions were given verbally prior to the start of the experiment and included the following: 1) Prosaccade: ‘’from central fixation look towards the square as quickly as possible when it appears. Look back to the fixation cross when the square disappears and the fixation cross reappears in the center.’’ 2) Antisaccade: ‘’when the square appears, look in the opposite direction as quickly as possible, without looking towards the square. Look back to the fixation cross when the square disappears and the fixation cross reappears in the center’’. Task instructions preceded each new block of ten stimuli. There were four blocks per task making a total of eight, which were

orderly alternated. Eye movements were recorded during the entire oculomotor-task using an MR-compatible eyetracker (Cambridge Research Systems Ltd., Rochester, UK (Kimmig et al., 1999) in combination with Labview (National Instruments Corporation, Austin, USA) acquisition software on a PC with a multifunctional I/O Board (National Instruments Corporation, Austin, USA).

Analysis:
Data analysis of fMRI scans was done with custom-written programs in IDL. All fMRI volumes where registered to the last functional volume using a least squares differences criterion (Thevenaz et al., 1998). The structural scan was also registered to the FA30-scan thereby providing spatial alignment between the structural scan and the functional volumes. A 3D-gaussian filter (8mm full width at half max) was applied to all fMRI volumes.

Data for each task were first submitted to linear multiple regression analysis. The factor matrix contained factors for stimulus related changes in BOLD-signal for prosaccades, antisaccades, retrosaccades following prosaccades, retrosaccades following antisaccades, and reading of the instructions. Low frequency noise was modeled with additional factors i.e. the mean signal intensity of each scan, and 88 discrete cosine functions forming a high pass filter with a cut-off at $3.73*10^{-2}$ Hz to correct for low frequency scanner and physiological noise but also for differences in initial activity level between conditions. All events in the design-matrices were convolved with a predefined hemodynamic response function (Friston et al., 1995).

The t-statistics of the regression coefficients were calculated for every voxel. The t-volumes representing activation during the different trial types were included in further analysis. Subsequently these volumes were spatially normalized in Talairach orientation to enable group-wise comparisons (Collins et al., 1994). The effects of the task were analyzed using the normalized t-volumes and the pooled standard deviation, which constitutes a random effects analysis (Worsley, 1994). The Bonferroni correction for the number of tests in all voxels resulted in a critical t-value of 4.52 for each voxel.

2.3 Results:

In 99% of the presented stimuli subjects made as saccade within 600 ms after stimulus presentation. The group results for activation during prosaccades and antisaccades (i.e. the contrast [1,1,0,0] with positions in the

contrast meaning [prosaccades, antisaccades, retro during pro, retro during ant]) are displayed in figure A1, page 68. Both tasks activated an extensive network of occipital areas including Brodmann area (BA) 17, 18 and 19, and the superior parietal lobe (BA 7). Activation of in the frontal lobes included the FEF in the lateral parts of BA 6, the SEF in the medial part of BA 6, the striatum, the insula (BA 13), and parts of BA 9 and the Anterior Cingulate (AC) (BA 32). The contrast between antisaccades and prosaccades ([-1,1,0,0]) (Figure A1, page 68) revealed increased activation during antisaccades relative to prosaccades in restricted portions of nearly all these regions.

The results of the comparison between the testsaccades and the retrosaccades ([1,1,-1,-1]) are displayed in figure A2, page 68. All regions that were activated during prosaccades and antisaccades were significant in this contrast as well, indicating generally more saccade related activation for testsaccades than for retrosaccades. More specifically, occipital and parietal areas had lower positive responses. Three regions within this contrast exhibited a significant negative BOLD response during retrosaccades. These were a combination of the AC and the SEF, the striatum, and the insula (Figure 2.2).

In all these three regions, the average amplitude of the BOLD response across the region was larger during antisaccades than during prosaccades (t_{37}=4.23;p<0.001 for the striatum, t_{37}=5.521;p<0.001 for the AC and SEF, t_{37}=5.836;p<0.01 for the insula). The negative response associated with the retrosaccade differed across these regions. For the SEF & AC the amplitude of the negative response associated with the retrosaccades was equally large following prosaccades and antisaccades (t_{37}=.342;p=.734, no difference). In the insula the negative response of the retrosaccades was slightly stronger following prosaccades than following antisaccades (t_{37}=1.215;p=.232), while in the striatum the negative response of the retrosaccades was larger after antisaccades than after prosaccades (t_{37}=4.173;p<0.001). Moreover, in the striatum the amplitudes of the testsaccades and the retrosaccades were equally large for both prosaccades (t_{37}=.138;p=.891) and antisaccades (t_{37}=-.-660;p=.513), which effectively cancelled out any global signal change when averaging over the entire length of the prosaccade or antisaccade condition.

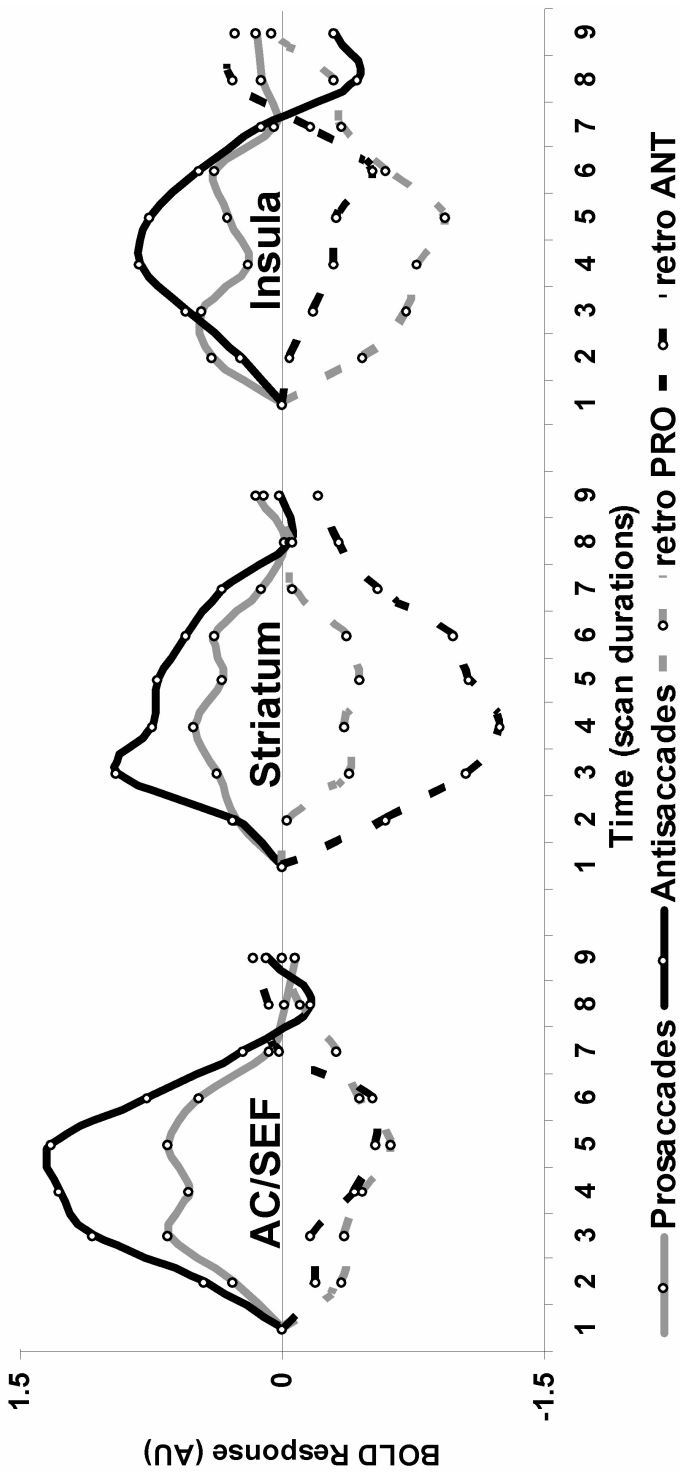

Figure 2.2. Averaged BOLD responses for testsaccades (prosaccades & antisaccades) and retrosaccades (retro PRO & retro ANT) in the SEF/AC, the striatum, and the insula. The time-axis is in single scan durations (1.49 s) The temporal offset of the retrosaccade has been shifted 2 scan durations to align with the onset of the testsaccade.

2.4 Discussion:

With this experiment we replicated the finding of our previous study in which we detected stronger involvement of the striatum, the FEF and the SEF in antisaccades relative to prosaccades (Raemaekers et al., 2002a). Activation during testsaccades was larger in all oculomotor related brain areas. We found robust negative BOLD responses in the striatum, the SEF and AC, and in the insula during retrosaccades. In the striatum this negative response was larger following antisaccades than following prosaccades.

The negative responses that were found in a number of brain regions would effectively mask the response of interest when the time-interval between the events is short, as is typically the case in a blocked design. Surprisingly, the negative and positive responses in the striatum were equal in proportion during both prosaccades and antisaccades. As a result, no significant activation will be detected in the striatum in a blocked design, when making a comparison to a rest condition, or when comparing prosaccades to antisaccades.

A few explanations remain for the observed signal differences between testsaccades and retrosaccades. The spatial predictability of retrosaccades may reduce attentional demands, which could attenuate the level of activation compared to testsaccades. Alternatively, central fixation may represent a special physiological baseline, requiring very little effort to return to (Evdokimidis et al., 1991). Perhaps even the brainstem could execute such a movement by itself. These possibilities should be addressed in future research. However, both these alternatives cannot explain why the BOLD signal would show a phasic decrease compared to the initial level of activity at the beginning of the trial.

It has been argued that negative BOLD signal is more likely to arise as a result of an elevated physiological baseline level of activity during a resting state, instead of having a hemodynamic origin (Gusnard et al., 2001). Recent evidence shows that sustained negative BOLD signal is indeed largely accounted for by a reduction in neural activity (Shmuel et al., 2002) as opposed to possible effects of vascular stealing or increased deoxyhemoglobin in the absence of increased flow. We therefore trust this negative response to be associated with an actual decrease or inhibition of neural activity compared to our baseline, i.e. fixating passively either in the center or in the periphery between the saccadic events.

Interestingly, phasic decreases in activation have been observed in the striatum in non-human primates using single cell recordings (Apicella et al., 1991). These decreases involve tonically active neurons (TANs), which

have a regular tonic firing rate of 3-9 Hz in the absence of movement, and are thought to be cholinergic interneurons (Pisani et al., 2001). TANs respond to conditioned sensory stimuli that elicit behavioural reactions. The response of these neurons generally consists of a brief depression of firing lasting 200-300 ms. The number of TANs showing a depression increases with duration of conditioning (Aosaki et al., 1994) in a learning task. In the present study, the disappearance of the peripheral stimulus and reappearance of the fixation cross may constitute a conditioned stimulus or trigger for a predefined eye movement back to the center. Thus, the drop in activity in TANs may cause the negative BOLD response. It should be noted however, that although TANs are widespread in the caudate and the putamen, they account for only less than 2% of the striatal population in monkeys (Pisani et al., 2001). Furthermore, the brief depression can be followed by rebound excitation (Aosaki et al., 1994). Taken together, we regard the brief depression of TANs as a potential cause of the negative BOLD response, but it is clear that this notion requires more understanding of the origins of the BOLD signal before it can be substantiated. In addition, it is not clear why the negative response is stronger in antisaccade than in prosaccade blocks.

Conclusion:
Saccadic fMRI paradigms can be confounded by the presence of retrosaccades. The pattern of activation in relation to the retrosaccade probably reflects neural processes related to anticipation, due to predictability of the stimulus. This study demonstrates the importance of fully modeling all experimental events, even those events that are deemed irrelevant by the researchers at first sight.

Neuronal substrate of the saccadic inhibition deficit in schizophrenia investigated with 3-dimensional event-related functional magnetic resonance imaging

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Raemaekers, M., Jansma, J.M., Cahn, W., Van der Geest, J.N., van der Linden, J.A., Kahn, R.S., Ramsey, N.F. Neuronal substrate of the saccadic inhibition deficit in schizophrenia investigated with 3-dimensional event-related functional magnetic resonance imaging. *Arch Gen Psychiatry.* 2002 Apr;59(4):313-20.

Abstract:
Background: Several studies have shown that the ability to suppress automatic saccadic eye movements is impaired in patients with schizophrenia as well as in their first degree relatives, and suggest that this impairment is a potential vulnerability marker for schizophrenia. The neurobiological mechanisms underlying normal saccade production and inhibition, revealed in primate studies, indicate that the impairment may result from a failure of the oculomotor system to effectively exert inhibitory control over brainstem structures. Functional localization of the affected brain structure(s) potentially provides a physiological measure for the investigation of vulnerability markers in schizophrenia.

Methods: The hemodynamic response to discrete visual stimuli was measured during prosaccades (saccades toward a peripheral stimulus), antisaccades (saccades toward a position opposite to a peripheral stimulus) and active fixation (holding fixation and ignoring a peripheral stimulus) in 16 patients with schizophrenia receiving atypical neuroleptics and 17 healthy controls subjects, using an event related functional magnetic resonance imaging task design.

Results: Brain responses were detected in the frontal and parietal regions of the oculomotor system in all 3 tasks. Patients made more errors during inhibition tasks, and exhibited a selective failure to activate the striatum during inhibition of saccades. In other regions that were active during inhibition, including the supplementary and frontal eye fields, no difference was found between patients and control subjects.

Conclusions: A fronto-striatal network is engaged in the suppression of automatic eye movements. The results indicate that abnormalities in this network, rather than the selective dysfunction of prefrontal brainregions, underlie the saccade inhibition deficit in schizophrenia.

3.1 Introduction:

Smooth pursuit eye movement (Campion et al., 1992)and the inhibition of automatic saccades (Everling et al., 1999) is impaired in patients with schizophrenia, their healthy first-degree relatives (Rosenberg et al., 1997a,Crawford et al., 1998), and healthy subjects with high scores on a measure of schizotypy (O'Driscoll et al., 1998). This suggests that eye movement deficits are a vulnerability marker (Holzman et al., 1988,Ross et al., 1998), or reflect an endophenotype (Clementz et al., 1998) for schizophrenia. A common feature of both smooth pursuit eye movements and antisaccade tasks is the requirement to avoid automatic intruding saccades. The failure to effectively inhibit saccades may explain the oculomotor impairments observed in patients with schizophrenia (Matsue et al., 1994,Levy et al., 1993). A potential link has been reported between the antisaccade deficit and a genetic marker on chromosome 22q (Myles-Worsley et al., 1999). The neural correlate of the antisaccade deficit in schizophrenia has not yet been identified. The elucidation of the neural correlate could provide a physiological measure of saccade inhibition, which could in turn prove to be a better endophenotype marker than the behavioral measure.

In the anti-saccade task (Hallett and Adams, 1980) subjects have to suppress saccades that are triggered by a peripherally presented stimulus (i.e. ‘prosaccades’), and instead have to generate a saccade toward a location in the opposite direction (hence ‘antisaccade’). Patients with schizophrenia fail to suppress the reflexive saccades more often than healthy controls and exhibit longer saccade onset latencies of correct responses, whereas prosaccade performance is well within normal range (Fukushima et al., 1990a,Rosse et al., 1993,Crawford et al., 1995,Karoumi et al., 1998). The deficit in saccade suppression in schizophrenia has been attributed to prefrontal dysfunction, a postulated key feature of schizophrenia (Weinberger et al., 1986). Some reported correlations between saccade inhibition, dorsolateral prefrontal cortex (DLPFC) function and working memory support this theory (Nieman et al., 2000,Park and Holzman, 1993,Roberts et al., 1994). However the notion of DLPFC involvement in saccade inhibition is primarily based on lesion studies (Guitton et al., 1985,Pierrot-Deseilligny et al., 1991a).

Alternatively, studies in non-human primates indicate that saccade inhibition involves more complex hierarchical networks (Berthoz, 1996). In a recent study on functioning of the superior-colliculus (SC) in primates, neuronal activity in the SC was reduced immediately before antisaccades but not before prosaccades, indicating that the SC must be suppressed by

this network to prevent an automatic saccade (Everling et al., 1999). The failure to inhibit a saccade can be caused by any one of the brainstructures forming the neural network that projects to the SC. The saccadic inhibition deficit in schizophrenia could therefore be associated with cortical as well as basal ganglia dysfunction, or disconnection, because both have been implicated in schizophrenia (Frith and Done, 1988) (Buchsbaum et al., 1999).

To our knowledge, only 2 functional imaging studies have investigated the saccade inhibition deficit in schizophrenia. The deficit was associated with reduced bloodflow in the DLPFC and frontal eye fields (FEF) in one study (Nakashima et al., 1994) and with reduced blood flow in the insula, anterior cingulate cortex and striatum in the other study (Crawford et al., 1996). In both studies, single measurements of brain perfusion required sustained periods (minutes or longer) during the task. Because the responses to stimuli are brief, such prolonged measurements are predominantly sensitive to tonic levels of activity. Brain regions that exhibit a change in tonic activity may be directly involved not in saccade inhibition, but rather in sustained attention and effort.

To investigate the specific neuronal correlates of the saccadic inhibition deficit in schizophrenia, we used a new experimental design that differs from the previous studies in 2 ways. First, to focus on the inhibition of saccades, an active fixation task was added to the experiment (Hikosaka and Wurtz, 1983); subjects were required to ignore distracting visual stimuli. The antisaccade task is not a sufficient because it involves other processes in addition to inhibition, fore example those that are required for deliberate initiation of a saccade toward another predetermined location (Passingham, 1993,Evdokimidis et al., 1996). Patients with schizophrenia are more easily distracted by stimuli when the task requires active fixation (Fukushima et al., 1990a). Second, the tasks were adapted for event related functional magnetic resonanceimaging (ERfMRI). This technique has the advantage of coupling changes in the BOLD (blood oxygenation level dependent) signal with specific events in time, which provides the opportunity to distinguish brain activity related to the test saccade from that related to the returning saccade.

Brain responses for each of the tasks were assessed for both groups with ERfMRI, and were subsequently compared. To measure performance, electro-oculography (Stevens et al., 1979) recordings (EOG) were done immediately after functional magnetic resonance imaging (fMRI) scans. We hypothesized that patients with schizophrenia would exhibit reduced

brainresponses specifically during the inhibitory tasks either in brain areas involved in the generation of an inhibitory signal, i.e. the DLPFC, FEF and supplementary eye fields (SEF), or in the transmission of this signal toward brainstem structures, i.e. basal ganglia connections (Anderson et al., 1994,Law et al., 1997,Connolly et al., 2000,Sweeney et al., 1996,O'Driscoll et al., 1995,Funahashi et al., 1993,Schlag-Rey et al., 1997).

3.2 Patients and Methods:

Sixteen patients with schizophrenia (13 men and 3 women, mean ± SD age, 27.9 years ± 5.5 years) from the Department of Psychiatry at the University Medical Center of Utrecht, the Netherlands, participated in this study. All patients met the criteria for schizophrenia according to the DSM IV (American Psychiatric Association., 1994) as assessed with the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al., 1992) (1 patient had disorganized schizophrenia, 11 had paranoid schizophrenia, 3 had schizophreniform disorder, and 1 was undifferentiated; mean ± SD duration of illness 29±19 months) and were screened for severity of present symptoms using the Positive And Negative Syndrome Scale (PANNS) (mean ± SD sum of positive items: 13.3±3.9; sum of negative items: 15.2±3.9; sum of generalized items: 29.9±6.6) (Kay et al., 1987). Every patient was taking a stable dose of atypical neuroleptic medication (clozapine: n=7 with a mean ± SD daily dose of 200±87 mg; olanzapine: n=8 with a mean ± SD daily dose of 11.3±6.4 mg; quetiapine fumarate: n=1with a daily dose of 450 mg). The control group consisted of 17 healthy subjects (10 men, 7 women mean ± SD age, 25.9 ± 4.1 years). None of the subjects in the control group exhibited any signs of a major psychiatric disorder according to the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998a). All participants were right handed according to the Edinburgh Handedness inventory (Oldfield, 1971b) (mean ± SD patients, 0.84 ± 0.14; mean ± SD controls, 0.84 ± 0.18). There was no significant difference in educational level between the groups (mean ± SD patients, 12.1 ± 2.3 years; mean ± SD controls, 13.1 ± 2.0 years). A history of substance abuse or major neurologic illness resulted in exclusion from the experiment, as did metal implants. All subjects gave their informed consent for participation (approved by the Human Ethics Committee of the University Medical Center Utrecht).

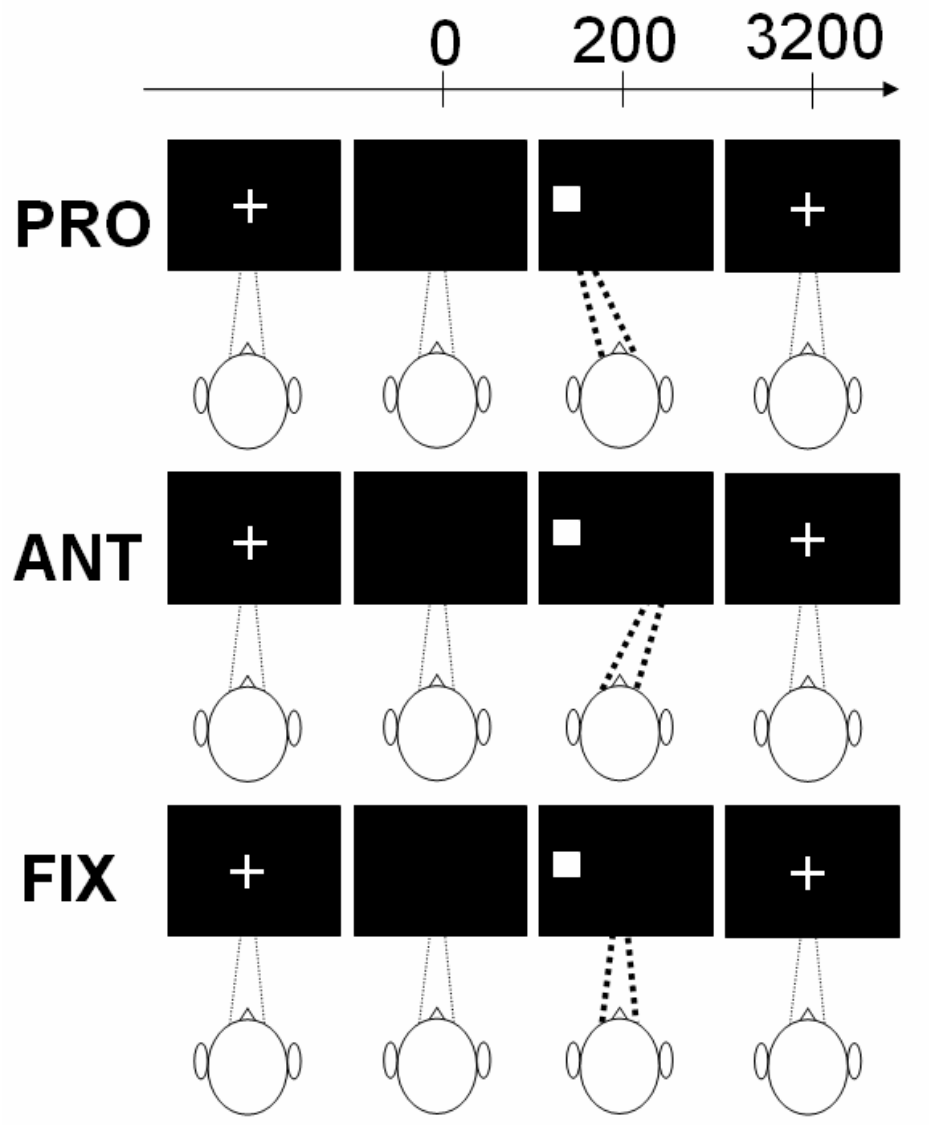

Figure 3.1. Schematic display of tasks in scanner. Each trial consisted of one saccadic event. The four black frames (from left to right) show the computer display changes within one trial, with the timing (in ms) shown at the top. A trial started with the disappearance of the cross (1.2 ° visual angle) at t=0, which was followed by a white square (1.2 ° visual angle) after 200 ms, appearing randomly 9.0° to the left or right. After 3000ms the square disappeared and the cross reappeared. Dotted lines shown the required viewing direction. All trials followed this scheme, but the instruction changed every 17 trials. The three instructions (tasks) are shown above each other: PRO, prosaccade, ANT antisaccade and FIX fixation. Note that the difference was only present during appearance of the square (third frame from the left).

