

**THE ROLE OF THE POSTERIOR PARIETAL CORTEX IN THE
PLANNING OF SACCADIC EYE MOVEMENTS**

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**Thesis submitted to the University of Nottingham
for the degree of Doctor of Philosophy**

May 2007

Abstract

This thesis aimed to investigate the role of posterior parietal cortex (PPC) in relation to saccade planning and more specifically the spatial remapping processes essential to this behaviour. These experiments begin through the use of transcranial magnetic stimulation (TMS) on a version of the classic double-step saccade paradigm (Chapter 2). TMS was not found to disrupt spatial remapping on this task and a potential explanation for this in terms of task specifications was proposed. In Chapter 4 this theme was explored further through a series of variations on the double-step saccade task, in which the order of target presentation was manipulated; these led to the conclusion that both target encoding and spatial remapping are influenced by such task-related factors.

In Chapter 3, a second set of TMS experiments is discussed, which investigated the updating of saccade plans in response to a change in target location, rather than eye position.

Finally in Chapters 5 and 6 neuroimaging studies that aimed to evaluate the cortical areas involved in these processes are discussed. The first of these (Chapter 5) was an extension of the behavioural studies previously conducted in Chapter 4. The second employed a novel saccade paradigm to investigate the effect of intervening saccades made between the time of target encoding and execution (Chapter 6). The findings from these experiments supported the idea that the PPC is important for representing saccade goals and updating these following a change in the spatial relationship between the centre of gaze and the target location for a future saccade.

In Chapter 7 the findings from the aforementioned studies were discussed in relation to current debate within this area of research, concerning in particular the functional significance of saccade-related neuronal activity in PPC, as were suggestions for future studies that might help provide further insight into these issues.

Acknowledgements

There are a number of people who have been integral in helping me to achieve this goal and I would here like to thank them for their various contributions:-

I would first like to thank my supervisor, Stephen Jackson, for encouraging me to do a PhD in the first place, for his supervision and guidance with my research, and in addition to this, for teaching me to ski. Thanks also to Roger Newport for instigating the weekly highlight that was ‘cake day’ and for always being willing to provide an honest opinion and a listening ear, in exchange for chocolate.

Thanks to Sally Pears, for her advice and support in the early days when all this seemed a distant dream, and for her company throughout. Thanks too to Åsa Pellijeff, for many long discussions over coffee, or occasionally wine, and for her much valued friendship. Thanks to Leo Bonilha, for encouraging me to aim higher (and run faster!), and for his advice on fMRI design and analysis and much more besides. Thanks to all the friends in the lab that I’ve met along the way, who shared with me both the highs and the lows of the PhD experience: Dan Smith, Carl Jackson, Sven Müller, Kirsten McKenzie, Catherine Preston, Duncan Astle and Violet Yang.

Many thanks to all those that over the years gave up far too much of their time to sit in front of the eye tracker, under the TMS coil or to lie in the scanner and thus aid me with my data collection, your patience was much appreciated.

And last, but by no means least, thanks to my family and my friends, for all their support and encouragement in this endeavour, and especially to Stevie, for always believing in me.

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Chapter 1: Literature Review

1.1. Introduction

The accurate planning of movements is intrinsic to our ability to interact effectively with the environment. Reaching out and picking up an object, for example, requires precise arm movement control in terms of direction, extent and velocity. Eye movements similarly require planning in order to effectively shift gaze towards an object of interest. The focus of the experiments described in this thesis has been to further our understanding of how saccades are planned with a particular emphasis on investigating the role of the posterior parietal cortex (PPC) in this process.

The importance of the PPC in the planning and execution of saccadic eye movements has been well established by previous research, however the precise nature of its role is still a matter of debate, and a variety of different saccade-related functions have in fact been attributed to this area (Section 1.2). Saccadic eye movements themselves are not unitary in nature and can be divided into a number of subtypes, for example on the basis of how they are generated, i.e. internally or externally. This is important when considering the cortical areas responsible for oculomotor control since certain types of eye movement may involve the PPC to a greater extent than others (Section 1.3).

Investigations into the neural areas associated with these different types of eye movements have made use of a range of different methodologies. These have included: single-unit recording and inactivation studies in non-human primates, neuropsychological studies of patients with parietal lesions, and eye-tracking, transcranial magnetic stimulation (TMS) and functional magnetic resonance imaging (fMRI) paradigms in healthy participants (Section 1.4).

Studies such as these have helped identify a network of areas, including the PPC, that contribute to the control of saccades. Subregions of the PPC have also been classified, both in relation to their location and also the different aspects of oculomotor behaviour they are primarily involved in. The lateral intraparietal area (LIP), for example, has been shown in monkeys to be particularly active during eye movements and is thus often referred to as the ‘parietal eye field’ (PEF) (Andersen et al., 1992; 1997). The ‘parietal reach

region' (PRR), in contrast, although also found on the banks of the intraparietal sulcus (IPS), is more greatly associated with reach-related activity (Batista & Andersen, 2001) (Section 1.5).

Although the amount known about the cortical processes involved in the planning and execution of saccades has increased greatly over recent years, a number of debates in the literature still remain. The functional significance of neuronal activity in area LIP has, for example, been contested by different research groups. It is variously argued to be visual in nature and related to attention (Colby & Duhamel, 1996) or alternatively indicative of motor intention (Snyder et al., 1997) (Section 1.6).

The experiments described in this thesis have used a number of techniques in an attempt to investigate unresolved issues in this area of research. The spatial remapping of visual target locations, and the importance of this process to saccade planning, has been a particular focus of interest in all of the studies discussed here. Eye-tracking paradigms, with the additional employment of both TMS and fMRI, have been utilised as a means of examining these behaviours and the cortical areas associated with them (Section 1.7).

1.2. Proposed functions of the PPC

Before attempting to investigate posterior parietal contributions to oculomotor behaviour, it is useful to first consider the nature of saccade-related functions that have been linked to this area on the basis of previous research. These consist of a wide variety of processes, including the combination of sensory information from different modalities to build up a representation of the world around us, which may perhaps be expressed in terms of a map of motor intention or spatial attention. In relation to such representations, the PPC may also be responsible for converting between information stored in different frames of reference (i.e. sensorimotor transformations) and also for ensuring that these representations are continuously updated (spatial remapping). Further to these processes a role for the PPC in saccade initiation has also been put forward. The role of the PPC in these behaviours is not yet

precisely defined, but an overview of some of the studies that provide support for these functions is given below.

1.2.1. Multi-Sensory Integration & Multimodal Space Representation

Findings from single-unit recording studies have led to the suggestion by Colby and Duhamel (1996) that the parietal cortex contains multiple spatial representations for action. They suggested that spatial perception is modular in nature and that a single object will be represented multiple times according to the actions that can be performed on it. Andersen et al., (1997) similarly proposed that the main function of the PPC is one of multimodal space representation. Information from different sensory modalities including vision, audition and somatosensation converges here, along with efference copy from bodily movements making it well placed to carry out processes requiring the manipulation of information from different sensory modalities. This information is integrated to form abstract spatial representations, both of the world and our body, which can be used for movement guidance. These spatial representations could be coded in gaze-centred (retinotopic), eye-centred (oculocentric), head-centred (craniotopic) or body-centred coordinates. Alternatively they could also be coded in an exocentric or world-centred frame of reference.

This idea that a single-object may be simultaneously encoded in a variety of reference frames is also linked to another proposed function of the parietal lobe, that of sensorimotor transformations, i.e. converting between coordinate systems for the control of movement. This process is discussed in more detail in section 1.2.4, below.

1.2.2. Motor Intention / Visuospatial Attention

While the use of a single unimodal map of the world is generally agreed to be unlikely, the functional significance of these multiple representations is however still under debate. Snyder et al., (1997) have argued that posterior parietal cortex codes motor intention for planned movements rather than spatial attention to sensory stimuli. Further to this, Andersen & Buneo (2002) have

suggested that the PPC is involved in the formation of motor intentions and that a map of these early movement plans is held within the PPC, with specialised regions for particular movement types (see Section 1.5.1 for more details).

In contrast to this however, Colby & Goldberg (1999) have proposed that parietal activity instead reflects visuospatial attention. Maps in the parietal lobe are thought to represent the location of salient objects and the actions that can be performed on them (Colby & Duhamel, 1996). They argue that parietal neurons, whilst representing objects in motor coordinates, in fact exhibit visual responses that are independent of specific motor intentions. This debate is discussed in more detail in section 1.6.

The use of visual search tasks has also suggested a role for the PPC in visuospatial attention. Poor performance on such tasks by a patient with bilateral parietal lesions has for example been demonstrated (Friedman-Hill et al., 1995), as has a disruption to task execution following parietal TMS (Ashbridge et al., 1997; Ellison et al., 2003; Walsh et al., 1998; 1999). Studies making use of functional imaging have however suggested extensive overlap in terms of the parietal areas involved in covert shifts of attention and those accompanying saccadic eye movements (Corbetta et al., 1998; Nobre et al., 2000), making it difficult to distinguish neural activity related to attention and intention.

1.2.3. Saccade Triggering

Another proposed role for the PPC in relation to oculomotor control is that of saccade triggering. Evidence to support the idea that posterior parietal areas may be responsible for the initiation of reflexive saccades in response to newly presented visual (or auditory/somatosensory) stimuli is discussed by Leigh & Kennard (2004). Increased latencies for visually guided saccades have for example been shown following lesions to the PEF, particularly on the right, whereas this was not seen for the frontal eye fields (FEF) and supplementary eye fields (SEF) (Pierrot-Deseilligny et al., 1991).

This idea has also been supported by findings from TMS studies that have similarly demonstrated increased reaction times for reflexive saccades

following parietal stimulation (e.g. Elkington et al., 1992). Increased latencies on memory-guided saccade tasks have also been shown however, suggesting that the PPC may additionally be involved in the triggering of saccades to remembered locations (Müri et al., 1996b; 2000). Others have argued that intentional saccades are in fact controlled by the FEF rather than parietal areas and that the superior colliculus (SC) may also be involved in saccadic initiation (see Leigh & Kennard, 2004).

In a recent review however, Rafal (2006) showed that this increase in reaction time, ostensibly due to a slowing of saccadic initiation, is additionally present for key press responses. He has thus argued that the primary function of the PPC is not in fact the initiation of either voluntary or reflexive saccades, but rather the computation of sensorimotor transformations.

1.2.4. Sensorimotor transformations

Sensorimotor transformations, i.e. converting between reference frames associated with different body parts, are necessary for action since limb movements may need to be coded in different spatial coordinates than those used for vision. The location of the PPC between the primary visual areas of occipital cortex and the primary motor cortex, and its connections to other oculomotor areas such as the frontal lobe, make it ideally placed to mediate these transformations (Rizzolatti et al., 1997). Support for this idea has come from single-unit recording studies making use of anti-saccade tasks. Zhang & Barash (2004), for example, recorded from neurons in monkey area LIP during a memory-guided anti-saccade task, in which visual information on a target location must be transformed in order to plan a motor command away from the visual target. They concluded that the activity of these neurons during this task corresponded to working memory for the computation of the required sensorimotor transformation. A disruption in this process of converting information from the visual environment into a code that is suitable for action, was proposed to be responsible for the slower performance on an anti-saccade task of patients with parietal lesions demonstrated by Machado and Rafal (2004a; 2004b) (see section 1.3.3.).

The parietal cortex is not the only area that has been implicated in this process however, with evidence from both single-unit recording studies in monkeys (Graziano, Yap and Gross, 1994; Graziano and Gross, 1997; 1998; Graziano, 1999) and TMS in humans (van Donkelaar et al., 2002) additionally implicating the premotor cortex (PMC). Given its location between prefrontal and primary motor cortex, the premotor cortex is similarly well-placed for converting sensory information into a form appropriate for movement guidance, and Graziano and colleagues have demonstrated arm-centred coding of visual objects by neurons in this region. This idea has since been supported by work from van Donkelaar et al., (2002) who compared the effects of applying TMS to this area and to the PPC during a visually-guided reaching task. The results supported the hypothesis that reaches may be coded simultaneously in limb-centred coordinates in the PMC and eye-centred coordinates in the PPC.

1.2.5. Spatial Remapping

In order to maintain an up-to-date representation of the relationship between different body parts and our environment, remapping of spatial coordinates must take place to account for the eye and body movements that we constantly perform. This is important both for acting effectively within the environment, for example in planning motor commands to achieve goals, and also for the sake of perceptual continuity (Ross et al., 2001).

Recordings from LIP neurons have suggested a role for this area in the process of spatial remapping. A shift in the receptive field of these neurons has been shown to occur in advance of an eye movement, suggesting therefore that it might in fact be triggered by motor intention (Duhamel et al., 1992a). However, such behaviour is not exclusive to this area and evidence for presaccadic remapping has similarly been observed in FEF (Bruce and Goldberg, 1985; Goldberg and Bruce, 1990) and the superior colliculus (SC) (Mays and Sparks, 1980).

When more than one saccade is to be performed spatial remapping is also required in order to update remembered target locations. This is important both in the planning of a saccade sequence, in order to account for intended eye

displacements and also for accurate execution in terms of compensation for any inaccuracy in the end-point of the previous saccade. Extraretinal information regarding the metrics of the first saccade is thought to be used in updating the visual representation of the second saccade target when double-step saccade sequences are performed (Schlag and Schlag-Rey, 2002). Evidence for parietal involvement comes from neurophysiological recording studies (Mazzoni et al., 1996) and inactivation (Li and Andersen, 2001) of parietal neurons in monkeys, neuropsychological studies of patients with parietal lesions (Heide et al., 1995) and both TMS (van Donkelaar & Müri, 2002) and functional imaging (Heide et al., 2001) studies in humans.

1.3. Types of Saccadic Behaviour

As mentioned above, the involvement of the PPC may not be equal for the planning and execution of different kinds of saccadic behaviour. In order to understand more fully the reasons for this and to focus in on those to which the PPC seems most closely associated and which will therefore be the subject of investigation in the experiments discussed in this thesis, a short overview of the different subtypes and their neural bases will be provided here. (For a more thorough discussion see Leigh and Kennard, 2004).

1.3.1. Reflexive/Visually-Guided Saccades

Early evidence suggesting a role for parietal neurons in the generation of reflexive saccades came from Mountcastle (1976), who noted a discharge of these neurons prior to saccade onset. This, it was proposed, might reflect a ‘command function’ for the execution of reflexive saccades.

More recent studies in patients with cortical lesions have also suggested parietal involvement in these types of saccades (Pierrot-Deseilligny et al., 2001; Gaymard et al., 2003) and this has been further supported by findings from TMS studies (Elkington et al., 1992; Kapoula et al., 2001). It has alternatively been argued however that this may in fact be a more general problem of attention rather than a specific deficit in saccade generation (Rafal, 2006).

1.3.2. Anti-Saccades

In addition to prosaccades, eye movements executed away from a visual stimulus (anti-saccades) may also involve some degree of parietal control. Gottlieb and Goldberg (1999), for example, showed that intraparietal neurons were activated by the presentation of contralateral visual targets regardless of whether monkeys were instructed to perform pro- or anti-saccades, with responses actually found to be greater in the anti-saccade task.

Saccadic latencies for patients with parietal lesions performing an anti-saccade task were assessed by Machado and Rafal (2004a; 2004b), who found reaction times to contralesional targets to be more affected for anti-saccades than prosaccades. This was explained by the fact that anti-saccades require a sensorimotor transformation in order to execute a movement to a location away from the visual target; this is a function thought to be performed by LIP neurons, which are damaged in these patients. This has been supported by single-unit recording studies in monkeys (Zhang & Barash, 2004). Parietal and frontal TMS have additionally been shown to result in increased latencies on an anti-saccade task (Terao et al., 1998).

1.3.3. Memory-Guided Saccades

Memory-guided saccades appear to involve a network of areas, including the dorsolateral prefrontal cortex (DLPFC), FEF and PPC (Pierrot-Deseilligny et al., 1993). Deficits in terms of latency and accuracy of memory-guided saccades have been demonstrated in both patients with parietal lesions (Duhamel, et al., 1992b; Heide et al, 1995) and in healthy subjects following parietal TMS (Müri et al., 1996b; 2000).

1.3.4. Multi-Step Saccade Sequences

1.3.4.1. Double-Step Saccade Paradigm

The double-step saccade task is a classic paradigm requiring two memory-guided saccades (Hallett & Lightstone, 1976; Mays & Sparks, 1980).

It is useful in that it allows a dissociation to be made between the location of the retinal stimuli (i.e. the two visual targets) and the metrics of the saccades executed in response to them, and can thus be used to investigate spatial remapping (Heide et al., 1995). Schlag and Schlag-Rey (2002) describe this task in detail: the subject starts each trial by fixating a central point, two targets are then presented in quick succession within a period of ~150ms (i.e. less than saccadic latency period). Subjects are instructed to look toward the remembered locations of the targets in the order they were presented. The saccade towards the first target is simple, and can be performed on the basis of retinal information about target position, i.e. the retinal vector from the fixation point to target one. The saccade to target two is slightly more complicated however, since the start of this movement is no longer from the fixation point and thus it cannot be planned through use of the retinal vector alone. Schlag and Schlag-Rey discuss two possible solutions to this problem; the first is allocentric in nature, and requires the subject to memorise the spatial relationship between targets 1 and 2, and use this to calculate the vector between them. Alternatively an egocentric method could be used to solve this problem, which involves integrating the remembered retinal vector from the fixation point to target 2 with information on the eye displacement brought about by the first saccade in order to obtain the coordinates of target 2 in space. The authors suggest that the second solution is most likely, and propose information on eye displacement is provided by an eye position signal (EPS), an internal, or 'extraretinal' signal, possibly arising from an efference copy (motor command) or corollary discharge (neuronal signal) for the eye movement, or alternatively a signal of intended movement.

The functional importance of the PPC to the double-step saccade task has been supported by investigations using various experimental techniques including single-unit recording in monkeys (Mazzoni et al., 1996), studies of patients with parietal lesions (e.g. Duhamel et al., 1992b; Heide et al., 1995), functional imaging of healthy subjects on an extended triple-step version of the task (Heide et al., 2001), and parietal TMS (e.g. van Donkelaar & Müri, 2002). These studies are discussed in more detail in the corresponding methodological sections below (section 1.4).

1.3.4.2. Saccade Sequences

Since the PPC has been consistently implicated in double-step saccades, it seems reasonable to assume that it would be at least as involved in longer saccade sequences if not more so. On the basis of this idea, a triple-step paradigm was used by Heide et al., (2001) in their fMRI study in an attempt to increase the extent of parietal activity observed in a memory-guided saccade task. The planning and execution of saccade sequences has also been investigated behaviourally through eye-tracking studies; in order to investigate certain aspects of oculomotor behaviour it is useful to have more than two saccades in a sequence. Such studies have, for example, considered the extent to which a complete series of movements is planned prior to saccade initiation, the allocation of attention to multiple upcoming saccade goals, and our ability to perform online compensation for errors made in the execution of previous saccades in a sequence (e.g. Zingale and Kowler, 1987; Bock et al., 1995; Godijn & Theeuwes, 2003).

1.4. Experimental Methodologies for Assessing the Role of the PPC

Previous studies investigating the function of the PPC in relation to saccadic behaviour have made use of a wide variety of methodologies. A brief description of these techniques is given below, with examples of some of the key studies that have made use of them to investigate pertinent issues in this research area. More detailed descriptions of TMS, fMRI and eye tracking have been provided since these techniques constitute those employed in this thesis in attempting to assess parietal contributions to saccade control.

1.4.1. Animal Studies

Animal studies can provide an informative way of investigating the role of the PPC in saccadic behaviour that is not possible in humans due to the invasive nature of such techniques. The majority of such studies have been conducted on macaque monkey since these are the group of non-ape primates

most closely related to humans. The findings from these studies are of course limited in terms of extrapolation to humans; the difference in body size can make comparisons problematic, since this factor has been shown to be important in determining brain size. Further to this it is also quite possible that some areas seen in macaque monkeys may not have a direct homologue in humans, or alternatively a set of areas might in fact be duplicated in one species compared to the other (Sereno and Tootell, 2005). Despite the existence of anatomical differences in this region, the human area PEF, in the IPS, is believed to correspond to the monkey area LIP. It is therefore useful to consider findings from neurophysiological studies in non-human primates when evaluating the contribution of parietal areas to oculomotor control.

1.4.1.1. Single-Unit Recording

In single-unit recording studies, electrophysiological activity from a single neuron is recorded by means of a microelectrode, inserted into the brain of a living animal. Such studies have both good spatial and temporal resolution, however they are only able to sample a tiny percentage of the cortex at a time, and do not take into account possible interactions between areas. Inferring the role of a region of cortex on the basis of single neuron (or group of neurons') activity in one particular area to a specific stimulus type may therefore be problematic.

The findings from such studies have however proved informative in terms of gaining greater insight into saccade planning, through assessing neuronal activity during the presaccadic period. Duhamel et al., (1992a), for example, made use of single-unit recording in monkeys performing a saccade task. This task involved a jump in the fixation target occurring concurrently with the brief presentation of a visual stimulus. The visual stimulus was located in such a position that the endpoint of the saccade would bring its previous location within the receptive field of a particular LIP neuron. The neuron would be expected to discharge at around 70ms after the stimulus enters the receptive field. Results showed, however, that the discharge actually began 150ms before this, i.e. 80ms prior to saccade onset. On the basis of this study a role for anticipation in the parietal cortex, in terms of the predicted outcome

of a saccade, was put forward. It was proposed that saccadic anticipation leads to a shift in the parietal representation of visual space before the eye movement is executed. This, it was suggested, could underlie our ability to maintain an up-to-date visual representation of the world, despite almost constant movements of the eyes.

Single-unit recording was also used by Kusunoki and Goldberg (2003) in order to investigate the time course of this receptive field shift in LIP neurons. Results indicated a drop in the visual responsiveness in the current receptive field of these neurons at the same time as perisaccadic activity (i.e. for the future receptive field) increases.

While Duhamel et al. had claimed that predictive remapping only occurred when the monkey actually intended to make a saccade to a particular location, and was not seen to accompany covert shifts of attention, Colby et al., (1996) disagreed with this. They showed activity from the same neurons at the time of stimulus presentation regardless of whether saccades were allowed or not, thus suggesting a sensory, as opposed to presaccadic, nature. Presaccadic activity was however seen in memory-guided saccade tasks, in which case activity rose at the start of the trial during fixation, whilst the monkey anticipated the onset of a behaviourally relevant stimulus. Visual activity was enhanced in response to behaviourally relevant stimuli, i.e. when a saccade was to be made to the location of the stimulus, or the monkey covertly attended the stimulus without looking at it, in comparison to the responses during a straightforward fixation task. The authors concluded that LIP neurons respond under a number of different conditions that are not solely sensory or motor in nature and that parietal activity may also be influenced by cognitive factors such as attention and anticipation.

Mazzoni et al., (1996) similarly concluded that the function of LIP neurons is not entirely sensory or attentional in nature. A delayed double-saccade task was used, and the majority of neuronal activity seen in this area during the delay before the first saccade was shown to code the location of the first target, as opposed to the location of the most recently displayed one, i.e. target 2, thus supporting the idea that these neurons are also involved in saccade planning.

1.4.1.2. Reversible Inactivation

Reversible inactivation can be useful in terms of assessing the importance of a particular cortical area to performance on a task. Its usefulness is however limited to some extent in terms of distinguishing whether a region is itself vital to the performance of that task or instead serves as a connection to other task-essential brain areas. Discrete cortical structures of the brain can be reversibly inactivated by the injection of a local anaesthetic. This blocks action potentials in the axons passing in and out of that region, giving the effect of a temporary lesion. Alternatively, cooling brain tissue in a specific region produces similar results. This involves surgically implanting a cryode, a device through which chilled liquid can be circulated within stainless steel tubes, whilst the monkey is conscious and alert.

A third method used to bring about a temporary lesion is through the injection of muscimol. Muscimol is a Gamma-Amino Butyric Acid (GABA) agonist; through its function as a receptor for GABA, the major inhibitory neurotransmitter in the brain, it is able to bring about effective inactivation of selective parts of the brain. This approach was used by Li et al., (1999) in macaques, to investigate the function of area LIP. Following the injections a hypometria was noted for memory-guided saccades directed into the upper half of contralesional space, whereas ipsilesional saccades, in contrast, showed a slight but significant hypermetria. An increased scatter in terms of saccade end-points was also noted for these types of eye movements, whereas the metrics of visually-guided saccades appeared to be relatively unaffected. An increased latency was however seen for both visual and memory-guided saccades directed into the contralesional visual hemifield. From the results it was concluded that this area is important for coding target locations for movement planning and perhaps also in the process of target selection, i.e. deciding where to look prior to saccade onset, hence inactivation of this area affected both saccade latency and metrics.