Tasks:

The oculomotor task consisted of 3 parts (Figure 3.1), i.e. prosaccade (PRO), antisaccade (ANT) and active fixation (FIX). It was performed in the scanner and during additional EOGs (electro-oculograms). A personal computer, rear projection screen and a video-projector system were used for task assessment. The beginning of each trial was time-locked to the fMRI scans. By doing this, the return saccade was delayed enough to separate the corresponding BOLD response from that of the response of interest, namely the first eye movement (in the image analysis the 2 BOLD curves were mathematically uncorrelated). A new stimulus was triggered by the scanner for every ninth scan, thereby generating a fixed stimulus interval of 12.9 seconds and giving the stimulus related BOLD signal time to return to baseline (Bandettini and Cox, 2000).

Instructions for all tasks were given verbally prior to the start of the experiment and included the following: (1) PRO: ‘’From central fixation look toward the square as quickly as possible when it appears. Look back to the fixation cross when the square disappears and the fixation cross reappears in the center.’’; (2) ANT: ‘’When the square appears, look in the opposite direction as quickly as possible, without looking toward the square. Look back to the fixation cross when the square disappears and the fixation cross reappears in the center’’; (3) FIX: ‘’Keep looking at the location of the fixation cross when it disappears, and do not move toward the square’’. At the beginning of each new block of 17 stimuli the task instructions were changed. There were 3 blocks per task making a total of 9 blocks, which were presented in a semi-randomized sequence (PRO-ANT-FIX-ANT-PRO-FIX-PRO-FIX-ANT).

A similar oculomotor-task with EOG-recordings immediately followed the fMRI session, to measure subject task performance. Stimulus visual size was slightly reduced (0.8° visual angle) as was visual distance between central fixation and peripheral square (7.0° visual angle). To avoid fatigue, the inter-stimulus-interval was shortened to 3 seconds of which 1.8 seconds were used for central fixation and 1.2 seconds for display of the peripheral square. All 51 stimuli for each task were presented in 1 block. Blocks were presented in a PRO-ANT-FIX sequence. Subjects were seated in a near dark room, in front of a VGA monitor. Electro-oculographic data for horizontal saccades were acquired using surface electrodes at a sampling rate of 500 Hz. Data were processed offline with the aid of a custom, non-automated EOG-analysis program. Onset latencies were determined for all saccades. Any initial movement of the eyes in the wrong direction (depending on the task) with a latency longer than 100 ms was counted as an error. Any stimulus-related saccades during FIX were counted as errors.

All images were obtained with a clinical scanner (ACS-NT 1.5T; Philips Medical Systems, Best, the Netherlands) with fast gradients (PT6000). The head was held in place with a strap and padding. Structural and functional images were acquired in transverse orientation, from the same section of the brain (Figure 3.2). For functional scans, a 3D-PRESTO pulse sequence (Ramsey et al., 1998a) was used with following parameters: echo time, 36 milliseconds, repetition time, 24 milliseconds, flip angle, 10º, matrix 48*64*24, field of view, 192*256*123 mm, voxelsize 4 mm isotropic, scan duration 1.43 s per 24-slice volume. Immediately after functional scans, an additional PRESTO scan of the same volume of brain tissue was acquired with a high (30º) flip angle (FA30) for the image registration routine. A total of 1620 functional volumes were acquired per subject.

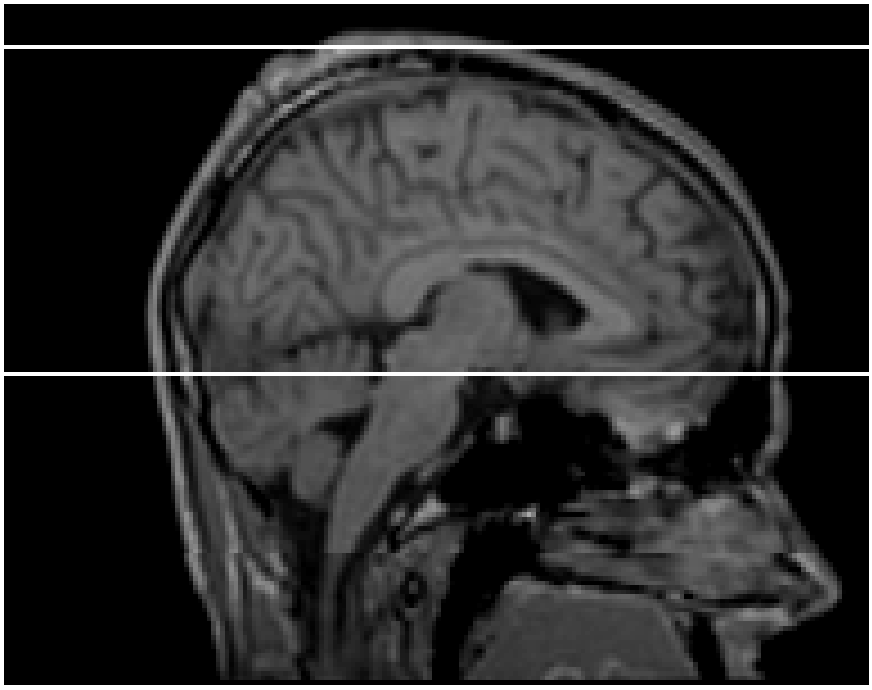

Figure 3.2. Volume of brain scanned with functional MRI. Typical fMRI scan volume of 24 contiguous slices, shown on top of the midsagittal slice of the anatomical volume for one subject. FMRI scans were acquired as 3-dimensional volumes, in transverse orientation, and covered almost the whole brain.

Analysis:

The last functional volume was registered to the FA30 volume. Next, all fMRI volumes were registered to the last functional volume using a least squares difference criterion (Thevenaz et al., 1998). The structural scan was also registered to the FA30-scan thereby providing spatial alignment between the structural scan and the functional volumes (Ramsey et al., 1998a). Only the scans acquired during stimulus trials were analyzed. A 3D-gaussian filter (8mm full width at half maximum) was applied to all fMRI volumes. To decrease low frequency noise, for each voxel the offset and linear trend were calculated for every set of 9 scans belonging to a single stimulus, using a standard regression procedure. The residuals were used for further analyses.

Next, 3 t-values per voxel were obtained from a standard linear multiple regression procedure with a factor matrix that contained the 3 factors representing stimulus related changes in BOLD-signal during PRO, ANT and FIX respectively. For each trial in the matrix the BOLD curve was modeled using a curve derived from a previous (unpublished) study. This curve has a skewed gaussian shape (with a kernel of 6 seconds) that peaks at 5 seconds. Thus, the 3 t-values for each voxel represented the presence and magnitude of the stimulus-contingent BOLD response during the 3 tasks for each subject. Finally, all the now co-registered t-volumes and structural scans were spatially normalized in Talairach orientation to enable group-wise comparisons (Collins et al., 1994).

To test for specific effects of inhibition, and of illness on inhibition in the brain activity maps, a multivariate repeated measures analysis was applied. Four independent comparisons were tested for significance for each voxel by first calculating the within a subject contrast value, and subsequently comparing these contrast values between the groups. Four comparisons of interest represented overall activity (activity during all conditions for both groups), overall effect of illness (differences between groups for all conditions), overall effect of inhibition (ANT and FIX combined vs. PRO for both groups) and effect of illness on inhibition (differences in effect of inhibition between groups). The group effects were converted to t-values (Worsley, 1994) and were subsequently tested for significance ($P<.05$) with the Bonferroni correction for the number of voxels (approximately 14 000, resulting in a critical t-value of 4.51 for each voxel). Thus, for the assessment of significant effects a threshold of t=4.51 was applied to all of the image analysis results.

3.3 Results:

EOG performance:
The EOG results indicate that patients did not perform worse than controls on the PRO task, neither in terms of reaction time (t_{29}=0.38; *P*=.71), nor error rate (t_{29}=-0.23; *P*=.82). However, in the ANT task the error rate was significantly increased (figure 3.3a), in the window of 100 to 180 milliseconds (t_{28}=1.90; *P*=.04), but not in other time windows. In addition, patients made more saccades (distraction) during FIX (t_{29}=1.70; *P*=.03) (figure 3.3b). The reaction time of correct antisaccade responses was increased (t_{29}=1.92; *P*=.05) (figure 3.3c). Thus, patients were moderately impaired in inhibiting saccades. When the data were summed across all tasks, patients made more eye movements overall within a time window of -100 to 500 milliseconds (Mann-Whitney U test : z=2.36, *P*=.02).

FMRI: Saccade-related activity:
The maps corresponding to the tested contrasts are shown in figure B, page 69. The overall activity pattern, including all tasks and subjects, is extensive (Figure B1, page 69). In addition to extensive occipital activation, the detected regions of the oculomotor system included the PEF in Brodmann Area (BA) 39 and 40, FEF at the intersection between the superior frontal and precentral fissure extending dorsally into the lateral part of BA 6, and SEF in the medial part of BA 6. Further activity was found in the anterior cingulate and anterior insula.

Compared with controls, patient brain activity was significantly reduced in visual cortex, and to a lesser extent in all other oculomotor regions (PEF, FEF, SEF) as well as the anterior cingulate (Figure B1, page 69). However, when examining the overall group t-map of the patients, significant activity was found in all of these regions indicating that activity was relatively reduced but not absent. This is shown for the visual cortex in figure 3.4.

Functional MRI: Inhibition-related activity:
In the overall inhibition map, a significant BOLD response occurred in the lateral occipital lobe (V5), FEF and SEF (Figure B, page 69). Interestingly, the BOLD response decreased in some subregions of the occipital lobe (V1 and V2). The interaction between inhibition and illness was significant only bilaterally in the striatum. More specifically, 4 areas were found, with the following Talairach x, y and z coordinates: left putamen (-23,8,7); right putamen (26,4,-3); left caudate body (-10,1,10); right caudate body (13,1,10).

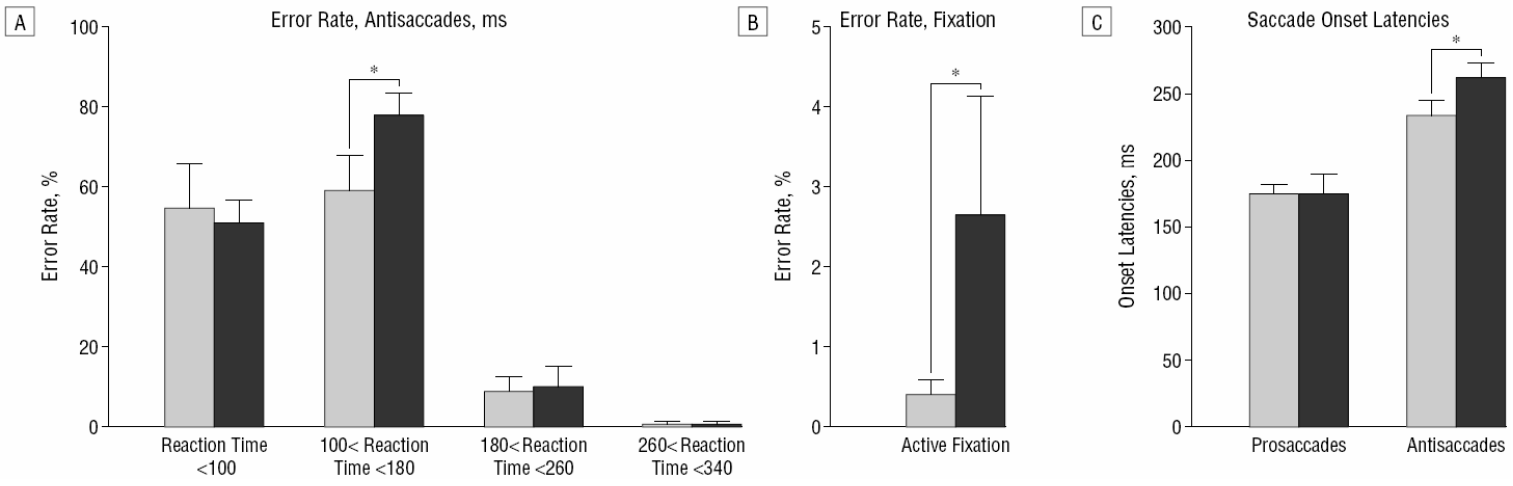

Figure 3.3. Performance on the tasks, assessed with EOG for patients (n=15) and control subjects (n=14). a. Antisaccade distractibility expressed as percent of the number of trials in which the first eye movement occurred, for each time-window. Error rate across all trials was 18% for control subjects and 21% for patients. b. Distractibility rates of patients and control subjects during active fixation. c. Reaction time of patients and control subjects on antisaccades and prosaccades (rt > 100 ms). Asterisks denote a significant difference between patients and controls (P<0.05). Performance of controls is shown in light bars, performance in patients in the dark bars. Error bars represent standard error of the mean.

Further inspection revealed that in controls the striatum responded (selectively) to the inhibition tasks, but that this response was absent in patients (Figure 3.5). This difference was also present in the individual inhibition t-maps. Whereas 12 of 17 control subjects showed activated voxels (t>3.0) in the striatum, the same was true for only 5 out of 16 patients (χ^2_1 =3.69; p=0.03). In further exploring the data, we looked at lower thresholds in the inhibition maps (t_{31}>3.5), and observed additional reduced responses in patients, in the thalamus, intraparietal area, and BA 44.

Clinical variables:
Clinical variables, i.e. positive, negative and general PANSS scores as well as type and dose of medication, did not correlate significantly with measures of performance or striatal activity.

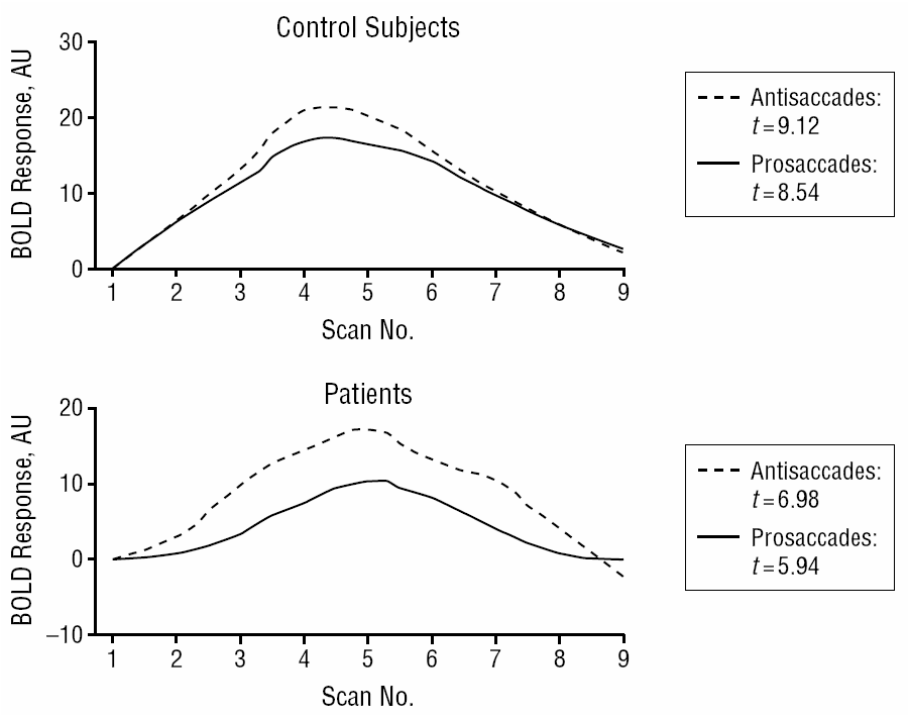

Figure 3.4. Averaged BOLD responses and corresponding t-values during prosaccades and antisaccades averaged over the activated voxels in the occipital lobe, displayed in figure B1, page 69, for control subjects (n=17) and schizophrenic patients (n=16). Due to normalization of values in multiple regression analyses, the BOLD response amplitude is presented in arbitrary units (A.U.).

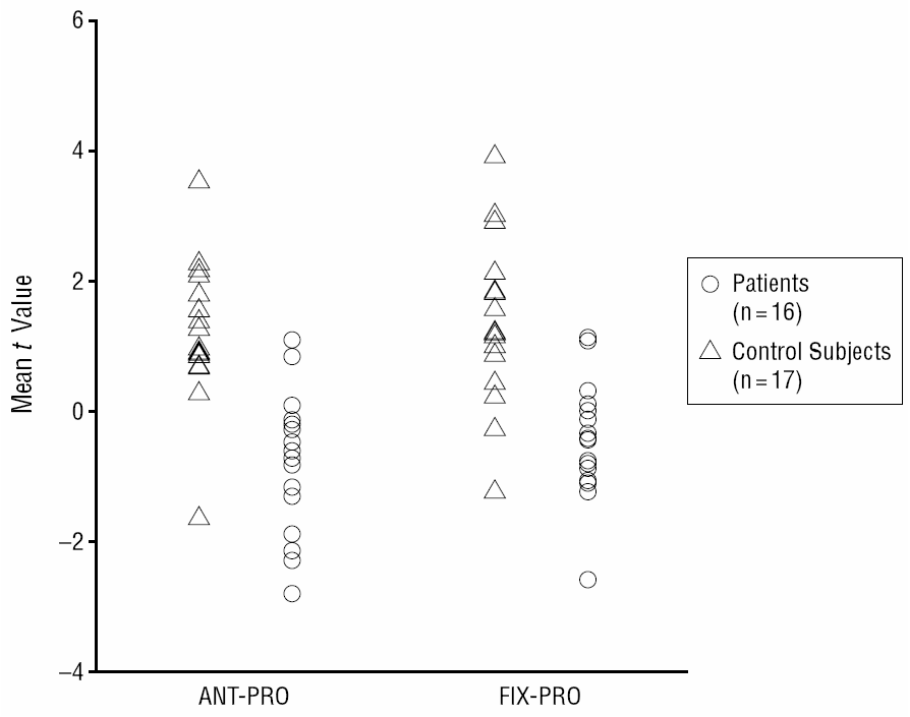

Figure 3.5. The scatterplot shows the mean t-value in striatum, of the difference in brain activity between antisaccades and prosaccades and between fixation and prosaccades for each subject. For these mean t-values, only voxels were selected where an interaction between illness and inhibition was found in the group-comparison (yellow in figure B2, page 69). The plots show that the patients fail to activate the striatum during both inhibition tasks (antisaccade and fixation). Controls are indicated with triangles, and the patients with squares.

3.4 Comment:

In accordance with other studies, patients with schizophrenia were impaired on the ANT task, which requires the inhibition of prepotent saccades. The imaging results suggest that this impairment is associated with a failure to engage striatal structures. In healthy volunteers we demonstrated significant activation in a network of regions that are known to subserve saccadic eye movements (Berthoz, 1996) (Schlag-Rey et al., 1997,Anderson et al., 1994,Law et al., 1997,Sweeney et al., 1996,O'Driscoll et al., 1995). Furthermore, the inhibition of saccades was found to involve the SEF (the anterior aspect of the supplementary motor area), FEF, and the striatum, confirming previous studies (Anderson et al., 1994,Law et al., 1997,Sweeney et al., 1996,O'Driscoll et al., 1995,Connolly et al., 2000).

Thus, the obtained brain activity maps for each task demonstrated that the event-related fMRI procedure was adequate.

Although patients with schizophrenia showed activity in the same regions combined as the controls during all tasks, overall the magnitude of activity was smaller. Because reduced activity occurred in all oculomotor-related areas, patients with schizophrenia generally exhibited either lower amplitudes of, or more variability in, the BOLD response. The latter explanation is supported by the finding that the patients made more eye movements in the period between 100 milliseconds before and 500 milliseconds after a stimulus during the EOG test. A higher incidence of random eye movements during the fMRI experiment may have increased variability by adding BOLD responses to the data, resulting in increased noise. The fact that inhibition-related brain activity was present in FEF and SEF in both patients and controls indicates that patients complied with the tasks when undergoing scans. This further indicates that the observed difference between patients and control subjects in the striatum does not reflect merely a behavioral difference caused by a higher percentage of incorrect prosaccades in patients during inhibition.

The relevance of the striatum for oculomotor functioning has been studied extensively(Alexander et al., 1986,Alexander and Crutcher, 1990). The striatum represents the major input site of the subcortical oculomotor circuit. These subcortical regions transfer input from the frontal cortex downward to the substantia nigra pars reticulata (SNr) (Berthoz, 1996,Alexander et al., 1986,Alexander and Crutcher, 1990). From the SNr ascending fibers feed back to the cortex through the thalamus, whereas the descending efferents provide an escape route from the basal ganglia-thalamocortical loop by projecting to the superior colliculus superior colliculus (Parent, 1990,Gerfen, 1992,Hikosaka and Wurtz, 1983).

The striatum is capable of exerting both an inhibitory and an excitatory influence on the SNr by means of 2 parallel pathways. The inhibitory pathway projects to the SNr directly (Brandt et al., 1998), and the excitatory pathway passes through the external globus pallidus and subthalamic nucleus (Gerfen, 1992,O'Connor, 1998).With these 2 connections the striatum can facilitate or suppress overt oculomotor behavior, and does so by transiently modulating the tonic inhibitory control that is exerted by the SNr on the SC (Hikosaka and Wurtz, 1983,Kato et al., 1995). The suppressed neuronal activity in the SC during saccadic inhibition reflects an effort to avoid fast reflexive saccades triggered by the SC through afferents of the nuclei of the optic tract or parietal regions (Everling et al., 1999).

Accordingly, inhibition of saccades requires adequate input from a network that feeds into the SNr, which is most likely initiated in the frontal cortex.

Indirect evidence for the striatum's possible role in saccade inhibition in human subjects is provided by the fact that distractibility rates are increased in patients with degenerative diseases affecting the basal ganglia such as Huntington disease (Lasker and Zee, 1997). This indicates that although direct connections also exist between the frontal cortex and the SC (and the reticular formation), providing an alternative means of inhibitory control (Berthoz, 1996), impaired striatal function does affect saccadic inhibition. Tardive dyskinesia in patients with schizophrenia has been shown to enhance distractibility, which has been ascribed to altered γ-aminobutyric acid and dopamine function in the basal ganglia (Thaker et al., 1989,Jeste and Caligiuri, 1993). The striatum has been implicated in schizophrenia in other studies. The clinical efficacy of neuroleptics is linked to dopamine D_2 receptors, which are present in high concentrations in the striatum, and both medicated and unmedicated patients demonstrate abnormal striatal metabolic rates (Cohen et al., 1997,Buchsbaum et al., 1999).