A later study by Li & Andersen (2001) similarly made use of muscimol injections to selectively inactivate area LIP and observe monkeys' performance on a double-step saccade task. They were interested specifically in the type of extraretinal signals utilised in this task and attempted to determine whether the

direction, or the hemifield of the end-point of the first saccade were most important in terms affecting performance of the second saccade. They reasoned that if eye displacement is the more important factor, then greater impairment of the second saccade would be seen following a first saccade directed contralesionally, regardless of the exact end-point. Alternatively if eye position signals are dominant, then a first saccade ending in the contralesional visual field will lead to a disruption of the second saccade, whatever its direction. The results supported the second of these two mechanisms and the authors therefore concluded that eye position rather than eye displacement is the dominant extraretinal cue used for spatial computations in the PPC. They suggested a mechanism whereby information on target location and current eye position are combined to form a head-centred representation of space. Following the first saccade, information on the updated eye position is subtracted from this craniotopic representation and the second saccade can therefore be computed. Although LIP inactivation was also seen to result in an impairment in terms of latency and amplitude of single visual and memory-guided saccades, the extent of this impairment was not influenced by varying the initial eye position. This suggested that eye position signals are only made use of when retinotopic coding of the target alone is insufficient, i.e. for double but not single saccades. Based on these findings the authors put forward the idea that area LIP contains multiple representations of visual space and that the particular reference frame utilised can vary in a task-dependent manner.

A different role for LIP was suggested by Wardak et al., (2002) however who similarly used muscimol injection to reversibly inactivate neurons in this region. This study failed to show any effects on either latency or accuracy of single saccades to either visual or remembered target locations. When bilateral targets were presented, a decrease in the frequency of contralateral eye saccades was seen, as was an increased search time for contralateral targets in a visual search task. On the basis of these results, a role for LIP in representing and selecting between salient targets as goals for upcoming saccades was suggested.

Two possible explanations for the difference in results in Wardak et al.'s and Li et al.'s (1999) study were put forward by the authors; it was suggested,

that either the level of inactivation might be responsible, with saccade programming and target selection sharing the same neural substrate, but with saccadic deficits being produced only by higher levels of inactivation, whereas a moderate level would be enough to impair target selection. The alternative explanation was that these two functions are actually served by distinct subsystems within LIP making it possible for them to be separately disrupted.

1.4.2. Neuropsychological Studies

By studying the behaviour of patients with lesions in particular regions of the brain insight can be gained into the cortical areas that are fundamental to a specific task. One problem with neuropsychological studies however is that lesions resulting from trauma or disease can be quite diffuse, affecting a number of areas of the brain; this can make it hard to draw conclusions about the precise area responsible for any observed deficits. Further to this, in the case of chronic lesions it is not possible to ascertain the extent of cortical reorganisation that may have occurred to compensate for these effects. Such studies are however useful in that they offer the chance to evaluate to some extent the functional importance of the cortical areas involved, and the results from single patients can be compared both to other case-studies in which similar areas have been affected, as well as lesion studies in non-human primates such as those discussed above.

Oculomotor control in a patient with a right fronto-parietal lesion was assessed by Duhamel et al., (1992b). They found that although the patient was able to accurately make saccades to a rightward followed by a leftward target, performance of the reverse sequence of movements was disrupted; the first contralesional saccade was carried out appropriately but the second one was never properly performed. This deficit could not be accounted for in terms of an encoding failure in a particular frame of reference or the inability to make saccades in a particular direction. It was instead attributed to a problem with corollary discharge. It was suggested that, as a result of the lesion, the patient was unable to make use of information on the direction and amplitude of the first contralesional saccade. This information would be vital in order to update the spatial representation of the location of the second target.

A later study by Heide et al., (1995) also used a double-step saccade task to investigate the performance of patients with either unilateral frontal or posterior parietal lesions. Whilst those with lesions to frontal areas such as the FEF, prefrontal cortex (PFC), and the supplementary motor area (SMA), showed deficits in the temporal order and the triggering of saccadic sequences, those with parietal lesions showed impairments that were more spatial in nature. In particular, saccadic dysmetria (over or under-shooting the target) or failure of the second saccades was seen, although only when the first target was presented in the contralesional hemifield; this was, in agreement with Duhamel et al., (1992b) similarly attributed to an inability to compensate for the displacement of the eye by making use of corollary discharge. Delayed latencies and hypometria (under-shooting the target) was also observed for contralesional first saccades in the patients with PPC damage. This was suggested as a confirmation of its role in the control of visually triggered saccades. The authors suggest that the results of this study indicate that the impairments shown by the patient in the study by Duhamel et al., (1992b) must be attributed to the parietal rather than frontal damage, as these effects were not observed in the patients with lesions restricted to the FEF. They propose a functional specialization, in which neurons in the FEF code saccadic target location in an eye-centred reference frame and the PPC is responsible for remapping retinal coordinates prior to an eye movement.

From an analysis of saccade studies involving patients with various cortical lesions, Pierrot-Deseilligny et al., (2002) conclude that three main areas are involved in saccade triggering. The exact function of each of these areas is thought to differ depending on the type of saccade that will be made; reflexive saccades, for example were thought to be controlled by the PEF, concurrent with Heide et al.'s (1995) claim that the PPC is responsible for visually triggered saccades. The FEF, on the other hand was thought to be more involved in intentional saccades, and the SEF in motor programs, either including a sequence of several saccades or a combination of eye and limb movements. Pierrot-Deseilligny et al., point out however that although the PEF and FEF may be specialised in such a way they are also likely to work together to some extent, as shown by the fact that only bilateral lesions of both

the PEF and FEF result in chronic disturbances in the triggering of saccades (Pierrot-Deseilligny et al., 1988).

1.4.3. Behavioural Studies: Eye Tracking

All of the experiments discussed in this thesis have made use of eye tracking as a method of behaviourally evaluating performance on a task. For this reason an overview of eye tracking in terms of its use as an experimental technique has been provided below.

Eye movements are made in order to bring a specific area of the visual field into central or foveal vision so that it can be viewed in high resolution. Although it is possible to consciously direct attention to targets in peripheral vision, as in a covert shift of attention, in order for an object to be seen in fine detail, an eye movement (i.e. an overt shift of attention) must be made. The point at which a scene is foveated is thought to give some indication of where visual attention is being directed, and may therefore provide information on regions of interest to the viewer. Studying eye movements may also give some insight into the neurological mechanisms responsible for controlling how visual attention is directed.

Light is reflected from each surface of the eye that has a change in refractive index; therefore reflections can similarly be seen from the back surface of the cornea, and the front and rear surfaces of the lens. These reflections, along with that from the front surface of the cornea, are known as the Purkinje images. The fourth Purkinje image, which comes from the back surface of the lens, is the second brightest, and thus the relative displacement between the first (front corneal) and fourth images can be measured to give an indication of the orientation of the eye in space, which is independent of head position (Young, 1976). Two points of reflection are needed in order to separate eye movements from movements of the head, this is because the difference between these two reflections changes with rotations in eye position, but remain stable with small head movements (Duchowski, 2003). Dual-Purkinje image (DPI) eye trackers, such as the ones used in the experiments in this thesis, are able to separate translational and rotational movements of the eye, by measuring the first and fourth Purkinje images; both these images

move through the same distance with eye translations, but different distances when the eye rotates. DPI eye trackers use an infra-red light source set at a fixed position relative to the eye, and can provide a fairly precise method of eye tracking, although in order to achieve this accuracy it may be necessary to stabilise the position of the head. One of the benefits of an infra-red eye-tracking device is that this light source is invisible to the eye and therefore is not distracting to the participant (Duchowski, 2003).

In the analysis of eye movement data one of the first stages is to discriminate between fixations and saccades. Fixations occur when the eye is basically stationary, whereas saccades are defined by rapid reorienting movements (Jacob and Karn, 2003). A number of methods can be used in order to make this discrimination. One of these is to use the velocity of the eye in order to compute its change in position over time (Jacob and Karn, 2003). As Salvucci and Goldberg (2000) point out, there is no standard technique for differentiating fixations and saccades; a variation in the methods used can however lead to differences in the analysis of the eye movement recordings.

The signal-to-noise ratio of a system is important in order for the recordings to be precise; artefacts that may occur, for example through blinking can often be eliminated (Duchowski, 2003).

Behavioural studies are useful since they can be used to give a clear indication of behaviour in the normal healthy brain and can also be used in combination with other methods, for example TMS or fMRI and in neuropsychological studies of patients.

1.4.4. Transcranial Magnetic Stimulation

A number of the eye tracking tasks discussed in this thesis were carried out in combination with transcranial magnetic stimulation to posterior parietal cortex in an attempt to evaluate the importance of this area to task performance. An overview of TMS as an experimental procedure has therefore been provided below.

1.4.4.1. What is TMS?

Transcranial magnetic stimulation involves the application of a short magnetic pulse to the scalp in order to stimulate a particular region of the cortex. A TMS machine consists of stimulating coil and a main unit, which is made up of a charging system, one or more energy storage capacitors, a discharge switch and circuits for pulse shaping, energy recovery and control functions (Pascual-Leone et al., 1999). The capacitors generate a pulse, causing a current in the coil and in turn a powerful, rapidly-changing magnetic field below the coil. This reaches a strength of almost 1.5 Tesla, tens of thousands of times the strength of the earth's magnetic field, but with a duration of less than a millisecond (George, 2003). The magnetic field passes into the cortex without attenuation from the skin or scalp (Walsh & Rushworth, 1999), inducing the flow of small electrical currents in the resting nerve cells it encounters. The brain is basically an electrical organ that transmits electrical signals from one cell to the next and TMS works by exploiting this (George, 2003). The electrical field induced in the brain tissue is proportional to the rate of change of the magnetic field with respect to time, and the speed of the magnetic field rise time, i.e. the time taken for the magnetic field to develop (Barker, 1999). The rate of change and the rise time are both critical to the effectiveness of the magnetic stimulation (Pascual-Leone et al., 1999).

Inhibitory effects of TMS result from a disruption of normal cortical processing, causing delayed or poorer performance on the task in hand. The current can thus be thought of as a kind of 'neural noise', or the addition of random activity to a particular cortical region. This disruption is however temporary in nature, and does not lead to any lasting damage; the effect of TMS has therefore been described as a 'virtual lesion' (Walsh & Rushworth, 1999).

Facilitatory effects of TMS include for example the induction of phosphenes following stimulation to the occipital cortex or the parietal area V5 (Stewart et al., 2001) and muscle contractions through stimulation of primary motor cortex (Walsh & Rushworth, 1999). A further facilitatory effect is the 'paradoxical' improvement in performance that can occur on some tasks (e.g.

Walsh & Rushworth, 1999). This might be due to the existence of competing mutually inhibitory systems in the brain, so that when one is ‘knocked out’ as a result of TMS the other can perform at an increased level. An example of this is that stimulation to area V5, which controls motion processing, decreases performance on a search task that requires this form of processing, whilst improving performance on a search task based on form and colour processing (Walsh et al., 1988).

Single-pulse TMS is useful for delivering stimulation at a precise point in time, whereas the delivery of multiple pulses has lower temporal resolution but can be useful for localising brain regions (Walsh & Rushworth, 1999). Single pulse TMS has a duration of ~1 millisecond, and is generally limited to a rate of 0.3-0.5Hz. Repetitive TMS (rTMS), on the other hand consists of a high frequency train of pulses at a rate of up to 60Hz, and can last for thousands of milliseconds (Pascual-Leone et al., 1999). Single pulse TMS has been used to induce errors on sensory detection tasks, but has not been as effective on more cognitive tasks, for which rTMS tends to be more successful. In the experiments discussed in this thesis it was decided that double-pulse TMS would be used, since it was hoped that this would be more likely to be effective at disrupting task performance than single pulse stimulation.

1.4.4.2. Advantages and Disadvantages of TMS as a Methodology

Although TMS has only intermediate temporal and spatial resolution (Stewart et al., 2001), the ‘virtual lesions’ brought about by it are reversible in nature, and thus the effects of disruption can be investigated without any chance of cortical reorganisation, which might occur following brain damage. For these reasons Walsh and Rushworth (1999) argue that TMS can be said to have good functional resolution. Diaschisis, the change in activity and function at sites anatomically connected to a lesion, is something that must be taken into account in TMS as in classical lesion studies and has been put forward as a possible explanation for different effects that are sometimes observed between real and virtual lesions of the same cortical area.

One advantage of TMS over imaging techniques such as PET, fMRI and EEG is that by disrupting activity in a particular cortical region (as opposed to

measuring it), it can be used to assess the necessity of that area to a given task (Walsh & Rushworth, 1999). There is however a certain amount of inter-individual variability in brain-scalp relationships, and thus the positioning of the coil on the scalp on the basis of bony landmarks can lead to variations in the area of the brain targeted by TMS (Meyer et al., 1991). For this reason in the TMS experiments discussed in this thesis, attempts were made to functionally localise suitable stimulation sites. This is discussed in more detail in the relevant experimental chapters.

1.4.4.3. Safety

While the safety of single-pulse TMS has been well established, the safety of rTMS is not as well documented. Repetitive TMS has in fact been known to cause epileptic seizures in those with a personal or family history of the disorder, and for this reason it is recommended that these individuals are excluded from such studies. Seizures may also rarely occur in participants without a history of epilepsy. A number of safety guidelines should therefore be taken into consideration when using rTMS; these are discussed in a paper by Pascual-Leone et al., (1993). Walsh and Rushworth (1999) also recommend a TMS website (<http://pni.unibe.ch>), which provides up to date safety information. Since little is known concerning the potential long-term effects of rTMS, it is advisable that participants should not take part in repeated experiments over a short period of time.

In some individuals TMS can also have less serious effects such as headaches and nausea, and it is also possible that some may find facial twitches too uncomfortable to continue; participants should thus be made aware of their right to withdraw from the study at any point.

1.4.4.4. Studies Using TMS to Disrupt Eye Movements

The TMS studies conducted in this thesis attempted to disrupt the planning and execution of eye movements. In this section therefore I will discuss some of the previous TMS studies with similar experimental aims. Oyachi and Ohtsuka (1995), for example, used single pulse TMS to the PPC

during a task involving single memory-guided saccades. Right hemisphere TMS delivered 100ms after the disappearance of the central fixation caused a decrease in the accuracy to both rightward and leftward targets, whereas left hemisphere TMS had no effect on saccadic error. The authors interpreted these findings in terms of a role for the human right PPC in maintaining the spatial accuracy of remembered target locations. The differences between right and left hemisphere stimulation were explained in terms of a greater specialisation for visuospatial functions within the right hemisphere. TMS was thought to decrease saccadic accuracy by briefly activating parietal neurons and changing the motor planning signal, without actually eliminating it entirely.

A significant delay in saccade onset following stimulation to both hemispheres of the PPC was found in a study by Terao et al., (1998) using an antisaccade task. This effect was dependent on the time of stimulation, occurring earlier for TMS to the PPC than to frontal areas. This was thus thought to reflect the flow of information from posterior to anterior cortical regions prior to saccade onset.

A later study by Müri et al., (2000) also found increased saccadic latency following TMS to the left PPC delivered 100ms after the central fixation offset in a memory-guided saccade task equivalent to that used by Oyachi and Ohtsuka (1995). Müri et al., (1996b) had previously found TMS to the right PPC, delivered 260ms after target presentation, resulted in a greater amplitude error for contralateral saccades, and an increased latency for stimulation to left and right hemisphere, when delivered 100ms after the go-signal. From these two studies it was concluded that the contribution of both hemispheres to the preparation of memory-guided saccade amplitude, in the early part of the sensorimotor integration process, may differ and that the triggering of memory-guided saccades is controlled bilaterally by the PPC (Müri et al., 2000).

van Donkelaar and Müri (2002) similarly found that the time at which TMS was delivered to the PPC in a double-step saccade task can alter the effects on task performance. TMS delivered just before the onset of the second saccade disrupted craniotopic coding of target locations. This disruption only occurred for saccade sequences initially made contralateral to stimulation site, followed by an ipsilateral eye movement, and was not seen when TMS was applied at the onset of the first saccade, or even 100ms into it. In order to code

in craniotopic coordinates both retinal and extraretinal information must be combined, and the authors suggest that it is in fact the extraretinal signals that were interrupted by the TMS in this study, as it appeared that the retinal information was being used by the participants.

TMS during a double-step saccade task was also used by Tobler & Müri (2001), but instead of looking at the role of PPC they assessed the function played by the right FEF and the SEF in this task. TMS was delivered prior to the execution of the first saccade in this study, and for FEF stimulation, greater amplitude errors in the contralateral second saccade were seen. This was attributed to a disruption in retinotopic rather than craniotopic coding and led to the suggestion that the FEF may be important for remembering target locations. SEF stimulation, conversely, increased the number of order errors, i.e. executing the sequence of double-step saccades to the remembered target locations in the wrong order.

1.4.5. Functional Magnetic Resonance Imaging

In addition to TMS, another experimental technique employed in two of the experiments discussed in this thesis is functional magnetic resonance imaging (fMRI). A brief overview of the basic principles underlying this technique and a discussion of some of the issues related to its use are therefore provided in the following section. A more in-depth discussion of the principles involved can be found from Jezzard et al., (2001) and an fMRI guide written by de Haan and Rorden (available online:

http://www.sph.sc.edu/comd/rorden/fmri_guide/index.html).

1.4.5.1. What is fMRI?

Functional MRI provides a method of observing metabolic activity in vivo in the healthy human brain. It works by measuring the response of hydrogen molecules (in water in the brain) to a perturbation, brought about by the application of a brief radiofrequency (RF) pulse whilst in a magnetic field. As these nuclei return from the perturbed to their original orientation, in alignment with the magnetic field (relaxation), they emit energy, and it is this

energy that can be measured as a radio signal. This signal is then transformed in order to obtain a three dimensional image of the brain.

fMRI makes use of the fact that local blood flow increases in active areas of the brain. Haemoglobin, which carries oxygen in the bloodstream, has magnetic properties that cause inhomogeneities in the surrounding magnetic field. Its paramagnetism is high when deoxygenated, and very low when oxygenated, and it is therefore possible to measure the ratio of oxygenated to deoxygenated haemoglobin; this is known as the blood oxygenation level dependent (BOLD) effect (Detre & Wang, 2002; Heeger & Rees, 2002; Ogawa et al., 1990; 1992).

Activity within a brain area leads to an increased flow of oxygen-rich blood to that area. The supply of oxygen outweighs the demands of the neural tissue, which is thus unable to completely absorb it. The ratio of oxygenated to deoxygenated blood is thus seen to increase, although this is only evident after a delay of a couple of seconds (during which a small dip occurs), and peaks at around six seconds, returning to baseline at around 24 seconds. The function of the fMRI BOLD signal over time in response to a temporary increase in neural activity is known as the haemodynamic response function (HRF) (Heeger & Rees, 2002).

Time-locking of the BOLD effect to specific events can provide insight into the time course of the observed neural activity; this is known as event-related fMRI (Aguirre & D'Esposito, 2000; Donaldson & Buckner, 2001). Although metabolic changes in response to a single event would be hard to detect against a background of fluctuations in the haemodynamic response, averaging over multiple incidences of the same event does allow a clearer signal to be obtained. Event-related fMRI has advantages over functional imaging experiments with block designs since it is possible for experimental trials to be randomised. This increases the likelihood that participants' attentional state will be similar in all cases and thus that any differences seen are genuinely related to variations in processing demands as opposed to a reflection of a more general change in arousal level. The fMRI experiments discussed in this thesis have therefore made use of event-related paradigms.

1.4.5.2. Advantages and Disadvantages of fMRI as a Methodology

One of the major advantages of fMRI over other methodologies in cognitive neuroscience is its excellent spatial resolution (2-5mm), which thus allows a detailed evaluation of the areas that appear to be active during a particular task of interest. In terms of temporal resolution, however, fMRI compares rather poorly (5-8 seconds), this is due to the inherent slowness of the haemodynamic response in comparison with the underlying cortical activity (Horwitz et al, 2000; Menon, 2001). Another problem associated with imaging techniques lies in the fact that a correlation observed between activity in an area and a particular task, does not necessarily imply causation. An area could show task-related activity without being vital to task performance. Further to this, the chance of false negatives (due to the small size of the signal changes) and false positives (due to the extremely large number of voxels being considered) are both high. The chance of these can be controlled through the use of a significance level for activation that reflects the aims of the investigation, i.e. lower when attempting to identify all areas involved in a particular task, but more stringent when determining only the areas showing the greatest amount of task-related changes in activity.

1.4.5.3. Studies Investigating Saccade-related Activity through fMRI

Attempts have been made in previous studies to investigate saccade-related activity through the use of fMRI. I will therefore now provide a brief review of some of the most relevant of these studies and their findings in relation to the aims of the experiments presented in this thesis.

Cortical activity on an eye-movement task requiring remapping of visual signals was assessed by Merriam et al., (2003). Participants fixated a cross on the right of the screen while a central stimulus was presented. They then made a leftward saccade towards a cross on the left side of the screen. The location of the previously presented stimulus was thus brought into the right visual field. Results showed parietal activity first in the hemisphere contralateral to the stimulus and subsequently in the ipsilateral hemisphere, presumably demonstrating the remapping of its retinal location. This study differed from

localisation tasks that use fMRI to look for areas of activity related to particular tasks, in that it investigated whether the activity of voxels in the IPS corresponded to responses of single neurons within the parietal lobe. It was thought that the region of interest identified in this study was likely to contain the human homologue of area LIP, along with other parietal regions.

In an earlier fMRI study, Sereno et al., (2001) had identified more specifically a region they believed might correspond to the macaque area LIP. A delayed saccade task was used to record activity as participants made saccades to remembered target locations; an area in the superior parietal lobe was found to show activity that corresponded to a systematic map of remembered contralateral target locations in retinotopic coordinates, i.e. a map of visual information spread across the surface of the brain.

Areas believed to correspond to the monkey area LIP and a parietal reach region (PRR), were also identified by Medendorp et al., (2003) using event-related fMRI and both memory-guided arm and eye-movements. The results suggested that representations for targets in both of these types of movements appear to be coded in a gaze-centred frame-of-reference, and also that the PPC is responsible for the spatial updating of these representations that occurs across eye movements. It is suggested that if the PPC were responsible for selecting targets for action (Snyder et al., 1997) then it would be more effective to code eye and limb movements in a common coordinate system.

An extension of the double-step saccade task mentioned previously was used in an imaging study by Heide et al., (2001); this task was a triple-step paradigm designed to elicit higher levels of activation. Using fMRI, strong activation during the triple-step saccades was found in the middle and posterior portion of the right intraparietal sulcus, which the authors suggest probably corresponds to the parietal eye field. This area, along with the FEF and SEF, was found to be significantly more active compared to activity during other saccade tasks. The right IPS activity was suggested to be related to the updating of spatial representations based on corollary discharge from the previous eye movement, whereas the SEF, it was proposed was more important in terms of triggering memory-guided saccades.

1.5. Areas involved in saccade control

In order to investigate more specifically the role of the PPC in the planning of saccades it is useful to consider also the contributions made by other cortical areas to this behaviour (see Leigh and Kennard, 2004, for a more detailed review). A short discussion of these areas is therefore given below, followed by an overview of some important subdivisions of the PPC itself. This is of interest since the anatomical divisions also display functional differences in terms of their importance to saccade control.

According to Leigh and Kennard (2004), the superior colliculus (SC) seems to be important for the release of fixation, which is necessary for making saccadic gaze shifts, whereas the PEF, which projects to the SC, plays a role in the initiation of reflexive visually-guided saccades. The frontal eye fields, on the other hand, are more involved in voluntary saccades, whilst memory-guided saccades require the involvement of a network of four main cortical areas including the DLPFC, FEF, PEF and SEF. The SEF also seem to have a role in internally guided target selection and self-control when switching motor responses. Figure 1, below, shows the locations of a number of cortical areas that participate in the generation of saccades (taken from Leigh & Kennard, 2004).

Colby and Goldberg (1999) have argued that one of the main differences between area LIP (PEF) and the more obviously oculomotor FEF and SC can be seen in the response of their neurons to the appearance of a visual stimulus within their receptive fields during a fixation task. In all three areas this response is increased if the stimulus is behaviourally relevant, but unlike the FEF and SC, this enhanced response in the LIP is independent of the action planned towards the stimulus, for example a reach or a saccade, and even whether an action is intended or not.

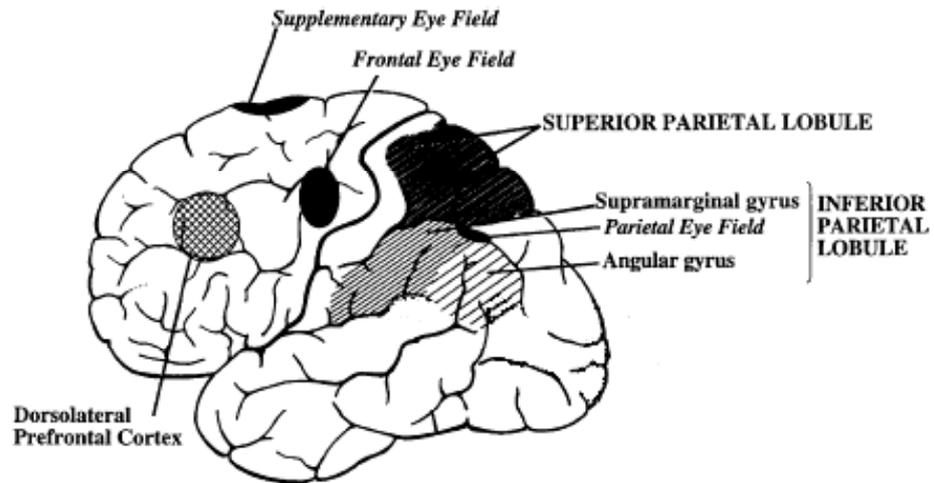


Figure 1: Cortical areas that participate in saccade generation (Taken from Leigh & Kennard, 2004).

1.5.1. Subdivisions of the PPC

1.5.1.1. Superior Parietal Lobe (SPL)

Consisting of Brodmann areas (BA) 5 and 7 in humans and areas 5a and 5b in monkeys, the SPL appears to have a mainly somatosensory role (Rizzolatti et al., 1997), but has also been shown to receive visual information in its posterior and mesial parts. The anterior bank of the parieto-occipital sulcus within the SPL consists of a number of visual areas, including areas V6/PO and V6A. These two areas show distinct properties, V6/PO is a purely visual area and forms a fairly direct route between the occipital and parietal lobes, with information leaving this area being sent to regions of the intraparietal sulcus (IPS), such as areas VIP (ventral intraparietal area) and LIP (lateral intraparietal area). Area V6A is distinguishable from V6/PO in that it also contains non-visual neurons, which appear to be involved in the control of the hand during reaches, with or without visual feedback (Fattori et al., 2001). Lesions of the SPL have been shown to be related to optic ataxia (De Renzi, 1982), in which patients show deficits for reaching movements performed under peripheral visual guidance; the SPL thus appears to be important for the control of the arm during the transport phase of reaches, and in particular when

the arm itself is not being foveated (Rizzolatti et al., 1997). It has also been suggested that deficits resulting from damage to the SPL may reflect an impaired ability to maintain internal representations of the body's state, in relation to the fact that the SPL is believed to be critical for the process of sensorimotor integration (Wolpert et al., 1998).

1.5.1.2. Inferior Parietal Lobe (IPL)

The IPL, containing BA 39 and 40 in humans, and areas 7a and 7b in monkeys, is more visual in function than the SPL. It can be separated into an anterior part, known as the supramarginal gyrus (SMG) and a posterior part, the angular gyrus (AnG). Damage to the IPL, in distinction from the SPL has been shown to cause the spatial disorder of neglect (De Renzi, 1982). In light of clinical evidence such as this, it has been proposed by Rizzolatti et al., (1997) that the IPL may be the anatomical substrate at the basis of space perception.

1.5.1.3. Intraparietal Sulcus (IPS)

The intraparietal sulcus provides an anatomical division of the PPC, with the SPL located above it, and the IPL below. Within the IPS a number of subdivisions can be distinguished, including areas AIP (anterior intraparietal), VIP and LIP (Rizzolatti et al., 1997). AIP seems to be involved in grasping movements, and the coding of 3D object features prior to gripping them, whereas VIP and LIP both appear to be involved in coding target location. The main distinction between VIP and LIP, however, is that whereas the somatomotor area VIP does not encode in retinotopic coordinates, the oculomotor area LIP does (Rizzolatti et al., 1997) and has thus been labelled the 'parietal eye field' or PEF (Andersen et al., 1992; 1997). Another region, also found on the banks of the IPS, has been shown in contrast to be more greatly associated with reach-related activity and is thus referred to as the 'parietal reach region' (PRR), (Batista & Andersen, 2001). The TMS and fMRI experiments discussed in this thesis have primarily aimed to further

assess the role of the human parietal eye fields, i.e. the purported homologue of area LIP, in relation to saccade planning.

1.6. Functional Significance of Neuronal Activity in Area LIP

Some of the most convincing evidence for posterior parietal involvement in saccade planning has come from neurophysiological studies in monkeys recording from neurons in this area. These have in particular demonstrated presaccadic changes in neural activity thought to be indicative of spatial remapping. The functional significance of the activity of neurons within LIP is however still a matter of debate. The alternative positions within this debate are outlined briefly below since it may be useful to consider them when attempting to investigate the manner in which a program for an impending saccade is made and stored, and how parietal areas are involved in carrying out these functions.

1.6.1. Visuospatial Attention

Neuronal activity within in LIP has been proposed to reflect encoding of the spatial location of the object of visual attention in terms of distance and direction from the centre of gaze (Colby & Duhamel, 1996). In other words, information on the vector of the saccadic eye movement necessary to acquire the visual stimulus. This idea was based on findings from a study by Duhamel et al., (1992), who showed that when a monkey intends to make a saccade, neurons in area LIP become responsive to visual stimuli in the region of the saccade goal. The fact that this shift in the receptive field of these neurons is anticipatory, i.e. it occurs in advance of a movement, led the authors to suggest that these neurons predict the ‘sensory consequences’ of an intended saccade. This predictive response was thus argued to be visual in nature, and not related to motor planning.

Previous work by Bushnell et al., (1981), also supported this idea; they concluded that the behavioural enhancement of visual responses of neurons within monkey area 7 was not dependent on the specific movement planned towards a visual stimulus. Further support for this theory has come from a

study by Bisley & Goldberg (2003) who claimed that while activity in LIP for a single location within the visual field did reflect increased attention at this location, it was not predictive of a monkey's intention to make an eye movement in that direction.

1.6.2. Motor Intention

Work by Snyder et al., (1997), similar to that described above, has led to the contrasting conclusion that motor intention is in fact reflected in the pre-movement activity of LIP neurons. They argue that the anticipatory nature of this process does not necessarily indicate *sensory* remapping as Colby and Duhamel had suggested. They propose that predictive behaviour is as likely to occur in motor planning as it is in sensory pathways. This agrees with earlier work by suggesting an anatomical specialization for movement planning within the PPC (Mountcastle et al., 1975; Andersen et al., 1987). This has led to the later proposal by Andersen and Buneo (2002) that intention is in fact an early plan for movement, coded in visual coordinates within the PPC, and that activity here reflects the goal of a movement as opposed to the exact muscle activation required to reach that goal. In the absence of any specific intention to make a movement, they argued that 'default plans' are formed to stimuli of interest.

The activity of a population of neurons in the posterior parietal cortex was assessed by Quiroga et al., (2006) who attempted to predict target location based on both the locus of attention and movement plans on a trial-by-trial basis. As noted by List & Landau (2006) this study is important in terms of the attention vs. intention debate, since if cells in LIP and PRR code only attention to a location, then the type of movement made, i.e. a saccade or a reach, should have no effect on neural activity. Predictions of target locations as markers of attention were significantly worse than predictions based on either saccades or reaches for the same target locations. This was argued by the authors to provide conclusive evidence for the role of the PPC in movement planning. However, as List and Landau point out, predictions based on attentional signals were also above chance, suggesting that PPC activity may encode both location- and action-predictive information.

1.7. Overview of Thesis

Despite ever-increasing knowledge regarding the role of the PPC in relation to saccadic behaviour, debates still remain regarding in particular the functional significance of activity within this region. The experiments presented here in this thesis will attempt to investigate further the role of the parietal lobes in relation to saccade planning. More specifically they will focus on the process of spatial remapping essential to the planning and execution of certain saccadic movements. These begin through the use of TMS on a version of the classic double-step saccade paradigm, in which remapping is required to account for displacement of the eye (Chapter 2). In Chapter 3, a second TMS study is presented in which the updating of saccade plans in response to a change in target location, rather than an eye position, is investigated.

A series of variations on the double-step saccade task, in which the order of target presentation is manipulated with the aim of assessing the effect of this on processing complexity and the task-dependent nature of spatial remapping, are next discussed (Chapter 4).

Finally the findings from neuroimaging studies investigating the cortical areas involved in eye movement planning and spatial remapping are presented. The first of these is an extension of the behavioural studies previously conducted (Chapter 5). The second makes use of a novel saccade paradigm to investigate the effect of intervening saccades made between the time of target encoding and execution (Chapter 6). The findings from these studies will be discussed in relation to unresolved issues within this area of research.

Chapter 2: The effect of parietal TMS on spatial updating of a visual target representation in response to an eye movement

2.1. Introduction

The experiments described in this chapter aimed to investigate the role of the parietal eye fields (PEF) in terms of the spatial remapping associated with saccades. Based on previous research in this area it was decided that TMS would be an effective technique to use for such an investigation (e.g. Müri et al., 1996b; 2000; Oyachi and Ohtsuka, 1995; van Donkelaar and Müri, 2002). A double-step saccade task was chosen since spatial remapping is required for accurate performance. By using TMS to induce a temporary disruption to the neural activity in the PEF, it was hoped that an indication of its importance to this type of behaviour could be gained.

In designing a TMS study it is important to consider both where and when the TMS should be delivered. Previous literature suggests that the PEF may be responsible for spatial updating on a double-step saccade task (Duhamel et al., 1992b; Heide et al., 1995; Li & Andersen, 2001), but in order to stimulate this area a method of defining this site in terms of the corresponding scalp location has to be employed. A TMS localiser task was chosen (c.f. Ashbridge, Walsh & Cowey, 1997) since this is a systematic way of functionally assessing the effects of TMS at sites on the scalp approximately above the anatomical region of interest and should thus provide an effective guide for where best to place the TMS coil. Once a suitable stimulation site has been defined for each participant, this site can then be used in the double-step task. If it was to disrupt spatial remapping, a decrease in compensation for error in the first saccade would be expected in the metrics of the second saccade when TMS was delivered here compared to stimulation at a control site.

Previous studies involving visual and memory-guided saccades have shown that the time of TMS delivery can lead to significant differences in its effect on task performance (e.g. Müri et al., 1996b; 2000; van Donkelaar and Müri, 2002). In order to decide the optimum time at which the TMS should be applied during the double-step saccade task, it was decided that a sample of

latencies for single saccadic eye movements should first be collected. It was thought, based on previous studies, that the remapping in this task would occur just before the start of the second saccade and therefore it would be most effective to deliver the TMS at this point. van Donkelaar and Müri (2002) for example, found a disruption in performance on a double-step task when TMS was delivered 150ms after the onset of the first saccade. Stimulation at the onset of the first saccade, or 100ms following it was not however found to be effective.

Accuracy has been shown to decrease with each successive saccade in a memory-guided sequence (Bock et al., 1995). This seems to be a result of error propagation, where errors made on one saccade are only partially corrected for (through remapping) in the following saccade. The extent of this correction, i.e. how much an error in the end-point of the first saccade is compensated for in the metrics of the second saccade, can therefore be useful as a measure of spatial remapping. If, as proposed, TMS to the PEF is able to disrupt this process, this should be evidenced by a decreased amount of compensation. In other words the metrics of the second saccade would be expected to more closely reflect that required if there was no error at all in the first saccade, than those required if the error was taken into account. This measure of compensation has previously been used by van Donkelaar and Müri (2002). Their study however only considered the process for a sequence of two horizontal eye movements, whereas the current study uses a task in which saccade direction is considerably more varied. Since the eye movements we make everyday are not restricted to the horizontal plane, it is expected that spatial remapping must account for error in terms of saccade direction as well as amplitude. How a saccade vector is coded in terms of motor coordinates is not yet known, nor how end-point error for a saccade is calculated, although this presumably requires a comparison of actual and predicted end-points. By considering both the extent to which compensation for error in the amplitude *and* angular direction of the first saccade can be affected by TMS, it may be possible to gain further insight into these processes.

2.2. Experiment 1: Determining the Latency of Single Saccadic Eye Movements

Methods

Participants

Five participants (2 female) aged 22-25 years (mean 23.2 years) took part in this study. All had normal vision.

Materials

A pupil and dual first Purkinje image Video Eyetracker (Cambridge Research Systems) with a sampling frequency of 50Hz and an accuracy of 0.5-0.25 degrees of visual angle was used. Calibration was performed using a built-in procedure in which 20 small white dots (0.25 deg arc) appeared on the screen one at a time at positions around a 5x4 grid scaled to 90% of the display size. The dots remained on for 500ms each and the participant's accuracy in foveating these was assessed, this procedure was repeated as necessary until all dots had been accurately fixated. During the experimental session a video image of the eye could be seen by the experimenter on a separate computer screen, this made it possible to monitor the participants' position in the eye-tracker throughout the progress of the experiment. Participants viewed the stimuli binocularly, although only the left eye was tracked. An EyeLock headrest (Cambridge Research Systems) attached to the eye tracker was used to keep participants' heads in position, and this was placed on a Vision Science height-adjustable workbench (Cambridge Research Systems).

The eye tracker was set in front of a 19in NEC MultiSync Monitor with a spatial resolution of 1024 x 768 pixels at a frame rate of 60Hz, on which visual stimuli were displayed, at a viewing distance of 80cm. Stimuli were generated using the MATLAB (The MathWorks) Psychophysics Toolbox (Brainard, 1997; Pelli, 1997). A speaker was used to play auditory beeps, and the study was carried out in a darkened room.

Procedure

Oculomotor Task: Three beeps were used to signal the start of each trial, a central fixation cross was then displayed on the screen; participants fixated this for a variable duration (mean = 2000ms, s.d. = 200ms) at which point a black circular target 8mm diameter (0.57degrees of visual angle, deg) was briefly displayed on a grey background (250ms) at an amplitude of 8.4cm (6deg) from the fixation point. The target could appear at locations within a circle around the fixation point (i.e. 0°-360°); the exact angle was pseudo-randomly determined by the computer on a trial-by-trial basis. The appearance of the target (and the simultaneous offset of the fixation point) was the cue to initiate a saccade to the location of the target. Each participant performed 30 trials in total.

Data Analysis: Plots of eye movement traces using x and y coordinates from eye-position data recorded every 20ms were analysed. Trials showing artefacts in the eye movement trace, such as blinks were rejected. The latency was defined as the time at which the absolute change in eye position from the start position (calculated as: $\sqrt{(\text{latest}(x)^2 + \text{latest}(y)^2)} - \sqrt{(\text{previous}(x)^2 + \text{previous}(y)^2)}$) exceeded a threshold of 25mm. Data from all participants was grouped to obtain an estimate of the mean and standard deviation (s.d.) saccadic latency for an eye movement of this amplitude.

Results

The mean and s.d. saccade latency were 217.9ms and 34.2ms respectively.

2.3. Experiment 2: Parietal Eye Field Localiser Task

A number of previous TMS studies investigating parietal contributions to saccadic control have centred the TMS delivery at the P3 and P4 sites of the international 10-20 electrode system, (e.g. Elkington et al., 1992; Müri et al.,

1996b; 2000; Kapoula et al., 2001; van Donkelaar and Müri 2002; Yang and Kapoula 2004). The locations of P3 and P4 can be determined in relation to landmarks on the scalp such as the vertex (e.g. van Donkelaar and Müri, 2002), which is itself found using the nasion-inion line and the line between the preauricular points. Coil placement made on the basis of such bony landmarks may lead to problems in terms of the specific brain region targeted by TMS (Pascual-Leone et al., 1999) and does not allow for potential inter-participant variability in either the anatomical location of the IPS in relation to the scalp or in the functionally effective site of stimulation. The use of digital co-registration to aid coil-positioning allows for individual differences in brain size and anatomy by employing each participant's magnetic resonance imaging (MRI) scan to determine scalp location. Nevertheless, this technique still fails to take into account the functional significance of a cortical area in relation to task demands (Pascual-Leone et al., 1999).

An alternative to using a small number of fixed scalp locations, e.g. P3 and P4, is to systematically sample across a number of parietal locations. Oyachi & Ohtsuka (1995), for example, were able to identify, using a grid of stimulation sites and coregistration with 3D MRI, the most effective site of stimulation for a memory-guided saccade task. This site was taken as the one that produced the greatest decrease in saccadic accuracy, however, the existence of individual differences in the location of this site were not reported. Likewise, Ashbridge, Walsh & Cowey (1997) used a 'hunting' paradigm to determine coil position on a visual search task. The behavioural effects of TMS to a particular scalp location were assessed and this process repeated as necessary at adjacent locations until either a 'hot spot' is determined or a certain threshold number of trials reached without a site being found for that participant.

On the basis of previous studies, it was therefore decided that the location of PEF should be systematically determined on an individual basis. The same task as that used in Experiment 1, i.e. a single reflexive saccade, was chosen for this purpose, but with the addition of TMS to a number of scalp locations over the PPC prior to the onset of the eye movement. By comparing the effect of TMS on these saccades to trials with sham TMS at the same site, it was

hoped that a measure of the functional importance of that particular site to the task could be obtained.

Methods

Participants

18 healthy participants (10 females) aged between 19 and 54 (mean 25.7 years) took part in this study. All had normal vision.

Materials

A Magstim Rapid TMS machine (The Magstim Company Ltd.) with a double 70mm coil was used, along with the eye tracker, computer screen and speaker as described above. Participants also wore surgical hoods, on which the grid of stimulation points were marked, as described in the procedure below.

Procedure

TMS: During real stimulation the coil was placed flat and tangential to the scalp surface at each of the grid points. During sham TMS trials, in contrast, the coil was held perpendicular to the scalp with one end of the coil positioned at the centre of the grid. Thus although a magnetic field was no longer induced in the cortex the participants still heard the clicking sounds accompanying the magnetic pulse and still felt the coil against their head. This procedure controls for the accessory cues provided by sensory inputs accompanying TMS, such as the click sounds, which may themselves affect saccadic reaction time (Terao et al., 1998); the contraction of muscles in the scalp, however, would not be felt during sham TMS. The wand was always held with the handle at the back of the head, so that the current would flow in a postero-anterior direction, which has been shown to be most effective for a Magstim Rapid coil (Kammer et al., 2001). Stimulation was set to 120% of the motor threshold determined for each participant.

Ten of the participants received TMS to the right hemisphere and eight to the left hemisphere. Each participant wore a securely fitting surgical hood, on which a grid of stimulation points was drawn on the appropriate side. The nasion, inion and pre-auricular points were first marked on the hoods and lines were then drawn through these to locate the vertex. The grids were 16cm^2 , made up of $4 \times 4\text{cm}^2$ squares, with a centre at P3 (on the left) or P4 (on the right), i.e. 3cm lateral and 3cm posterior to the vertex. Nine points on each grid were used as stimulation sites, i.e. 3 on each row of the grid, each spaced 2cm apart (see Figure 2.1). During the study TMS was delivered to each of these 9 points in a predefined order pseudo-randomly determined by the computer at the start of each session. Thirty trials of real TMS and 30 of sham TMS were delivered to each of the stimulation points in an ABBA pattern. Each participant therefore took part in 540 trials in total for this task (60 for each of the 9 stimulation sites).

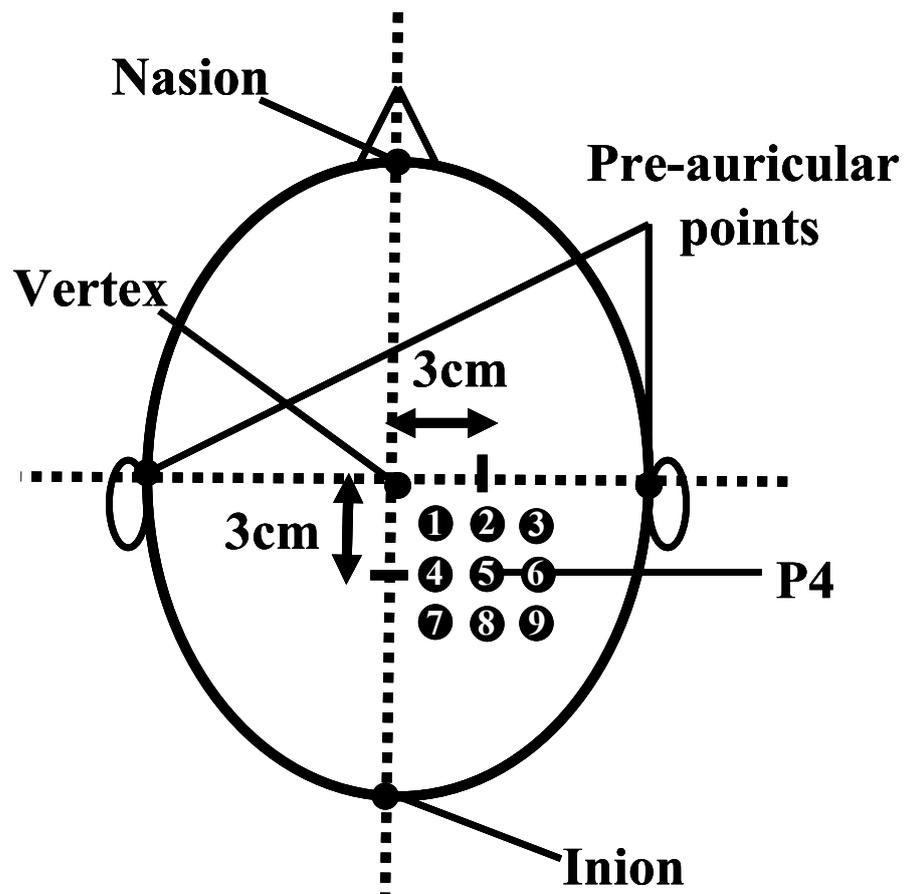


Figure 2.1: Grid of stimulation points for the right-hemisphere, with 9 stimulation points centred on P4.

Oculomotor Task: The task was essentially the same as that described above. A double-pulse of 25Hz TMS was delivered 100ms after the appearance of the visual target, i.e. ~118ms before expected eye movement onset, based on the mean latency found in Experiment 1. Any disruption caused by TMS should thus be during the period of saccade preparation, which might be expected to lead to delays in saccade initiation. Müri et al., (1996), for example, had previously found increased saccadic latency following TMS to both the right and left PPC delivered at this time (100ms after the go-signal) using a memory-guided saccade task.

Data Analysis: The eye-tracking data was analysed in the same manner as in Experiment 1 in order to determine mean saccadic latency for real and sham TMS at each of the stimulation sites. Noisy trials due to excessive blinking or head movements were discarded, as were those in which the time for saccade onset was incorrectly identified by the algorithm. At some sites the TMS induced facial twitching and for this reason no data was collected for that participant at that particular site. All datasets that were analysed had a minimum of 7 trials per condition (mean = 26.4). In order to identify a test site, i.e. a site at which real TMS significantly increased saccadic latency compared to sham TMS, and a control site, i.e. a site at which TMS did not significantly affect saccadic latency compared to sham TMS, one-tailed two-sample Student's t-Tests were performed on the data for all nine stimulation sites in each participant. It was predicted that the mean saccadic latency would be greater for trials with real TMS compared to sham TMS.

Results

Mean and standard error latencies for real and sham TMS at each of the stimulation sites for each participant in the right and left hemisphere TMS conditions are shown in the graphs in Appendix 1 as are the results of the statistical analysis for this data. Table 2.1 below provides a summary of the results, listing the sites chosen as 'test' and 'control' stimulation sites for each

participant. For participants 5 and 7 in the right hemisphere TMS condition, the mean latency for the real TMS trials was not found to be significantly greater than for sham TMS trials at any of the stimulations sites. The sites with the largest difference between real and sham TMS were therefore chosen as the test sites for these participants. In the left hemisphere TMS condition, no suitable sites could be determined as test sites for participants 1 and 2. Therefore, these participants did not take part in the memory-guided double-saccade task that followed.

Participant	Test Site	Control Site		Participant	Test Site	Control Site
Right Hemisphere TMS				Left Hemisphere TMS		
1	7	2		1	-	-
2	9	5		2	-	-
3	7	8		3	3	1
4	9	2		4	4	1
5	1*	7		5	2	8
6	9	1		6	2	6
7	7*	6		7	2	1
8	7	5		8	7	8
9	2	4				
10	1	6				

Table 2.1: Test and control sites chosen for each of the participants in the right and left hemisphere TMS conditions. *Difference between latency for real and sham TMS not statistically significant.

The frequency of significant TMS effects found at all sites for all participants was calculated. The results of this are shown in Figure 2.2 below, in which the sites are displayed in terms of their scalp locations (see Figure 2.1.). N.B. It is important to note that less participants were tested using left hemisphere (eight participants) compared to right hemisphere TMS (ten participants) when considering the number of significant sites found in each hemisphere.

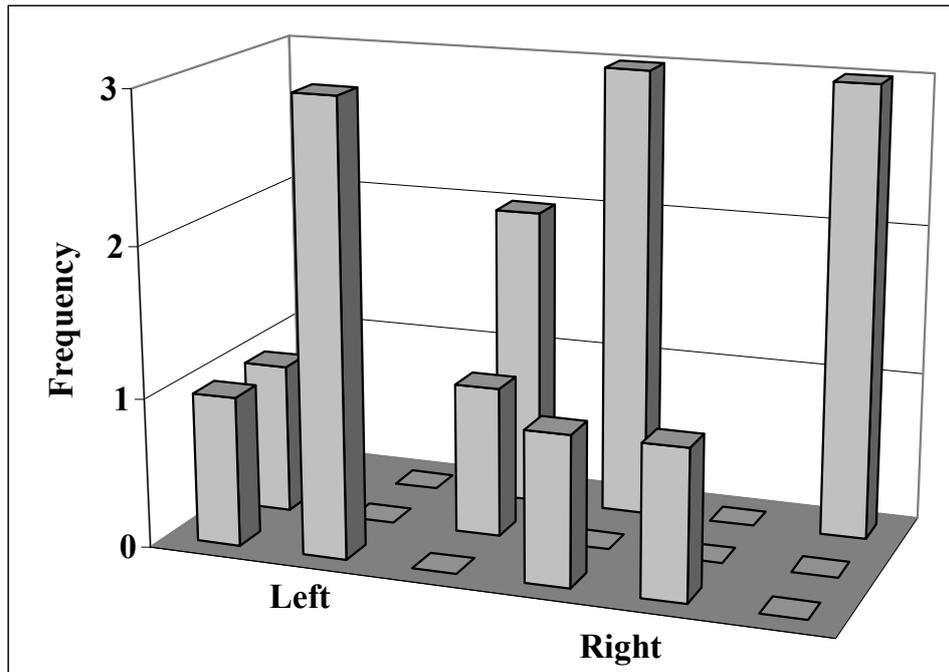


Figure 2.2: Frequency of stimulation sites at which saccade latency was found to be significantly affected by TMS to the left (8 participants) and right (10 participants) hemisphere. Sites are displayed in terms of the relative scalp locations at which TMS was delivered.