Alternatively, dysfunction of the fronto-striatal-thalamic loops could contribute to cognitive and psychotic symptoms of schizophrenia (Willner, 1997). Because the frontal regions are involved in the generation of a ''stop'' signal, frontostriatal connections play an important role in downward transmission of this signal (Guitton et al., 1985,Pierrot-Deseilligny et al., 1991a). A deficit in functional connectivity between the frontal lobes and the striatum has been demonstrated in schizophrenia in concordance with a reduction in interconnecting white matter between these areas (Buchsbaum et al., 1998).

Surprisingly, in this study there was no indication that abnormal activity in the frontal lobes contributed to the deficit. Healthy volunteers did not exhibit a BOLD response in DLPFC, in spite of its postulated involvement in saccade inhibition (Nakashima et al., 1994,Funahashi et al., 1993,Walker et al., 1998). Because the DLPFC did not respond to the stimuli in a transient manner, it may have been constantly active during inhibition tasks, thereby remaining unnoticed in this experiment and making the detection of abnormal activity in patients difficult. However, the role of the DLPFC in saccadic inhibition has not been consistently confirmed by imaging studies (Muri et al., 1998,Nakashima et al., 1994,Crawford et al., 1996,O'Driscoll et al., 1995).

Our study is limited in several respects. For one, eye movements were not recorded with the scanner. Performance measurements were acquired

without the scanner immediately after the fMRI session, because at the time of the fMRI experiment we did not have access to an MRI-compatible eye-tracking device. Also the task was not the same as in the scanner; the interstimulus interval was shortened in order to minimize the overall length of the experimental session. This shorter interval may have affected performance, because it may be more stimulating and alerting for subjects. However, the main reason for the off-line recording of eye movements was to determine whether all subjects were capable of performing the task as intended, and whether the patient group would display a deficit as was expected on the basis of reported findings in the literature. The results indicated that all subjects were capable of performing the tasks and that the patients made more errors. The brain activity maps showed that most of the brain regions that were active in controls were also active in patients during all tasks, albeit at lower levels, providing indirect evidence that patients did perform the tasks and that the difference in brain activity was not due to non-compliance. Another limitation is that in maximizing sensitivity to transient brain responses to examine the dynamics of the involved network, we did not measure sustained brain activity during the tasks. It would be worthwhile to design studies that allow the assessment of both types of responses simultaneously. Responsivity of one region can conceivably depend on the tonal activity of another (Moore et al., 1999).

Finally, because we tested patients on medication, one could argue that the results are associated with medication effects. Although we do not know whether the observed abnormalities of brain function in the striatum in our patients are the result of medication, we do know from the literature that the *behavioral* abnormalities observed in patients with schizophrenia is not explained by medication (Clementz et al., 1994,Katsanis et al., 1997,Crawford et al., 1995). Therefore, if the brain activity effect were attributed to medication, the observed abnormality in brain function would bear no relevance to the behavioral deficit in saccadic inhibition. However, we show that the striatum is actively involved in saccade inhibition in healthy subjects, so any medication effects on this region would be expected to have behavioral consequences.

In summary, we have shown that the network that subserves inhibition of prepotent saccades may be dysfunctional in schizophrenia at the level of the striatum. Because saccade inhibition deficits may be regarded as biological markers for schizophrenia (Clementz et al., 1994,Katsanis et al., 1997), fMRI images of the neuronal circuits underlying saccade inhibition could be a useful tool to identify those at risk for schizophrenia (Clementz et al., 1998,Freedman et al., 2000).

Brain activation during antisaccades in unaffected relatives of schizophrenic patients

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Abstract:
Background: Schizophrenia patients have difficulty inhibiting automatic saccades. Many studies have failed to resolve whether healthy first-degree relatives share the same deficit. Measures of brain activity may be more sensitive than behavioral measures. In patients, the saccadic inhibition deficit has been related to impaired frontostriatal functioning. This study attempts to establish whether this abnormality is also present in unaffected relatives of patients.

Methods: Functional brain images were acquired during prosaccades and antisaccades in sixteen control subjects and sixteen unaffected sibling of schizophrenia patients using an event related functional Magnetic Resonance Imaging design. Eye movements were measured during scanning.

Results: The task activated a network of regions corresponding to the oculomotor system. Siblings and controls did not differ during execution of prosaccades. During antisaccades, siblings did not activate the caudate nucleus. Siblings and controls subjects did not differ on the percentage of errors during antisaccades

Conclusions: Siblings did not appropriately activate the striatum during antisaccades, similar to what has been reported in patients. Siblings, however, did not make significantly more errors during antisaccades, indicating that they were able to compensate for the inactive caudate. Future research is needed to assess the potential of this striatal deficit as (genetic) risk factor for schizophrenia.

4.1 Introduction:

Endophenotypes have emerged as an important concept in the study of complex neuropsychiatric diseases. The endophenotype approach attempts to reduce the complexity of the phenotype-genotype relationship by choosing traits which are located more 'upstream' in the process by which genes ultimately contribute to overt traits, thereby increasing the power of linkage analyses. For schizophrenia, a number of traits have been proposed as candidate endophenotypes. They do not only have an increased incidence in patients, but also in their unaffected relatives. Although many endophenotype definitions typically involve performance measures on cognitive or motor tasks, measures of the actual brain activation may be more intimately related to an underlying genetic defect. Brain imaging methods could thereby further close the gap between phenotype and genotype and help to define more powerful endophenotypes.

Various eye movement abnormalities have been proposed as candidate endophenotype for schizophrenia. Among other things, patients with schizophrenia have difficulty suppressing reflexive saccadic eye movements. One way to measure the ability to inhibit reflexive saccades is the antisaccade task, where subjects have to inhibit an eye movement towards a novel stimulus (prosaccade) and instead make an eye movement in the opposite direction (antisaccade) (Hallett, 1978a). All reported experiments on antisaccade performance in schizophrenia indicate an increased percentage of inhibition errors compared to healthy control subjects, while prosaccade performance remains largely intact (see Brownstein 2003 for a review) (Brownstein et al., 2003). The deficit is present irrespective of medication, clinical subtypes and variations in the task related variables. The fact that test-retest reliability is high, confirms that antisaccade performance is a stable trait (Calkins et al., 2003a).

Experimental data on antisaccade performance in non-schizophrenic relatives of patients is far less consistent. Whereas six studies provide support for increased percentage of inhibition errors during antisaccades in relatives (Clementz et al., 1994,Curtis et al., 2001,Ettinger et al., 2004,Karoumi et al., 2001,Katsanis et al., 1997,McDowell and Clementz, 1997), two studies fail to do so (Brownstein et al., 2003,Crawford et al., 1998). Two studies found an antisaccade deficit only in relatives with schizophrenia with high levels of schizotypy, but not in relatives without schizotypal symptoms (Thaker et al., 1996,Thaker et al., 2000). The origin of the inconsistency in the data remains as yet unknown (Levy et al., 2004,Calkins et al., 2004a).

It is possible that measuring antisaccade behavior is a relatively insensitive method to detect underlying brain abnormalities. Direct measures of brain function may be more sensitive, as they are more intimately related to a biological deficit. Using functional Magnetic Resonance Imaging (fMRI), increased functional activation has been reported in the dorsolateral prefrontal cortex (DLPFC) during a spatial (Callicott et al., 2003b) and a verbal (Thermenos et al., 2004) working memory task in non-schizophrenic relatives of patients. In addition, reductions in activation have been found in the thalamus, cerebellum, and medial prefrontal cortex during a sentence completion task (Keshavan et al., 2002). Importantly, all these studies detected neurofunctional abnormalities in relatives in the absence of behavioral differences. Brain imaging methods therefore seem to provide more sensitive means to detect abnormalities than behavioral measures.

The present study attempts to establish whether the use of functional brain images could also improve sensitivity for detecting abnormalities in antisaccades in relatives of patients. In a previous study, we linked the saccadic inhibition deficit in schizophrenic patients to a failure to engage the striatum as measured with event related fMRI(Raemaekers et al., 2002a). Using a largely similar design, we now investigated brain activity during prosaccades and antisaccades in unaffected relatives of patients, in order to assess the presence or absence of the deficit. Presence of the striatal dysfunction in unaffected siblings would indicate that the dysfunction observed in schizophrenic patients is unlikely to be a secondary effect of illness or medication, and could be a risk factor for the development of schizophrenia.

4.2 Methods:

Subjects:
16 healthy control subjects (8 male, 8 female; mean ± SD age, 33.4 ± 13.6 years) and 16 unaffected and unrelated siblings (8 male, 8 female; mean ± SD age 33.9 ± 11.3 years) of patients with DSM IV (American Psychiatric Association., 1994) schizophrenia (as assessed with the Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992) participated in the experiment. None of the subjects had any signs of present or past major psychiatric illnesses according to the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998a). A history of major neurological illness resulted in exclusion from the experiment, as did metal implants. Subjects were screened for the presence psychiatric illnesses in

first and second degree relatives using the family interview for genetic studies (Maxwell, 1992).
All subjects gave informed consent for participation (approved by the Human Ethics Committee of the University Medical Center Utrecht).

Scanning protocol:
All images were obtained with a Philips ACS-NT 1.5T MRI scanner (Philips Medical Systems, Best, the Netherlands) with fast gradients (PT6000). The head was held in place with a strap and with padding. Structural and functional images were acquired in transverse orientation, from the same section of the brain. For functional scans, a navigated 3D-PRESTO pulse sequence (Van Gelderen et al., 1995,Ramsey et al., 1998a) was used with following parameters: TE 37 ms, TR 24 ms, flip angle 9.5 degrees, matrix 48*64*24, FOV 192*256*96 mm, voxel size 4 mm isotropic, scan duration 1.49 s per 24-slice volume. Immediately after functional scans, an additional PRESTO scan of the same volume of brain tissue was acquired with a high flip-angle (30 degrees, FA30) for the image coregistration routine (see below). Finally, a T1 weighted structural image was acquired.

Task design:
The fMRI design used a PC, a rear projection screen and a video-projector system for presentation. All stimuli were projected in white on a dark background. All events were time-locked to the fMRI scans. Instructions were given verbally, prior to the start of the experiment. With the aid of a laptop, a limited number of test trials were presented until subjects indicated they understood the task. The design consisted of two tasks, that is prosaccades and antisaccades, which had identical stimuli. Whether subjects were requested to make prosaccades or antisaccades depended on a short summary of the instructions at the beginning of each new block of ten stimuli. Instructions were presented for a duration of three scans, followed by a 6 scan period of central fixation. Each new trial started with the disappearance of a fixation cross (0.9 ° visual angle) at central view. After a 200 ms gap period, a square (0.9° visual angle) was presented semi-random 8.7° to the left or right of central fixation. If the instructions were prosaccades, subjects had to make a saccade towards the square as quickly as possible. If the instructions were antisaccades, subjects had to avoid an automatic eye movement towards the square, and instead make a saccade towards the opposite direction. The square was extinguished after 3240 ms, simultaneously with the reappearance of the fixation cross at central view.

This signaled the subjects to refixate in the center of the screen. A new stimulus was triggered 10.16 s. after central refixation, thereby generating a fixed stimulus interval of 13.4 s giving stimulus related BOLD signal time to return to baseline (Bandettini and Cox, 2000). Stimulus related changes in BOLD signal were thus measured relative to fixating in the center or in the periphery. The two events, e.g. the prosaccade/antisaccade and the refixation in the center, were placed in time in this way, in order to keep the correlation between the two associated BOLD-responses to a minimum. There were four blocks per task making a total of eight, which were orderly alternated. Each subject made 40 prosaccades and 40 antisaccades in total, during the 20 minute functional imaging session.

Eye Movements:
Eye movements were recorded during the entire oculomotor-task using an MR-compatible infrared limbus eyetracker (Cambridge Research Systems Ltd., Rochester, UK (Kimmig et al., 1999) in combination with Labview (National Instruments Corporation, Austin, USA) acquisition software on a PC with a 6032E multifunctional I/O board for PCI Bus (National Instruments Corporation, Austin, USA). This acquisition-PC was linked to the Stimulus-PC by a parallel cable to synchronize the eye-recordings and the task presentation. The calibration and adjustment of the sensor were done during a five minute period prior to scanning. The sampling rate of the recording was 500 Hz. For each saccade in the time window of 200 ms before until 600 ms after the stimulus presentation, the latency and the direction were determined using a custom nonautomated analysis program in the Interactive Data Language (IDL) (Research Systems Inc., Boulder, USA). Trials with saccade onset latencies shorter than 100 ms were marked as anticipatory and excluded from further analysis. An error-trial was counted if the first saccade was in the wrong direction. Trials with uncorrected errors were also excluded.

Analysis:
Data analysis of fMRI scans was done with custom-written programs in IDL (Research Systems Inc. Boulder, USA). The last functional volume was registered to the FA30 volume. Next, all fMRI volumes where registered to the (now registered) last functional volume using a least squares differences criterion (Thevenaz et al., 1998). The structural scan was also registered to the FA30-scan thereby providing spatial alignment between the structural scan and the functional volumes. A 3D-gaussian filter (8mm full width at half max) was applied to all fMRI volumes.

Data for each subject were submitted to a linear multiple regression analysis. The factor matrix contained factors for stimulus related changes in BOLD-signal for prosaccades, antisaccades, refixation in the center during prosaccades, refixation in the center during antisaccades, and reading of the instructions. For both designs, low frequency noise was modeled with additional factors i.e. the mean signal intensity of each scan, and 88 discrete cosine functions forming a high pass filter with a cut-off at $3.73*10^{-2}$ Hz to correct for low frequency scanner and physiological artifacts but also for differences in baseline activation between conditions. All events in the design-matrices were convolved with a predefined hemodynamic response function (Friston et al., 1995). Parallel to this first regression model, there was a more elaborate model which included separate factors for correct and incorrect prosaccades and antisaccades. This model allowed to contrast brain activation associated with correct trials, to brain activation associated with incorrect trials. The sole purpose of the second analysis was the detection of effects of task performance, which could potentially confound group differences.

The t-statistics of the relevant contrasts (e.g. prosaccades, antisaccades, and antisaccades vs. prosaccades) were calculated for every voxel. Subsequently, the t-volumes were spatially normalized in Talairach orientation to enable group-wise comparisons (Collins et al., 1994). The effects of the task were analyzed using the normalized t-volumes of prosaccades and antisaccades (Worsley, 1994). Bonferroni correction for the number of tests for all brain voxels resulted in a critical z-value of 4.52 for each voxel. For the a priori test that siblings shared the same abnormality as the patients during saccadic inhibition(Raemaekers et al., 2002a), the critical z-value for a region corresponding to the Talairach definition of the Caudate Nucleus and the Putamen (Talairach and Tournoux, 1988) was established at 3.66 (Bonferroni correction for 200 voxels). Effects of task performance were analyzed post hoc, using the regression-coefficients of the elaborate design for correct and incorrect prosaccades and antisaccades.

4.3 Results:

There was a trend for increased saccadic onset latencies of correct prosaccades (t_{30}=1.68; P=0.052) and antisaccades (t_{30}=1.44; P=0.080) in siblings compared to controls (Table 4.1). There were no group differences in the percentage of errors for both prosaccades (t_{30}=0.99; P=0.166) and antisaccades (t_{30}=0.57; P=0.288) (Table 2). Both groups corrected nearly all erroneous saccades within the 600 ms time window after trial presentation. To confirm that the oculomotor task was adequate for detecting saccade

related brain activation, we tested overall eye movement activation (i.e. the combined activation of prosaccades and antisaccades). The activity map revealed large portions of visual cortex, and a network of regions corresponding to the oculomotor system including, the parietal, frontal, and supplementary eye fields (PEF, FEF, SEF) (Figure C1, page 69). To test whether the paradigm was sufficiently sensitive to pick up saccadic inhibition related activation, we tested the contrast between antisaccades and prosaccades. Activity was higher during antisaccades than during prosaccades within restricted portions of nearly all visual and saccade related brain areas, ($z>4.52$; $P<0.05$) (Figure C1, page 69).

Prosaccade activation in siblings was normal, as it did not differ from control subjects in any voxel in the entire scanned volume (not shown). Subsequently, we tested for differences between the groups on the contrast between prosaccades and antisaccades, with a restricted focus on the striatum (Raemaekers et al., 2002a). This revealed a difference between the groups in two regions with the following Talairach x,y and z coordinates: right caudate body (13,-2,18), and the left caudate body (-16,-2,18) ($z>3.66$; $P<0.05$) (Figure C2, page 69). The difference was caused by an absence of activation in the caudate in siblings compared to controls during antisaccades. Neither group activated the caudate during prosaccades. No differences between the groups were detected on the antisaccade vs. prosaccade contrast outside the striatal volume when correcting for the whole brain (not shown)($z>4.52$;$P<0.05$). The averaged blood oxygenation level dependent (BOLD) responses demonstrated a similar pattern in siblings and control subjects in all saccade regions (SEF and visual cortex are shown in figure 4.1), but not in the caudate body. However, as different thresholds were used inside and outside the striatum, the conclusion that the striatum is selectively implicated is not warranted. Figure 4.2 displays the individual averaged z scores in the caudate body for the contrast between antisaccades and prosaccades.

	Control Subjects (n = 16)	Siblings (n = 16)	Effect Size (d)
Latency Prosaccades (ms)	183.8 ± 30.1	205.3 ± 41.4	.59
Latency Antisaccades (ms)	251.9 ± 42.9	281.0 ± 68.4	.51
Errors Prosaccades (%)	.5% ± 1.5%	1.2% ± 2.2%	.37
Errors Antisaccades (%)	22.8% ± 18.6%	26.8% ± 21.4%	.2

Table 4.1. Average scores with standard deviations on the four measures of task performance.

Subsequently we tested whether the activation differences in the striatum could also be a secondary result of the percentage of errors the subjects made. We reanalyzed the data of the caudate body, with the activation for correct and incorrect trials as separate factors in the regression analysis for each subject. Performance (i.e. correct versus incorrect antisaccade responses) did not affect activity (t_{31}=0.499; p=0.621 for the left and t_{31}=0.00; p=1.00 for the right body of the caudate). Furthermore, the average reaction time that it took for the subjects to make a correct antisaccade also did not affect activity, as there were no correlations in the caudate between average reaction times of correct responses and the level of activation in the left (r=0.063; p=.733) or right caudate body (r=-0.050; p=0.786; n=32) during antisaccades. There was also no significant correlation between the average level of inhibition related activation (antisaccades vs. prosaccades) and the proportion of errors during antisaccades (r=.07; p=.80 for siblings and r=.40; p=.13 for control subjects). These findings indicate that performance differences between groups are unlikely to have caused a bias in the group comparisons.

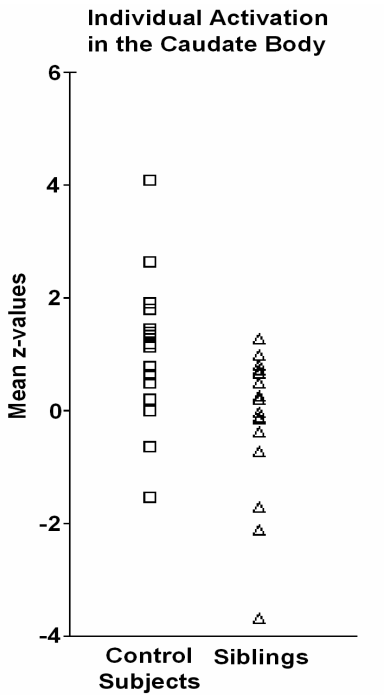


Figure 4.2. Scatter plot of the averaged z value over voxels in the caudate body that significantly distinguished siblings from control subjects on the contrast between antisaccades and prosaccades (displayed in figure 4.1).

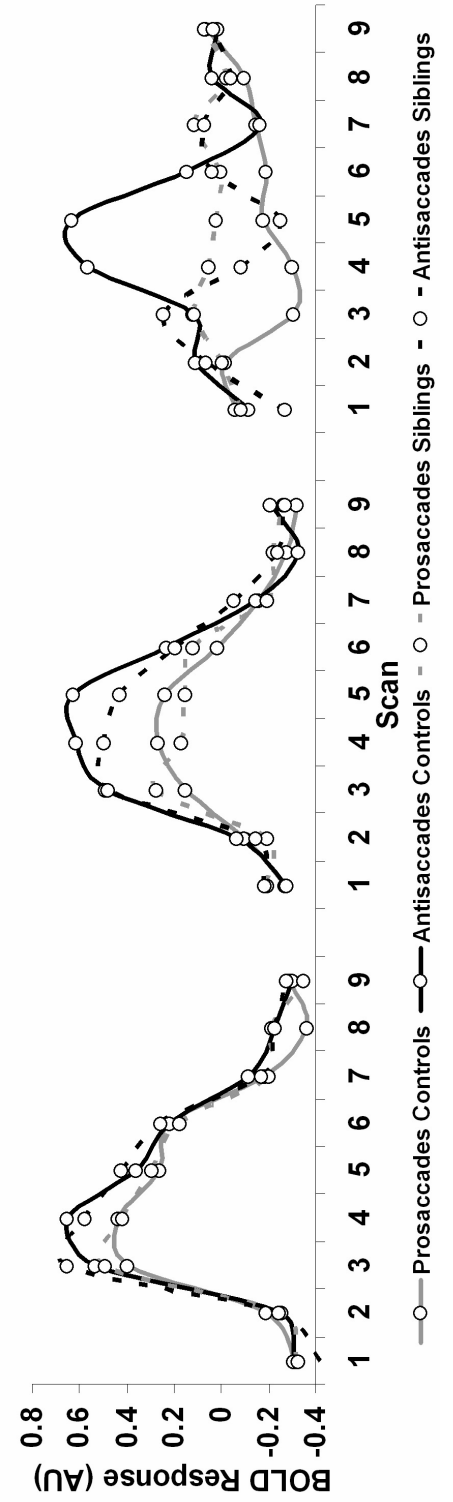


Figure 4.1. Averaged BOLD-responses related to prosaccades and antisaccades for control subjects and siblings in visual cortex and SEF, and the voxels of the striatum that demonstrated a difference between siblings and controls on the contrast between antisaccades and prosaccades (regions displayed in Figure 2). Bold responses are corrected for the effects of the refixation movement towards the center (see Methods). Because of the normalization of values in multiple regression analysis, the BOLD response amplitude is presented in arbitrary units (AU).

4.4 Discussion:

The present study was conducted to investigate whether healthy siblings of schizophrenic patients exhibit the same brain dysfunction during inhibition of eye movements, that has been reported in schizophrenic patients (Raemaekers et al., 2002a). We found that healthy siblings display normal brain activity during reflexive eye movements made in a prosaccade task. During inhibition of eye movements, however, siblings differed from controls in that they failed to activate the body of the caudate nucleus appropriately. This finding matches results of the previous study in which schizophrenic patients failed to recruit the striatum during saccade inhibition (Raemaekers et al., 2002a). In spite of differences in brain activity, siblings did not make significantly more errors during antisaccades, suggesting that they were able to compensate.

To our knowledge, no anatomical abnormalities have ever been detected in the striatum in relatives of schizophrenic patients (Lawrie et al., 2001,Seidman et al., 1999,Staal et al., 2000,Steel et al., 2002). This makes the absence of activation in the caudate body in unaffected siblings more likely to reflect abnormal connectivity between the frontal areas of the oculomotor system and the striatum. The presence of a frontostriatal deficit in schizophrenia has been demonstrated both functionally and anatomically (Buchsbaum et al., 1992). It is thought to underlie many of the cognitive deficits that have been observed in schizophrenia (Pantelis et al., 1997).

The fact that we did not observe significant differences in the frequency of errors on the antisaccade task, confirms the notion that behavioral measures of saccade inhibition are not very sensitive, and as such are at best a weak indicator of risk. FMRI revealed a clear functional brain abnormality in the striatum. This discrepancy between behavioral measures and measures of brain function has been shown in other domains, such as working memory and language processing tasks (Callicott et al., 2003b,Thermenos et al., 2004,Keshavan et al., 2002). The present study indicates that direct measures of brain function can provide a more sensitive means to detect abnormalities than behavioral measures.

The finding that brain function was abnormal whereas actual performance was within a normal range in siblings could be explained in terms of compensatory neural mechanisms. Such mechanisms could provide backup for functional brain abnormalities and thereby normalize task performance. Compensatory mechanisms can thereby effectively mask a neurophysiological abnormality, rendering the behavioral measure as unreliable to detect abnormal brain function.

Little is known about compensatory mechanisms in the human brain. Knowledge about the saccade systems in the primate brain, however, provides some clues as to how a striatal deficit could be compensated. The striatum is a major input site of the cortical areas of the oculomotor system (Alexander et al., 1986,Alexander and Crutcher, 1990,Berthoz, 1996). Through the striatum, cortical areas can exert inhibitory control over midbrain structures (Hikosaka et al., 2000). By means of these connections, the cortex can inhibit saccade neurons in the midbrain, and thereby prevent the midbrain from triggering a reflexive saccade (Everling et al., 1999). An abnormality in the striatum, or frontostriatal connections, as observed in this study, would reduce the ability of the cortex to inhibit the midbrain, which could then result in an increased number of reflexive saccadic eye movements. Support for a similar role of the striatum in saccade inhibition in humans comes from studies finding increased distractibility during antisaccades in patients with degenerative diseases of the basal ganglia like Huntington's disease (Lasker et al., 1987,Blekher et al., 2004) and Parkinson's disease (Armstrong et al., 2002,Briand et al., 1999).

Compensation may involve other pathways linking cortex and midbrain. For example, there is evidence for existence of projections from the cortex to the midbrain, which bypass the striatum, and allow the cortex to exert direct control over the midbrain. Both direct projections from the FEF, (Sommer and Wurtz, 2000) and from the DLPFC (Gaymard et al., 2003) to the midbrain have been implicated in inhibition of saccadic eye movements. These connections could act as compensatory pathways for suppressing reflexive eye movements when the corticostriatal circuit is impaired. Although the siblings were apparently able to compensate, we did not detect a functional brain correlate of this compensation. This either suggests that compensation does not require significant extra activation as a result of overcapacity of the system, or that it is too widely distributed or too heterogeneous across subjects to be detected with fMRI.

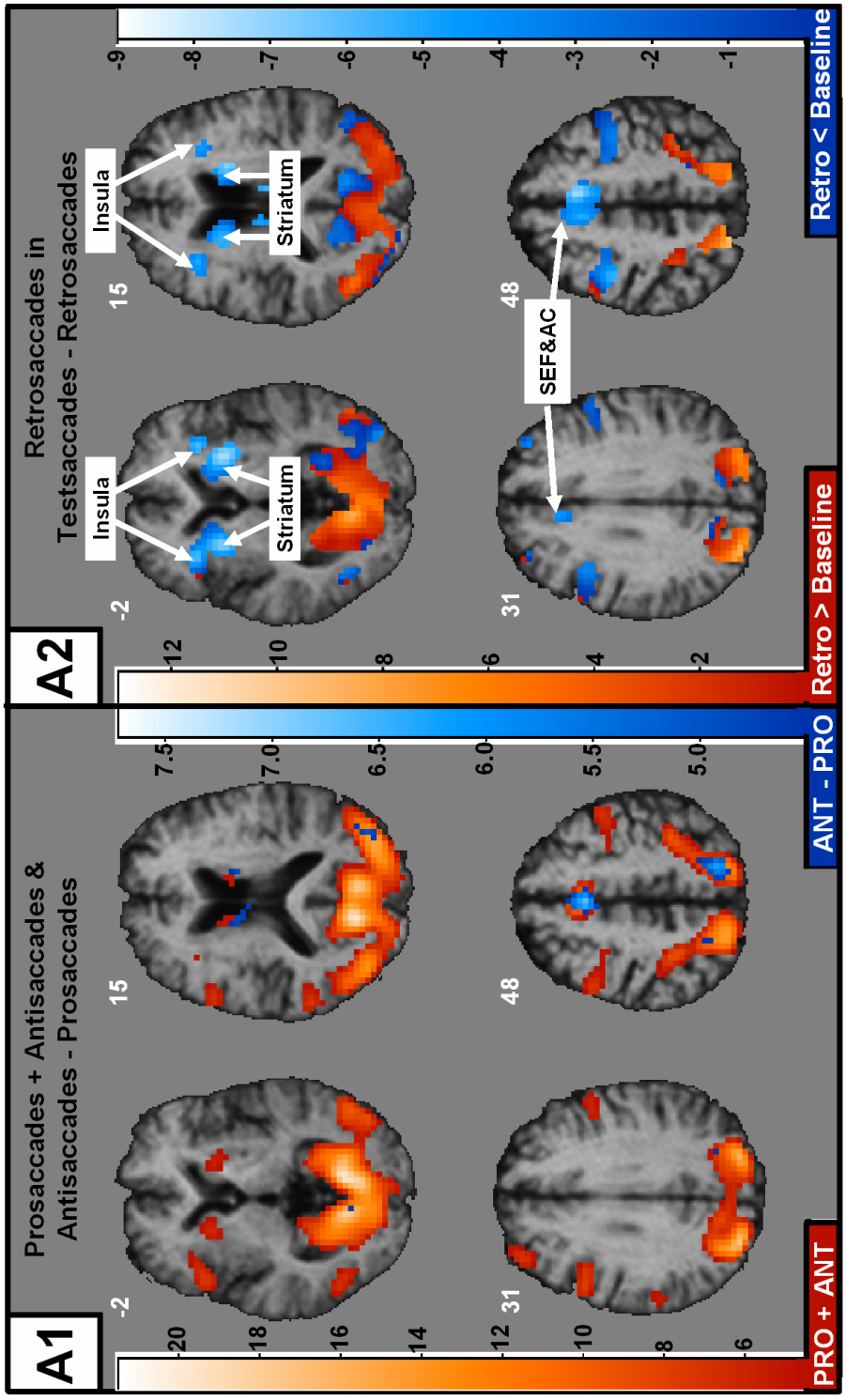
The Event Related fMRI design we used in this, and our previous, experiment (Raemaekers et al., 2002a), did not detect activation in the DLPFC, in spite of its previously reported involvement in saccadic inhibition and the saccadic inhibition deficit in schizophrenia (McDowell et al., 2002,Nakashima et al., 1994). We chose an event-related design in order to be able to control for effects of task performance on brain activation and to filter out the effects of the eye movement towards the center after each prosaccade or antisaccade. A shortcoming of this technique, however, is that it is insensitive to sustained activation. A large proportion of the difference in activation in the DLPFC when comparing prosaccades and antisaccades,

can be accounted for by sustained activation (Connolly et al., 2002,Desouza et al., 2003), and this is best measured with a so-called block design as opposed to an event-related design. Because of the insensitivity of event related fMRI to sustained types of activation, the possibility remains that we missed functional abnormalities in DLPFC in siblings and patients.

Although the striatum is most likely involved in saccadic inhibition, the contrast between antisaccades and prosaccades can as well represent areas that are involved in voluntary saccade initiation. There is also some evidence for impaired voluntary saccade initiation in siblings (Myles-Worsley et al., 1999,Thaker et al., 1996,Thaker et al., 2000). In this study, siblings had a tendency for longer saccade onset latencies for both antisaccades and prosaccades. This may suggest that they have a behavioral abnormality in the initiation of eye movements, but not specifically voluntary eye movements. As the abnormal brain activation was observed for the contrast between the two conditions, it is unlikely that the striatal deficit actually represents a deficit in saccade initiation that is present in both conditions. More data is needed to assess the nature of increases in saccadic onset latencies in siblings.

In summary, the present study reveals a failure to activate the striatum during antisaccades in unaffected siblings. This abnormality was previously demonstrated in schizophrenic patients, and most likely marks a deficit in the frontostriatal circuitry. As such, future research is warranted to further investigate the usability of this deficit as a vulnerability marker for schizophrenia. The fact that behavioral measures of saccade inhibition were not affected in siblings indicates that other pathways can compensate for the attenuated striatal activity. Further research is needed in more genetically informative samples to establish whether this deficit qualifies as a candidate endophenotype for schizophrenia.

Color illustrations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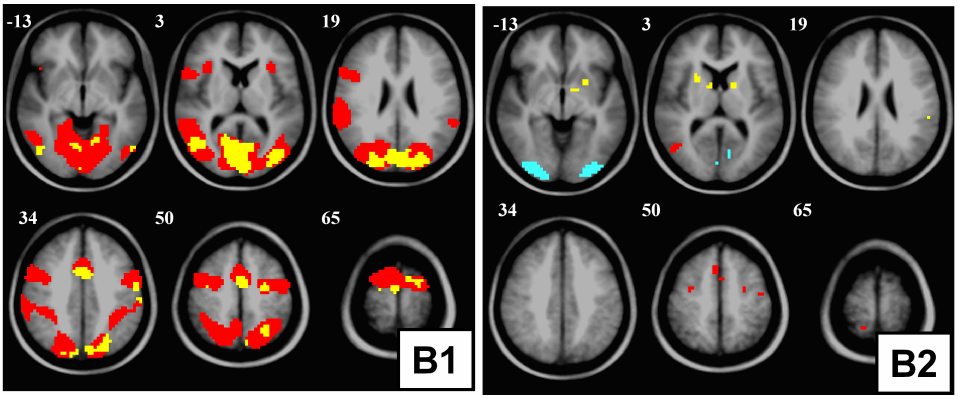

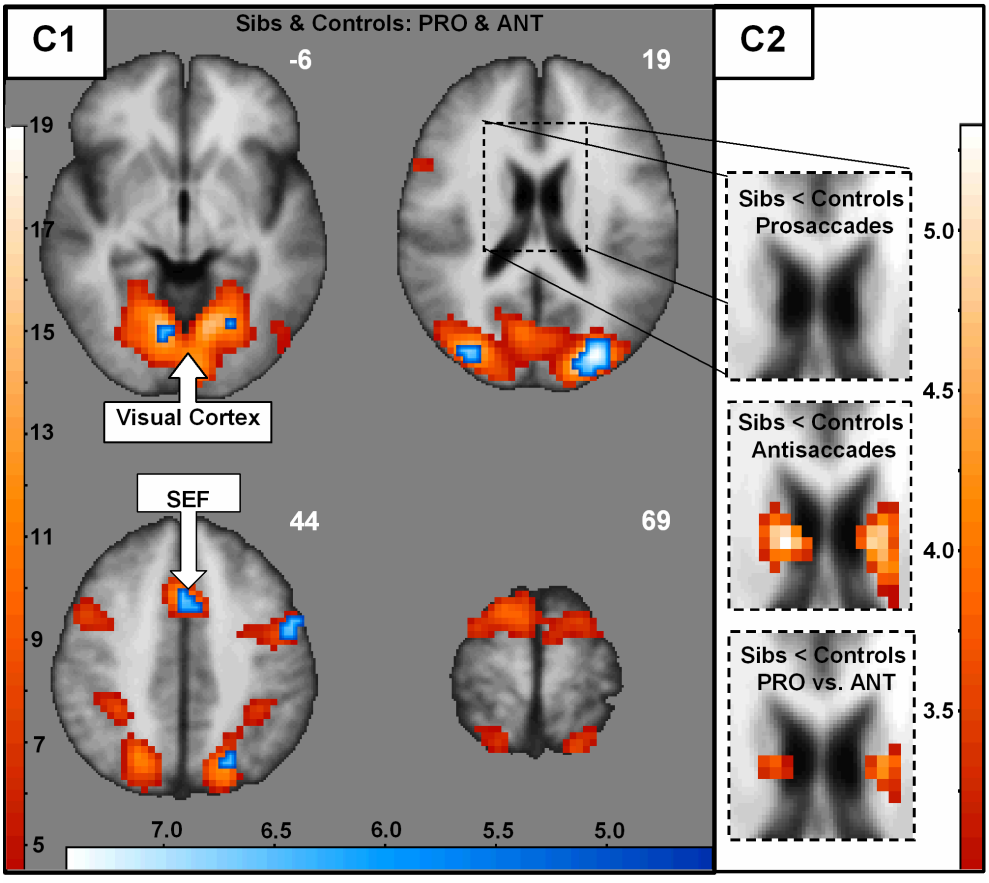

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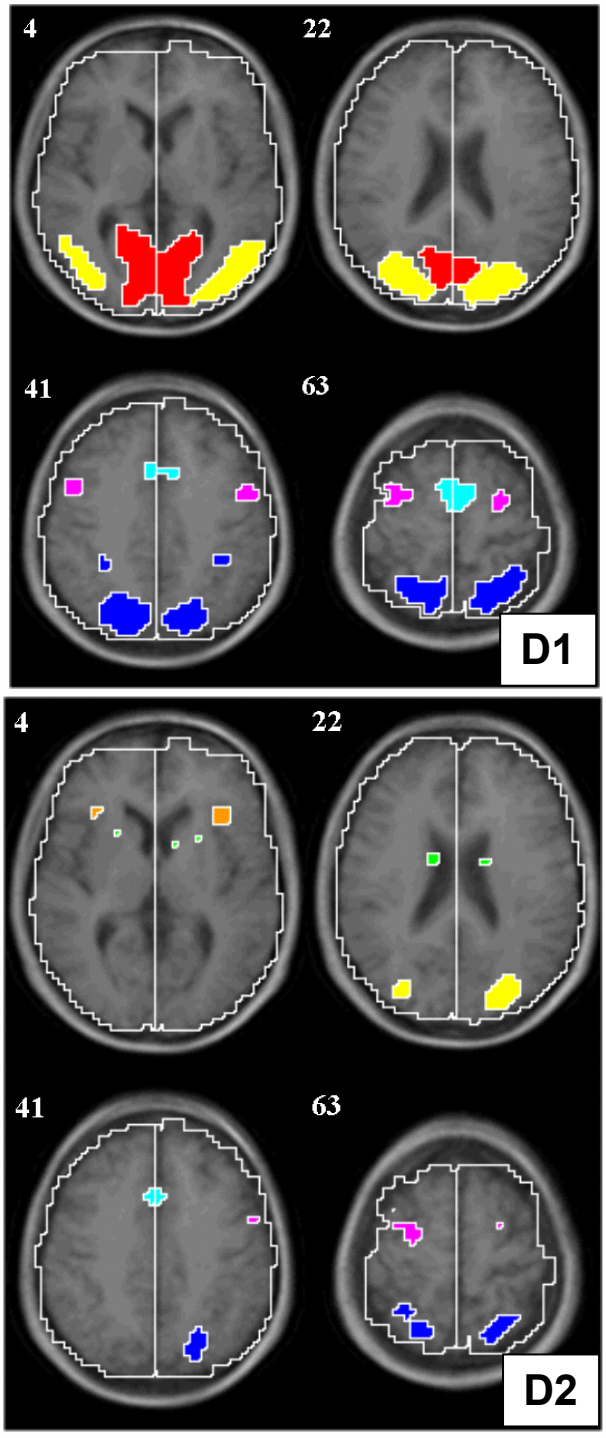
Figure A. Group results projected on the averaged normalized anatomical scans. The white number on the top left of each slice indicates the z-coordinate in MNI space.
1. Group results for activated voxels during prosaccades and antisaccades. Red-white shows the voxels that were active during both conditions. Superimposed in blue-white are the voxels which were more strongly activated during antisaccades than prosaccades.
2. Group results when contrasting testsaccades to retrosaccades. All the displayed voxels have increased activation during testsaccades relative to retrosaccades ($z > 4.52$). The color of the voxels indicates the level of activation during the retrosaccades in relation to baseline (red-white=positive; blue-white=negative)

Figure B. Active voxels superimposed on the averaged anatomical scan. The numbers displayed in the top left corner of each slice correspond to Talairach z-coordinates. Only the most informative slices are shown. Coloured voxels represent significant effects at $P<0.05$ (Bonferroni corrected). 1. Response pattern for all three tasks combined. Red voxels are those that were active in both groups (patients and controls, main effect of visual processing). In yellow, regions are shown where there is a difference between patients and controls (controls > patients). Analyses per group showed that patients exhibited significant activity in the yellow regions, but less strongly than the controls. 2. Response pattern for the inhibition conditions. Red voxels are those that were active selectively during saccadic inhibition (as opposed to prosaccades), in both patients and controls. Blue indicates the regions where activity decreased during inhibition in both groups. Yellow voxels represent the key finding, namely the significant difference between patients and controls during inhibition of saccades (patients < controls).

Figure C. 1. Active voxels superimposed on the average anatomical scan. The numbers displayed on the top left corner of each slice correspond to the Talairach z coordinates. Only the most informative slices are shown. Colored voxels represent significant effects at $P<0.05$. Red-yellow, indicates increased activation related to both prosaccades and antisaccades. Blue-white indicates areas with increased activation during antisaccades, relative to prosaccades. 2. Close-up of the striatal area. Colored voxels represent more activation in controls compared to siblings during, prosaccades (upper window), antisaccades (middle window) and the contrast between antisaccades and prosaccades (lower window).

Figure D. Regions of interest based on the voxels activated during prosaccades (1) and during saccadic inhibition (2) for all subjects (z > 3.5). ROI's are projected on an averaged anatomical image. The ROI's include V1&V2 (red), V5 (yellow), SPL&IPS (blue), FEF (purple), SEF (cyan), Insula (orange), and the striatum (green). ROI's are separated for the right and left hemisphere for prosaccades, but not for saccadic inhibition. Talairach z-coordinates are displayed on the top left of each slice.

Figure E. Voxels that were active in the group comparisons during either session one (blue), session two (yellow), or both sessions (red), superimposed on the average anatomical scan. The numbers displayed on the top left corner of each slice correspond to the Talairach z coordinates. Only the most informative slices are shown. Colored voxels represent significant effects at P<0.001 (uncorrected). Figure 1 depicts the results for prosaccades, 2 for antisaccades, and 3 for inhibition. The white lines encircle the regions of interest that are defined based on the results of previous studies and involve V1, V2/V5, Parietal, Frontal Eye Fields (FEF), the insula, striatum, and the Supplementary Eye Fields (SEF).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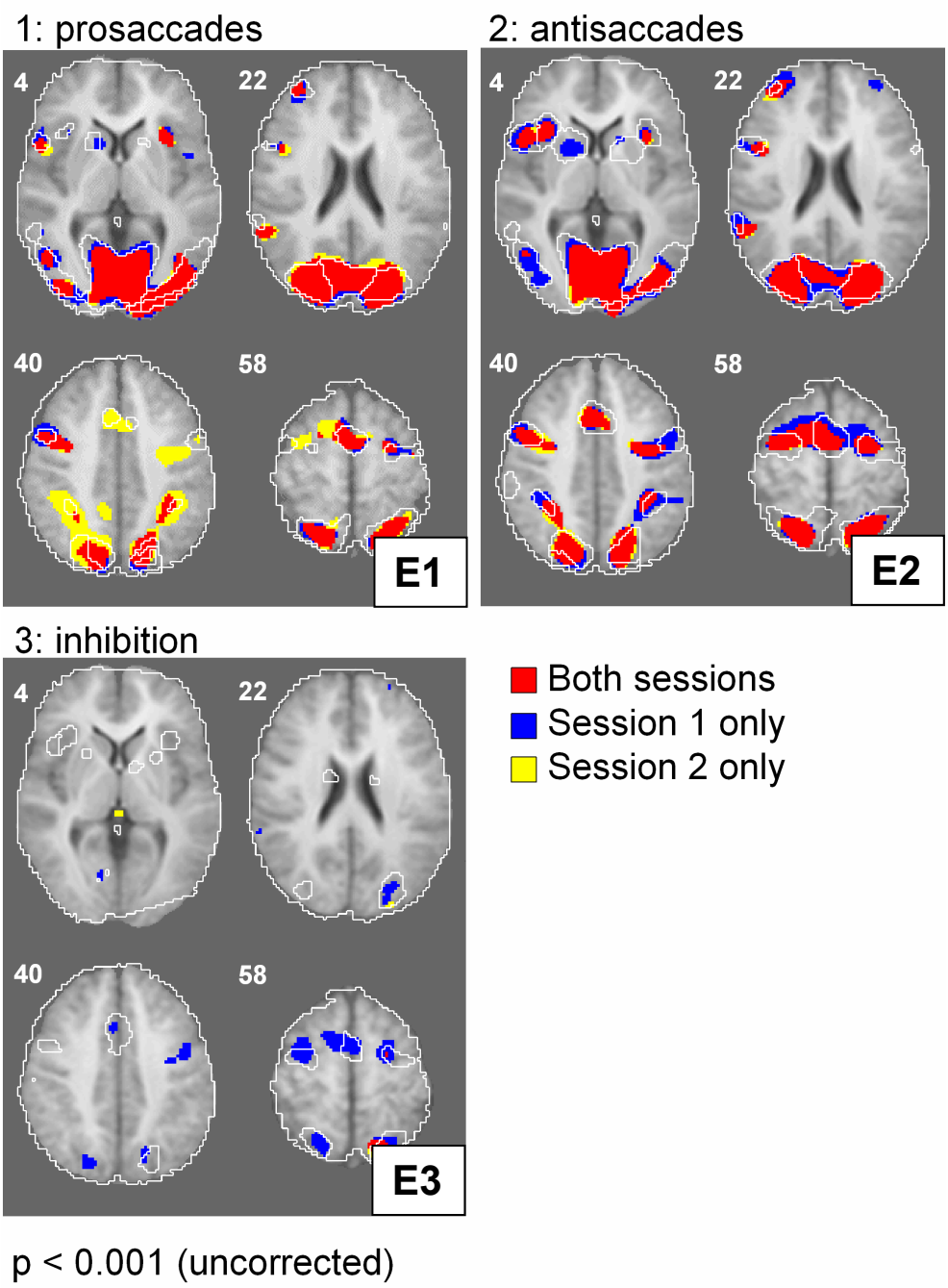

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Effects of aging on BOLD fMRI during prosaccades and antisaccades.