Discussion

For a few of the participants no site at which TMS significantly affected saccadic latency (or at least showed a trend in the right direction, as in the case of participant 5 in the right hemisphere TMS group) could be identified. However, this was the case only for two out of the 18 participants, and thus overall this appeared to be an effective method of defining both test and control sites for the delivery of TMS in the double-step saccade task.

2.4. Experiment 3: Double-Step Saccade Task

Once the timing of the TMS and the sites to be used for stimulation, i.e. the test and control sites for individual participants, had been determined, the double-step saccade task was then carried out as described below.

Methods

Participants

Sixteen of the participants (9 females) who had previously taken part in Experiment 2 also took part in the double-step saccade task; these were the participants for whom a suitable test site could be determined (i.e. ten right hemisphere and six left hemisphere TMS).

Materials

These were the same as those described previously, including the TMS machine, TMS coil, surgical hoods, eye tracker, computer screen and speaker. The same surgical hoods worn for Experiment 2 were repositioned on the basis of bony landmarks (i.e. vertex, nasion, inion and preauricular points).

Procedure

TMS: TMS was delivered using the same intensity as for Experiment 2. The wand was held tangential to the head throughout this task; half the subjects were stimulated at the test site first followed by the control site and vice-versa for the other half. Both TMS and no TMS trials were included at the test site and the control site; this was pseudo-randomly determined by the computer on a trial-by-trial basis so that there were equal numbers of both trial types (i.e. 60 TMS and 60 no TMS for each site). For each participant, the hemisphere of TMS stimulation was the same as that in the localiser task (Experiment 2).

Oculomotor Task: A central fixation cross was displayed, followed by the simultaneous presentation of two 8mm circular targets (one black, one white) for 250ms. Participants were instructed to make saccades to the remembered locations of these targets when they disappeared from the screen (half white first then black, the other half black first then white). Targets could be presented at nine possible locations in each quadrant of the computer screen. These positions were 3cm (2.15deg), 5cm (3.58deg) or 7cm (5deg) to the left

or right of the centre of the screen and 3cm, 5cm or 7cm above or below the centre, there were therefore 36 possible target locations in total.

Targets could appear either in the same hemifield (e.g. right-right, or left-left), in this case they were selected from quadrants above and below each other, or in different hemifields (e.g. right-left or left-right), in this case quadrants adjacent to each other. The hemifield order was pseudo-randomly selected by the computer on a trial-by-trial basis from a pre-defined index of possible locations for the separate trial types (i.e. same/ different hemifield trials).

On TMS trials, a double-pulse of 25Hz TMS was delivered at a variable delay following target presentation (mean = 368ms, s.d. = 34 ms, based on the previously determined latency data (218ms), plus 150ms, so that it should occur just prior to onset of the second saccade (see van Donkelaar and Müri, 2002).

Data Analysis: The eye movement data were analysed in the same manner as before; noisy trials, and trials in which participants had performed the task incorrectly e.g. by looking to the targets in the wrong order or starting the trial too early, were discarded. All datasets that were analysed had a minimum of 7 trials per condition (mean = 28.89).

The end point accuracy of the saccades to the second target was determined in order to assess the amount of disruption caused by TMS to the test and control sites. The participant was taken to be fixating when the change in eye position over two samples remained stable (i.e. <25mm), using the same algorithm previously employed to determine latency. Coordinates for x and y eye position at fixation, i.e. the end-point of the saccade, were obtained from the eye tracker and compared with the x and y coordinates for the target positions so that a measure of error could be calculated using the following equation (N.B. a positive y value is ‘up’ for the stimulus presentation software, but ‘down’ for the eye tracker software):

$$\text{Error} = \sqrt{[(x(\text{target}) - x(\text{fixation}))^2 + (y(\text{target}) - (-y(\text{fixation})))^2]}$$

An example trial is shown in Figure 2.3, in which eye position over time (red line for horizontal position, blue line for vertical position) is displayed in

the left-hand plot. Horizontal and vertical target positions and the TMS and go-signal times are also shown. Saccade end-points, as determined by the algorithm, are also shown on this plot, and again on the right-hand plot, which allows an easy comparison with the target locations.

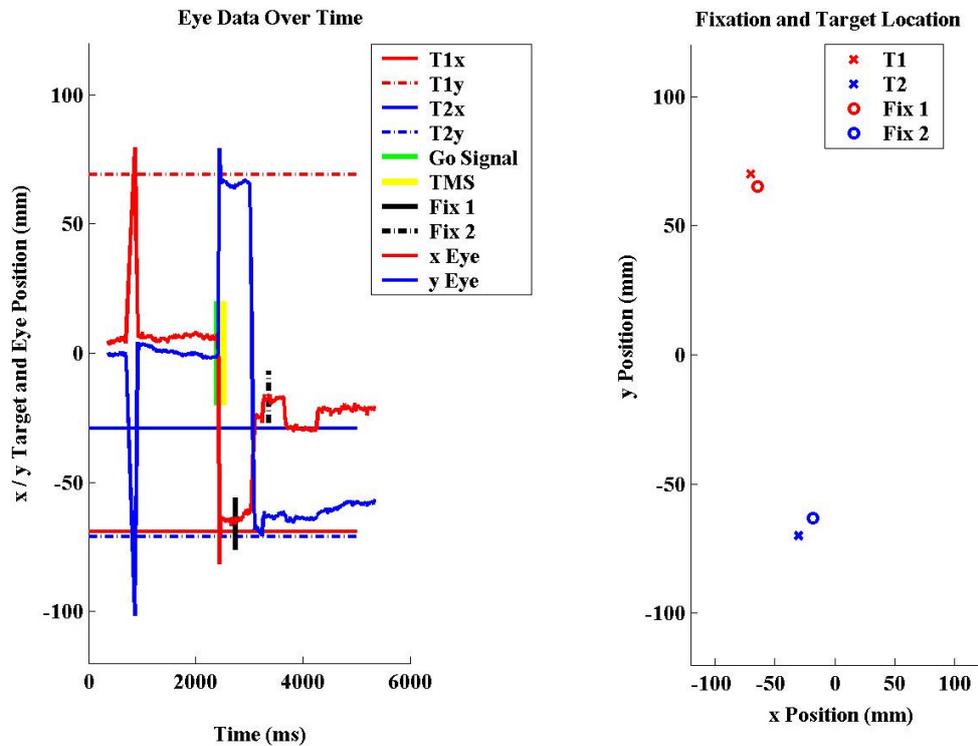


Figure 2.3: Plot of eye movement trace using x (red) and y (blue) coordinates (in mm) from eye-position data over time (ms) for one trial (left plot). The horizontal red bars represent the x (solid) and y (dashed) location of target 1, and the horizontal blue bars those of target 2 (solid, x; dashed, y). The participant can be seen to be fixating at the centre of the screen (0 on y axis) until the go-signal (vertical green bar), with a blink present at ~1000ms. TMS delivery is marked by a vertical yellow bar and the two saccades and fixations (solid vertical black bar = fixation 1, dashed vertical black bar = fixation 2) can be seen following the go-signal. The end point of these saccades is also plotted in relation to the target positions on the screen (right plot), with 'x's representing the target locations (red = target 1, blue = target 2) and the 'o's showing fixation locations (red = fixation 1, blue = fixation 2).

In order to evaluate the influence of TMS on spatial remapping it is useful to consider the metrics of the second saccade in relation to those required given any error in the first saccade. In the analyses detailed above, the end-point error for the second saccade does not take into account any potential

error in the end point of saccade 1. Gain, however, a measure employed by van Donkelaar and Müri (2002), can provide an indication of the relationship between saccade 1 amplitude error and saccade 2 amplitude error. It is defined as the amplitude of the saccade executed, divided by the amplitude required. For the first saccade therefore this would be the distance between the central fixation cross and target 1, whereas for saccade 2 it would be the distance between target 1 and target 2. If compensation for error in the first target were taking place, then a positive linear relationship would be expected between first and second saccade gain. van Donkelaar and Müri (2002) state, however, that if such updating was not occurring, due to the use of a more object-based coding of the target locations, then the value of the slope for first saccade gain plotted against second saccade gain would be closer to zero.

To further quantify the extent to which end-point error in saccade 1 is accounted for in the exhibited amplitude of saccade 2, a measure known as compensatory gain can also be computed. This is defined as the amplitude of saccade 2 divided by the amplitude required *given saccade 1 error*. The end-point of saccade 1 therefore has to be calculated first, and this can then be used to calculate the amplitude of the vector from this end-point to the location of target 2, i.e. the amplitude required to accurately saccade to the previous location of target 2. This measure was also used by van Donkelaar and Müri (2002), who observe that perfect compensation (for the error in saccade 1) would result in a compensatory gain value of one, which they state would reflect the use of retinotopic coding. A value of less than 1, however, would indicate that less account is being taken of the error in saccade 1, which they suggest would indicate the use of a more object-based frame of reference.

Results

Although initially it had been intended that results from right and left hemisphere stimulation would be considered separately, ultimately this was not possible in terms of the data collected. During the analysis stage, data from a large number of the participants (5 right hemisphere and 4 left hemisphere) had to be discarded due to the high incidence of noisy trials. Thus it was decided to

combine the data from the remaining participants (2 left and 5 right hemisphere TMS) for the following stages of the analysis.

End Point Error: Mean end-point error was calculated for all conditions in each participant, and a 2 x 2 within-subjects ANOVA, with the factors trial type (no TMS and TMS) and stimulation site (test and control), was used to analyse the data. A main effect of trial type was found ($F_{(1,6)} = 7.040$, $p < 0.05$), however end-point error was actually shown to be higher overall for the no TMS trials compared to the TMS trials (no TMS: mean = 18.72mm, s.d. = 3.22mm; TMS: mean = 16.34mm, s.d. = 4.05mm). There was no significant main effect of stimulation site ($F_{(1,6)} = 2.706$, N.S.) and the interaction did not reach significance ($F_{(1,6)} = 3.822$, N.S.). The graph below (Figure 2.4) illustrates these results.

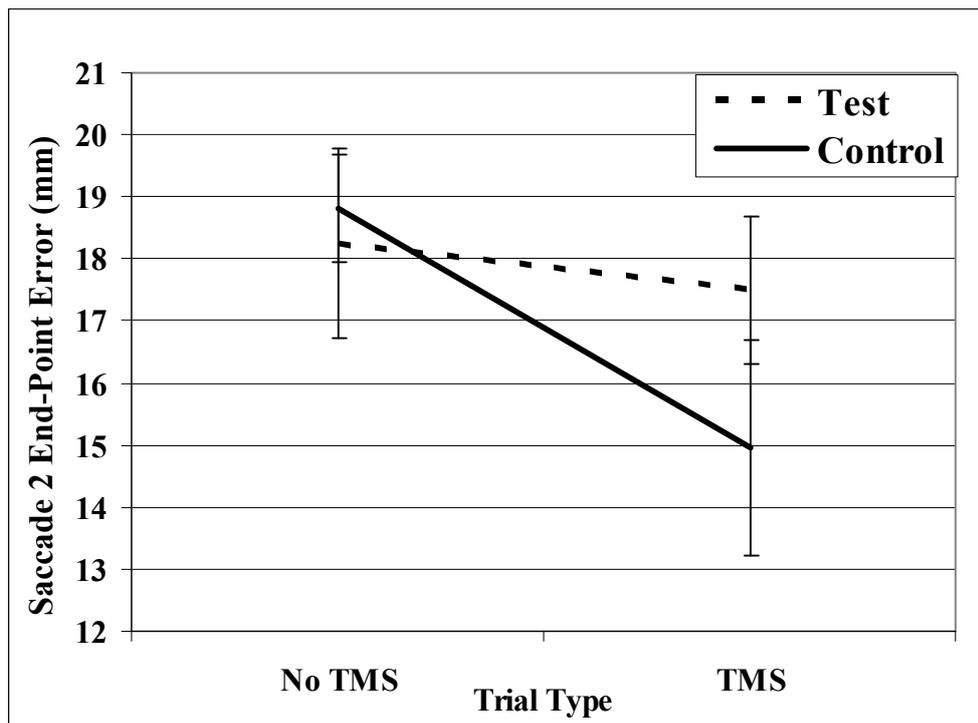


Figure 2.4: Mean end point error (in mm) for saccade 2 on TMS and no TMS trials at both the test site (dashed line) and the control site (solid line). Error bars show standard errors.

A paired-sample one-tailed Student's t-Test was used to test the prediction that saccade 2 error would be greater for TMS trials at the test site

compared to the control site. Error was in fact shown to be greater at the test site compared to the control site for TMS trials ($t_{(6)} = 2.790, p < 0.05$). No difference however was found between the means for TMS and no TMS trials at the test site, for which it was predicted greater error would be seen on saccade 2 for the TMS trials ($t_{(6)} = 1.721, N.S.$).

In order to further assess these results in terms of spatial updating, the data was next considered in terms of saccade gain.

Gain and Slope Values: Gain values were calculated for each trial for all participants as described in the data analysis section above, these were then used to compute a slope value for gain 1 (x axis) plotted against gain 2 (y axis), for each condition for all participants. These slope values were then entered into a 2 x 2 within-subjects ANOVA, with the factors trial type (No TMS and TMS) and stimulation site (test and control). No significant main effects of stimulation site ($F_{(1,6)} = 0.049, N.S.$) or trial type ($F_{(1,6)} = 0.235, N.S.$) were found, nor was a significant trial type x stimulation site interaction ($F_{(1,6)} = 0.037, N.S.$). The graph below (Figure 2.5) illustrates these results.

A paired-sample one-tailed Student's t-Test was used to test the prediction that the slope value for TMS trials at the test site would be significantly lower than that for TMS trials at the control site. No difference was seen for this comparison ($t_{(6)} = 0.735, N.S.$). Similarly, no difference was found between the means for TMS and no TMS trials at the test site, for which it was predicted a lower slope value would be seen for the TMS trials ($t_{(6)} = 1.299, N.S.$).

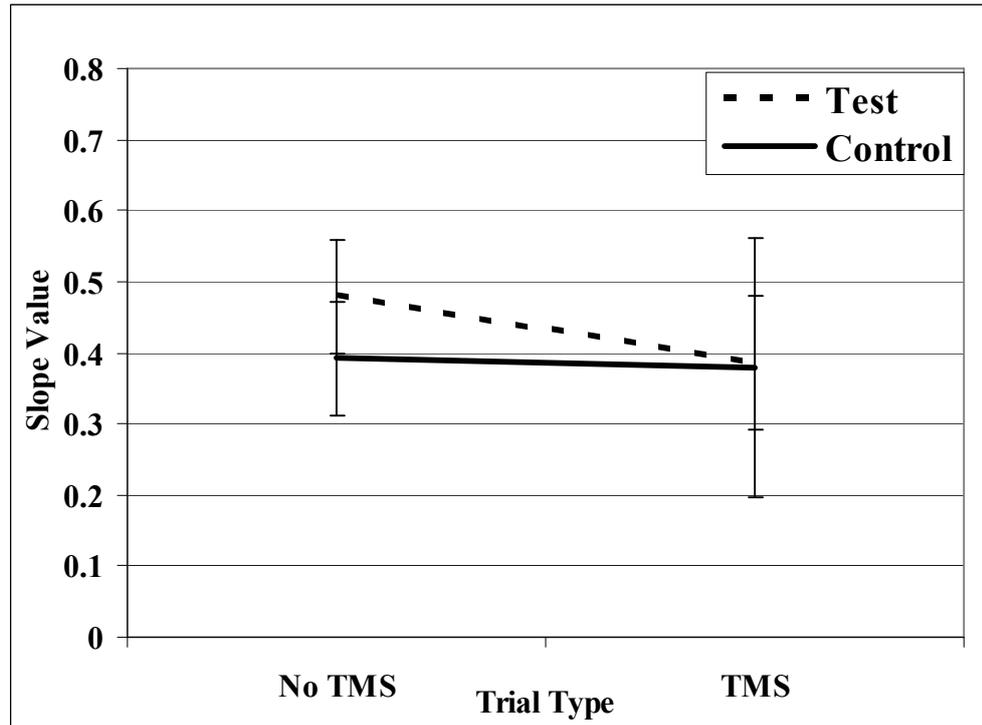


Figure 2.5: Slope values for gain 1 plotted against gain 2 on TMS and no TMS trials at both the test site (dashed line) and the control site (solid line). Error bars show standard errors.

Compensatory Gain: Compensatory gain was also calculated for every trial and a 2 x 2 within-subject ANOVA was conducted on the means for each participant in each condition. No main effects of stimulation site ($F_{(1,6)} = 0.972$, N.S.) or trial type ($F_{(1,6)} = 1.068$, N S.) were seen and the interaction was also not found to be significant ($F_{(1,6)} = 2.630$, N S.). The graph below (Figure 2.6) illustrates these results.

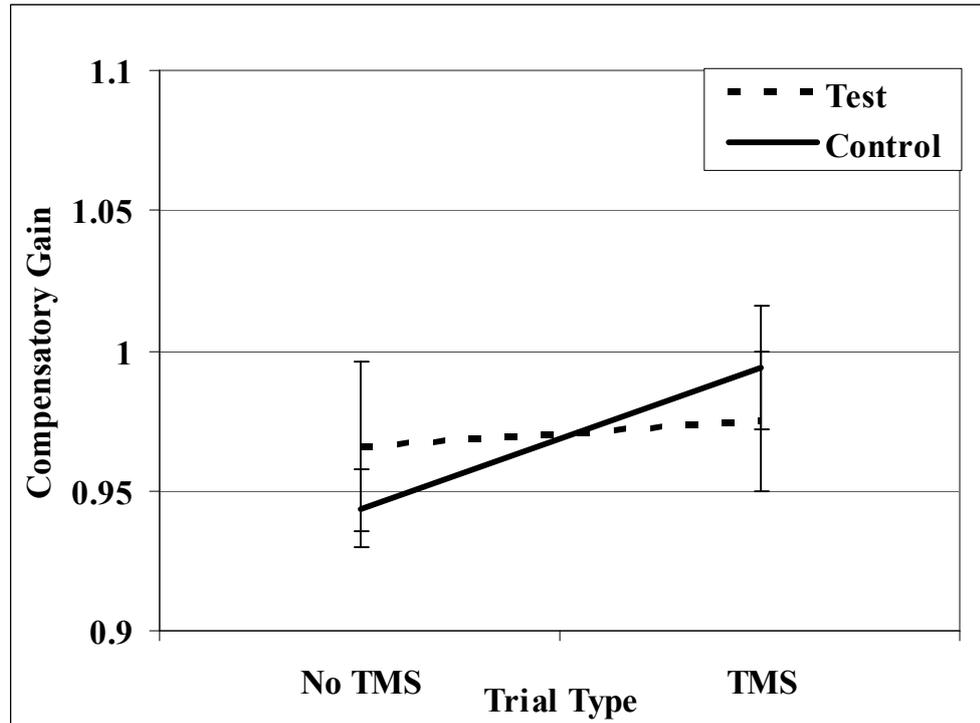


Figure 2.6: Mean compensatory gain on TMS and no TMS trials at both the test site (dashed line) and the control site (solid line). Error bars show standard errors.

A paired-sample one-tailed Student's t-Test was used to test the prediction that compensatory gain for TMS trials at the test site would be significantly lower than that for TMS trials at the control site. No difference was seen for this comparison ($t_{(6)} = 1.646$, N.S.). Similarly, no difference was found between the means for TMS and no TMS trials at the test site, for which it was predicted a lower compensatory gain would be seen for the TMS trials ($t_{(6)} = 1.217$, N.S.).

Since the significant difference in saccade 2 error for TMS trials at the test site compared to the control site could not be explained in terms of a difference in compensatory gain (which only assesses saccade amplitude), it was decided that angular compensation should also be considered. The compensatory gain measure does not however work as well for compensatory angular gain as for compensatory amplitude gain. This is due to the values involved in each case, for example with compensatory amplitude gain, a typical value for the amplitude required for perfect compensation, would be, for example, 105mm, whereas the typical value for the actual saccade

amplitude might be, for example, 100mm, this would give a value for the compensatory amplitude gain of 0.95 (i.e. 100/105). However, for compensatory angular gain, the values used are different in nature, for example the angle required for the saccade for perfect compensation could be anywhere between 0° and 360°. If, for example, the angle required was 1° and the actual angle of the saccade executed was 2° this only reflects a difference between them of 1°, and yet the compensatory angular gain would be 0.5 (1/2). The same value of compensatory gain would be found on a trial where the angle required was 16° and the angle actually executed was 8°, which does not really reflect the fact that the difference here is much larger, i.e. 8° rather than 1°. Similarly a difference of only 1° with higher values, e.g. 354/355 would give a value of almost 1 for the compensatory angular gain.

Clearly therefore, in order to quantify angular compensation a different measure would be preferable, so instead the absolute difference between the angle of saccade 2 and the angle required given error in saccade 1 was calculated for all trials in each condition for every participant. The means were entered into a 2 x 2 within-subjects ANOVA, with the factors trial type (no TMS and TMS), and stimulation site (test and control). A main effect of trial type was found ($F_{(1,6)} = 6.731$, $p < 0.05$), however the difference between saccade 2 angle and the compensatory angle was actually shown to be larger for the no TMS trials compared to TMS trials (no TMS: mean = 6.93°, s.d. = 1.37°; TMS: mean = 5.72°, s.d. = 2.02°). There was no significant main effect of stimulation site ($F_{(1,6)} = 0.769$, N.S.) and the interaction did not reach significance ($F_{(1,6)} = 3.875$, N.S.). The graph below (Figure 2.7) illustrates these results. A paired-sample one-tailed Student's t-Test was used to test the prediction that angular difference would be greater for TMS trials at the test site compared to the control site. No difference however was found for this comparison ($t_{(6)} = 1.400$, N.S.) or for that between the means for TMS and no TMS trials at the test site, for which it was predicted a greater angular difference would be seen for the TMS trials ($t_{(6)} = 0.947$, N.S.).

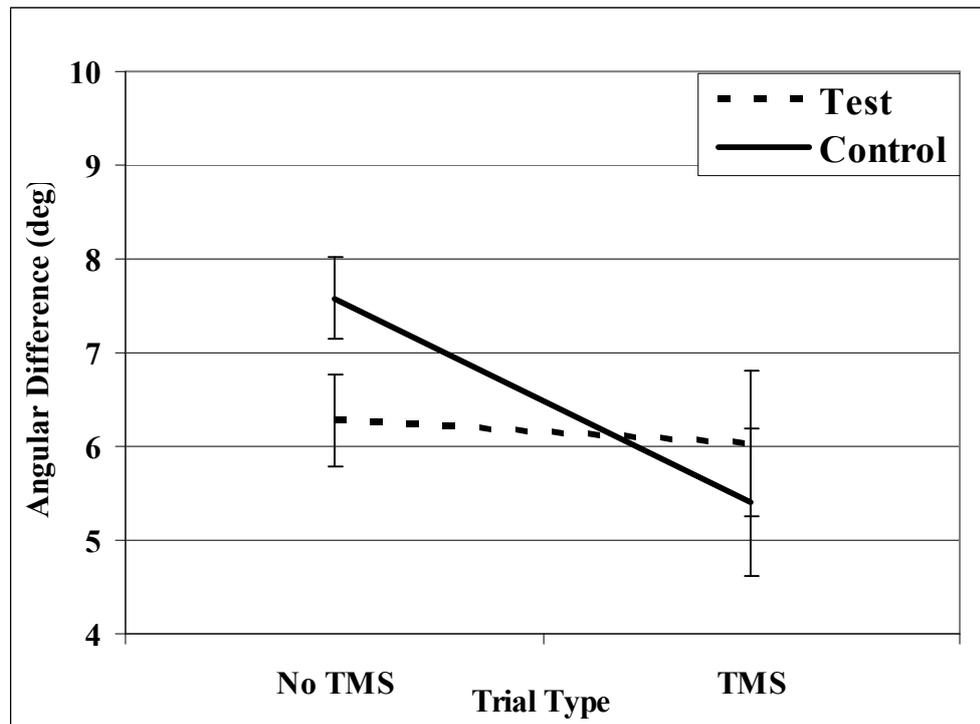


Figure 2.7: Mean absolute difference between the angle of saccade 2 and the angle required given error in saccade 1 on TMS and no TMS trials at both the test site (dashed line) and the control site (solid line). Error bars show standard errors.

Discussion

From the results, therefore, it does appear that TMS is having an effect on saccadic error in this task. The effects seen, however, were not in line with those predicted. An overall effect of TMS, rather than an interaction, suggests that the effects of TMS were the same across both sites, whereas an effect had been predicted at the test site but not the control site. Further to this, these effects were in fact in the opposite direction to that expected, with an apparent paradoxical improvement in accuracy seen for TMS compared to no TMS trials. Although pre-planned comparisons did reveal significantly greater error for trials with TMS at the test site compared to the control site, from an examination of the graph, this appears to be the result of reduced error for trials with TMS at the control site rather than an increase in saccadic error resulting from TMS at the test site.

Considering saccadic error alone, however, can't tell us a great deal about the spatial remapping thought to be taking place in this task. To investigate this, gain, a measure of compensation was also calculated. From this it seemed that in terms of the amplitude of the second saccade at least, an equivalent amount of compensation was occurring across all of the experimental conditions. This measure therefore provides no evidence to suggest spatial remapping in this task is disrupted by parietal TMS.

The slope values calculated for the relationship between the amplitude errors (gain) in the two saccades were shown to be around 0.5 in the study by van Donkelaar and Müri (2002). This is slightly higher than the group mean slope values seen in the current study (around 0.4). As the authors point out, this suggests the use of a more object-based frame of reference and that the amplitude error of the first saccade is less accounted for in the second saccade in the current study. This observed difference between the two studies might be best explained in terms of task specifications. Whereas in van Donkelaar and Müri's study, the targets were shown sequentially, with a variable delay (500-1500ms) between the two targets, in the present study the targets were displayed concurrently. The appearance of both targets on the screen at the same time might have encouraged the use of a more object-based frame of reference, i.e. the coding of one target location in relation to the other.

The authors also note however, that the use of this slope value as a measure is problematic, since a single slope value is calculated from a number of trials, whereas as compensation is something that would occur within a trial. This suggests therefore that a measure such as compensatory gain would be more appropriate for considering the extent of spatial updating taking place in this task. The data from van Donkelaar and Müri's study exhibited generally high values of compensatory gain, which the authors explained as evidence for the use of craniotopic updating; a value of 1 reflecting perfect compensation. The compensatory gain values seen in the present study were similarly generally high, with the means for all conditions ranging from 0.94 to 0.99, suggesting that participants may in fact have been using a craniotopic frame of reference and were in fact compensating for error in saccade 1 as far as the amplitude of saccade 2 was concerned. Another important difference between the current study and that of van Donkelaar and Müri, however, is that their

task only used horizontal saccades. The fact that saccade direction in the current task was not restricted in this way means that compensation for error in saccade 1 needs to be assessed in terms of both amplitude and direction; this is not accounted for in their measure of compensatory gain.

By considering compensation in terms of the angular direction of the saccade, a TMS effect was found, although, as for end-point error, this was again seen as a lower level of compensation on the no TMS trials (i.e. greater difference between the actual angle of saccade 2 and the compensatory angle required) compared to TMS trials. From the graph it also appeared that, as for end-point error, the effects of TMS on this measure were more evident at the control site than at the test site.

Although the test and control sites were chosen on the basis of a presence/ absence of a TMS effect on saccades, this was done only in terms of latency. Therefore TMS at these sites may additionally affect a different aspect of the saccade e.g. the stored saccade plan, or the spatial memory for the target location, and hence other saccade metrics such as accuracy in terms of angle or amplitude. Oyachi and Ohtsuka (1995), for example, suggest that the human PPC maintains the spatial accuracy of remembered target locations for memory-guided saccades, and hence that TMS can result in decreased saccadic accuracy by activating neurons in this cortical area for a brief period and changing the motor planning signal without actually eliminating it altogether. If this was the case it could perhaps go some way towards explaining these unexpected findings.

Another potential explanation for the results might be in terms of the reference frames used by participants for this particular task. Without TMS participants might, for example, make use of an object-based frame of reference to a certain extent, such as the representation of the spatial relationship between the targets. Theoretically this might be encouraged through their concurrent presentation, since it would be easier to establish the spatial relationship between them when both targets are visible together on the screen compared to the situation when spatial information must be integrated across sequential target presentations.

Schlag and Schlag-Rey (2002) discuss two possible solutions for accurately performing the double-step task; the first is allocentric in nature and

requires the participant to store in memory the spatial relationship between targets 1 and 2. Alternatively, participants may use an egocentric method to solve this problem, which would involve integrating the remembered retinal vector from the fixation point to target 2 with information on the eye displacement brought about by the first saccade. If, as a result of task specifications, participants were using an allocentric method to complete the double-step task in this study, the mental representation of the spatial relationship between the two targets might be disrupted by TMS. This could in turn lead to a reliance on a more retinotopic frame of reference, such as would be used in the egocentric solution. Retinotopic coding of target locations would, as van Donkelaar and Müri point out, result in greater spatial updating of the target location following the first saccade, which could potentially explain the overall decrease in error and improved angular compensation for TMS compared to no TMS trials. The feasibility of this potential explanation for the findings will need to be further evaluated.

2.5. General Discussion

2.5.1. The Use of TMS in a PEF Localisation Procedure

The results from the PEF localiser task seemed to suggest that such a localisation procedure may be a useful method of mapping the effects of TMS over a region of interest, such as the posterior parietal cortex. Within individuals it provides a way to determine functionally effective TMS sites for a particular task, whilst across a group it allows the opportunity to evaluate the existence of possible between-participant variation.

No single site stood out across the group as consistently affected by TMS in the same way in terms of increased latency. Sites at which a significant difference in latency between real and sham TMS was seen were found for both left and right hemisphere stimulation, without major differences in terms of frequency. Within participants, however, it is not possible to say from the data collected so far whether any inter-hemispheric differences might exist in terms of TMS effects. This is therefore something that would be interesting to consider given the debate in the literature concerning the relative roles of the

two hemispheres in terms of saccade-related behaviours. TMS studies by Müri et al., (1996b), Oyachi & Ohtsuka (1995) and Rushworth et al., (2001; 2003) have all, for example, noted differences between parietal TMS to the left and right hemispheres.

Various studies have reported an effect of parietal TMS on latency (Elkington et al., 1992; Terao et al., 1998; Muri et al., 2000; Kapoula et al., 2001; Yang and Kapoula, 2004) and saccadic accuracy (Oyachi and Ohtsuka, 1995; Müri et al., 1996b; van Donkelaar and Müri, 2002) using memory-guided saccade tasks, reflexive saccades and anti-saccades. However whether TMS to the same site will influence both of these variables has not yet been conclusively determined. In terms of error, certain studies have particularly noted an effect on saccade amplitude (e.g. Müri et al., 1996b; Oyachi and Ohtsuka, 1995); there is less information in the literature however on whether TMS to this same parietal site also disrupts the angular direction of the saccade.

It is also unclear from previous research whether TMS to a particular parietal site would have an effect on different types of saccades. For example, would a site for which TMS resulted in increased latency on a single reflexive saccade, as used in the localiser task, also be expected to affect accuracy on a memory-guided double-step saccade task. If single visually-guided saccades are being used in the localiser task, it could perhaps be more appropriate to use a task that more closely resembles this when attempting to disrupt the remapping process. It might also be useful to consider the effects of TMS in terms of both latency and accuracy of saccades in the localiser task, when attempting to determine the location of the PEF in individual participants.

2.5.2. Role of the Posterior Parietal Cortex in Saccade-Related Spatial Remapping

Despite the evidence in the literature to support the idea that the PPC is critically involved in the spatial remapping required to perform saccade sequences (e.g. Heide et al., 1995; 2001; van Donkelaar and Müri, 2002), the results from the double-step saccade task discussed here failed to provide any further evidence to support this. Rather, it appeared that parietal TMS, if

anything, might indirectly be bringing about greater levels of spatial remapping by disrupting the spatial representation of the remembered target locations. This is however only one experiment and it will therefore be important to assess the evidence for a parietal locus for the spatial remapping process to a greater extent through additional studies.

2.6. Conclusions

On the basis of the experiments discussed in this chapter, it is not possible to draw any firm conclusions regarding the role of the PPC in the spatial remapping of target locations to account for eye displacement. The failure of parietal TMS to show any evidence of disrupted remapping could in fact be due to a range of experimental variables as discussed above. The experiments discussed in the next chapter continue the attempt to investigate parietal involvement in this process, by assessing the effects of parietal TMS on the updating of a saccade plan in response to a change in target location. Other issues raised in the current Chapter, such as the potential for interhemispheric differences and whether TMS at a single site influences multiple saccade metrics, will also be addressed.

Chapter 3: The effect of parietal TMS on the spatial updating of a saccade plan in response to a change in target location

3.1. Introduction

The preparation and execution of saccades to environmental stimuli requires a number of different stages; these are discussed by Pierrot-Deseilligny et al., (2003), who state that this starts with the perception stage following exposure to the stimulus. Perception itself requires attention and perhaps selection if there is more than one salient stimulus present. According to the authors this is then followed by spatial integration, a process thought to be carried out by the PPC, which involves defining the location of the stimulus in relation to the body. A memorization stage occurs next, if necessary, which can vary in length depending on the situation and requires the formation of a spatial memory of the stimulus location prior to the use of this information in the movement stage. The authors suggest that reflexive saccades in particular might be triggered by the PEF, immediately following the spatial integration stage, whereas the FEF is responsible for the initiation of intentional saccades following a delay.

TMS could therefore be used in an attempt to disrupt different but related stages within the process of saccade preparation and execution, which may or may not share common anatomical loci. The idea that the PPC may be important in relation to reflexive saccades, has been backed up by Elkington, Kerr and Stein (1992), who delivered TMS to this area during a visually-guided saccade task, and demonstrated effects on both latency and accuracy in terms of saccade amplitude. They concluded that the PPC plays an important role in the programming of accurate saccades to visual stimuli. The idea that this area is important for movement planning is also supported by Andersen et al., (1997), who conclude that in particular, the PPC contributes to this process through the coding of spatial locations of the goals for movements in terms of motor coordinates.

In the double-step saccade task described in Chapter 2, a final plan for the second saccade has to account for error in saccade 1. In other words the original saccade plan, made before the first eye movement is initiated, must be

updated as a result of this change in eye position and any accompanying end-point error.

An alternative situation in which a saccade plan must be updated is in response to a change in the location of the goal for the movement, for example following a visual target 'jump' or perturbation. It has been suggested that parallel saccade preparation may occur both in situations when, due to a programming error, predictive feedback suggests that the current saccade will end in the wrong location, and also in the case when a second, more important target appears, such as is the case with a target jump (Becker & Jürgens, 1979). Becker & Jürgens particularly investigated corrective saccades; some later studies have however suggested that in-flight changes in the direction and amplitude of the primary visually-directed saccade itself may be possible if a target jump occurs during the reaction time period (e.g. Van Gisbergen et al., 1987).

Findlay and Harris (1984) similarly concluded that saccades may not, as had previously been thought, be completely ballistic in nature, but may to a certain extent be open to mid-flight modification. They investigated target perturbations occurring during the saccade preparation period and concluded that both the amplitude and direction of a saccade can be modified as long as information concerning the target jump is available to the visual system at least 80ms prior to saccade initiation. They also questioned whether saccade amplitude and direction were programmed independently of one another, but found no real evidence to confirm this.

A certain amount of saccadic flexibility has also been noted by Gaveau et al., (2003) for undetected intra-saccadic target perturbations; these modifications were shown to be direction-specific depending on the target jump, and could not therefore be explained as a general change to the saccade in the presence of perturbations.

In contrast to the idea that saccade plans can be updated prior to saccade initiation or even later during the saccade itself, Becker (1991) argues that a 'retinal comparison' of current and intended eye locations, even if it occurred at the start of a saccade, would be too late to affect the course of that saccade.

In terms of the cortical areas thought to be responsible for the updating of a motor plan in response to a perceptual change, such as a change in the

location of a visual target for a saccade, a single-unit recording study in monkeys carried out by Bracewell et al., (1996) suggests that this might be a function of neurons in LIP. They demonstrated an alteration in neuronal activity in this area associated with changes of motor intention. It therefore seems likely that the PEF, the purported human homologue of area LIP could be responsible for this behaviour in humans.

The current study will use parietal TMS in an attempt to disrupt the updating of a plan for a single reflexive saccade in response to a visual target jump occurring in the reaction time period. In doing this, the role of the PPC, and in particular the PEF, in this type of behaviour can be evaluated, since if it is important to this updating process, then TMS here would be expected to result in reduced compensation for the target jump in terms of saccade metrics.

Given the evidence that changes to a saccade can occur even after initiation, TMS will be delivered at two different times during the trial. Early TMS will be given following the presentation of a visual target, but prior to expected saccade onset, i.e. the latency period, during which the target jump itself will occur; this is thus when the updating of the original saccade plan (or programming of a new plan) might take place. Late TMS, conversely, will be triggered by the start of the saccade at which point it might be possible to disrupt the execution of the updated plan.

As in the double-step saccade task, an attempt will be made to identify sites in individual participants that might correspond to the location of the PEF on the basis of the effects of TMS on a single reflexive saccade. This time however three saccade metrics: amplitude error, angular error and latency will all be taken into consideration during the localisation procedure as opposed to latency alone. This also affords the opportunity therefore of assessing the extent to which these saccade metrics may be controlled by a common area, or alternatively programmed independently.

Given the proposed additional role of LIP in detecting salient visual stimuli (Gaymard et al., 2003), it will also be important to check that any failure to update a saccade plan following a target jump cannot alternatively be explained as a result of TMS disrupting the perception of the perturbation. Thus participants will also be required to respond as to whether or not they perceived a target jump on that trial. Since there has previously been some

debate as to whether the amplitude and direction of a saccade may be programmed independently, this task will make use of both types of target perturbation and evaluate the effects of parietal TMS to updating of the movement plan for each of these.

To increase unpredictability in terms of the size of the target jump, a range of different size perturbations will be used. The size of the target jump would be expected to affect ease of detection, and thus detection thresholds for the range of target jump sizes will be assessed to ensure they are roughly equivalent for amplitude and angular perturbations.

3.2. Experiment 4: The Effect of Varying Target Jump Size on Detection Thresholds

It would be expected that for a range of different size perturbations, the percentage of times participants thought that they had detected a target jump would increase linearly with size, i.e. smaller target jumps would be harder to detect and therefore elicit a lower percentage of 'yes' responses than larger target jumps. This task aimed to determine a range of target jump sizes, for which the ease of detection was roughly equivalent within each of the size brackets (e.g. small, medium or large) for both amplitude and angular perturbations. This range of target jump sizes could then be used with TMS in the single-saccade target perturbation task.

Methods

Participants

Three participants (2 female) aged 20-23 (mean 21.3 years) completed this task. All had normal vision.

Materials

Eye tracking was performed using the same method as described in Experiment 1 (Chapter 2). Stimuli were displayed using a 20in Dell Trinitron

Monitor with a spatial resolution of 800 x 600 pixels at a frame rate of 100Hz and a viewing distance of 55cm. Stimuli were generated using the MATLAB (The MathWorks) Psychophysics Toolbox (Brainard, 1997; Pelli, 1997).

A speaker was used to play auditory beeps, and the study was carried out in a darkened room. Participants' responses were recorded using a standard computer keyboard.

Procedure

Oculomotor Task: This task required participants to execute a single reflexive eye movement towards a visual stimulus. An auditory beep was used to signify the start of each trial, a black fixation cross on a grey background was then displayed on the screen, and remained on until the eye-tracker determined that the participant was correctly fixating on the cross, i.e. the pupil was directed to a region of the screen 15mm (1.56deg) around the centre. A single circular black target of 3mm diameter (0.31 deg) was briefly displayed at an amplitude of around 90mm (9.29deg), based on a normal distribution, mean = 90mm s.d. = 5mm (0.52deg), in order to reduce predictability. The orientation of the target varied between 0-360°, and was pseudo-randomly determined by the computer. After 200ms the target was extinguished and a second identical target was briefly displayed (10ms).

On half of the trials the amplitude of the second target location was altered and on half the orientation of the second target varied compared to the first target. There were seven possible perturbation types: 3 positive perturbations, and 3 negative perturbations as well as a no perturbation condition (i.e. the second target was displayed in the same location as it had been originally). For amplitude, a positive perturbation meant increased amplitude and a negative perturbation meant decreased amplitude; there were 3 possible sizes for each of these, small (3.2mm), medium (8mm) and large (12.8mm). For the angular perturbations these values corresponded to either a clockwise (positive) or anti-clockwise (negative) change in target location (in degrees of orientation); as for amplitude there were 3 possible perturbation sizes, small (2°), medium (5°) and large (8°). The size of these perturbations, as seen on the computer screen, were roughly equivalent for each size bracket

across the amplitude and angular perturbations (small =0.33deg, medium = 0.83deg, large =1.33deg). Participants were instructed to initiate an eye movement towards the target as soon as it appeared. They were also asked to indicate whether they had perceived a change in the target location; this was done through a response on the keyboard: 'j' for 'yes', and 'f' for 'no'. Participants were positioned with their right and left index fingers resting on these keys (i.e. right index finger on 'j', left on 'f'), so that they did not have to look at the keyboard to make the response. Participants were informed that this was not a reaction time task. Each participant completed 196 trials in total; 14 of each perturbation type and size, with 28 of these being no-perturbation trials.

Data Analysis: The percentage of 'yes' responses was calculated for each participant for each of the amplitude and angular perturbation types and sizes and these values were used to find a group mean.

Results

The graphs below (Figures 3.1 and 3.2) show the mean percentage of 'yes' responses; these indicate that target displacements became progressively easier to detect with an increase in size, with a value of ~40% for the small target jumps (mean = 42%), ~75% for the medium (mean = 76%) and ~95% for the large target jumps (mean = 96%). The main exception to this was the large positive amplitude perturbation, which showed a notably lower mean percentage of yes responses (59%). This might result from the relationship between the direction of the perturbation and the direction of the eye movement, i.e. both would be moving outwards from the centre of the screen, which might somehow have masked detection of the target jump.

Alternatively, this could perhaps be explained by the fact the second, perturbed target is presented even further into peripheral vision. At this increased eccentricity retinal sensitivity in terms of spatial detail will be worse compared to that for a target presented closer to foveal vision. The no-perturbation conditions showed a mean 'yes' response of around 20% (mean = 22%),

showing participants were not always completely sure that the target remained stationary on these occasions.

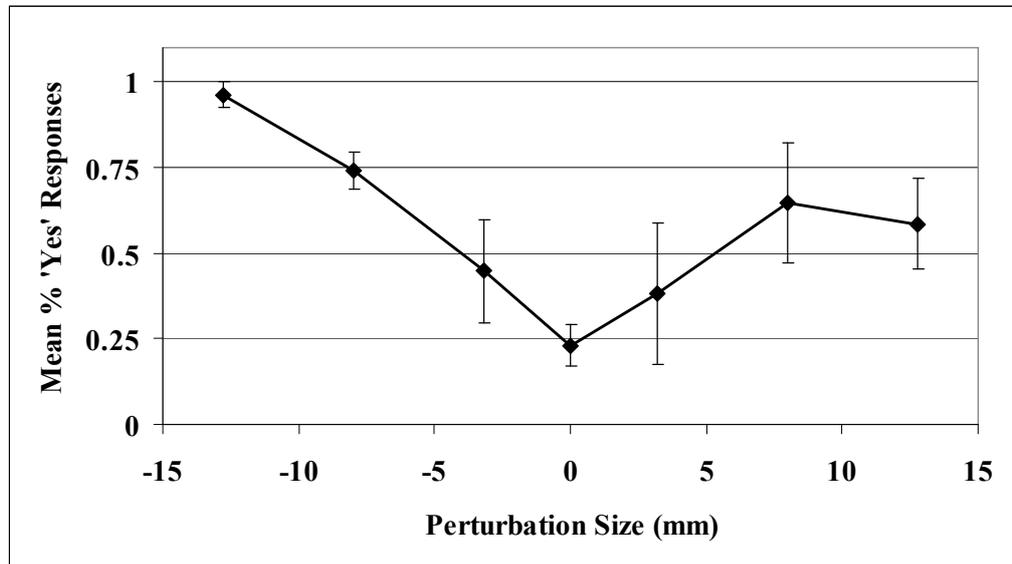


Figure 3.1: Group mean percentage of 'yes' responses for each of the target perturbation sizes in the amplitude perturbation condition. Error bars show standard errors.

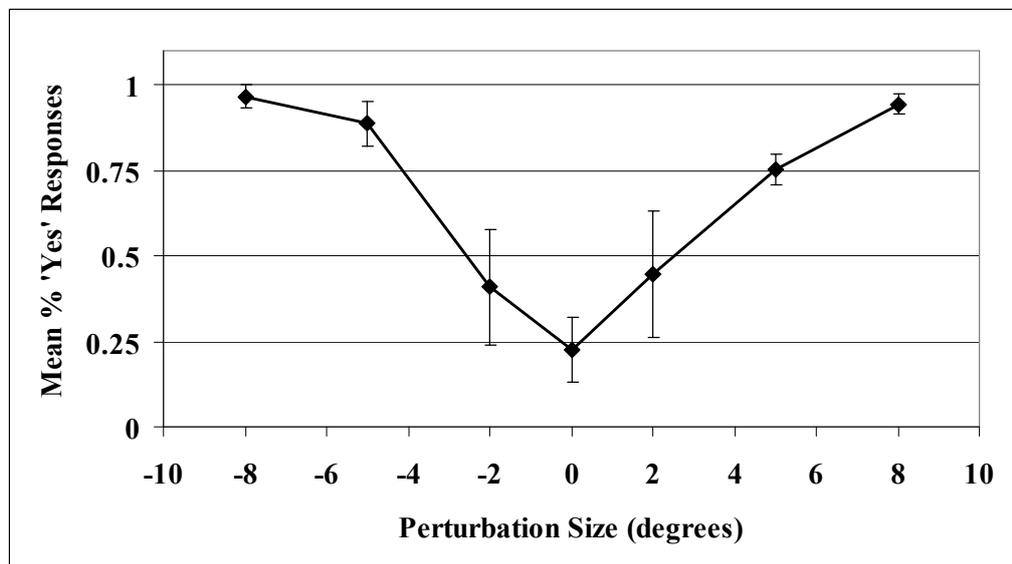


Figure 3.2: Group mean percentage of 'yes' responses for each of the target perturbation sizes in the angular perturbation condition. Error bars show standard errors.

Discussion

The target perturbation sizes used in this task appeared to provide a suitable range of performance levels that were roughly comparable across the amplitude and angular perturbation types.

3.3. Experiment 5: Parietal Eye Field Localiser Task

Methods

Participants

Ten healthy adults (6 females, mean age: 21.2) participated in this task. All had normal vision.

Materials

Stimulus presentation and eye tracking were carried out in the same way as in Experiment 4. A Magstim Rapid TMS machine (The Magstim Company Ltd) with a double 70mm coil was used to deliver TMS. Participants wore securely fastened surgical hoods throughout the experiment.

Procedure

TMS: The procedure for TMS delivery was the same as that described in Experiment 2 (Chapter 2). Two grids of stimulation sites were marked on the surgical hoods worn by the participants, one on the right and one on the left, in the same manner as described in Experiment 2.

The order in which participants received left and right hemisphere stimulation was counterbalanced across individuals. Blocks of sham TMS were completed at the start and end of each session. Experimental trials involving stimulation at each of the 9 sites within a hemisphere took place between the sham blocks. The order of stimulation for these sites was pseudo-randomly determined by computer. There were therefore 18 blocks of real

TMS, each consisting of 15 trials. Each sham block also contained 15 trials. Participants completed 300 trials in total.

Oculomotor Task: Participants were required to make a single reflexive eye movement towards the target. A beep was used to signify the start of each trial, at which point a black fixation cross appeared on the screen against a grey background. This remained on until the eye-tracker determined that the participant was correctly fixating it, i.e. the pupil was directed to a region of the screen 15mm (1.56deg) around its centre. A single black peripheral target was then presented and participants were instructed to execute a saccade toward it as soon as it was detected. One hundred milliseconds after the appearance of the target a double-pulse of 25Hz TMS was delivered. The target remained on the screen for a total of 200ms, after which the screen went blank and the eye tracker continued to record for a further 2000ms. The trial then ended and the fixation cross reappeared for the start of the next trial.

Data Analysis: Plots of eye movement traces using x and y coordinates from eye-position data recorded every 20ms were analysed. Trials showing artefacts in the eye movement trace, such as blinks were rejected. Three dependent variables were collected from the eye-movement data: latency, amplitude error, and angular error. The latency was defined as the time at which the absolute change in eye position from the start position (calculated as: $\sqrt{(\text{latest } (x)^2 + \text{latest } (y)^2)} - \sqrt{(\text{previous } (x)^2 + \text{previous } (y)^2)}$) exceeded a threshold of 25mm. The end-point of the saccade was determined using a similar algorithm; the participant was taken to be fixating when the change in eye position over two samples remained stable (i.e. <25mm). Coordinates for x and y eye position obtained from the eye tracker were converted to obtain the amplitude and orientation of the end point and this was compared to the target position to obtain error data for these measures.

A bootstrapping resampling method with 5000 iterations was used to statistically assess the probability that the difference between the median for the sham condition and the medians for the TMS conditions at each of the sites were due to chance. Medians were used since this minimises the influence of

any outliers in the data that might otherwise have biased the results and led to the selection of inappropriate stimulation sites. This was done separately for the latency, amplitude error and angular error data. From the data, a test site was determined for each participant based on the site that appeared to be most disrupted by TMS. This was evaluated first in terms of the latency data, although error data were also taken into consideration, in particular the amplitude error. For example, if none of the sites appeared to show a significant delay due to TMS in terms of the latency data, the site with the greatest TMS effect on error was chosen. If possible the chosen site was one that was found to be significantly different from the sham condition at the 0.05 alpha level; if this was not possible then the site with the p-value closest to significance was used.

Results

The effects of right and left hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in each of the participants are shown in the graphs in Appendix 2 in terms of significant differences (1 - p-value) for the comparison of the median latency or error for real and sham TMS trials.

Table 3.1, below, provides a summary of the results, listing the sites chosen as 'test' and 'control' stimulation sites for each participant.