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Abstract
Age affects the ability to inhibit saccadic eye movements. According to current theories, this may be associated with age-induced neurophysiological changes in the brain, and with compensatory activation in frontal brain areas. In the present study, the effects of aging are assessed on brain systems that subserve generation and inhibition of saccadic eye movements. For this purpose, an event related fMRI design was used in adults covering three age ranges (18-30, 30-55 and 55-72 years). Group differences were controlled for task performance. Activity associated with saccadic inhibition was represented by the contrast between prosaccade and antisaccade activation. The tasks activated well documented networks of regions known to be involved in generation and inhibition of saccadic eye movements. There was an age-related shift in activity from posterior to frontal brain regions after young adulthood. In addition, old adults demonstrated an overall reduction in the BOLD signal in the visual and oculomotor system. Age, however, did not affect saccade inhibition activity. Mid and old adults appear to increase frontal activation to maintain performance even during simple prosaccades. The global reduction of the BOLD response in old adults could reflect a reduction in neural activity, as well as changes in the neuronal-vascular coupling. Future research should address the impact of altered vascular dynamics on neural activation and the BOLD signal.

5.1 Introduction:

Advances in neuroimaging techniques allow for increasingly sophisticated measurements of brain activity. Functional Magnetic Resonance Imaging (fMRI) has proven to be a valuable tool not only for mapping of brain functions, but also for explaining behavioral differences between groups in terms of neurofunctional differences. FMRI is increasingly being used to investigate the effects of aging on brain activation. Many physiological changes occur in the brain during the transition from young to old adulthood. Many of these changes have been associated with cognitive decline. Imaging studies have investigated age related impairments in various brain functions including memory, perception, and motor action. This study addresses the effects of ageing on brain activation during execution and inhibition of saccadic eye movements.

Performance on saccade tasks declines with age. There is a linear relationships between age and the onset latencies of visually guided saccades (Abel et al., 1983,Carter et al., 1983,Olincy et al., 1997,Tedeschi et al., 1989,Carter et al., 1983,Tedeschi et al., 1989). Elderly subjects also demonstrate impaired antisaccade performance. For the generation of antisaccades subjects have to inhibit a saccade towards a novel peripheral stimulus and instead make a saccade in the opposite direction (Hallett, 1978a). Old adults make more erroneous saccades towards the stimulus and exhibit increased onset latencies of correct antisaccades when compared to young adults (Nieuwenhuis et al., 2000,Olincy et al., 1997,Butler et al., 1999,Klein et al., 2000,Sweeney et al., 2001). Some studies only report increased onset latencies of antisaccades in elderly subjects (Eenshuistra et al., 2004,Fischer et al., 1997a,Munoz et al., 1998).

The neural substrate of the oculomotor impairment remains unknown. A widespread network has been unveiled that subserves the generation and suppression of saccadic eye movements, and encompasses eye fields in all the major cortices including not only the parietal eye fields (PEF) the frontal eye fields (FEF), supplementary eye fields (SEF), but also portions of visual cortex and the basal ganglia (Cornelissen et al., 2002) (Desouza et al., 2003) (Kimmig et al., 2001) (Raemaekers et al., 2002a,Matsue et al., 1994). Impaired inhibitory control over saccades in elderly subjects has been attributed to impaired function of the frontal lobes, but this notion is mainly based on the finding that patients with lesions affecting the dorsolateral prefrontal cortex (DLPFC) demonstrate similar deficits (Pierrot-Deseilligny et al., 1991a). The existing fMRI literature demonstrates substantial agreement with the models of the oculomotor system that are based on cell recordings in non-human primates (Berthoz, 1996). The extensive

knowledge of this network provides a reliable background for investigating differences between age groups.

As of yet, there has been no study that addressed the changes in brain activity that occur in the oculomotor system with aging. Both global and regionally specific changes may occur in the system during the lifespan. Elderly subjects are thought to engage compensatory mechanisms, supposedly to counteract age induced physiological changes. A possible mechanism for compensation is described by the HAROLD-model (Hemispheric Asymmetry Reduction in OLD adults) (Cabeza et al., 2002). This model states that frontal activity tends to be less lateralized in older than in younger adults, which may reflect compensatory processes. Support for the HAROLD-model has also been found for control over motor functions. During a go/nogo task elderly subjects exhibit a more bilateral pattern of frontal recruitment during correct inhibition than young adults (Langenecker and Nielson, 2003) (Nielson et al., 2002).

Whether it is essentially hemispheric asymmetry reduction that is reflected in these studies is unclear. The apparent hemispheric asymmetry reduction could also represent a net increase in activation in frontal brain areas with increasing age. A recent study on motor inhibition during stroop interference reported more activation in the task specific areas of the frontal lobe in elderly subjects (Langenecker et al., 2004,Milham et al., 2002). This indicates that elderly subjects may compensate through increasing engagement of task specific frontal brain areas. This implies that aging may be accompanied by changes in the anterior-posterior asymmetry instead of hemispheric asymmetry. A shift towards more frontal activation could reflect an increase in effort to maintain task performance.

In this study we assess age related changes in the oculomotor system by measuring BOLD responses during saccadic eye movements in a sample of subjects covering a broad age range. Differences between the age groups are assessed for prosaccades, and the contrast between antisaccades and prosaccades (saccadic inhibition) in task related brain areas. We expect increasing compensatory activation to arise in frontal brain areas with increasing age during either prosaccades or saccadic inhibition, or during both. This additional activation would shift the anterior-posterior asymmetry. Such compensation may arise quite early in the lifespan as age-related increases in activation (e.g. related to working memory) have already been observed within a group of only younger adults (Adler et al., 2001a). To minimize the confound of differences in performance between young and

old subjects, we applied an event-related paradigm that allows us to base group comparisons on correct responses only.

5.2 Methods:

Subjects:
31 subjects (16 male) participated in the experiment. The subjects were evenly distributed between 18 and 72 years of age (mean, 41 years; SD, 19 years). For group wise comparisons, subjects were categorized in young adulthood (18-30 years), mid adulthood (30-55 years), and old adulthood (55-72 years). All were right handed according to the Edinburgh Handedness inventory (Oldfield, 1971b)(mean, 0.84; SD, 0.18). A history of substance abuse or major neurological illness resulted in exclusion from the experiment, as did metal implants. All subjects gave informed consent for participation (approved by the Human Ethics Committee of the University Medical Center Utrecht).

Scanning protocol:
All images were obtained with a Philips ACS-NT 1.5T clinical scanner (Philips Medical Systems, Best, the Netherlands) with fast gradients (PT6000). The head was held in place with a strap and with padding. Structural and functional images were acquired in transverse orientation, from the same section of the brain. For functional scans, a navigated 3D-PRESTO pulse sequence (Van Gelderen et al., 1995,Ramsey et al., 1998a) was used with following parameters: TE 37 ms, TR 24 ms, flip angle 9.5 degrees, matrix 48*64*24, FOV 192*256*96 mm, voxel size 4 mm isotropic, scan duration 1.49 s per 24-slice volume. Immediately after functional scans, an additional PRESTO scan of the same volume of brain tissue was acquired with a high flip-angle (30 degrees, FA30) for the image coregistration routine (see below). Finally, a T1 weighted structural image was acquired.

Task design:
The fMRI design used a PC, a rear projection screen and a video-projector system for presentation. All stimuli were projected in white on a dark background. All events were time-locked to the fMRI scans. The design consisted of two tasks, that is prosaccades and antisaccades. These two conditions had identical stimuli. During prosaccades, subjects had to make eye movements towards peripherally presented stimuli. During antisaccades, subjects were requested to suppress these stimulus triggered eye movements and instead make saccades towards the opposite direction.

Each new trial started with the disappearance of a fixation cross (0.9 ° visual angle) at central view, and was. After a 200 ms gap period, a square (0.9° visual angle) was presented semi-random 8.7° to the left or right of central fixation. The square was extinguished after 3240 ms, simultaneously with the reappearance of the fixation cross at central view. A new stimulus was triggered by the scanner every ninth scan, thereby generating a fixed stimulus interval of 13.4 s giving stimulus related BOLD signal time to return to baseline (Bandettini and Cox, 2000).

Instructions were given verbally prior to the start of the experiment and included the following: 1) Prosaccade: ‘’from central fixation look towards the square as quickly as possible when it appears. Look back to the fixation cross when the square disappears and the fixation cross reappears in the center.’’ 2) Antisaccade: ‘’when the square appears, look in the opposite direction as quickly as possible, without looking towards the square. Look back to the fixation cross when the square disappears and the fixation cross reappears in the center’’. Task instructions preceded each new block of ten stimuli. There were four blocks per task making a total of eight, which were orderly alternated.

Eye Movements:
Eye movements were recorded during the entire oculomotor-task using an MR-compatible eyetracker (Cambridge Research Systems Ltd., Rochester, UK (Kimmig et al., 1999) in combination with Labview (National Instruments Corporation, Austin, USA) acquisition software on a PC with a multifunctional I/O Board (National Instruments Corporation, Austin, USA). This acquisition-PC was linked to the stimulus-PC by a parallel cable to synchronize the eye-recordings and the task presentation. Calibration and adjustment of the sensor were done during a five minute period prior to scanning. The sample frequency of the recording was 500 Hz. For each saccade in the time window of 200 ms before until 600 ms after the stimulus presentation, the latency and the direction were determined using a custom analysis program in IDL (Research Systems Inc., Boulder, USA).

Analysis:
Data analysis of fMRI scans was done with custom-written programs in IDL. The last functional volume was registered to the FA30 volume. Next, all fMRI volumes where registered to the (now registered) last functional volume using a least squares differences criterion (Thevenaz et al., 1998). The structural scan was also registered to the FA30-scan thereby providing spatial alignment between the structural scan and the functional volumes. A

3D-gaussian filter (8mm full width at half max) was applied to all fMRI volumes.

Preprocessing of individual subjects datasets:
Next, a standard linear Multiple Regression analysis was conducted for each subject. Several factors were generated for each subject individually, based on trial type (prosaccade/antisaccade), accuracy of the trials (first saccade in correct/incorrect direction), whether a potentially false saccade was corrected by a saccade towards the opposite direction, and anticipation (first saccade before or after 100 ms). This resulted in a maximum of 6 factors per condition (Figure 5.1). Two separate factors represented the eye movement which returned the view to central gaze for the two conditions. All events in the design-matrices were convolved with a predefined haemodynamic response function (Friston et al., 1995). Additional factors included the average intensity of each scan, and 88 discrete cosine functions forming a high pass filter with a cut-off at $3.73*10^{-2}$ Hz to correct for low frequency scanner and physiological artifacts but also for differences in baseline activation between conditions. After voxelwise regression analysis, 3D volumes were created of the regression coefficients. Only the volumes containing the regression coefficients representing the activation during the different trial types were included in further analysis. Subsequently, these volumes were spatially normalized in Talairach orientation to enable group-wise comparisons (Collins et al., 1994). To assess brain activation for the different conditions in the entire group, voxelwise t-tests were performed using the normalized volumes containing the regression coefficients and the pooled standard deviation (Worsley, 1994). Bonferroni correction for the number of tests resulted in a critical t-value of 4.52 for each voxel.

Group-wise analyses:
To assess age related changes in brain activation, two different strategies were employed. The first strategy involved a voxel-based analysis of aging effects. For this analysis, an independent variable was created that contained the age in days for all the subjects. This independent variable was used as a regressor in a voxel wise regression analysis of age over the volumes containing the regression coefficients of correct prosaccades, and the contrast between prosaccades and antisaccades (saccadic inhibition). Subsequently, the two regression coefficients were tested for significance. The resulting statistical t-maps represent the linear effects of age on regional brain activation for prosaccades and saccadic inhibition. The second strategy was a region of interest based approach. Two sets of regions of interest (ROI's) were defined, one for prosaccades and one for saccade inhibition.

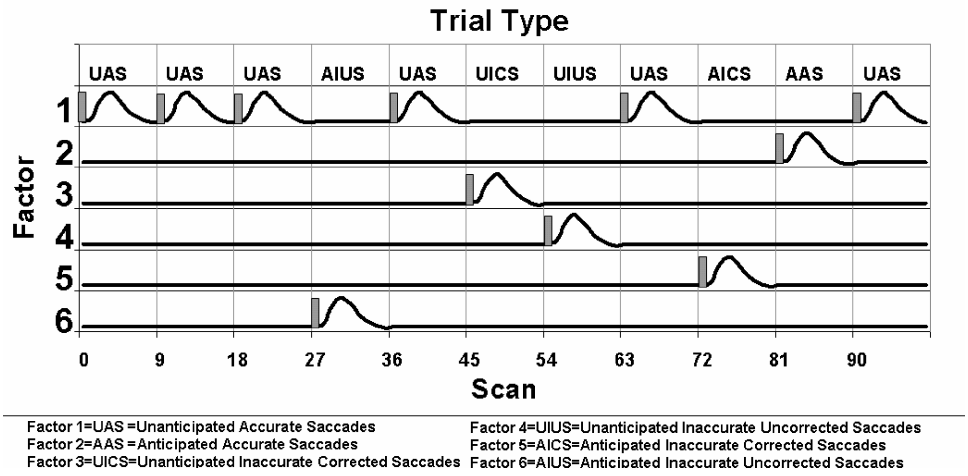


Figure 5.1. Schematic of the factors for one condition. Results per trial were subdivided in 6 categories. These categories represented the 6 different behavioural outcomes per trial (see the abbreviations). The different categories were subsequently represented in separate factors in the regression model.

Prosaccade ROI analyses:

The first set was based on the t-map for the group during prosaccades. A mask was created by applying a threshold of $z=3.5$ ($p<0.0005$ uncorrected) on this t-map, yielding the Regions of Interest for further analyses. Borders between interconnected regions were drawn using a watershed algorithm and a priori knowledge of functional localization. The resulting ROI's are displayed in figure D1, page 72. Magnitude of activation within each ROI was calculated for each subject by averaging the regression coefficients of prosaccades over the voxels within the regions of interest. Group differences between the three age groups during prosaccades (i.e. young, mid, and old adults) were estimated using a repeated measures GLM which included one within-subject factor (the activation during prosaccades in the ROI's for prosaccades).

Saccadic Inhibition ROI analysis:

The second set of ROI's was based on the t-map for saccadic inhibition (antisaccades vs. prosaccades). ROI's were defined using the same procedure as for prosaccades. The resulting ROI's are displayed in figure D2, page 72. The magnitude of activation in each subject was calculated for prosaccades and antisaccades by separately averaging the regression coefficients for the two conditions over the voxels within the regions of interest. The repeated measures GLM included two within subjects factors

(the activation during prosaccades and during antisaccades in the ROI's for saccadic inhibition).

5.3 Results:

Behavioral results:
Separate ANOVA's, with age (3 groups) as an independent variable and the measures of task performance as the dependent variables, revealed no significant effect of age on saccade onset latencies during prosaccades ($F_{(2,28)}$=1.54; p=.233), nor during antisaccades ($F_{(2,28)}$= 1.98; p=.157) (Table 5.1). The same was true for the effect of age on the error rates during prosaccades:($F_{(2,28)}$=1.73; p =.196) and during antisaccades: (F<1) (Table 5.1).

However, there was a near-significant correlation between age and the onset latencies of prosaccades (r=0.29; p=0.055 one tailed; effect size d=.64) and a moderate positive correlation between age and saccade onset latencies of antisaccades (r=0.34; p=0.030 one tailed; d=.72). The number of errors on prosaccade trials increased with age (r=0.35; p=0.027 one tailed; d=.75). We did not find a relationship between age and number of errors on antisaccade trials (r=.21; p=0.126 one tailed; d=.43).

	Young Adults (n = 12)	*Mid Adults (n = 9)*	*Old Adults (n = 10)*
Reaction times (msec)			
Prosaccades	178.4 (18.3)	176.3 (32.5)	195.0 (27.9)
Antisaccades	250.4 (33.7)	245.6 (43.4)	281.8 (54.6)
Errors (%)			
Prosaccades	0.0 (0)	1.1 (2.1)	2.1 (4.0)
Antisaccades	24.9 (15.7)	24.2 (20.6)	35.7 (26.7)

Table 5.1. Averages with standard deviations of the behavioural results.

Brain Imaging data:

Voxel based analysis:
For all fMRI analyses, group comparisons were based only on correct responses. To test whether brain activation was adequately measured, the group results for prosaccades were inspected. The task activated an

extensive network of brain areas known to be involved in visual and oculomotor processing. These regions included extensive portions of visual cortex, superior and inferior parietal areas, and the medial and lateral premotor cortex (Figure D1, page 72). In the voxel-based analysis, we found no brain regions that exhibited a linear relationship between age and level of activation during correct prosaccades, nor during correct saccadic inhibition at the significance threshold of $p<0.05$ (corrected).

Region Of Interest analysis:

Prosaccades:
Ten regions of interest were derived from the group results for prosaccades. The ROI's included the combination of V1 and V2, an area within Brodmann area 19 corresponding to V5, a combination of the superior parietal lobe and the intraparietal sulcus (SPL&IPS), the frontal eye fields (FEF), and the supplementary eye fields (SEF). These five regions were separated for the left and right hemisphere (Figure D1, page 72). A multivariate repeated measures analysis of variance of the prosaccade data, with region (10 levels) as a within subject factor, and age-group membership as between subject factor (3 levels) revealed a modest interaction effect between region and group ($F_{(18,42)}=1.86$; $p=.05$), indicating a difference between the groups in the distribution of activation across the regions of interest. Subsequently, the group differences in anterior-posterior asymmetry were tested by contrasting activation in the frontal regions (FEF, SEF) to the posterior (V1&V2, V5, IPS&SPL) regions (average activity across ROI's)(Figure 5.2a). A one-way ANOVA revealed a significant effect of group ($F_{(2,28)}=3.93$; $p=0.03$). Further tests indicated that this effect could be explained by relatively more posterior compared to frontal activation in young adults than in the two older groups ($t_{30}=2.31$; $p=.03$; $d=.88$). In addition, there was an effect of group ($F_{(2,28)}=6.52$; $p=.005$), indicating a difference between the groups in the average activation in all the ROI's. Using the post hoc Tukey HSD method we found that elderly subjects had lower overall activation than young adults ($p=0.036$) and mid adults ($p=0.005$) (Figure 5.3). To test whether this reduction could have arisen as a result of a difference in goodness of fit with the canonical HRF between the groups, we correlated the averaged BOLD response over all the ROI's with the canonical HRF. The correlation was high for all three groups ($r=.99$ for young, $r=.97$ for the mid, and $r=.96$ for old adults). The differences between these correlations are too small to explain the nearly 40% reduction in signal that was observed between old adults and the two younger groups. The use of ROI's and smoothing of the statistical maps

could also have contributed to group differences due to effects of partial voluming. Therefore we reanalysed the data without spatial smoothing, and tested the average peak activations in the 10 ROI's between the groups. This also revealed a reduction in old adults compared to the two younger groups (t=2.46; p=0.02).

Saccadic Inhibition (Antisaccades vs. Prosaccades) :
Regions of interest were also defined for the saccadic inhibition contrast (antisaccades vs. prosaccades). There was no activation in the V1&V2 region in this contrast. However, there was additional activation in the striatum and the anterior parts of the insula, making a total of 6 ROI's (Figure D2, page 72). A repeated measures GLM was conducted to check for lateralization effects in these ROI's. As this test revealed no differences between the groups in hemispheric asymmetry for separate ROI's ($F_{(2,28)}$=1.287; p=.263), nor for all ROI's combined(($F_{(2,28)}$=.195; p=.824), we collapsed ROI's across hemispheres to increase statistical power of the GLM. The multivariate test for saccadic inhibition included task as an additional within-subject factor (2 levels, i.e. prosaccades and antisaccades). Average regression coefficients were calculated for both correct prosaccades and correct antisaccades within the ROI's for saccadic inhibition. None of the interactions with group were significant, indicating that age did not significantly affect the distribution of activation. We also did not find any difference between the groups in the anterior-posterior asymmetry (i.e. the difference between prosaccades and antisaccades in the contrast V5, SPL&IPS vs. FEF, SEF, Striatum, and Insula, $F_{(2,28)}$=.370; p=0.694) (Figure 5.2b). There was, however, a significant overall effect of group ($F_{(2,28)}$=4.04; p=0.02). Post hoc tests using the Tukey HSD method revealed that activation in the mid adulthood group was higher in the saccadic inhibition areas, than in old adults (p=0.023).

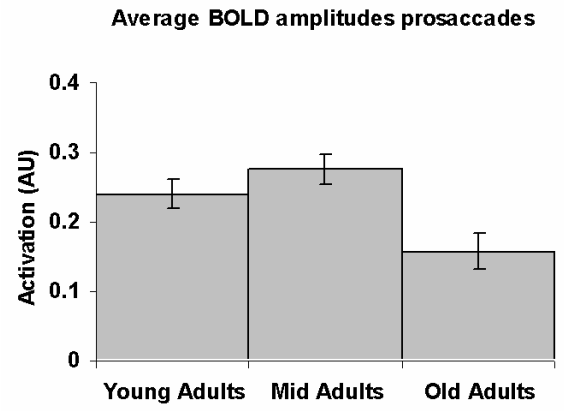


Figure 5.3. Average activation in all the prosaccade ROI's in the three groups. Bars indicate standard errors.

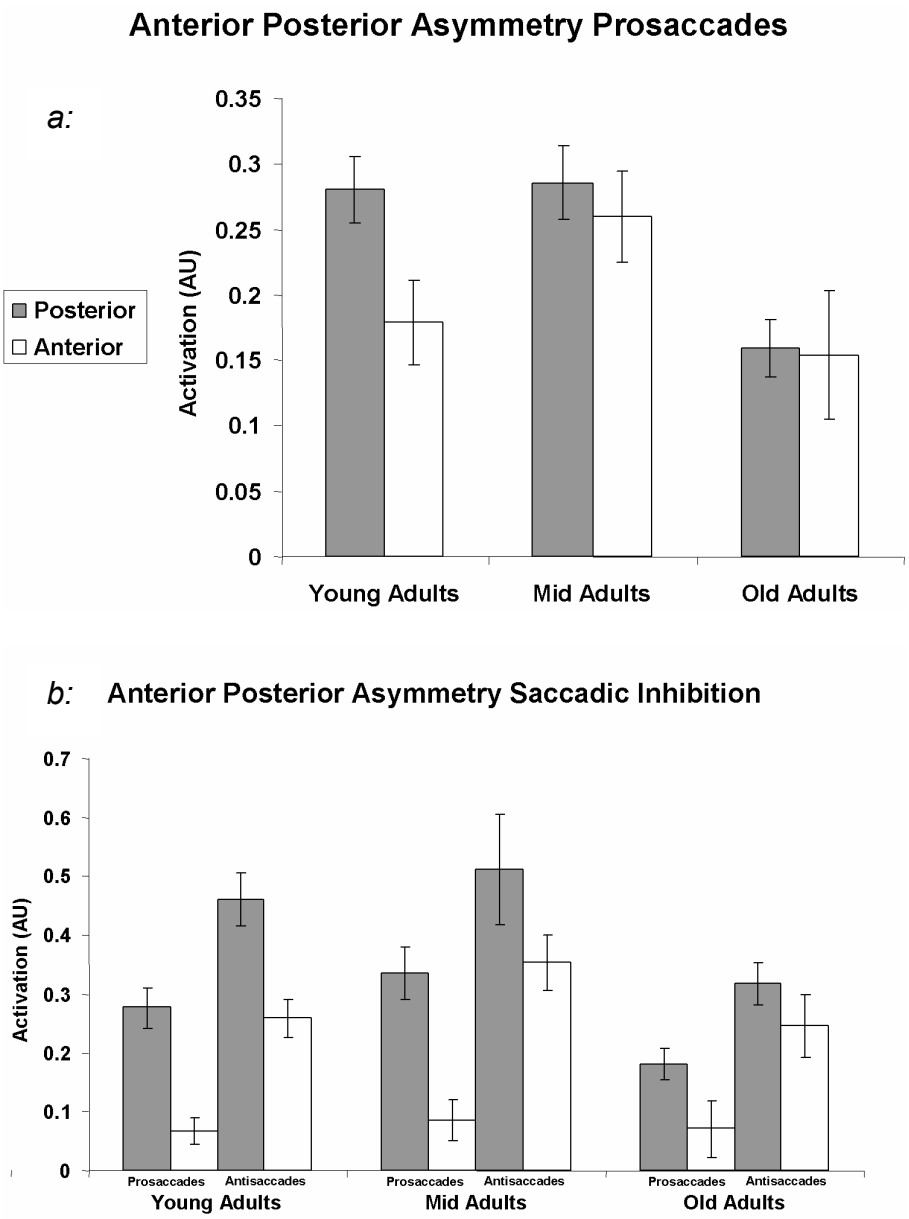


Figure 5.2. a. Average activation during prosaccades in the posterior (V1&v2,V5,SPL&IPS) and the anterior (FEF&SEF) prosaccade ROI's in the three age groups. b. Average activation during prosaccades and antisaccades in the posterior (V5, SPL&IPS) and anterior (FEF, SEF, Striatum, Insula) saccadic inhibition ROI's in the three age groups. Bars indicate standard errors.