Participant	Right Hemisphere TMS		Left Hemisphere TMS	
	Test Site	Control Site	Test Site	Control Site
1	8	5	7	4
2	8	7	6	4
3	1	8	1	9
4	3	1	3	4
5	5	4	1	5
6	1	6	1	6
7	7	6	1	6
8	7	5	9	2
9	1	5	2	6
10	4	9	2	1

Table 3.1: Test and control sites chosen for the right and left hemisphere TMS conditions in each of the participants.

Group Effects

The frequency of sites on the left and the right hemisphere that showed a significant effect of TMS on each of three saccade metrics is shown in Figure 3.3. In total across all 10 participants statistical analyses revealed a significant effect when TMS was applied at 14 sites for latency (2 left hemisphere, 12 right hemisphere) (Figure 3.3 a), 23 sites for amplitude error (8 left, 15 right) (Figure 3.3 b) and 14 sites for angular error (6 left, 8 right) (Figure 3.3 c). Frequencies are shown as a percentage of the maximum number of times a site could possibly be found significant, which was ten since each site was tested once in each of the 10 participants.

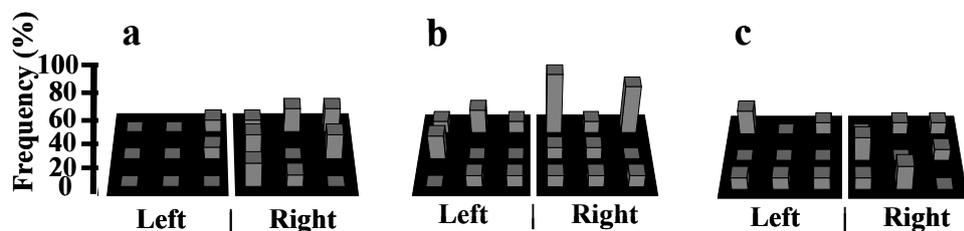


Figure 3.3: Frequency of significant TMS sites for a. latency, b. amplitude error and c. angular error collapsed across participants.

Discussion

Overall, therefore, a large number of the 18 TMS sites showed significant effects for each of the saccade metrics. However, specifically at which site TMS was found to most disrupt eye movements was not uniform across participants. In fact, a large amount of individual variability in the effects of TMS at each site was apparent. Within individual participants no one site on the left or right hemisphere was consistently found to disrupt both latency and error (amplitude or angular). The results of this experiment are presented in a paper, Ryan et al., (2006), which is included in Appendix 3.

3.4. Experiment 6: Single Saccade Target Perturbation Task

Methods

This part of the study followed a very similar procedure to Experiment 4, with the addition of TMS to a certain percentage of the trials.

Participants

The same participants took part as in Experiment 5, with the exception of participant 9 who could not be tested due to technical difficulties with the equipment.

Materials

The same experimental setup was used as in Experiment 5 described above, including the eye tracker, TMS machine, surgical hoods, computer screen for stimulus display and keyboard to record participants' responses.

Procedure

TMS: All participants were stimulated on both the right and left hemispheres (240 trials on each). This included 120 trials at the test site and

120 at the control site on each hemisphere, based on the sites determined for individuals by the localiser task. TMS was delivered on two thirds of the trials at each site, whilst the other third were no TMS trials; the stimulation type was pseudo-randomly determined by the computer on a trial-by-trial basis. For half of the TMS trials (i.e. 40 at each of the 4 stimulation sites for each participant), TMS was delivered 100ms after the target presentation, this was termed 'early' TMS. For the other half of the TMS trials, stimulation was driven by the start of the saccade; this was defined as a change in the position of the eye that was >15mm, and was termed 'late' TMS.

Oculomotor Task: This was essentially the same as that described above in Experiment 4; participants were asked to make a single reflexive saccade towards the final location of a visual target and to say whether or not they had perceived a target jump. Half of the trials involved an amplitude perturbation and half an angular perturbation. As before there were 3 perturbation sizes, small medium and large; each of these perturbation types could either be positive or negative. For 25% of the trials in each perturbation type, the perturbation size was zero, i.e. there was no target jump for a quarter of the total trials at each stimulation site. As mentioned above, there were 240 trials at each stimulation site; participants therefore completed 960 trials each in total. The order of stimulation to each of the hemispheres and to the test and control sites was counterbalanced across participants.

Data Analysis: As for the previous experiment, plots of eye movement traces using x and y coordinates from eye-position data recorded every 20ms were analysed. Trials showing artefacts in the eye movement trace, such as blinks were rejected. The end-point of the saccade was determined using an algorithm that looked for absolute changes in either the horizontal or vertical position over two samples; the participant was taken to be fixating when the change in eye position over two samples remained stable (i.e. <25mm). Coordinates for x and y eye position at the end of the saccade were converted to obtain the amplitude and direction of the saccade and this was compared to the target position to obtain error data for these measures.

Results

A large amount of the data from participant 5 had to be rejected, and therefore the data from this participant were excluded from the analysis.

Amplitude Error

Slope values were obtained for the plot of the required saccade amplitude (following the target perturbation) on the x-axis, against the actual amplitude of the saccade executed on the y-axis. A slope of 1 would thus indicate perfect compensation for the target perturbation, whereas a value less than or greater than 1 would reflect that the saccade plan was not entirely updated following the target jump. This was done for all perturbation sizes, for both the test and control site in each hemisphere for all participants.

Right Hemisphere TMS

Group mean slope values were obtained for the right-hemisphere stimulation and entered into a 3 x 2 within-subjects ANOVA, with the factors stimulation type (no, early and late TMS) and site (test and control). There was no main effect of stimulation type ($F_{(2,14)} = 0.328$, N.S.) and no significant interaction between the two factors ($F_{(2,14)} = 0.035$, N.S.). However, the main effect of site was found to be approaching significance (test site: mean = 0.821, s.d. = 0.277, control site: mean = 1.011, s.d. = 0.182; $F_{(1,7)} = 5.092$, $p = 0.059$). The results of the analysis can be seen in the graph below (Figure 3.4).

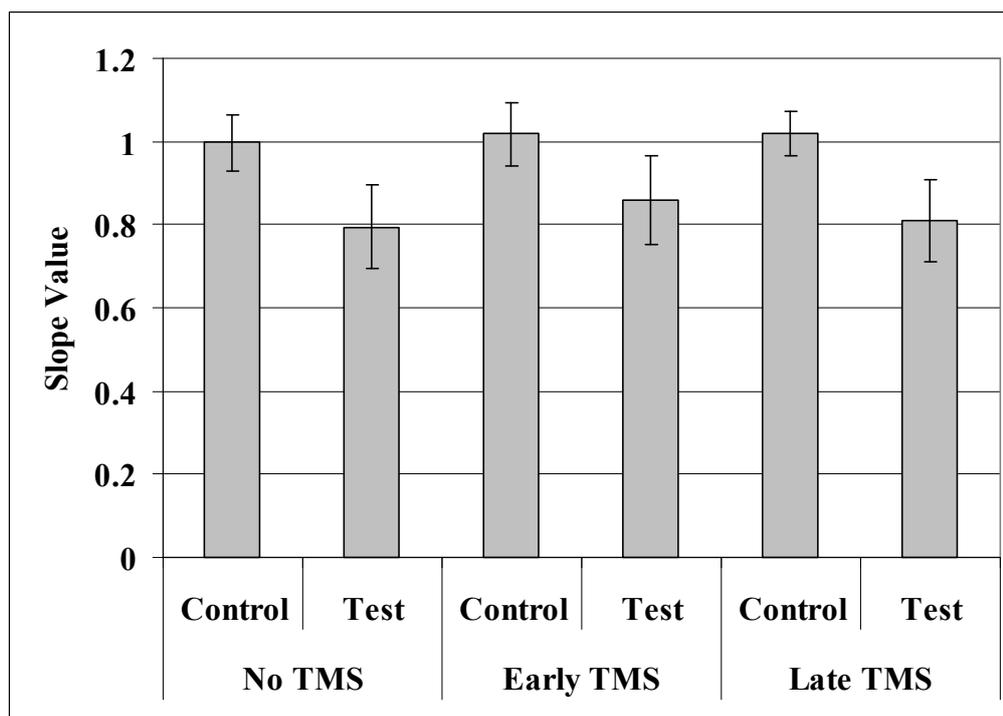


Figure 3.4: Group mean slope values for the plot of saccade amplitude required (after target perturbation) on the x-axis, against actual saccade amplitude on the y-axis, for all of the right-hemisphere stimulation conditions. Error bars show standard errors.

Left Hemisphere TMS

Group mean slope values were similarly obtained for the left-hemisphere stimulation and entered into a 3 x 2 within-subjects ANOVA, with the factors stimulation type (no, early and late TMS) and site (test and control). There was no significant main effect of stimulation type ($F_{(2,14)} = 1.305$, N.S.), no significant main effect of site ($F_{(1,7)} = 0.564$, N.S.) and no significant interaction ($F_{(2,14)} = 0.885$, N.S.). The results of the analysis can be seen in the graph below (Figure 3.5).

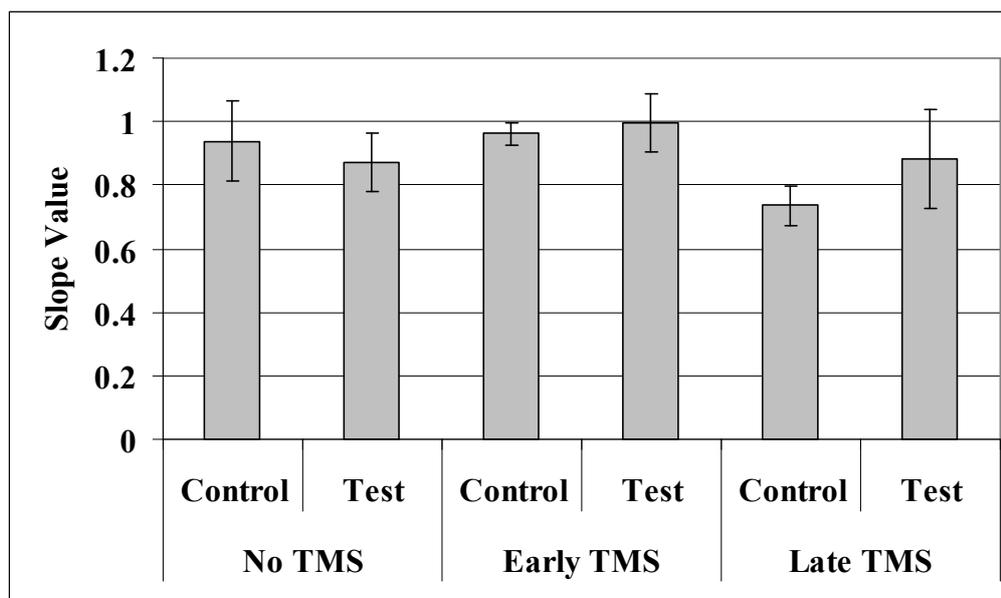


Figure 3.5: Group mean slope values for the plot of saccade amplitude required (after target perturbation) on the x-axis, against actual saccade amplitude on the y-axis, for all of the left-hemisphere stimulation conditions. Error bars show standard errors.

Angular Error

For the angular error data, slopes values were calculated for the plot of perturbation size on the x-axis against the difference between the actual angle of the saccade minus the angle required before the perturbation. A slope of 1 would thus indicate perfect compensation for the perturbation since the actual angle of the saccade minus the angle before the perturbation would be equal to the size of the perturbation. A slope value less than 1 would suggest that the saccade plan was not completely updated in terms of angular direction following the target jump. This was done for all perturbation sizes, for both the test and control site in each hemisphere for all participants.

Right Hemisphere TMS

Group mean slope values were then obtained for the right-hemisphere stimulation and entered into a 3 x 2 within-subjects ANOVA, with the factors stimulation type (no, early and late TMS) and site (test and control). There was no significant main effect of stimulation type ($F_{(2,14)} = 0.496$, N.S.), no

significant main effect of site ($F_{(1,7)} = 0.061$, N.S.) and no significant interaction ($F_{(2,14)} = 1.183$, N.S.). The results of the analysis can be seen in the graph below (Figure 3.6).

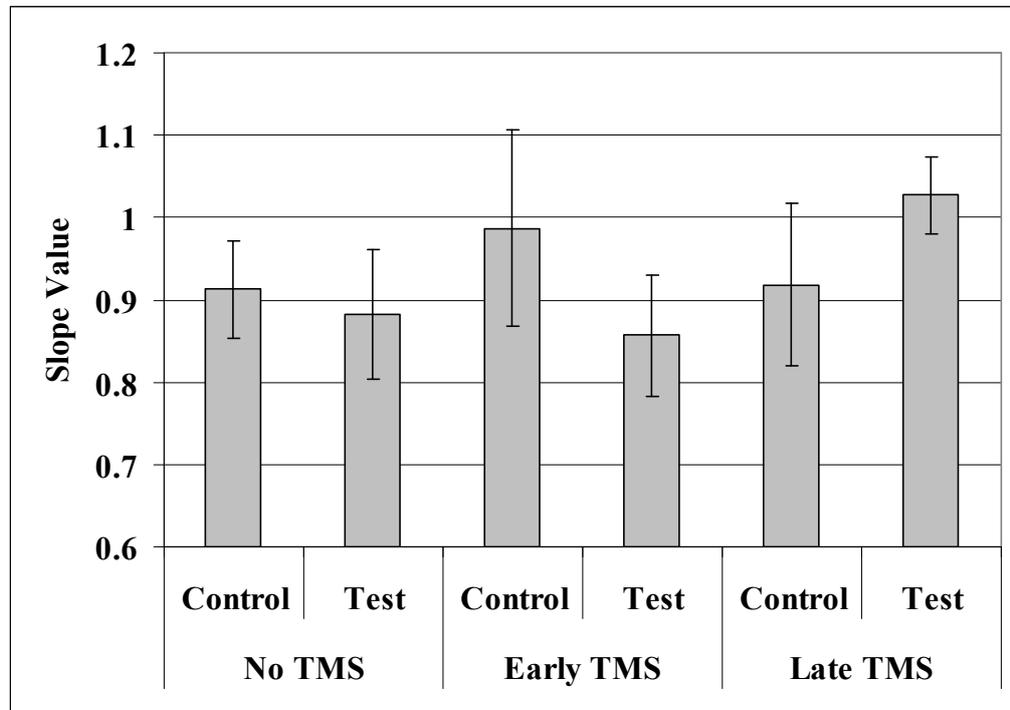


Figure 3.6: Group mean slope values for the plot of perturbation size on the x-axis against the difference between the actual angle of the saccade minus the angle required before the perturbation, for all of the right-hemisphere stimulation conditions. Error bars show standard errors.

Left Hemisphere TMS

Group mean slope values were similarly obtained for the left-hemisphere stimulation and entered into a 3 x 2 within-subjects ANOVA, with the factors stimulation type (no, early and late TMS) and site (test and control). There was no significant main effect of stimulation type ($F_{(2,14)} = 0.343$, N.S.), no significant main effect of site ($F_{(1,7)} = 0.002$, N.S.) and no significant interaction ($F_{(2,14)} = 0.012$, N.S.). The results of the analysis can be seen in the graph below (Figure 3.7).

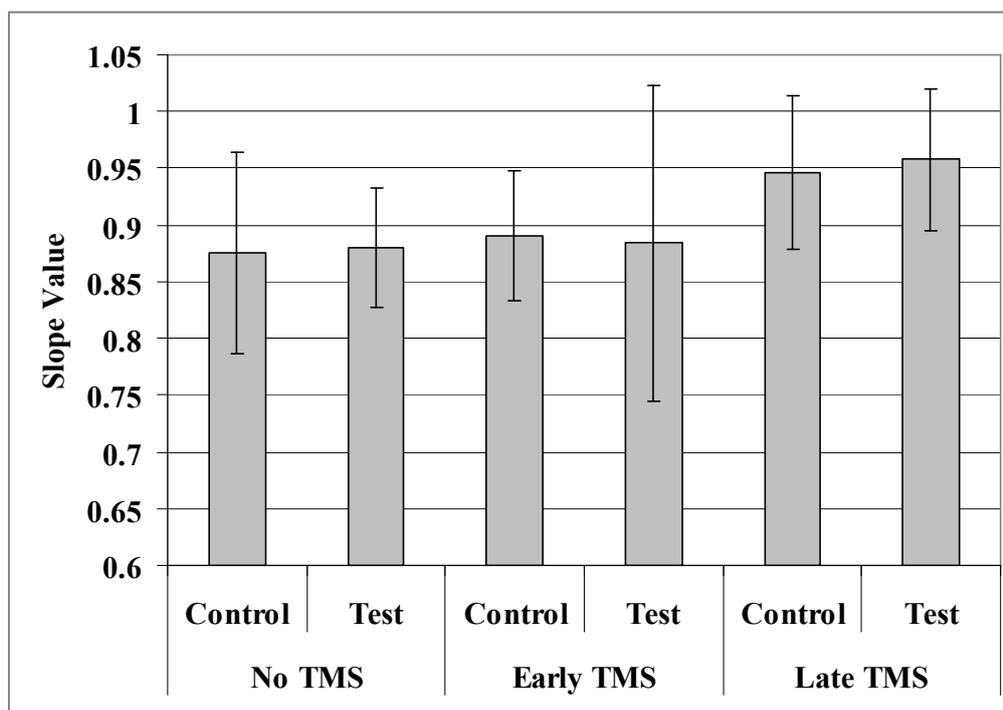


Figure 3.7: Group mean slope values for the plot of perturbation size on the x-axis against the difference between the actual angle of the saccade minus the angle required before the perturbation, for all of the right-hemisphere stimulation conditions. Error bars show standard errors.

Target Detection

In order to assess the extent to which participants had detected the target perturbations, mean percent correct scores were calculated on the basis of the responses for each of the conditions in each participant, i.e. right and left hemisphere TMS at the test site and the control site. Separate scores were calculated for the amplitude and angular perturbation trials.

Amplitude Perturbations: Right-Hemisphere TMS

Group mean percent correct scores were calculated for all conditions with right-hemisphere stimulation across participants. These were entered into a 2 x 3 within-subjects ANOVA, with the factors: site (test and control) and stimulation type (no TMS, early TMS and late TMS). No significant main effects of site ($F_{(1,7)} = 0.001$, N.S.) or stimulation type ($F_{(2,14)} = 0.298$, N.S.)

were found and no significant interaction ($F_{(2,14)} = 0.503$, N.S.). The graph below (Figure 3.8) shows the means and standard deviations for the data in this analysis.

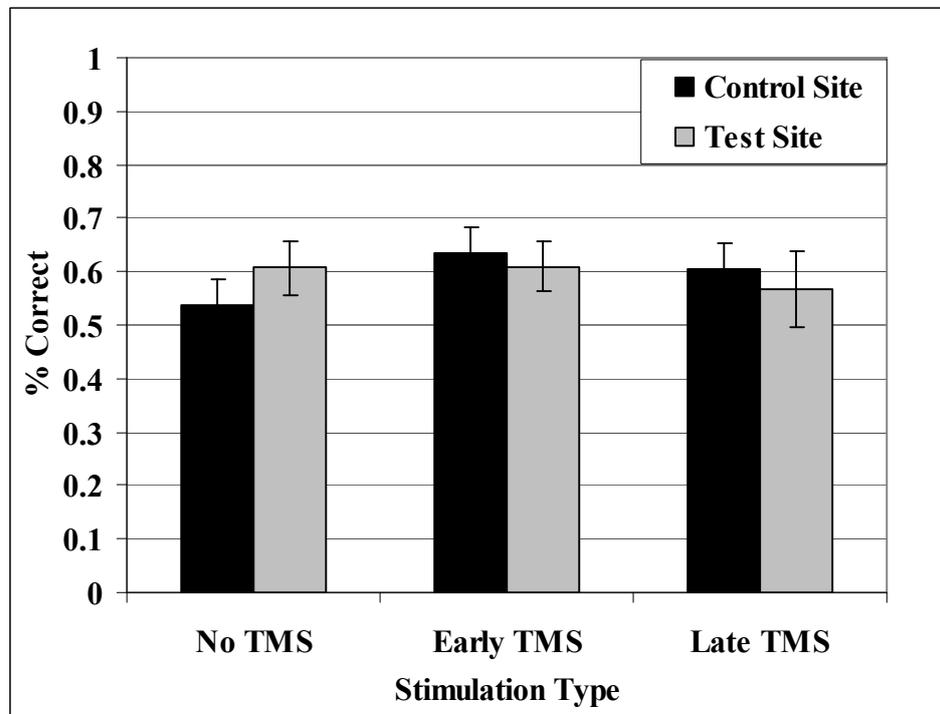


Figure 3.8: Group mean percent correct scores for the right-hemisphere stimulation conditions at the control site (black bars) and test site (grey bars) for trials with an amplitude perturbation. Error bars show standard errors.

Amplitude Perturbations: Left-Hemisphere TMS

Group mean percent correct scores were calculated for all conditions with left-hemisphere stimulation across participants. These were entered into a 2 x 3 within-subjects ANOVA, with the factors: site (test and control) and stimulation type (no TMS, early TMS and late TMS). No significant main effects of site ($F_{(1,7)} = 0.008$, N.S.) or stimulation type ($F_{(2,14)} = 0.425$, N.S.) were found and no significant interaction ($F_{(2,14)} = 3.038$, N.S.). The graph below (Figure 3.9) shows the means and standard deviations for the data in this analysis.

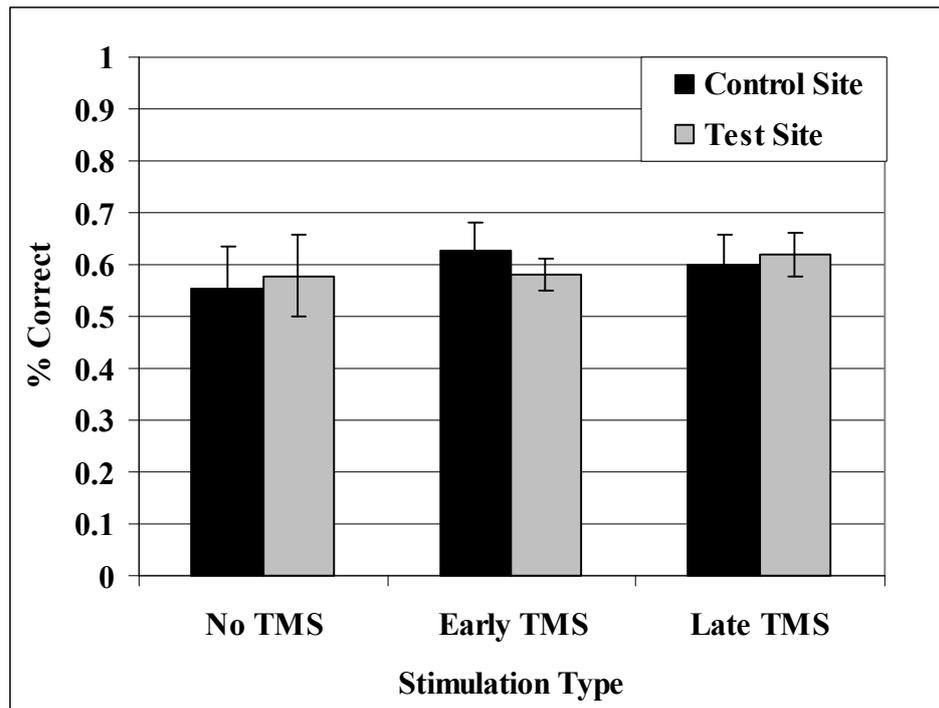


Figure 3.9: Group mean percent correct scores for the left-hemisphere stimulation conditions at the control site (black bars) and test site (grey bars) for trials with an amplitude perturbation. Error bars show standard errors.

Angular Perturbations: Right-Hemisphere TMS

Group mean percent correct scores were calculated for all conditions with right-hemisphere stimulation across participants. These were entered into a 2 x 3 within-subjects ANOVA, with the factors: site (test and control) and stimulation type (no TMS, early TMS and late TMS). No significant main effects of site ($F_{(1,7)} = 0.366$, N.S.) or stimulation type ($F_{(2,14)} = 1.077$, N.S.) were found and no significant interaction ($F_{(2,14)} = 1.223$, N.S.). The graph below (Figure 3.10) shows the means and standard deviations for the data in this analysis.

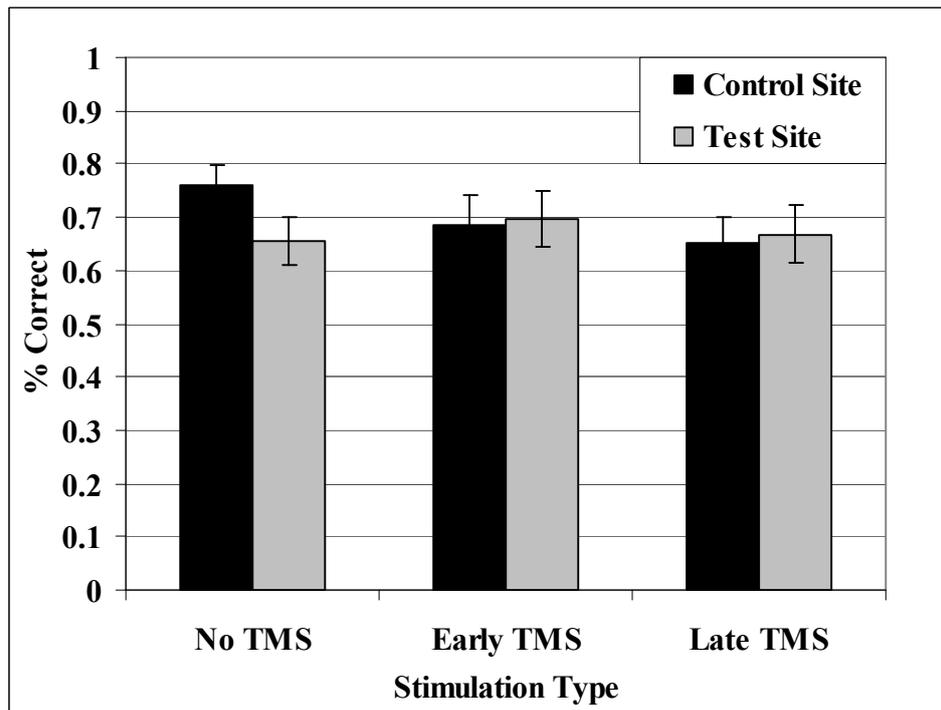


Figure 3.10: Group mean percent correct scores for the right-hemisphere stimulation conditions at the control site (black bars) and test site (grey bars) for trials with an angular perturbation. Error bars show standard errors.