5.4 Discussion:

This study addressed effects of age on brain activation during the generation and suppression of saccadic eye movements. Behavioral results demonstrated a moderate increase in reaction times for both prosaccades and antisaccades with increasing age. The number of errors increased for prosaccades, but not for antisaccades. Analysis of the fMRI data acquired during generation of prosaccades demonstrated a (well documented) network of visual and saccade related brain-areas (Raemaekers et al., 2002a). Generation of antisaccades gave rise to additional signal increases in the FEF, SEF, PEF, striatum, anterior parts of the insula, and V5. A region of interest analysis revealed that for prosaccades, the distribution of brain activation shifted with age. Old and mid adults demonstrated elevated activation in frontal brain areas relative to posterior brain areas, as compared to young adults. This relative enhancement of frontal activation during prosaccades was accompanied by an overall attenuation of brain activation in old adults in comparison to the two younger groups. Mid adults exhibited higher levels of activation in the brain areas associated with saccadic inhibition during generation of prosaccades as well as antisaccades. There were no differences between the age groups in brain activity associated with saccadic inhibition.

The observed effects of age on behavioral measures of prosaccades and antisaccades are smaller than what has been reported in other studies (Klein et al., 2000,Olincy et al., 1997). This may be associated with circumstances that are specific to this experiment, namely having to lie in an MRI scanner in darkness and with the loud noise generated by the scanner gradients, and the use of a long intertrial interval (13.4 seconds). This may differentially affect individual performance, thereby increasing the standard deviations of the mean estimates of the groups. Alternatively, the inclusion criteria of this fMRI experiment may have been more stringent than for behavioral experiments, which would disproportionally affect the elderly group in the sense that they are overall healthier than in behavioural studies. With regard to performance differences on the antisaccade trials, it is not quite clear what we should have expected. Support for a behavioral saccadic inhibition deficit in elderly subjects is equivocal, suggesting that the effect is rather small (see (Nieuwenhuis et al., 2000,Olincy et al., 1997,Butler et al., 1999,Klein et al., 2000,Sweeney et al., 2001), but also (Eenshuistra et al., 2004,Fischer et al., 1997a,Munoz et al., 1998)). Thus, elucidation of effects of age on saccade inhibition performance may require studies with large sample sizes to obtain adequate statistical power for the apparent small effect size.

The fMRI experiment demonstrated a shift in the anterior-posterior asymmetry from mid adulthood on(Cabeza et al., 2002). More specifically, the FEF and SEF increased in activity relative to the regions in the occipital and parietal lobes. The FEF and SEF have been associated with more cognitively demanding eye movements such as antisaccades (Raemaekers et al., 2002a,Muri et al., 1998,Sweeney et al., 1996)and newly learned sequences of saccades (Grosbras et al., 2001). Such an altered pattern of activation may therefore indicate compensatory processes or increased effort to maintain performance at least for a relatively simple task. Surprisingly, this shift towards frontal brain areas was not present for saccadic inhibition. It may be the case that increased compensatory frontal activation (with age) is not only present when cognitive demands are high, but also when the task is easy to perform. Even prosaccades may require more effort, or are executed in a more controlled manner, from mid-adulthood on. The increased brain activation in saccadic-inhibition areas in mid-adults may represent a similar process. As these brain areas represent a neuronal substrate of motor control, elevated activation towards mid adulthood in these areas could be indicative of increased reliance on controlled motor behavior.

In this study we did not find evidence for compensation through frontal hemispheric asymmetry reduction, but the oculomotor paradigm gave rise to little hemispheric asymmetry to begin with. It is still uncertain what the asymmetry reduction of the HAROLD model actually represents. Asymmetry could also be reduced as a result of a net increase in frontal activation in elderly subjects. Such a net increase may meet ceiling effects in the dominant, but not in the other hemisphere. Furthermore, it has been found for language that increasing the statistical threshold of the activation maps, increases hemispheric asymmetry (Rutten et al., 2002). Higher net activation could thus result in reduced hemispheric asymmetry.

The described effects of age on brain activity in essence reflect a reduced amplitude of the BOLD response following a saccade in old adults. Given that the amplitude can only be estimated with HRF functions, factors that affect the shape of the response could confound the results. However, we found that the amplitude reduction was not due to differences in shape of the BOLD response between the groups. We also show that the age effect is not due to partial voluming in the statistical maps, a potential confound that has been reported by others (Aizenstein et al., 2004). It is therefore likely that the effect of age indeed evidences an overall attenuation of activation. Others have also reported similar effects. Using a similar PRESTO technique, a negative correlation between age and the number of activated

voxels in the motor cortex was found during a simple fingertapping task (Hesselmann et al., 2001). D'Esposito and colleagues have reported that younger subjects demonstrated more than four times the number of suprathreshold voxels in the primary sensory motor cortex compared to elderly subjects, during a simple motor reaction time task (D'Esposito et al., 1999). Similar observations have been reported for visual processing during checkerboard presentation (Huettel et al., 2001) and perception of emotional faces (Iidaka et al., 2002) .

The origin of the observed global reductions in fMRI signal remains unclear as of yet. As there were no differences in compliance between the groups in this experiment, one would expect similar levels of neural activity at least in the brain regions that are involved in stimulus perception, such as V1 and V2. However, these regions also showed a marked reduction in fMRI signal. Loss of neural tissue may explain a part of this reduction, as there is evidence for cerebral atrophy in normal aging (Scahill et al., 2003,Grossman et al., 2002). However, considering the magnitude of the reduction in the fMRI signal we observed, it is unlikely it can be explained by loss of neural tissue only. It is possible that the reduction reflects an age-related alteration of cerebrovascular dynamics, but the present study does not provide direct evidence for this. There are, however, several indications provided by other studies that support this notion. For instance, a reduction of nearly 40% in total cerebral blood flow has been reported in aging subjects (80-88 years) as compared to young adults (19-29 years) using ungated two- dimensional phase-contrast magnetic resonance (MR) angiography (Buijs et al., 1998). Smaller reductions have been reported using single photon emission tomography (Larsson et al., 2001). Other indications that ageing may affect the neuronal-vascular coupling are findings that arteriosclerotic changes such as reduced elasticity and compliance of vessels, may attenuate the dynamic range of vascular reactivity in elderly subjects (D'Esposito et al., 2003). Two findings provide indirect evidence that reduced or inflexible blood supply in elderly may indeed affect the BOLD signal in fMRI. Firstly, older subjects exhibit an attenuated effect of hypercapnia during a breath-holding challenge compared to younger subjects (Riecker et al., 2003). Secondly, elderly subject exhibit a reduced increase in total hemoglobin during fingertapping, measured over the motor cortex with a combination of fMRI and near infrared spectroscopy. In that study, corresponding fMRI images showed smaller areas of cortical activation in elderly subjects (Mehagnoul-Schipper et al., 2002). In summary, there is indirect evidence from multiple studies to indicate that ageing may be accompanied by a

change in cerebrovascular dynamics that, in turn, may affect the BOLD response.

As in all cross-sectional studies, the results of this study could be confounded by group differences that result from historical influences such as educational opportunity, cultural factors and socioeconomic status. Unfortunately it is very difficult to control for these factors adequately, and they tend to result in overestimation of the age related differences between groups (Hedden and Gabrieli, 2004). Differences in intellectual ability between the groups could have arisen due to these factors. However, the significance of this for our study is not clear, given that intellectual ability does not affect saccadic reaction times, and only moderately influences the number of errors during antisaccades (Evdokimidis et al., 2002). Differences in the number of errors were further controlled for by the use of Event Related fMRI and analysis of correct responses only. Another issue is the possible incidence of mild cognitive impairment (MCI) or early dementia in the old adulthood group. Although all subjects clearly understood task instructions and performance was not severely impaired, the possibility remains that a few affected subjects in the elderly group caused the differences between the groups in behavioral and fMRI results. As of yet, it is not known how MCI affects oculomotor functioning, but there is evidence that impaired saccadic inhibition is the most specific oculomotor measure for Alzheimer's disease (Shafiq-Antonacci et al., 2003). Nevertheless. although the impairment in saccade inhibition in the elderly group in this study was mild at best, influences of MCI on fMRI results cannot be ruled out.

In summary, our study suggests that changes in brain function occur during aging even in the execution of simple eye movements. These changes appear to start during mid-adulthood and involve relative increases in activation in frontal brain areas of the oculomotor system. This increase does not appear to be specific for one particular oculomotor task tasks. In addition, we argue that neuronal-vascular coupling may be altered in elderly subjects, although the present study does not provide direct evidence for this notion. Future studies are therefore warranted to further explore the influences of age related changes in vascular dynamics on the BOLD signal and to subsequently develop techniques to account for these effects when comparing fMRI activation in subjects of different ages.

Test-retest reliability of fMRI activation during prosaccades and antisaccades.

Submitted as:
Raemaekers, M., Vink, M., Zamdbelt, B., van Wezel.R.J.A., Kahn, R.S., Ramsey, N.F. Test-retest reliability of fMRI activation during prosaccades and antisaccades.

Abstract:
Introduction: Various studies have investigated reproducibility of fMRI results. Whereas group results can be highly reproducible, individual activity maps tend to vary across repeated sessions. This type of reliability is of importance for application of fMRI for endophenotype research, where brain activity is compared to genetic polymorphisms. In this study, the reliability of activation maps during prosaccades and antisaccades is assessed by comparing individual results across two sessions in healthy subjects. Several measures of reliability were employed to not only assess reproducibility, but also to investigate factors by which it may be affected.

Methods: Functional brain images were acquired during two sessions for a prosaccade and antisaccade task in twelve subjects using an event related functional Magnetic Resonance Imaging design. Measures of reliability of both individual and group-wise activity included correlations of t-values between sessions, and relative amount of overlap activity. In addition, intraclass correlations in predefined regions of interest were assessed. Eye movements were measured during scanning.

Results: Classical measures of reliability indicated that groupmaps were quite reproducible. The reproducibility of individual maps was highly variable in terms of correlations of t-values and amount of overlap activity. We found that reliability of individual activity maps was explained to a large degree by individual sensitivity. Moreover, individual sensitivity also explained a large portion of the between subject variance in regional brain activation.

Discussion: Reliable fMRI results can be obtained in some, but not all subjects. This difference in reliability is caused by differences in sensitivity across individuals. Sensitivity itself is quite reproducible across sessions, indicating that it reflects an important feature that varies little across time. Thus, individual sensitivity may be an important issue for endophenotype research. Low sensitivity in some subject may be dealt with either by improving sensitivity of the fMRI procedure (fieldstrength, paradigm design, number of scans), or subject exclusion. Differences in sensitivity between subjects should be addressed before using regional brain activation as phenotype in genetic studies.

6.1 Introduction:

Functional magnetic resonance imaging (fMRI) has proven to be a valuable tool in revealing brain activation abnormalities associated with various forms of mental illness. The use of fMRI has also increased insight in the underlying mechanisms of small neurobehavioral abnormalities that are linked to these diseases (Raemaekers et al., 2002b). In the context of genetic determinants of human behaviour, these abnormalities are referred to as endophenotypes, and they are present in patients as well as in their healthy relatives. They are thought to have a more strongly genetically determined etiology than the disease itself, and could exhibit more straightforward genotype-phenotype relationships (Gottesman and Gould, 2003). Some studies have shown that the fMRI measurements of the neural mechanisms underlying endophenotypes, can be a more sensitive method for revealing abnormalities than the corresponding behavioral measures such as performance(Callicott et al., 2003a,Raemaekers et al., 2006a,Vink et al., 2006). The penetrance of the underlying genes may thus be higher when the expression is measured at the neurofunctional level, than when measured at the behavioral level. This suggests that fMRI images could allow for more accurate phenotyping than behavioral measures. However, one of the prerequisites of a good endophenotype is that it is state-independent and has a high test-retest reliability (Gottesman and Gould, 2003). In this study, we assess the reliability of brain activation maps that are associated with a well known endophenotype for schizophrenia, the antisaccade paradigm.

In the antisaccade task, subjects have to inhibit an eye movement towards a novel stimulus (prosaccade) and instead make an eye movement in the opposite direction (antisaccade) (Hallett, 1978b). Patients with schizophrenia (Fukushima et al., 1990b) and their relatives (Calkins et al., 2004b) have difficulty suppressing reflexive saccadic eye movements during the antisaccade task. The test retest reliability of reaction times of prosaccades and antisaccades ranges from fair to good across studies in healthy subjects (Ettinger et al., 2003,Harris et al., 2006,Klein and Berg, 2001,Roy-Byrne et al., 1995), and also in patients and their unaffected relatives (Calkins et al., 2003b) (Harris et al., 2006). Most, but not all of these studies report good reliability for antisaccade error rates as well.

The assessment of test-retest reliability in fMRI studies is a more complicated matter. Random and systematic differences in cognitive processes such as arousal, cognitive strategies, and small changes in performance could influence the measurements. In addition to actual differences in brain function between sessions, various non psychological factors may influence the individual measurements such as changes in the

position of the subject in the magnetic field of the MRI scanner and in the radiofrequency head coil, field inhomogeneities, image signal to noise ratios, and cardiac, respiratory or motion artifacts (McGonigle et al., 2000,Veltman et al., 2000). In summary, there are a lot of factors affecting the Blood Oxygenation Level Dependent (BOLD) response, so that one cannot expect exactly the same BOLD response in different measurements of the same subject on different occasions. In addition, data processing and analysis, statistical type I or II errors contribute to variable end-results.

Several measures are available to assess the reliability of fMRI results. The most common ones are discussed by Specht et al (Specht et al., 2003). These involve the calculation of the correlation of contrast t-values for pairs of activation maps, estimating the ratio of overlapping activation between sessions, and assessment of the intraclass correlations. Correlations of t-values and overlap of activation is typically used to assess within subject variability. For using fMRI images as endophenotype, the intraclass correlation (ICC) is most relevant (Shrout, 1979), where the variation of activity across subjects (for correlation with gene polymorphisms) is of critical importance. This measure reflects the ratio of the variance within subjects over the repeated measurements, and the variance between the subjects. A high ICC reflects a large between subject variability, and a small within subject variability. A good endophenotype therefore has a high ICC.

A limited number of studies have assessed the ICC of fMRI task activation. Poor reproducibility was found for a verbal working memory task which was repeated 9 times with an interval of 3 weeks (Wei et al., 2004). In contrast, Manoach et al. (Manoach et al., 2001) found moderate reliability in healthy subjects, also during a working memory task, and a recent study of Aron et al. (Aron et al., 2006) reported good to nearly perfect reproducibility in 8 subjects in a one year follow up study for a classification learning task, especially in the frontostriatal circuitry. The latter finding suggests that fMRI could already be used for tasks to classify subjects based on their pattern of regional brain activation.

These studies did not report estimates of the reliability for results of *individual* subjects. Although this seems trivial, as normally high ICCs cannot exist in the absence of reliable within subject measurements, the case may be different for fMRI. The first prerequisite for finding reproducible individual results in fMRI, is a good signal to noise ratio. Noisy data will by definition be less reproducible. The signal to noise or sensitivity of the measurement in a particular subject will determine the number of voxels passing the statistical threshold (extent of activity). However, fMRI

measurements of the same task within different subjects can substantially differ considering the extent of activation. On one hand this could indicate a high variability in the strategy that individuals follow in performing a particular fMRI task, but on the other hand it could mean that there is a large difference in the sensitivity of individual measurements between subjects. Such difference could have no relationship to actual differences in brain activation, but merely reflect differences in e.g. physiological noise, motion induced noise, or properties of the hemodynamic response. Differences between subjects in their extent of activation across the brain are much harder to interpret, as they could be caused by other factors than differences in brain activation. However, individual differences in physiological noise or shape of the hemodynamic response could very well be stable over time. A high ICC in a voxel or a region of interest could therefore reflect stable individual differences in the extent of activation across the whole brain, instead of regionally specific differences. For ICC estimation, it is therefore important to take the entire extent of activation into account.

In this study we address the reproducibility of brain activation maps during prosaccades and antisaccades and the contrast between prosaccades and antisaccades (saccadic inhibition), at the group level as well as at the individual level. This is done by calculating ratios of overlapping activation and correlations of group-wise t-maps, and t-maps of individual subjects. Group results of two sessions will be compared to detect systematic changes in brain activation between the sessions. ICCs will be calculated for brain activation in predefined regions of interest. In addition, we propose a method for assessing the sensitivity in individual subjects. The impact of the estimated sensitivity on measures of individual reliability is assessed, as well as the impact of individual sensitivity on the ICCs.

6.2 Methods:

Subjects:
12 healthy subjects (6 male, 6 female; mean ± SD age, 22.1 ± 1.75 years) recruited from the University of Utrecht participated in the experiment. None of the subjects had any signs of present or past major psychiatric illnesses according to the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998b). A history of major neurological illness resulted in exclusion from the experiment, as did metal implants. All subjects gave informed consent for participation (approved by the Human Ethics Committee of the University Medical Center Utrecht). All were right handed according to the Edinburgh Handedness inventory (Oldfield, 1971a).

The test and retest took place at the same time in the evening, with an interval of one week. All subjects were non-smokers and were asked to make sure that coffee intake and amount of sleep was equal for both days of scanning.

Scanning protocol:
All images were obtained with a Philips ACS-NT 1.5T MRI scanner (Philips Medical Systems, Best, the Netherlands) with fast gradients (PT6000). The head was held in place with a strap and with padding. Structural and functional images were acquired in transverse orientation, from the same section of the brain. For functional scans, a navigated 3D-PRESTO pulse sequence (Ramsey et al., 1998b,van Gelderen et al., 1995)was used with following parameters: TE 37 ms, TR 24 ms, flip angle 9.5 degrees, matrix 48*64*24, FOV 192*256*96 mm, voxel size 4 mm isotropic, scan duration 1.49 s per 24-slice volume. Immediately after functional scans, an additional PRESTO scan of the same volume of brain tissue was acquired with a high flip-angle (30 degrees, FA30) for the image coregistration routine (see below). Finally, a T1 weighted structural image was acquired.

Task design:
The fMRI design used a PC, a rear projection screen and a video-projector system for presentation. All stimuli were projected in white on a dark background. All events were time-locked to the fMRI scans. Instructions were given verbally, prior to the start of the experiment. With the aid of a laptop, a limited number of test trials were presented until subjects indicated they understood the task. The design consisted of two tasks, that is prosaccades and antisaccades, which had identical stimuli. Whether subjects were requested to make prosaccades or antisaccades depended on a short summary of the instructions at the beginning of each new block of ten stimuli. Instructions were presented for a duration of three scans, followed by a 6 scan period of central fixation. Each new trial started with the disappearance of a fixation cross (0.9 ° visual angle) at central view. After a 200 ms gap period, a square (0.9° visual angle) was presented semi-random 8.7° to the left or right of central fixation. If the instructions were prosaccades, subjects had to make a saccade towards the square as quickly as possible. If the instructions were antisaccades, subjects had to avoid an automatic eye movement towards the square, and instead make a saccade towards the opposite direction. The square was extinguished after 3240 ms, simultaneously with the reappearance of the fixation cross at central view.

This signaled the subjects to refixate in the center of the screen. A new stimulus was triggered 10.16 s. after central refixation, thereby generating a fixed stimulus interval of 13.4 s giving stimulus related BOLD signal time to return to baseline (Bandettini and Cox, 2000). Stimulus related changes in BOLD signal were thus measured relative to fixating in the center or in the periphery. The two events, e.g. the prosaccade/antisaccade and the refixation in the center, were placed in time in this way, in order to keep the correlation between the two associated BOLD-responses to a minimum. There were four blocks per task making a total of eight, which were orderly alternated. Each subject made 40 prosaccades and 40 antisaccades in total, during the 20 minute functional imaging session.

Analysis:
fMRI scans were analyzed with custom-written programs in IDL (Research Systems Inc. Boulder, USA) (Raemaekers et al., 2002a). The last functional volume was registered to the FA30 volume. Next, all fMRI volumes where registered to the (now registered) last functional volume using a least squares differences criterion (Thevenaz et al., 1998). The structural scan was also registered to the FA30-scan thereby providing spatial alignment between the structural scan and the functional volumes. A 3D-gaussian filter (8mm full width at half max) was applied to all fMRI volumes. Data for each subject were submitted to a linear multiple regression analysis. The factor matrix contained factors for stimulus related changes in BOLD-signal for prosaccades, antisaccades, refixation in the center during prosaccades, refixation in the center during antisaccades, and reading of the instructions. For both designs, low frequency noise was modeled with additional factors i.e. the mean signal intensity of each scan, and 88 discrete cosine functions forming a high pass filter with a cut-off at $3.73*10^{-2}$ Hz to correct for low frequency scanner and physiological artifacts but also for differences in baseline activation between conditions. All events in the design-matrices were convolved with a predefined hemodynamic response function (Friston et al., 1995).

The t-statistics of the relevant contrasts (e.g. prosaccades, antisaccades, and antisaccades vs. prosaccades) were calculated for every voxel. Subsequently, the t-volumes were spatially normalized in Talairach orientation to enable group-wise comparisons (Collins et al., 1994). The effects of the task for the whole group were analyzed for each session separately using one sample t-tests on the normalized t-volumes of prosaccades and antisaccades, and saccadic inhibition (Worsley, 1994). Systematic differences in brain activation between the sessions were estimated for the three conditions

separately by comparing average t-values in seven predefined regions of interest that are known to be involved in the oculomotor tasks (Raemaekers et al., 2005,Raemaekers et al., 2006b) (Figure E, page 73), with a repeated measures GLM (2x7 levels, i.e. two sessions and the seven regions of interest).

Analyses of Reliability:

Overlap in Activation:
This measure was used to assess the test-retest reliability of the brain activation of individual subjects, as well as the test-retest reliability of the brain activation found for the group-wise comparisons. This was done by calculating the relative amount of volume overlap in activation between the two sessions for the t-volumes of individual subjects, as well as for the t-volumes of the group wise comparisons. Overlap was estimated for prosaccades, antisaccades, and inhibition separately. The overlap between the sessions ($R^{12}_{overlap}$) was calculated by using the formula proposed by Rombouts et al. (Rombouts et al., 1998) and Machielsen et al. (Machielsen et al., 2000):

$$R^{12}_{Overlap} = \frac{2 * V_{overlap}}{V_1 + V_2}$$

V_1 and V_2 denote the number of voxels in the t-volume passing the threshold in session one and session two respectively, and $V_{overlap}$ the number of voxels that pass the threshold in both t-volumes. For estimating the $R^{12}_{overlap}$, a statistical threshold of $p<0.001$ (uncorrected) was used.

Correlation:
Another measure that can be used for estimation of reliability of fMRI results is the correlation measure (Tegeler et al., 1999). Like the $R^{12}_{overlap}$, this measure was used to assess the test-retest reliability of both individual subjects and the group-wise results. In contrast to the $R^{12}_{overlap}$, this measure is based on all the voxels in the brain, instead of only the voxels that have reached the significance threshold. All t-values in the t-volume of session one, are correlated with the t-values in the t-volume of session two (R^{12}). This was done for all conditions (prosaccades, antisaccades, and inhibition) and for the group t-volumes, as well as for the individual t-volumes. For averaging correlation coefficients across subjects, Fisher's z-transformation was used on the individually estimated R^{12} before group wise comparisons:

$$z' = \left(\frac{1}{2}\right)\log\left(\frac{1+R^{12}}{1-R^{12}}\right)$$

Intraclass correlations:
The measures of $R^{12}{}_{overlap}$ and R^{12} can be used to assess reliability within in a single measurement (either an individual or a group-wise measurement). Accurate classification or phenotyping of subjects does not only depend on the reliability of individual measurements, but also on the variance between subjects. Both the between and within subject variance is incorporated in the intraclass correlation (ICC) (Shrout, 1979) and is calculated for two within subject measurements as:

$$ICC = \frac{MS_{between} - MS_{within}}{MS_{between} + MS_{within}}$$

$MS_{between}$ and MS_{within} are the mean square errors for between-subjects and within-subjects variance respectively. The ICC thus represents the ratio of between-subject variance to total variance and is the appropriate metric for assessing within-subject reliability, rather than Pearson's R, because the observations are not independent. The ICC was determined for the average t-values in all the predefined regions of interest (Figure E, page 73).

Calculation of the sensitivity:

Mean square of the t-values:
The reliability measures that are discussed are all dependent on the signal to noise ratio, or sensitivity of the individual measurements. When the signal to noise ratio of an individual measurement is low, e.g. due to motion artifacts, scanner noise or physiological factors, the t-values in the statistical maps of individual measurements will be low and therefore irreproducible. This will result in a low $R^{12}{}_{overlap}$, and a low R^{12}. To estimate the average amplitude of the t-values, or the sensitivity of the individual measurements, for each subject and session, the average sums of squares of the t-values during prosaccades and antisaccades was calculated.

$$MS_t = \frac{1}{2*n}\sum_{i=1}^{2*n} t_i^2$$

In the formula, t depicts the t-value per voxel, and n the number of voxels. MS_t represents the total amount of stimulus related changes in the brain relative to the noise for prosaccades and antisaccades combined. The ICC of the MS_t was calculated to estimate the stability of sensitivity of measurements in individuals.

Subsequently, the MS_t for each subject was averaged over the sessions and was correlated with $R^{12}_{overlap}$ and R^{12} to estimate to what extent these measures were determined by the MS_t of the measurements. Finally, it was estimated whether intersubject differences in sensitivity could underlie part of the between subject variance when estimating the ICC for different regions of interest and task conditions. Therefore, ICCs were recalculated after removal of between subject variance due to differences in the average sensitivity over the two sessions.

Eye Movements:
Eye movements were recorded during the entire oculomotor-task using an MR-compatible eyetracker (Cambridge Research Systems Ltd., Rochester, UK (Kimmig et al., 1999) in combination with Labview (National Instruments Corporation, Austin, USA) acquisition software on a PC with a multifunctional I/O Board (National Instruments Corporation, Austin, USA). This acquisition-PC was linked to the stimulus-PC by a parallel cable to synchronize the eye-recordings and the task presentation. Calibration and adjustment of the sensor were done during a five minute period prior to scanning. The sample frequency of the recording was 500 Hz. For each saccade in the time window of 200 ms before until 600 ms after the stimulus presentation, the latency and the direction were determined using a custom nonautomated analysis program in IDL (Research Systems Inc., Boulder, USA). Reliability was estimated by the ICC of the behavioral measures.

6.3 Results:

Systematic Changes:
Subjects had an overall reduction in activation for prosaccades from session one to session two ($F_{(1,11)}$=11.05; p=0.007) (Figure 6.1) in the predefined regions of interest (Figure E, page 73). Although a reduction was also observed for antisaccades, the difference between the sessions was not significant ($F_{(1,11)}$=2.73; p=.13). There was no overall difference in inhibition related activation, nor were there any significant session by region of interest interactions in any of the three conditions.

Overlap Ratios:
The $R^{12}_{overlap}$ for the group t-volumes for prosaccades, antisaccades, and inhibition were .75, .80, and .18 respectively (Figure E, page 73). Thus, with 12 subjects, group results for prosaccades and antisaccades are highly reproducible. The lower overlap for activation during inhibition probably indicates lower statistical power than for the prosaccade and antisaccade condition. The average $R^{12}_{overlap}$ of the individual subjects were .30 (±.23) for prosaccades, .39 (±.32), for antisaccades, and .29 (±.17). The $R^{12}_{overlap}$ could substantially differ between subjects (00-.71 for prosaccades, .00-.90 for antisaccades, .02-.57 for inhibition).

Correlations:
The t-values of the group results of session one are plotted against the t-values of the group results of session two in figure 6.2 for the three conditions. The correlation in t-values between the sessions were all significant ($r=.87$; $p<0.001$ for prosaccades, $r=.93$; $p<0.001$ for antisaccades, and $r=.46$; $p<0.001$ for inhibition) (Figure 6.2). The average transformed correlations (z' values) were .23 (±.12) for prosaccades, .31 (±.12) for antisaccades, and .07 (±.07) for inhibition. For individual subjects, correlations differed substantially (.02-.77 for prosaccades, .29-.80 for antisaccades, -.12-.41 for inhibition) (see figure 6.3 for two examples)

Intraclass correlations:
Result for the ROI based ICC estimation are similar (Table 6.1a). Areas of reasonable and good reliability can be found mostly in occipital and parietal areas during prosaccades and antisaccades. Results in other areas are mixed, and differ between prosaccades and antisaccades. Reliability for brain activation during saccadic inhibition is poor in general.

Mean square t estimation:
The reliability of the MS_t was high over the two sessions (ICC=.73; p=0.002, 95% confidence interval=.30-.91)(Figure 6.4). When averaged over the sessions, there were large intersubject differences in MS_t (ranging between 1.47 and 3.75). The correlations between the average MS_t and the $R^{12}_{overlap}$, were significant for all conditions ($r=.82$; $p=0.001$ for prosaccades, $r=.87$; $p=0.000$ for antisaccades, $r=.62$; $p=0.033$ for inhibition). The correlations between the MS_t and z' were also significant for all conditions ($r=.86$; $p=0.000$ for prosaccades, $r=.87$; $p=0.000$ for antisaccades, $r=.75$; $p=0.005$ for inhibition). These correlations show that differences between subjects in overlapping activation over the two sessions ($R^{12}_{overlap}$), and differences in correlations of the individual t-values between subjects (z')

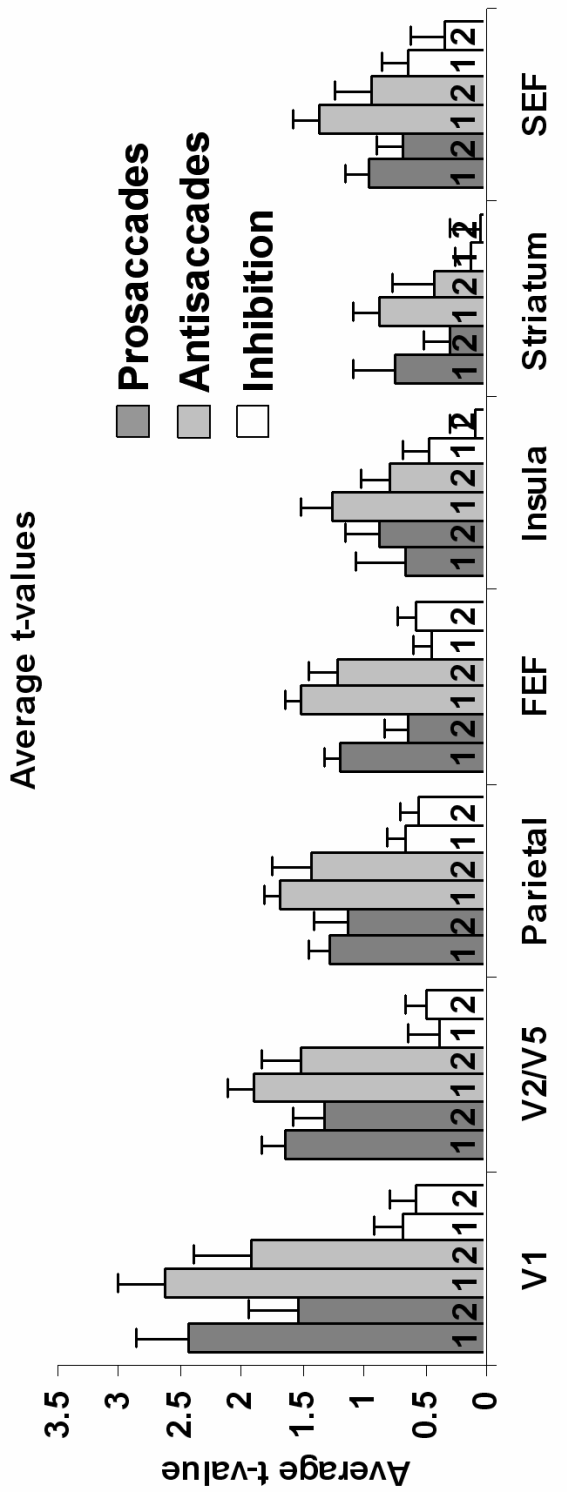


Figure 6.1. Average t-values for the three conditions in the relevant regions of interest (Figure E, page 73). The number at the bottom of each bar signifies the session number. Bars indicate standard errors.

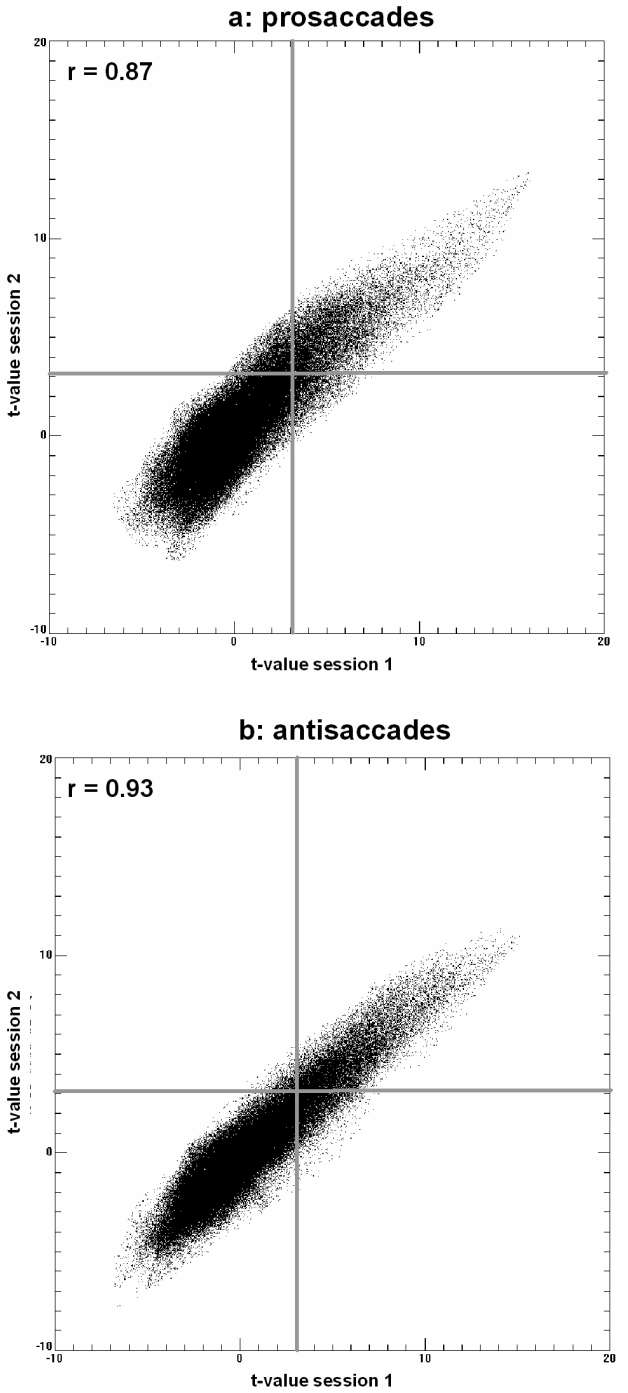
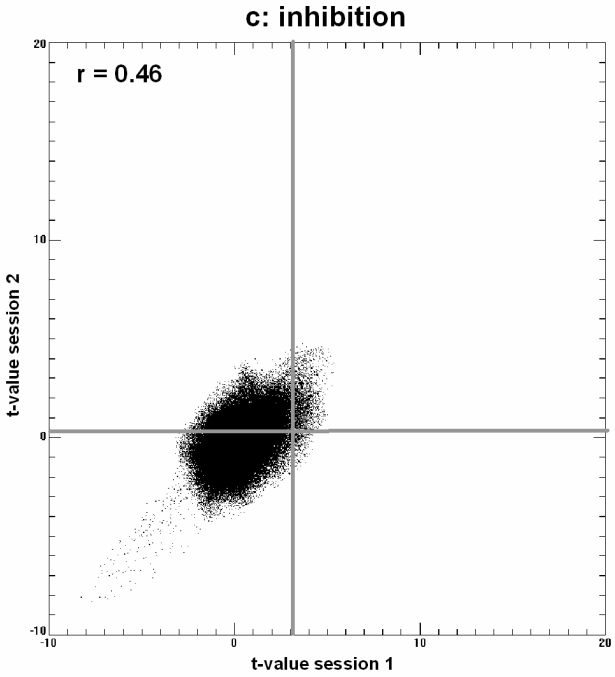


Figure 6.2. For the three conditions, the t-values group results of the first session plotted against the t-values for the second session. Grey lines indicate the statistical threshold that was used to calculate the $R^{12}_{overlap}$.



can be largely explained by intersubject differences in sensitivity of the measurements.

High correlations between MS_t and average t-values in the regions of interest show that a large proportion of the between subject variance in the regional brain activation could be explained by MS_t (Table 6.2). After removing the between subject variance that could be explained with MS_t using a linear regression, the ICC score for each region and condition was reassessed. Only the ICC of activation in the Supplementary Eye Fields during prosaccades remained significant (Table 6.1b).

Behavioral results:
When comparing the behavioral measures of the two sessions, there were no significant differences in performance (Table 6.3). The stability of the onset latencies of both correct prosaccades and correct antisaccades was high (prosaccades (Figure 6.5a); ICC=.85, 95% confidence interval=.55-.96, $p<0.001$; antisaccades (Figure 6.5b); ICC=.91, 95% confidence interval=.69-.98, $p<0.001$). The error rates for antisaccades were less stable over the two sessions (Figure 6.5c) (ICC=.50, $p<0.05$, 95% confidence interval=-.11-.85, $p<0.05$). The behavioral data in two subjects in session 1 were partially unusable due to noise. These two subjects had an error rate for antisaccades 70% and 61% in session 2. As these are much larger than

the average antisaccade error rate, the loss of the data may have resulted in an underestimation of the between subject variance, and subsequently the ICC. Errors during prosaccades were too sporadic to make a reliable ICC estimate.

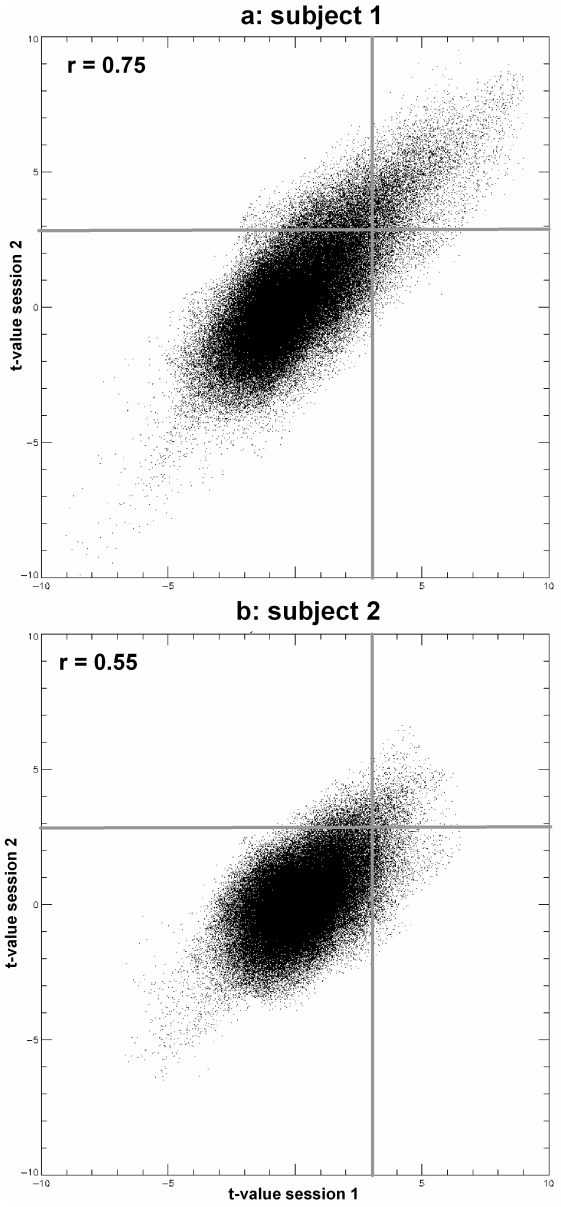


Figure 6.3. *For two individuals, the t-values for antisaccades in the first session plotted against the t-values for antisaccades in the second session. Grey lines indicate the statistical threshold that was used to calculate the* $R^{12}_{overlap}$.

		Prosaccades		Antisaccades		Inhibition	
	n	ICC	P	ICC	P	ICC	P
V1	12	.62*	.01	.70*	.00	-.09	.62
V2/V5	12	.58*	.02	.51*	.03	-.05	.56
Parietal	12	.61*	.01	.09	.37	-.10	.62
FEF	12	.14	.32	.19	.26	.05	.43
Insula	12	.43	.07	-.07	.59	-.30	.84
Striatum	12	.52*	.03	.01	.48	-.36	.90
SEF	12	.73*	.00	.40	.08	-.01	.51

		Prosaccades		Antisaccades		Inhibition	
	n	ICC_{cor}	P	ICC_{cor}	P	ICC_{cor}	P
V1	12	.42	.07	.45	.06	-.10	.62
V2/V5	12	.11	.35	.31	.14	-.05	.56
Parietal	12	.22	.23	.45	.06	-.10	.62
FEF	12	-.25	.80	.36	.11	.05	.43
Insula	12	.66*	.01	.03	.46	-.30	.84
Striatum	12	.31	.14	.07	.41	-.36	.90
SEF	12	.25	.20	.22	.23	-.01	.51

Table 6.1. ICC estimates with P values for average t-values in the seven predefined regions of interest (Figure E, page 73) for prosaccades, antisaccades, and saccadic inhibition. a: ICC estimates based on uncorrected average t-values; b: ICC estimates after removal of the between subject variance that could be explained by MS_t.

		Prosaccades		Antisaccades		Inhibition	
	n	R	P	R	P	R	P
V1	12	0.93*	0.00	0.89*	0.00	-0.05	0.87
V2/V5	12	0.85*	0.00	0.83*	0.00	0.47	0.12
Parietal	12	0.83*	0.00	0.64*	0.03	-0.12	0.72
FEF	12	0.87*	0.00	0.60*	0.04	0.02	0.96
Insula	12	0.21	0.51	-0.25	0.44	-0.44	0.15
Striatum	12	0.63*	0.03	0.37	0.23	-0.23	0.48
SEF	12	0.67*	0.02	0.67*	0.02	0.34	0.28

Table 6.2. Correlation estimates with P values for the correlation between MS_t and the average t-values in the seven predefined regions of interest (Figure E, page 73) for prosaccades, antisaccades, and saccadic inhibition.

		Session 1		Session 2		
	n	M	(S.D.)	M	(S.D.)	Paired t
Prosaccade latencies	11	177.42	19.00	181.91	22.34	-1.35
Prosaccade %errors	11	0.70	2.32	0.79	1.78	-0.15
Antisaccade latencies	10	243.24	37.97	238.81	35.39	0.88
Antisaccade %errors	10	16.36	9.27	18.42	15.14	-0.51

Table 6.3. Summary of the behavioral results of prosaccades and antisaccades for the two sessions.

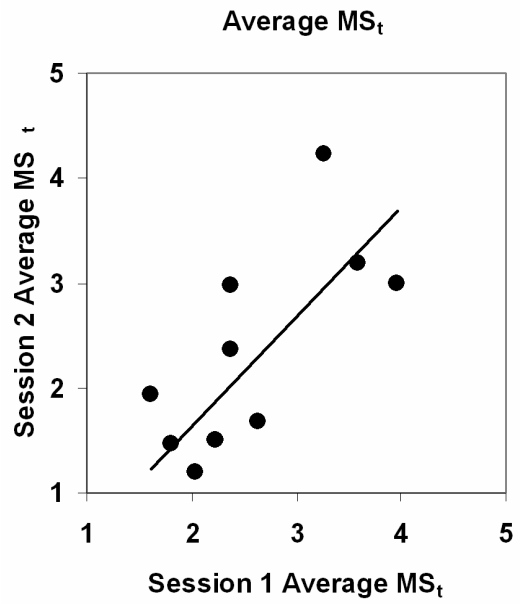


Figure 6.4. Scatter plot of the MS_t during session one, against the MS_t during session two.

6.4 Discussion:

In this experiment, the test-retest reliability of individual and group-wise fMRI activation maps during prosaccades, antisaccades, and saccadic inhibition was assessed. The group activation maps for prosaccades and antisaccades were very similar across the two sessions. The reliability of the group map for saccadic inhibition was considerably lower. Reliability of individual measurements was variable between subjects, and was highly correlated with the measure of sensitivity. In addition, intersubject differences in regional brain activation could be explained almost entirely by between subject differences in the total extent of activation, indicating that the strongest determinant of differences between subjects was the overall strength of activation as opposed to regionally selective strength of activation. In other words, some subjects display large regions of activations whereas others display small regions, and this feature is reproducible across sessions. Behavioral measures had moderate to good reliability.