Angular Perturbations: Left-Hemisphere TMS

Group mean percent correct scores were calculated for all conditions with left-hemisphere stimulation across participants. These were entered into a 2 x 3 within-subjects ANOVA, with the factors: site (test and control) and stimulation type (no TMS, early TMS and late TMS). No significant main effects of site ($F_{(1,7)} = 3.290$, N.S.) or stimulation type ($F_{(2,14)} = 1.650$, N.S.) were found and no significant interaction ($F_{(2,14)} = 0.167$, N.S.). The graph below (Figure 3.11) shows the means and standard deviations for the data in this analysis.

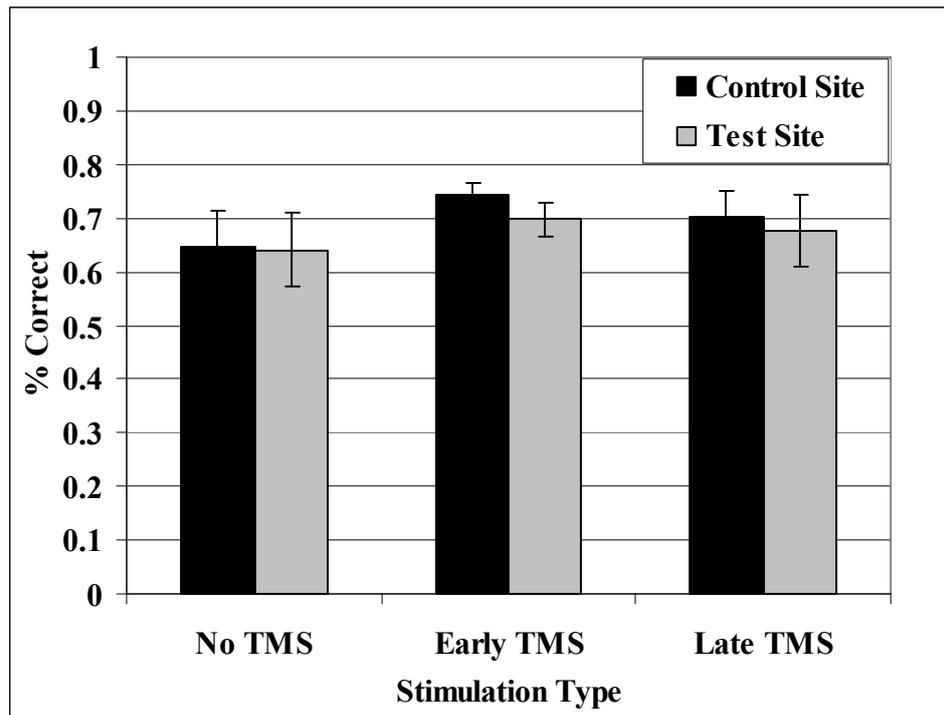


Figure 3.11: Group mean percent correct scores for the left-hemisphere stimulation conditions at the control site (black bars) and test site (grey bars) for trials with an angular perturbation. Error bars show standard errors.

Discussion

From these results, it appears that right-hemisphere TMS at the test site may be having an effect on the spatial updating of the saccade plan in response to a change in the location of the target for the saccade. The difference between the slope values for the conditions with TMS at the test site compared to the control site was shown to be approaching significance, and the direction of this difference indicated that the relationship between the amplitude of the saccade required and the amplitude executed was more discrepant when TMS was applied to the right test site. For the trials in which TMS was applied to the right control site, the slope values were around 1, suggesting perfect compensation for the target jump. However, this apparent difference between TMS at the control site and test site on the right hemisphere appeared to be true for all 3 stimulation types, i.e. early and late TMS as well as the no TMS trials. This result is unexpected and might perhaps be explained by the idea that the

effects of TMS at this site were longer-lasting than the length of a single trial, thus affecting all the trials within that experimental block. No such difference between stimulation at the test site and the control site was found for left-hemisphere TMS on the amplitude perturbation trials or for TMS to either hemisphere on the angular perturbation trials.

From the analysis of the target detection data, there was no evidence to suggest that TMS was disrupting participants' ability to detect the change in target location. Any effects of TMS can therefore not simply be explained in terms of an effect on visual perception.

3.5. General Discussion

3.5.1. Functional Localisation of PEF using TMS

An assessment of the effect of TMS on saccade metrics (latency, amplitude error and angular error) at a grid of locations over parietal cortex demonstrated a large amount of inter-individual variability in the site where TMS most affected saccades.

Interestingly, no one parietal site stood out across participants as consistently demonstrating a significant effect of TMS on any of the saccade metrics. Within participants it was also not possible to select a single site that affected all three saccadic measures.

In some participants no significant effects of real compared to sham TMS were found at any of the sites for any of the saccade metrics; a number of possible reasons could account for this. Firstly within the grid there were 2cm gaps between the stimulation sites used; although similar size grids have been used by previous studies (e.g. Terao et al., 1998) there is some evidence to suggest that the spatial resolution of TMS may be more focal than this, possibly as low as 0.5-1cm (Brasil-Neto et al., 1992). Using a grid with smaller distances between stimulation sites could potentially have revealed a site at which TMS was effective.

This study confirms the idea that it may be problematic to use a fixed scalp location on the basis of bony landmarks, such as an EEG site, for every participant. Given the individual variability demonstrated this is unlikely to be

the most effective method of determining a suitable TMS site. It may in fact be more appropriate to determine TMS sites functionally on an individual basis if possible. This idea and the results from Experiment 5 are discussed in Ryan et al., (2006) (see Appendix 3).

Another important issue to consider when using TMS is the difficulty in knowing the exact area of cortex targeted; the exact pathway taken by the current following cortical stimulation is not yet fully understood. The activation induced by TMS in terms of neuroanatomy may vary across both the area stimulated as well as across participants (Pascual-Leone et al., 1999). The results of the current study demonstrate variability in the effect of TMS when delivered to the parietal lobes. It is possible that TMS to other areas of association cortex, such as the prefrontal cortex would show a similar pattern of results; this could offer a potential explanation for inconsistent results in terms of the effectiveness of frontal TMS used clinically to treat depression (See e.g. Couturier 2005 for a review of such studies). The combination of neuropsychological tools such as functional imaging and TMS (e.g. Bestmann et al., 2004) may provide further insight into the resultant spread of activation and its associated cortical effects. This may eventually lead to a more clearly defined account of the function-anatomy relationship in this technique and prove useful in terms of optimal coil placement for investigating the functional significance of an area of cortex for a particular task.

3.5.2. The Role of the Parietal Lobe in Spatial Remapping

From the experiments in the previous Chapter, no real evidence was found to support the idea that parietal TMS is able to disrupt spatial remapping of target locations in response to an eye movement. However, there was some evidence to suggest that TMS to the PPC might be able to disrupt the representation of the spatial relationship between visual targets, such as the remembered spatial locations of the two targets in the double-step saccade task. A number of studies have proposed the idea of spatial ‘maps’ of the visual environment within the PPC; Sereno et al., (2001) for example recorded activity within this area whilst gradually altering the location of a peripheral target for a future saccade. The exact area of activity within this cortical region

was found to vary with changes in target location, leading the authors to conclude that the PEF is responsible for maintaining an up-to-date map of salient stimuli within contralateral space. However the target in their study was also the goal for a future saccade and thus it could also be argued that this apparent mapping of target location actually reflects a map of intended movement plans that represent the impending saccade (Andersen & Buneo, 2002), since this will also need to be updated following a change in target location.

In support of this idea, the experiments in this Chapter have indicated that TMS to the right PPC might be capable of disrupting the updating of a saccade plan following a target jump, either by preventing a modification to the original saccade plan or the programming of a new saccade plan. Elkington et al., (1992) have suggested that parietal TMS may induce an inability to program an upcoming saccade. They explained the delay they found in the initiation of reflexive saccades as an effect of TMS on the processing of visual information from extrastriate areas that is required to program the saccade. They suggested that the neurons in this area were refractory in the latency period as a result of recent depolarisation from the TMS, thus causing a delay in saccade onset. The effect seen in Elkington et al.'s study was found specifically for short-latency saccades that would require a faster rate of saccade programming. This explanation would therefore make sense in terms of the (re)programming required in response to a target jump, as this is likely to be a process that must be carried out rapidly. Although a refractory period induced by early TMS delivered after target presentation, i.e. following the formation of a saccade plan to the initial location of target presentation, might be able to explain a failure to modify the plan in response to a change in target location, it cannot help explain how similar effects are seen on trials with late TMS or even no TMS. It seems improbable that the refractory period would be long enough to affect more than one trial, since a single-pulse of TMS is only thought to disrupt cognitive functions for a few tens of milliseconds (Walsh & Rushworth, 1999). The current study made use of double-pulse TMS, for which the duration of the effects would be expected to be longer than for single-pulse. However, even if it were possible that the time-span of the TMS effects extended longer than a single trial, or that the double-pulse stimulation

to a particular cortical area over the experimental period induced more longer-lasting effects as can be seen with repetitive TMS (Walsh and Pascual-Leone, 2003), this would then raise the question of how new saccade plans were formed to the location of the original targets on the trials in that block. This could only be resolved if TMS induced a specific deficit in the alteration of the amplitude of a saccade plan, rather than in the generation of saccade plans per se.

In order to try and evaluate these possible explanations better, it would be useful to try and tease out the exact role of the PPC in saccade preparation and execution. Is its function, for example, the spatial representation of target locations for action, and if so are these in world-centred or body-centred coordinates, or in fact stored as a motor code for an intended movement? It might further be possible that all of these are the case, Andersen et al., (1997) have for example suggested that multiple reference frames may be in use within the PPC. In addition to its suggested role in spatial representations, there is also the question of whether the PPC is also the cortical area responsible for spatial remapping of the visual environment to update this representation following a movement of the eye or a visual change.

To try and answer these questions, it might be useful to make use of a task that dissociates the representation of a visual target location that can be stored as a motor code, from the representation of a visual target for which a saccade plan cannot usefully be formed. In the case of reflexive saccades, for example, or with the first target in the double-step saccade task, participants know at the time of presentation that they will have to make a direct saccade towards this target, and can thus plan the upcoming saccade immediately. However, if at the time of target presentation participants know that they will have to make a saccade towards the target and thus must remember its spatial location, but don't yet know the start point of that saccade, then a saccade plan for the intended movement cannot be formed in the same way. This would therefore seem less likely to encourage a coding of the target's spatial location in motor coordinates, but would presumably require some degree of spatial computation or remapping of the remembered spatial location when information on the future saccade's start point is made available.

In terms of methodology, TMS can be useful, since as discussed it can help determine the importance of a specific cortical area to a particular task; if task performance is disrupted following TMS to a particular area this leads to the assumption of its involvement in that specific task. However, in the reverse situation the conclusions that can be drawn are not quite so clear-cut. If, for example, TMS to an area is not found to result in a deficit in performance on a task, the most obvious conclusion would be that this area is not involved in this particular behaviour. However, this cannot be concluded definitively, since the failure to disrupt the process of interest might instead be due to technical issues such as the exact time of TMS delivery in relation to the task or the precise scalp location used for coil placement. The timing issue could perhaps to some extent be resolved through the use of repetitive TMS (Walsh and Pascual-Leone, 2003), but the site of stimulation is more problematic. Even if, for example, the appropriate site for coil placement could be accurately determined on one hemisphere, this still leaves the possibility that the particular process might be controlled bilaterally, so that if TMS was delivered to one hemisphere, the other side could compensate for this. As a result of these issues, it seems that it might therefore be useful to explore alternative methods of assessing remapping processes.

3.5.3. Task-Dependent Nature of Saccade Planning

From the results of the experiments discussed in this Chapter and the previous one, there is some evidence to suggest that saccade planning may not be a uniform process but may in fact vary according to the exact nature of the task. In the task in Experiment 6, for example, single saccades can be fully programmed as a motor code at the time of target presentation and it seems that TMS might be capable of preventing modifications to this motor code in response to a perceptual change. In the memory-guided double-step saccade task (Experiment 3), however, in order to be accurate, the spatial representation of target 2 must be updated following the execution of saccade 1, thus the motor program cannot be fully programmed in advance. However, the use of concurrent target presentation in this particular version of the task, as mentioned before, might have inadvertently encouraged encoding of the targets

in relation to each other and thus the planning of a complete double-step sequence that could in theory be executed without any of the expected spatial remapping. If in this case TMS was disrupting this ‘completed’ plan for the second saccade, this would then perhaps require reprogramming based on a memory of the target’s spatial location. Since this would occur after the start of the first saccade, its metrics might now be taken into account, or possibly a spatial representation of the target location encoded in a retinotopic rather than object-based frame of reference might be used. Either of these could potentially account for the greater amount of compensation observed.

So in Experiment 3, it was argued that TMS may cause saccade 2 to be reprogrammed, possibly using a different frame of reference for the target location, or at least an updated spatial representation. In Experiment 6, in contrast, it is argued that TMS is instead preventing reprogramming of the saccade. This difference might be related to the reason for the reprogramming in each case, i.e. in one it is in response to an action (the movement of the eye), whereas in the other it is due to a perceptual change (the target jump). From the detection data, it can be seen that this target jump was noticed by participants; it might therefore be that the ability to make use of this visual information was affected by TMS.

If, as suggested from the studies in this and the previous Chapter, saccade planning can be affected by task specifications, then it would be interesting to note how spatial remapping, in terms of compensation measures, is altered by changes to the task. For example, it would be predicted that if the simultaneous presentation of the targets was responsible for a reduced level of compensation in Experiment 3, then compensation should be higher if the targets were instead presented successively. This issue will be addressed in Experiment 7 of the next Chapter.

3.6. Conclusions

The experiments discussed so far in Chapters 2 and 3 have attempted to shed light on the role of the posterior parietal cortex, and in particular the parietal eye fields, in the preparation and execution of saccades, through the use of TMS. More specifically they have investigated the contribution of this

area to the spatial remapping process, both in terms of updating saccade plans to remembered visual targets following an eye movement and also in relation to the updating of a saccade plan following a change in the location of a visual stimulus. While the results so far have indicated that parietal TMS may have some effect on the tasks used, the exact nature of this effect still remains unclear. The problems associated with the use of a tool such as TMS have been considered and on the basis of these, in the experiments discussed in the next chapter, behavioural methods are instead employed in the attempt to investigate saccade-related spatial remapping processes and in particular how these are affected by the nature of the task.

Chapter 4: Behavioural Studies Investigating the Task-Dependent Nature of Saccade Planning and Spatial Remapping

4.1. Introduction

Previous studies have suggested that all saccades in a sequence are planned ahead of execution, although modifications to these plans are possible in response to execution errors (Zingale & Kowler, 1987). Further to this, single-unit recording studies in monkeys have demonstrated that neurons in the lateral intraparietal area (LIP) show predictive remapping based on motor intention, i.e. they are able to anticipate the ‘retinal consequences’ of a saccade (Duhamel et al., 1992). This is illustrated by their responsiveness to stimuli in the region of the future receptive field rather than the current one. It has since been shown that prior to the execution of a saccade sequence attention is allocated in parallel to all saccade goals, although more so to the first goal in the saccade sequence than to subsequent ones (Godijn & Theeuwes, 2003).

In certain situations, however, such processes may not be possible, for example when a prospective motor code for an intended movement cannot be formulated. The studies discussed in this chapter will compare saccade planning under conditions when participants are either able to plan a saccade to the location of a visual target on the basis of a retinal vector, to that when they are unable to do so and instead have to rely on information about the spatial location of the target coded in a non-retinotopic frame of reference.

By dissociating conditions when the location of the visual target can be stored as a motor code from those in which it cannot, it is hoped that these studies will provide further insight into how saccades are planned, specifically in terms of how targets for future saccade plans are encoded and the spatial computations that might then be needed to make use of this stored information. In the previous two chapters, attempts were made to disrupt the spatial computations used to alter the stored representations of target locations using TMS. The exact nature of these representations and how their coding might be affected by task requirements is not well understood. In Chapter 2, it was suggested that by displaying two targets concurrently, the use of an object-based frame of reference, in which the target locations were encoded in relation

to each other, might have been encouraged. For the double-step saccade study (Experiment 3), both saccades could be provisionally planned at the time of target presentation. The studies in this Chapter aim to investigate how motor intention, i.e. the action you intend to perform, might affect target encoding, e.g. whether you intend to make a saccade directly towards the target location from the current fixation, or whether you intend to make a saccade from an as yet unknown starting point. This difference in terms of the way the saccade sequences can be planned will be created by means of manipulating the order of target presentation.

The effects of this will be assessed behaviourally in terms of saccade metrics through the use of an eye-tracking paradigm. A difference in processing time might, for example, reflect the use of different strategies in terms of encoding targets for planning the saccades, which might also indicate the use of different oculomotor areas. Similar such findings have been seen previously in relation to the use of different representational codes for remembering target locations (Curtis et al., 2004).

The results of Experiment 3 led to the suggestion that saccade planning, and the spatial remapping of target locations, might be affected by the nature of the task. If, as argued in Chapter 2, less spatial remapping occurred to compensate for error in saccade 2 due to the concurrent presentation of the targets, then it would be expected that a sequential target presentation would lead to greater overall levels of spatial remapping. Other factors that make it harder for the targets to be encoded in relation to each another might also be expected to result in increased compensation e.g. a greater temporal gap between the targets, or the introduction of a third behaviourally-relevant target presented in between the targets for saccades 1 and 2. The extent of spatial remapping will be considered in the experiments in this Chapter, and will be quantified in terms of amplitude and angular compensation measures.

4.2. Experiment 7: Reverse Double-Step Saccade Study

The current study uses a memory-guided version of the double-step saccade task and compares the situation where participants are able to plan both saccades at the time of target presentation, to that when they must

maintain a memory of the spatial location of the first target seen for later use in a saccade plan (i.e. after target 2 presentation). This is achieved through adapting the paradigm so that in a 'forward' condition two targets are displayed successively in the same order as required in the memory-guided saccade sequence. In a 'reverse' condition, in contrast, the order is reversed so that participants are first presented with the target they will look to second, followed by the target for the first saccade. In this case they cannot plan an appropriate saccade to target 1 at the time of presentation, since they will not know the start point for this movement until target 2 is displayed.

It is of course possible that in the forward condition, target locations rather than saccade plans will be held in memory. Results from single-unit recording studies in monkeys, however, have suggested that although neurons in LIP do carry a signal coding a memory of the location of a sensory stimulus, this appears to be of lower prominence at the population level, than a signal of the intended movement plan (Mazzoni et al., 1996). They showed, through the use of memory-guided double-saccade experiments, that the delay period activity of the majority of LIP neurons represents the next planned saccade, and that neurons only begin to encode a new saccadic movement after the current motor plan has been executed. Xing and Andersen (2000) refer to this characteristic as the 'single-purpose' feature.

Behavioural differences between the forward and reverse conditions will be assessed by recording memory-guided eye movements and comparing a number of parameters, including saccadic latency, intersaccadic intervals mean endpoint accuracy and measures of amplitude and angular compensation.

It is predicted that latency will be increased in the reverse condition when the planning of the saccade sequence is not as straightforward. By making use of information on the end-point of an impending saccade, it is thought that participants may be able to plan a sequence of two successive saccades at an earlier point in the forward condition compared to the reverse condition. The spatial computations in the reverse condition would therefore be expected to be more complicated, since the second saccade must be planned to a remembered target location, rather than to one that is visually available at the time of programming.

The intersaccadic interval, that is the fixation time between the end of the first saccade and the start of the second saccade, will also be considered. It is possible that this variable might be indicative of the extent of processing that occurs immediately before initiation of the second saccade. The more spatial updating taking place at this point to account for any error in saccade one, the longer this might be expected to be. If on the other hand the entire saccade sequence is planned in advance and executed in full without modification, then it might be expected that this would be shorter.

This measure is therefore linked to another parameter, compensation, which looks at how the kinematics of the second movement compensate for any errors made in the first movement; this would therefore require updating rather than the unaltered execution of the whole pre-planned sequence.

In order to assess compensation it is first necessary to evaluate the end-point error of both the first and second saccades. When a memory-guided sequence of saccades is performed, error has been shown to increase with each successive saccade (Bock et al., 1995), it would thus be expected that accuracy for the second saccade would be worse than for the first. However previous studies have not looked at the effect of manipulating the order of target presentation on this parameter, so it is not known whether or not this will be true for both conditions. Viewing the second target first could improve saccade accuracy due to a primacy effect, i.e. no other targets are being held in memory when it is seen. Alternatively, because this target is not visible at the time of saccade planning but instead has to be held in memory, its accuracy might be worse due to degradation of the memory trace for this target location, i.e. a recency effect. The possibility of interacting influences of potential error accumulation and serial position effects will therefore be taken into account when considering the effect of target presentation order on saccadic accuracy.

Methods

Participants

Twelve healthy participants (7 females); aged 22-29 (mean 25 years) took part in this task. All had normal or corrected to normal vision.

Materials

Eye tracking was performed using the same method as described in Experiment 1 (Chapter 2). Stimuli were displayed using a 20in Dell Trinitron Monitor with a spatial resolution of 800 x 600 pixels at a frame rate of 100Hz and a viewing distance of 55cm. Stimuli were generated using the MATLAB (The MathWorks) CRS (Cambridge Research Systems) Toolbox. These stimuli consisted of a black central fixation cross, a circular black target of 6mm diameter (0.63deg) and a circular white target of the same size. A speaker was used to play auditory beeps and the study was carried out in a darkened room.

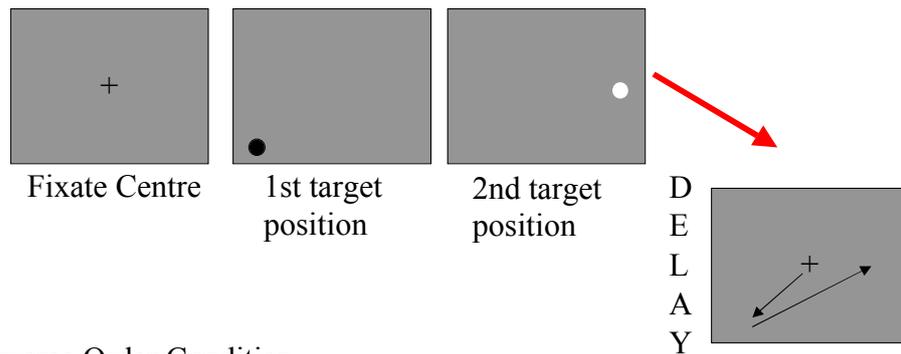
Procedure

Oculomotor Task: Participants were required to make a sequence of two memory-guided saccades towards the remembered locations of two visually presented targets. A black fixation cross was displayed on a grey background, which signified the start of each trial. This remained on until the eye-tracker determined that the participant was correctly fixating the cross, i.e. the pupil was directed to a region of the screen 15mm (1.56deg) around the centre. The first target was then presented and remained on for 1000ms, after which it was extinguished and the screen went blank for 500ms. The second target was shown, also for a duration of 1000ms, followed by a blank display for a period of 500ms. Participants were instructed to remain fixating centrally throughout the target presentation and during the ensuing delay period. This delay period had a variable duration based on a normal distribution (mean = 500ms, s.d. = 125ms) and was followed by an auditory beep (duration = 150ms). The beep was the cue for participants to start the saccades; using a variable delay served to ensure that the go-signal was not temporally predictable, which might have led to anticipatory saccades. The eye tracker continued to record for a further 3000ms to allow participants enough time to complete both saccades. The trial then ended and the fixation cross reappeared for the start of the next trial.

The screen was split into quadrants (top and bottom, left and right) and targets could appear at nine possible locations within each of these areas (see Experiment 3, Chapter 2 for details). On each trial an index of the 9 possible target positions for the pre-specified quadrants for targets 1 and 2 was shuffled, and target positions pseudo-randomly selected by the computer. The order in which the targets appeared in each quadrant was counterbalanced across the trials, so that on half the trials targets appeared in the same hemifield (i.e. both left or both right) and in the other half they were in different hemifields. Within these same/ different hemifield conditions, whether the targets appeared in the top or bottom quadrant first (same hemifield condition, i.e. both on the left, or both on the right) or in the left or right quadrant first (different hemifield condition, i.e. both bottom or both top), was also counterbalanced. On half the trials the black target was shown first followed by the white, and vice versa for the other half; there were therefore 16 possible trial order combinations.