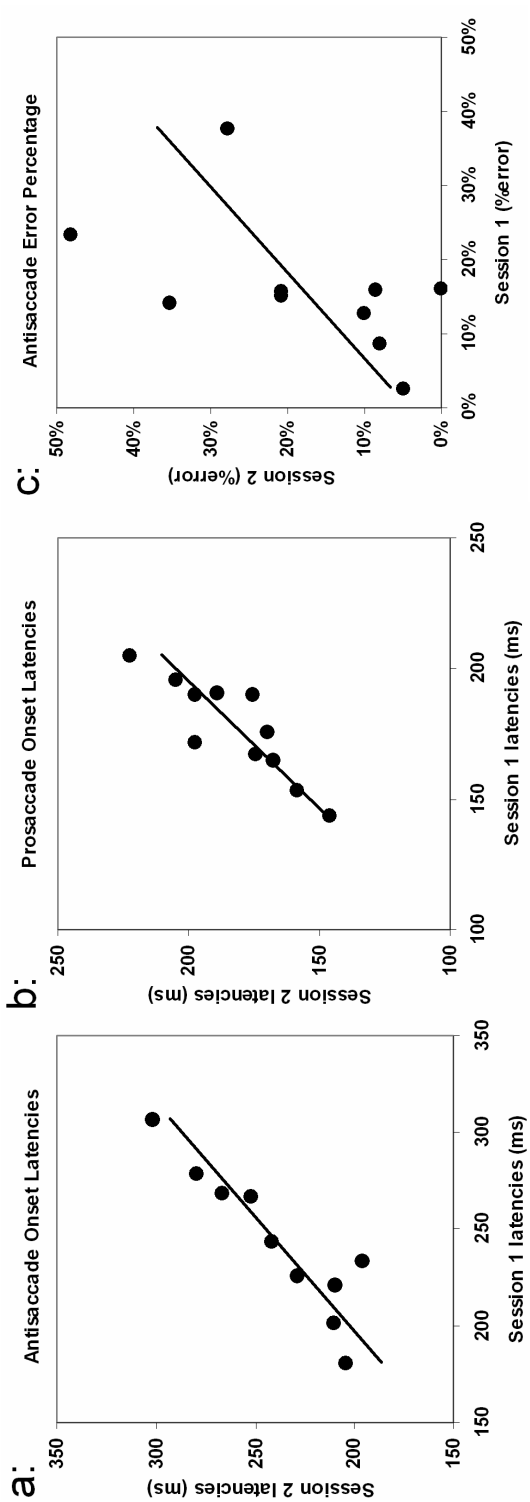


Figure 6.5. Scatter plots of the behavioral measures of the first session, against the behavioral measures of the second session. a: Saccade onset latencies for prosaccades; b: Saccade onset latencies for antisaccades; c: Error percentage during antisaccades.

As reported previously, fMRI group-wise results can be highly reproducible (Casey et al., 1998). Reliability of the group results for saccadic inhibition could still be considerably improved, however. In comparison to prosaccades and antisaccades, saccadic inhibition had lower effect sizes of individual measurements, which probably caused the lesser correlation and overlap of activation. When comparing the group-wise activation between the two sessions, there was an overall decrease in activation from session one to session two for prosaccades (the reduction during antisaccades did not reach significance). Reductions in activation have been associated with learning. It is well known that practice can lead to a reduction in functional activation over time (Chein and Schneider, 2005). Practice induced reductions in activation are thought to reflect functional trimming of neuronal ensembles that are involved in the task (Ramsey et al., 2004). Alternatively, familiarity with the fMRI procedure may play a role, in that subjects are less aroused in session two. Systematic changes over sessions can influence reliability measures. Although it will not much affect correlations, it will decrease the ICC and overlap ratio estimates.

Before estimating ICCs, the reliability of individual measurements was assessed, as this is one of the cornerstones of the reproducibility of between subject variation. We found a large variation in individual reliability, concerning both the overlap ratios ($R^{12}_{overlap}$), and the correlation of t-values (R^{12}). This variation in reliability could be largely explained by differences in the sensitivity of the individual measurements. Good individual reliability could thus be obtained, if the signal to noise ratio of the measurement was high enough (i.e. high t-values in individual t-maps). This suggests that individual reliability in this task is mostly a matter of signal to noise ratios, and not variability in brain activation between sessions. The average overlap ratios that we estimated for the three conditions were only moderate compared to the overlap ratios that have been reported in the literature for other fMRI paradigms (Machielsen et al., 2000,Rombouts et al., 1998,Specht et al., 2003). This is probably due to the fact that the other tasks were primarily visual, and were presented in a blocked design, a design type that is statistically very powerful. The current design is sparse event related, which may result in lower statistical power, and thus a lower overlap in activation

A large portion of the between-subject variance in brain activation in the predefined regions of interest can be explained by differences in the sensitivity of the individual measurements. Moreover, after removing the between-subject variance in regional brain activation that could be attributed

to differences in sensitivity between subjects, only one brain area during one condition remained that had an ICC that was significantly different from zero (Supplementary Eye Fields during prosaccades). We found evidence that the sensitivity of the measurement is quite stable in subjects (ICC=.73). Intersubject differences in sensitivity are not readably interpretable. They could be linked to intersubject differences in subjective attentional demand of the task (Adler et al., 2001b). On the other hand, they could also be related to global differences e.g. physiological noise, shape and size of the hemodynamic response, motion artifacts, etc. etc. Future studies should address the origin of individual differences in the extent of activation in fMRI measurements.

As a measure of sensitivity of the measurement, we used the mean square of all t-values (MS_t) during prosaccades and antisaccades. One could argue that regionally specific differences in brain activation will also result in differences in the MS_t. By correcting the average t-values in the regions of interest before ICC estimation, this regional between subject variation would be removed. However, the between subject differences in MS_t that we found could be so large (ranging between 1.47 & 3.75), that they can hardly be explained by regionally specific differences in activation. In addition, as MS_t correlates highly with the average t-values of nearly all areas of the visual and oculomotor system during prosaccades and antisaccades, it is clear that much of the individual variation in the regions of interest has a common source, and that MS_t reflects this common source. The contribution of regionally specific differences in brain activation to MS_t are thus at best minimal. However, to completely remove the contribution of regionally specific variations, the measure of sensitivity should be generated independently of the measurement. Recently proposed methods for individual calibration of the BOLD response using a breath holding challenge may become very useful in this context (Thomason et al., 2006).

As for the behavioral results, we found high test retest reliability of onset latencies of both prosaccades and antisaccades, similar to what is reported in the literature (Ettinger et al., 2003,Harris et al., 2006,Klein and Berg, 2001,Roy-Byrne et al., 1995). Reports on reliability of the percentage of erroneous distractions during antisaccades are less consistent (ranging between -.30 and .89). This measure may be more dependent on task specific variations (e.g. gap/overlap) between these studies which may affect intersubject variability, and thus ICC scores. The ICC score of .50 found in this experiment is, although significant, relatively small. Due to the loss of data of two subjects that had a very high error rate, the between subject variation may have been underestimated, which may have resulted

in a lower ICC. A larger sample size is needed to make a more precise estimate of the reliability of distractibility in this particular design.

In summary, group-wise t-maps of prosaccades and antisaccades can be highly reliable in a group with twelve subjects, but for contrasts with smaller effect sizes, like for saccadic inhibition, probably more subjects are required. As for individual maps, more statistical power is needed to make a more reliable estimate of the amplitude of the BOLD response, especially in individuals that have poor signal to noise. Individual reliability could thus benefit from increasing statistical power by e.g. going to higher field strengths, increasing the number of scans per measurement, and choosing task designs with a large effect sizes. In addition, future studies on test retest reliability should address differences in sensitivity between subjects, before estimating region of interest based ICCs. Endophenotyping based on brain function could benefit from incorporating differences in sensitivity as well.

Summary and discussion

The aim of this thesis was the assessment of the potential of using brain activation maps during the antisaccade paradigm as endophenotype for schizophrenia. Schizophrenia has a complex etiology, in which multiple genes act in concert with each other and the environment. As a result, detecting genes associated with the illness has proven to be a difficult challenge. Endophenotypes are more intimately linked with underlying genetics, and could thereby reduce the gap between genotype and phenotype (Gottesman and Gould, 2003). The use of endophenotypes can thereby increase the power of molecular genetic studies, but also increase insight in how genetic abnormalities lead to the development of schizophrenia.

The antisaccade paradigm may be a suitable endophenotype for schizophrenia. In the antisaccade task, subjects have to inhibit a saccade towards a new peripheral stimulus (i.e. prosaccade), and make a saccade in the opposite direction (i.e. antisaccade). Patients with schizophrenia and their healthy first degree relatives have difficulty suppressing eye movements towards the stimulus during antisaccades, while prosaccade performance is well within normal range (Calkins et al., 2004b). However, the behavioral measures of antisaccades are the output of a brain system that is quite extensive in the number of nodes and connections. The complexity of the underlying neural network implies that multiple elements contribute to performance, and constitutes a multifactorial link between genotype and phenotype, thereby reducing the effectiveness of the performance measure as endophenotype. By looking directly at the functional properties of the underlying neural network during antisaccades by using fMRI, the link between genotype and phenotype may be simplified.

Good endophenotypes should meet certain criteria. Not only should they be present in both patients and their healthy relatives, endophenotypes should also be measured with high reliability, sensitivity, and validity. The single important issue is that what we measure with fMRI in healthy subjects, patients, and their healthy relatives, is a strong and direct reflection of what is present or happening at the level of genes.

At first, we should have the ability detect brain activation in the neural network that is associated with prosaccades, antisaccades. In the first experiment, an event related fMRI design was used to measure brain activation during prosaccades and antisaccades (Raemaekers et al., 2005). This experiment demonstrated that is was possible to detect even the short neural events that are associated with saccadic eye movements and saccadic inhibition. The activated network showed a large overlap with brain regions that are known to be involved in oculomotor functioning from studies in

primates (Berthoz, 1996). Surprisingly, in some brain areas, i.e. the supplementary eye fields, the insula, and the striatum, there were reductions in brain activation associated with the saccade that returned the gaze back to the center after a prosaccade or antisaccade. In a blocked design, these reductions could mask activation in brain areas that are relevant to schizophrenia. Therefore, event related fMRI was used in all experiments.

The second study investigated if the newly developed event related antisaccade paradigm could also detect differences in activation between patients and healthy control subjects (Raemaekers et al., 2002b). We found that, compared to control subjects, patients did not activate the striatum properly during inhibition of saccadic eye movements. From primate research it is known that striatum is a major terminal through which the cortical structures of the oculomotor system can inhibit the midbrain and thereby prevent the execution of an automatic saccadic eye movement. An impairment in the striatum, or in the fibers connecting the cortex and the striatum, would impair the ability of the cortex to influence the midbrain and the ability to inhibit automatic saccadic eye movements. Hence, the antisaccade deficit in schizophrenia patients could be linked to a deficit in the striatum, or in the frontostriatal circuitry.

As it was possible to link the antisaccade deficit in schizophrenia to a specific functional deficit in the striatum, it was further investigated whether this abnormality was also present in healthy first degree relatives of patients (Raemaekers et al., 2006a). If the functional brain abnormality involving the striatum would qualify as endophenotype, the deficit had to be present in healthy siblings of patients as well. It was found that although siblings had abnormal striatal activation during antisaccades, the performance on the antisaccade task was within normal range. Hence, relatives were able to compensate for the striatal deficit. There is substantial overlap in function between the brain areas within the neural system subserving antisaccades. For example, apart from the connections between cortex and midbrain through the striatum, there are also direct connections between cortex and midbrain which bypass the basal ganglia. The midbrain could be inhibited through the direct connections, and leave the functional deficit in the striatum undetected at the behavioral level. An absence of a behavioral deficit would thereby mask the neurofunctional deficit, and thereby a possible genetic deficit. Functional brain activation measures may thus have an advantage over behavioral measures as endophenotype, in having a more penetrant genotype phenotype relationship.

A good endophenotype should also be a stable, triat-like characteristic, as high stability is indicative of a genetically determined trait. Although there is evidence that performance on the antisaccade task is relatively stable (Ettinger et al., 2003), it does not remain unchanged over the entire lifespan (Eenshuistra et al., 2004). If functional brain maps are to be used as endophenotype in linkage analysis, brain activation measurements have to be done in multiple generations. Age effects could substantially influence the individual measurements under these circumstances, which could lead to erroneous phenotyping. To detect and quantify effects of aging, the BOLD response during prosaccades and antisaccades was measured in a group covering a broad age range (Raemaekers et al., 2006b). It was found that from mid adulthood on, subjects tend to increase frontal brain activation relative to posterior brain activation. This may reflect an increase in voluntary control over saccade execution. However, age did not only affect the cognitive strategy of subjects in performing the task, but probably also the coupling between neural activation and the BOLD response. Both effects can influence the individual measurements. Hence, if functional activation maps are to be used as endophenotype, the age of the subject should be taken into account to avoid erroneous phenotyping.

For use as endophenotype in linkage analysis, it is also important to have reliable measurements over the short term. In the last experiment, the stability of the functional brain activation maps was measured over an interval of one week. In this study, reliability of individual results, as well as group-wise results were assessed. Reliability of group results was high for prosaccades and antisaccades. Reliability of individual results differed considerable between subjects. This variation in individual reliability was almost entirely caused by stable differences in the signal to noise ratio between the subjects. Reliable measurements were possible, if the signal to noise ratio in a particular subject was high enough. Although ICC scores indicated reasonable to good reliability in some brain areas during prosaccades and antisaccades, these were almost entirely caused by differences in signal to noise ratio between the subjects. For phenotyping individuals based on brain activation maps, it is thus important to control for individual differences in signal to noise. In addition, when using the same design, some but not all subjects may provide reliable results, dependent on their individual signal to noise ratio. With the current design, not all subjects could be phenotyped reliably.

Although there are still difficulties to overcome, endophenotyping of brain activation maps will undoubtedly become a very important aspect of the genetic research in schizophrenia. The results of the experiments in patients

and their relatives demonstrate that brain activation maps can give more detailed information than results obtained from behavioral experiments and diagnosis alone. However, there still is room for improvement, especially in increasing reliability of individual measurements. It can be expected that with increasing MR field strengths en faster scan acquisition times this problem can be mostly overcome. FMRI will prove to be a very useful tool, not only for detecting genes associated with schizophrenia, but also for giving more insight in how these genes play a role in the complex etiology of the illness.

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Nederlandse samenvatting

Schizofrenie is een complexe psychiatrische aandoening. Bij het ontstaan van schizofrenie spelen zowel meerdere genen als omgevingsfactoren een belangrijke rol. Deze complexe etiologie maakt het bijzonder lastig om genen te vinden die betrokken zijn bij het ontstaan van schizofrenie. Als alternatieve onderzoeksstrategie wordt daarom vaak gekeken naar kleine neurofysiologische afwijkingen die zowel bij patiënten met schizofrenie, als hun gezonde familieleden vaker voorkomen. Deze afwijkingen heten endofenotypen, en ze hebben een minder complex verband tussen genotype en fenotype. Een simpeler verband tussen genotype en fenotype vergroot de statistische power van moleculair genetische studies.

Er bestaat bewijs dat een afwijking bij het onderdrukken van automatische oogbewegingen een endofenotype is voor schizofrenie. Het vermogen tot onderdrukken van automatische oogbewegingen wordt gemeten met behulp van de antisaccade taak. Tijdens de antisaccade taak moeten proefpersonen een oogbeweging naar een nieuwe visuele stimulus onderdrukken, en zo snel mogelijk een oogbeweging naar de tegenovergestelde zijde van de stimulus maken. Patiënten met schizofrenie hebben meer moeite met het onderdrukken van een oogbeweging naar de stimulus dan gezonde proefpersonen. Bij de controle prosaccade taak, waar proefpersonen juist naar de stimulus moeten kijken, presteren patiënten normaal.

Uit onderzoek bij primaten blijkt dat bij het maken van antisaccades een extensief neuraal netwerk betrokken is. De betrokkenheid van een dergelijk uitgebreid netwerk zorgt er voor dat de relatie tussen een antisaccade afwijking en een genetische afwijking nog steeds bijzonder complex is. Het meten van hersenactiviteit met behulp van functional magnetic resonance imaging (fMRI) bij het maken en onderdrukken van oogbeweging, zou de relatie tussen genotype en fenotype kunnen vereenvoudigen. In dit proefschrift wordt de bruikbaarheid van hersenactiviteit bij antisaccades als endofenotype voor schizofrenie onderzocht.

Bij de eerste studie werd het antisaccade paradigma aangepast voor fMRI. De taak werd afgenomen in een ‘event related’ fMRI design. De taak bleek vrijwel alle hersengebieden te activeren waarvan bij primaten bekend is dat ze bij het generen en onderdrukken van oogbewegingen betrokken zijn. Uit dit onderzoek bleek dat ook de saccade die de ogen terugbracht naar het midden van het scherm na een prosaccade of antisaccade, significante

activiteit met zich meebracht. Deze activiteit kon de prosaccade of antisaccade activiteit maskeren in een traditioneel blokdesign.

Bij de tweede studie werd de antisaccade taak gebruikt om het neurale substraat van het saccadische inhibitie defect bij patiënten met schizofrenie te onderzoeken. Uit dit onderzoek bleek dat patiënten tijdens het onderdrukken van oogbewegingen minder activiteit vertoonden in het striatum dan de gezonde proefpersonen. Van primaatstudies is bekend dat de corticale gebieden van het oculomotor systeem via het striatum de hersenstam kunnen inhiberen, en daarmee een automatische oogbeweging kunnen onderdrukken. Een afwijking in het striatum, of in de verbindingen tussen de cortex en het striatum, zou het vermogen van de cortex om de hersenstam te inhiberen beperken. Het saccadische inhibitiedefect bij schizofrenie kon dus teruggevoerd worden op een afwijking in het striatum of in het frontostriatale circuit.

In de derde studie werd onderzocht of de afwijking in het striatum zoals gevonden bij schizofrenie ook aanwezig was bij gezonde broers en zussen van patiënten. Net als bij de patiënten bleek dat bij de familieleden het striatum tijdens het onderdrukken van oogbewegingen niet actief werd. Tegelijkertijd bleek dat bij familieleden in tegenstelling tot bij de patiënten, geen verschil waargenomen kon worden in de gedragsmaat van de taak. Familieleden bleken dus even succesvol in het onderdrukken van automatische oogbewegingen als gezonde proefpersonen. Blijkbaar zijn bij de familieleden van patiënten de hersenen in staat om de afwijking in het striatum te compenseren. Daardoor blijft de neurale afwijking in het striatum onzichtbaar als het op een gedragsniveau getest wordt. Dit zou dus ook een eventuele genetische afwijking die ten grondslag ligt aan de striatale afwijking maskeren. De hersenactiviteit tijdens het onderdrukken van oogbewegingen is dus een geschikter fenotype dan de gedragsmaat.

Aangezien een goed endofenotype grotendeels genetische bepaald is, zou het gedurende de levensloop vrijwel ongewijzigd moeten blijven. Uit studies naar leeftijdseffecten op de fMRI hersenactiviteit en prestaties op de antisaccade taak blijkt echter dat er over langere perioden toch veranderingen optreden. Als hersenactiviteit tijdens antisaccades als endofenotype gebruikt gaat worden, moet er voor deze effecten gecorrigeerd worden. In de vierde studie werd daarom de hersenactiviteit gemeten in een groep waarin een breed scala aan leeftijden vertegenwoordigd was. Uit deze studie bleek dat leeftijd een invloed had op de hersenactiviteit tijdens het maken van oogbewegingen. Proefpersonen van middelbare en oudere leeftijd waren, ten opzichte van de jongere groep, geneigd om de frontale

hersengebieden meer aan te spreken relatief ten opzichte van occipitale en pariëtale hersengebieden. Verder bleek dat fMRI activiteit in het gehele brein bijna 40% lager was in de oudere leeftijdsgroep. Gezien de omvang van de reductie, en gezien het feit dat de prestaties op de taak in deze groep niet drastisch afwijkend waren, moet geconcludeerd worden dat bij oudere mensen de koppeling tussen de neurale activiteit en de Blood Oxygenation Level Dependent signal, bij oudere mensen veranderd is. Als mensen gefenotypeerd worden op basis van hersenactiviteit moet er dus gecorrigeerd worden voor deze specifieke en globale effecten van leeftijd op het fMRI signaal.

Een goed endofenotype moet ook over de korte tijd stabiel gemeten kunnen worden binnen proefpersonen. Daarom werd in de laatste studie de test-hertest betrouwbaarheid van hersenactiviteit tijdens de antisaccade taak bepaald over de periode van een week. Het bleek dat groepsresultaten van de taak over het algemeen goed reproduceerbaar waren. De test-hertest betrouwbaarheid van de individuele resultaten verschilde echter substantieel tussen proefpersonen. Deze individuele verschillen in test-hertest betrouwbaarheid konden echter vrijwel geheel toegeschreven worden aan individuele verschillen in signaalruis verhouding. Deze verschillen in de signaalruis verhouding waren stabiel over de tijd. Stabiele verschillen in de signaalruis verhouding tussen subjecten resulteerde daardoor in een overschatting van de variantie tussen subjecten bij het bepalen van de intraclass correlation (ICC).

Uit deze studies is gebleken dat fMRI gebruikt kan worden bij het meten van hersenactiviteit bij het onderdrukken van oogbewegingen. Hierdoor kon aangetoond worden dat zowel patiënten met schizofrenie als hun familieleden een afwijkende hersenactiviteit hadden in het striatum tijdens het onderdrukken van oogbewegingen. De hersenactiviteitmaat bleek in familieleden zelfs aanwezig in afwezigheid van een gedragsafwijking. De hersenactiviteitmaat zou dus een sterkere koppeling met onderliggende genen kunnen hebben dan de gedragsmaat. Uit de test-hertest studie bleek echter dat de hersenactiviteit niet in alle subjecten betrouwbaar genoeg gemeten kon worden voor individuele classificatie. Deze situatie zal in de toekomst echter zeker verbeteren met hogere magnetische veldsterkten en meer verfijnde scan-acquisitietechnieken.

Curriculum vitae

Mathijs Raemaekers werd geboren op 30 december 1974 in Roermond. Nadat hij het V.W.O. diploma gehaald had aan de scholengemeenschap St. Ursula in Horn, begon hij in 1994 aan zijn studie Psychologie aan de Universiteit Utrecht. Naast zijn studie was hij actief bij de Studentengroep Sociale Wetenschappen. Na het behalen van zijn diploma in 2000, begon hij als assistent in opleiding bij de afdeling Volwassenen Psychiatrie in het Universitair Medisch Centrum Utrecht. Hier deed hij fMRI onderzoek naar de erfelijkheid van hersenactiviteit tijdens oogbewegingen bij schizofrenie. In 2005 begon hij als postdoc onderzoeker bij de leerstoelgroep functionele neurobiologie van de Universiteit Utrecht waar hij tot op heden fMRI onderzoek doet naar de vrijwillige controle over bewuste visuele waarneming.

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