At the start of each experimental session half the participants were instructed to look towards the black target 1st and the white target 2nd, regardless of the order the targets appeared in, and vice versa for the other half of the participants. Half of the trials were therefore ‘forward’ order, i.e. participants saw the targets in the order in which they had to look to them, and half of the trials were ‘reverse’ order (see Figure 4.1). Participants completed 80 trials in total.

Forward Order Condition



Reverse Order Condition

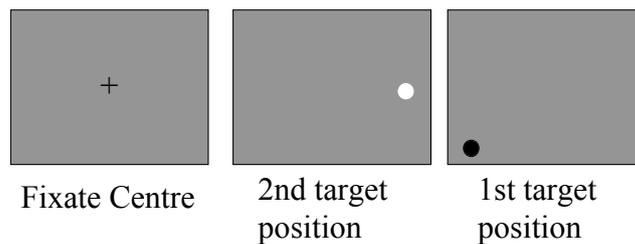


Figure 4.1: Comparison of forward and reverse order conditions, for a participant instructed to look at the black target first and the white target second.

Data Analysis: Plots of eye movement traces using x and y coordinates from eye-position data recorded every 20ms were analysed (see Figure 4.2). Trials in which participants started the eye movement before the go-signal, or looked to targets in the wrong order, were rejected. The fixation end-point of each saccade, latency and ISI was determined using an algorithm that calculated the absolute change in eye position for every sample recorded by the eye tracker (see Experiment 3, Chapter 2 for details). Coordinates for x and y eye position obtained from the eye tracker were compared with the x and y coordinates for the target positions to calculate a measure of error, using the following equations:

$$\text{Saccade 1 Error} = \sqrt{[(x(\text{target 1}) - x(\text{fixation 1}))^2 + (y(\text{target 1}) - (-y(\text{fixation 1})))^2]}$$

$$\text{Saccade 2 Error} = \sqrt{[(x(\text{target 2}) - x(\text{fixation 2}))^2 + (y(\text{target 2}) - (-y(\text{fixation 2})))^2]}$$

This gives an error value in terms of distance (in mm) of the fixation location from the target position.

An example trial showing the x and y position of the eye over time can be seen in Figure 4.2, below.

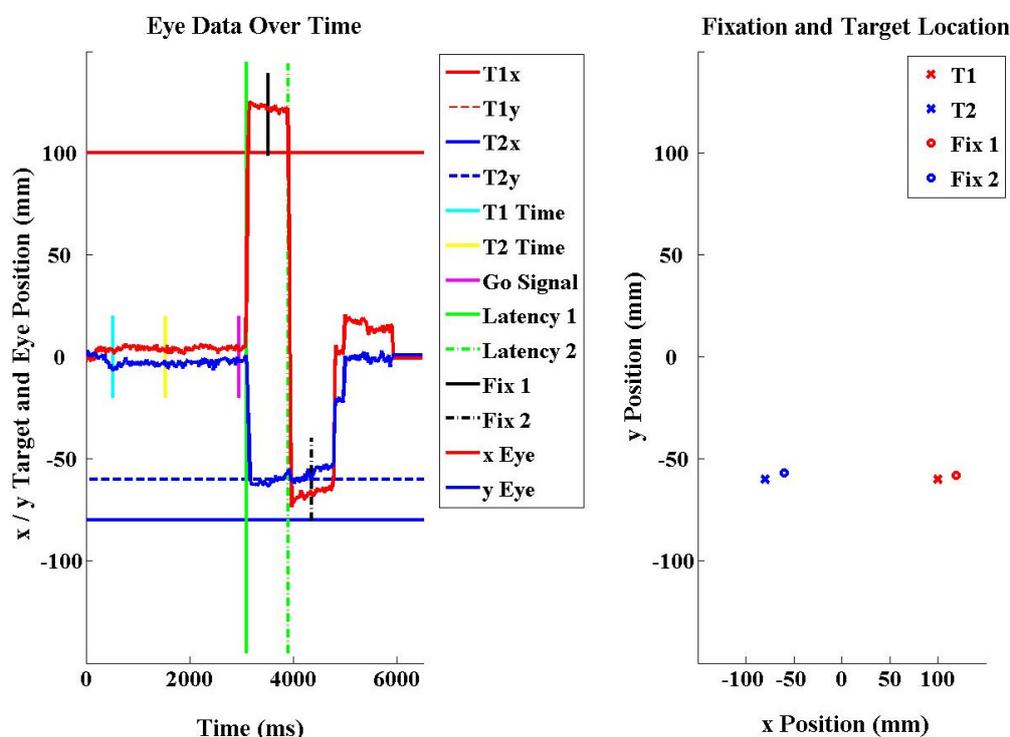


Figure 4.2.: Plot of eye movement trace using x (red trace) and y (blue trace) coordinates from eye-position data for one trial (left plot). The horizontal red bars represent the x (solid) and y (dashed) location of target 1, and the horizontal blue bars those of target 2 (solid, x; dashed, y). The participant can be seen to be fixating the centre of the screen (0 on y axis) until the go-signal (vertical pink bar). The two saccades and fixations can be seen clearly following the go-signal, after which the participant looks back to the centre of the screen. The times of the two target presentations are shown as light blue (target 1) and yellow (target 2) vertical bars. The vertical green bars show the latency of the 2 saccades, and the black vertical bars show the end-point fixations (solid, saccade 1; dashed, saccade 2). The end point of these saccades is also plotted in relation to the target positions on the screen (right plot).

The compensatory amplitude gain of the second saccade was calculated using a method similar to that set out in van Donkelaar and Müri (2002); it was determined by dividing the amplitude of the second saccade by the amplitude required given the fixation location of the first saccade. A value of 1 would therefore indicate perfect compensation for the error in saccade 1, whereas a value of less than or greater than 1 would indicate imperfect compensation (i.e.

as if the saccade was executed exactly as pre-planned with little or no modifications). As discussed in Experiment 3 (Chapter 2), compensatory angular gain would be problematic as a measure, thus instead, the absolute difference between the angle of saccade 2 and the angle required given error in saccade 1 was calculated for all trials in each condition for every participant.

Results

Results from two of the participants were excluded since a large number of trials had to be rejected during the data analysis stage. All remaining participants had a minimum of 12 trials per conditions (mean = 23.4).

Latency: A two-tailed paired sample Student's t-Test was used to assess for a difference in latency between the forward and reverse conditions. A significant difference was found, with the mean for the reverse condition being greater than for the forward (forward: mean = 245.45ms, s.d. = 83.79ms); reverse: mean = 300.96ms, s.d. = 96.22ms), $t_{(9)} = 4.865$, $p < 0.001$).

Intersaccadic Interval: A two-tailed paired-sample Student's t-Test failed to reveal a significant difference between the intersaccadic intervals for the two conditions (forward: mean = 943.25ms, s.d. = 164.69ms; reverse: mean = 931.78ms, s.d. = 152.92ms; $t_{(9)} = 0.988$, N.S.).

End-Point Error: A 2x2 within-subjects ANOVA with the factors order (forward and reverse) and saccade (saccades 1 and 2) was used to assess end-point error in the two conditions. A significant main effect of saccade ($F_{(1,9)} = 8.987$, $p < 0.05$) was found, however there was no main effect of order ($F_{(1,9)} = 2.975$, N.S.) and the interaction between these factors was not found to be significant ($F_{(1,9)} = 0.844$, N.S.). The graph below (Figure 4.3) shows the end-point error values for saccades 1 and 2 in the forward and reverse conditions.

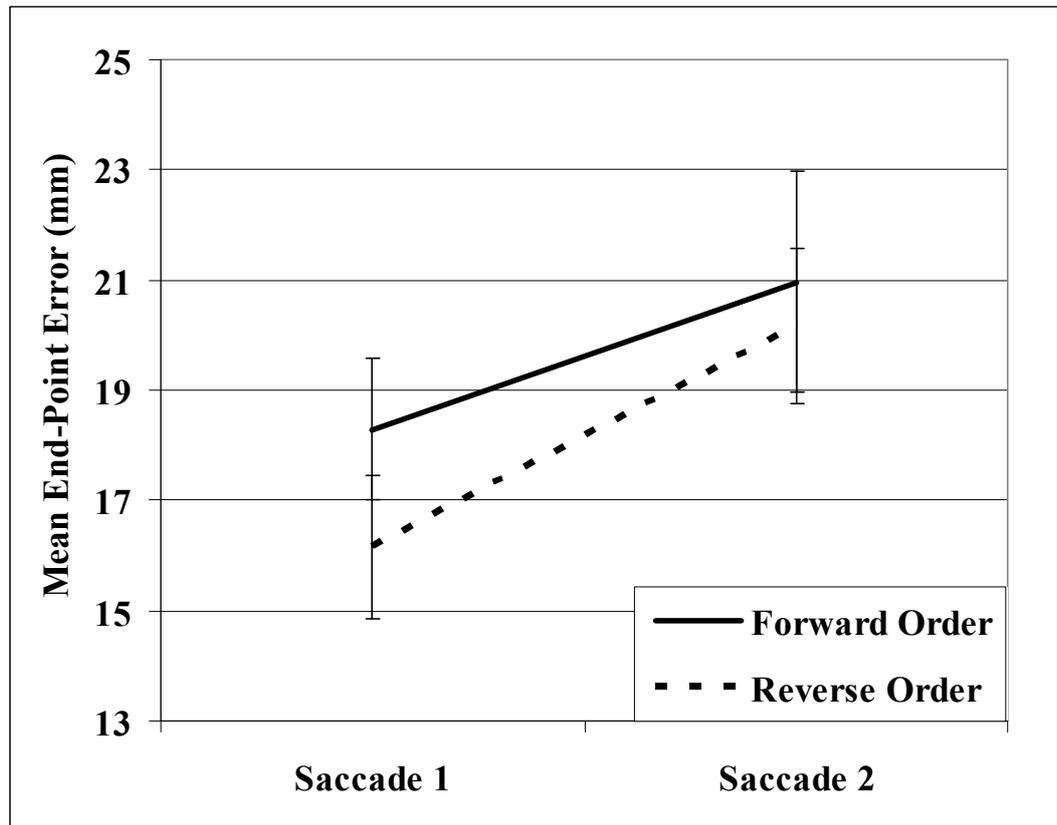


Figure 4.3: Group mean end point error for the first and second saccade in both the forward order (solid line) and reverse (dashed line) conditions. Error bars show standard errors.

It can be seen from this graph, that the main effect of saccade reflects an overall greater end-point error for saccade 2 than for saccade 1.

Amplitude and Angular Compensation Measures: A two-tailed paired-sample Student's t-Test failed to show a significant difference between the compensatory amplitude gain for the forward and reverse conditions (forward: mean = 0.98, s.d. = 0.07; reverse: mean = 0.99, s.d. = 0.06; $t_{(9)} = 1.240$, N.S.). Similarly, no significant difference was seen using a two-tailed paired-sample Student's t-Test to compare the mean angular difference (between angle required given error in saccade 1 and the angle executed) for the forward and reverse conditions (forward: mean = 4.24°, s.d. = 1.30°; reverse: mean = 4.54°, s.d. = 1.43°; $t_{(9)} = 0.843$, N.S.).

Discussion

The important finding from this task is that of a difference in latency between the forward and reverse orders of target presentation. This suggests that more complicated spatial computations may be required in the reverse compared to the forward order, thus leading to the need for increased processing time and hence a slower latency.

It would be interesting to investigate whether the observed difference in latency might also indicate the use of different brain areas to complete the task under the different conditions. Support for this idea comes from an imaging study by Curtis et al., (2004), in which delay-period activity in a memory-guided saccade task was assessed. They found that the role played by the different areas involved in oculomotor control was dependent upon whether or not a prospective motor code for an intended movement could be made. The authors state that when a saccade plan can be made in advance this is maintained by the FEF and when this is not possible the IPS instead maintains a retrospective spatial code for the retinotopic location of the stimulus. However, their study only used single saccades, whereas the double saccades used in the current study might mean that parietal activity would be expected in both conditions, since presumably both would require some degree of spatial computation. It might be possible that additional brain areas are required in the reverse order condition compared to the forward, since the saccade sequence cannot be planned as straightforwardly. Alternatively there might be greater activity in a common cortical area (such as the PEF) for the reverse condition compared to the forward order condition.

Although order of target presentation was not found to significantly affect end-point accuracy overall, from looking at the graph, end-point error seems to be generally worse in the forward than reverse order condition. This seems to be particularly the case for the first saccade and less so for saccade 2. This might be explained by fact that in the reverse condition, target 1 has just recently been viewed when the first saccade is executed, whereas in the forward condition another target is presented between viewing the first target and executing the saccade towards it, i.e. a recency effect.

If this idea of a recency effect was continued, it might be expected that end-point error for saccade 2 should be worse in the reverse order condition given that more time has passed since the target for this saccade was viewed than in the forward order condition. However, recency, or time since encoding, may not be the only factor that affects end-point error, it is possible for example that the number of targets held in memory might also be important, i.e. a primacy effect. The number of saccades executed between encoding the target and executing a saccade towards it might also have an effect on error, since the remembered target location would have to be remapped in response to these saccades and error might accumulate due to inaccuracies in this process.

As expected from the literature, end-point error for the second saccade was found to be worse overall than for saccade 1. From looking at the graph however, it seems that this is more apparent in the reverse order condition compared to the forward order. This result might be due to the influence of the recency effects discussed above.

From these results therefore, it is difficult to conclude whether end-point error is most affected by the order of target presentation, and thus a factor related to target encoding, either in terms of primacy (number of targets held in memory at time of encoding) or recency (number of targets viewed since encoding), or whether it is most affected by order of execution, i.e. an effect of error accumulation from a failure to compensate for inaccuracies in execution, or due to the greater spatial remapping required. These issues could be investigated through a follow-up study using three targets instead of two. This would provide six possible orders of target presentation, and the effect of this variable on end-point error (as well as the other saccade metrics considered in this study) could therefore be assessed. If the first and the last target encoded for use in a sequence of saccades are 'special' to some extent due to memory effects such as primacy or recency, then this might become more apparent when three targets are used.

In terms of compensation, the differences for forward and reverse orders of target presentation were not significant. Of interest, however in relation to this measure, is the fact that the extent of spatial remapping in this experiment (for both forward and reverse order trials) appears in general to be higher than in Experiment 3. In Experiment 3, compensatory amplitude gain (for the no

TMS trials only) had a mean of 0.95 (s.d. = 0.06) and the angular compensation measure had a mean of 6.93° (s.d. = 1.37°). In the current experiment, the mean compensatory amplitude gain (across both conditions) was 0.98 (s.d. = 0.06) and the angular compensation had a mean of 4.39° (s.d. = 1.25°), where greater compensation is reflected by a higher compensatory amplitude gain value, and a lower value for the angular compensation measure.

Given this apparent increase in the extent of spatial remapping when the two targets for a double-step saccade sequence are presented sequentially as opposed to simultaneously, it might be expected that other experimental manipulations that similarly make it harder for the targets to be coded in relation to each other would augment this effect. One such factor that might be expected to do this would be the addition of a greater temporal delay between the two targets. The next experiment will thus investigate whether saccade planning and the spatial remapping of target locations can be affected by this particular aspect of the task.

4.3. Experiment 8: Effect of Variable Delays on the Reverse Double-Step Saccade Study

Since a comparison of compensation measures in Experiments 3 and 7 indicates that spatial remapping in saccade planning may be affected by task specifications such as target presentation, it was decided that other factors that might also potentially influence this process should also be investigated. Going from simultaneous target presentation in Experiment 3, to successive presentation in Experiment 7, was equivalent to introducing a gap of 500ms between the target presentations. It was argued that this was enough to discourage to some extent the encoding of the targets in relation to one another, and thus result in a higher level of spatial remapping. Linearly increasing this temporal gap might therefore be expected to result in a linear increase in compensation, since it might become progressively harder to code the targets with respect to each other.

With greater time to plan the saccade sequence, it is possible that the latency difference between the forward and reverse order trials might become

less apparent, since there will be sufficient time to plan both despite the differing complexity. End-point error might also be expected to be affected, possibly in terms of memory-related factors such as the increase in time since viewing the target.

Methods

Participants

Twelve healthy participants (10 females); aged 22-29 (mean 24.8 years) took part in this task. Five of these had previously taken part in Experiment 7. All had normal or corrected to normal vision.

Materials

Eye tracking was performed using the same setup as described in Experiment 1 (Chapter 2). Stimuli were displayed using a 20in Dell Trinitron Monitor with a spatial resolution of 800 x 600 pixels at a frame rate of 100Hz and a viewing distance of 55cm. Stimuli were generated using Cogent Graphics (developed by John Romaya at the Laboratory of Neurobiology, Wellcome Department of Imaging Neuroscience, UCL, UK) implemented in MATLAB (The MathWorks). These stimuli consisted of a black central fixation cross, a circular black target of 6mm diameter (0.63deg) and a circular white target of the same size. A speaker was used to play auditory beeps, and the study was carried out in a darkened room.

Procedure

Oculomotor Task: This was essentially the same as that described in Experiment 7, the major difference being in terms of the length of the delay durations following each target presentation. Whereas in Experiment 7, the delay periods following target 1 and 2 presentation lasted for 500ms, in this experiment the duration was varied. There were four possible delay durations in total: 1000ms, 2000ms, 3000ms and 4000ms. The delay duration was

pseudo-randomly selected by the computer on a trial-by-trial basis, so that over the whole experiment there was an equal number of trials for each. The length of target presentation was the same as in Experiment 7 (1000ms). The duration of the delay following target 2 was always the same as that following target 1, although as before there was an additional delay of a variable duration (based on a normal distribution with a mean = 500ms and s.d. = 125ms). As before, this was followed by an auditory beep (duration = 150ms), which was the cue for participants to start the saccades. The eye tracker continued to record for a further 8000ms to allow participants enough time to complete both saccades. The trial then ended and the fixation cross reappeared for the start of the next trial.

Stimulus presentation and the instructions to participants were identical to that described in the Reverse Double-Step Saccade Study (Experiment 7). Participants completed 256 trials in total over two 128-trial sessions.

Data Analysis: As in the double-step saccade study, plots of eye movement traces using x and y coordinates from eye-position data recorded every 20ms were analysed. Trials in which participants started the eye movement before the go-signal, or looked to targets in the wrong order, were rejected. The fixation end-points, latencies and ISIs were determined using an algorithm, in the same way as for the double-step saccade experiment (see Experiment 3, Chapter 2 for details). Coordinates for x and y eye position obtained from the eye tracker were compared with the x and y coordinates for the target locations to calculate a measure of error, using the same equations as in Experiment 7. From this, error values in terms of distance (in mm) of the fixation locations from the target positions were obtained. Measures of compensation in terms of amplitude and angle of the second saccade were calculated using the same method as described in Experiment 7.

Results

Latency: A 4 x 2 within-subjects ANOVA, with the factors delay (1000ms, 2000ms, 3000ms and 4000ms) and order (forward and reverse) was

used to analyse the group mean latency data for each condition in this task. No significant main effect of delay was seen ($F_{(3,33)} = 0.552$, N.S.), and neither was a significant delay x order interaction ($F_{(3,33)} = 0.690$, N.S.). The main effect of order was however found to be approaching significance ($F_{(1,11)} = 4.665$, $p = 0.054$), this was in the direction expected, i.e. latency was greater for the reverse than the forward order condition (forward: mean = 290.09ms, s.d. = 82.76ms; reverse: mean = 303.34ms, s.d. = 90.67ms).. The data for these analyses are shown below in Figure 4.4.

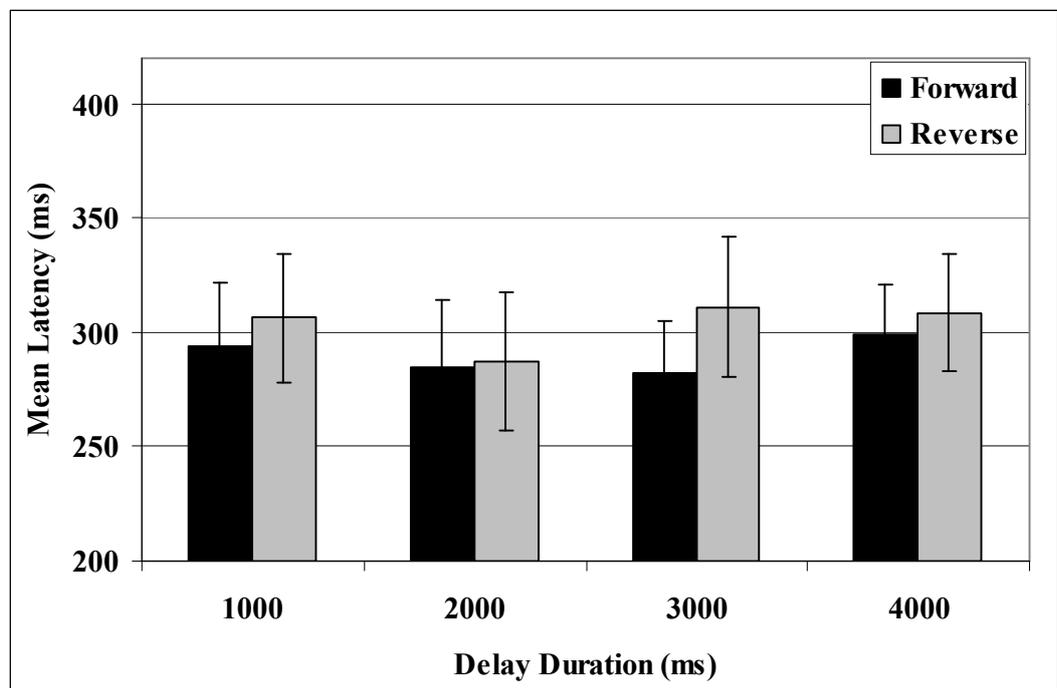


Figure 4.4: Group mean latency (in ms) for the forward and reverse trials with each of the four delay durations. Error bars show standard errors.

Intersaccadic Interval: Group mean ISI data was analysed using a 4 x 2 within-subjects ANOVA, with the factors delay (1000ms, 2000ms, 3000ms and 4000ms) and order (forward and reverse). No significant main effects of delay ($F_{(3,24)} = 1.476$, N.S.) or order ($F_{(1,8)} = 0.353$, N.S.) were found, and neither was a significant delay x order interaction ($F_{(3,24)} = 0.048$, N.S.). The data from this analysis are shown below in Figure 4.5.

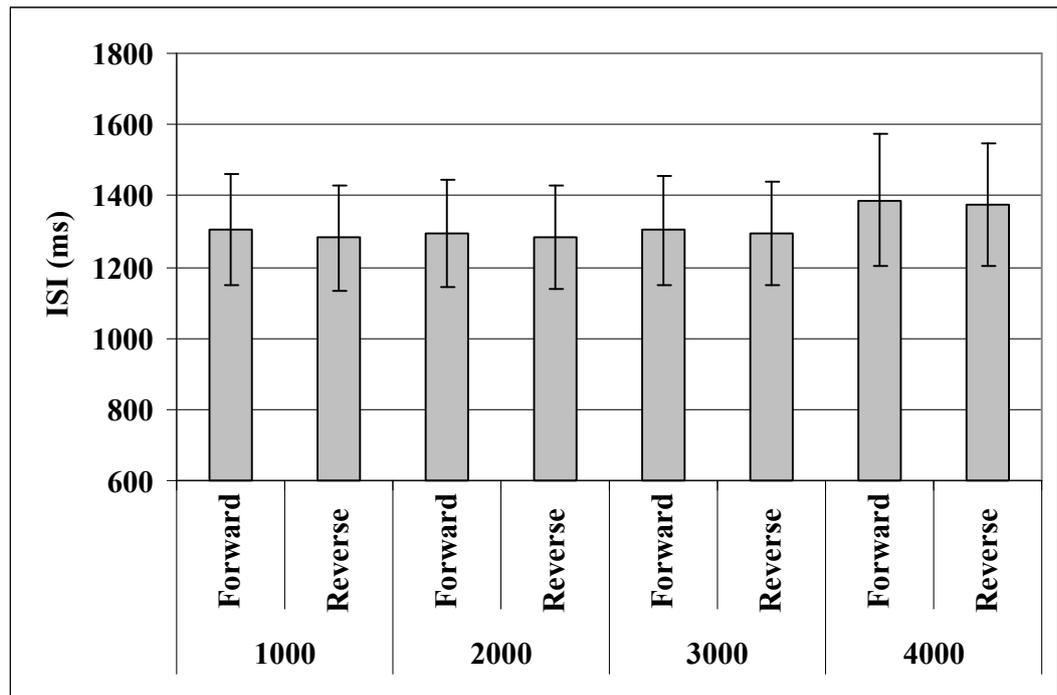


Figure 4.5: Group mean ISI (in ms) for forward and reverse trials at each of the four delay durations. Error bars show standard errors.

End-Point Error: Group mean end-point error data was analysed using a 4 x 2 x 2 within-subjects ANOVA, with the factors delay (1000ms, 2000ms, 3000ms and 4000ms), order (forward and reverse) and saccade (1 and 2). No significant main effect of delay ($F_{(3,33)} = 1.864$, N.S.) or order were found ($F_{(3,33)} = 0.236$, N.S.). There was however a highly significant main effect of saccade ($F_{(1,11)} = 16.685$, $p < 0.005$), with greater end-point error for saccade 2 than saccade 1 (saccade 1: mean = 25.89mm, s.d. = 3.77mm; saccade 2: mean = 29.62mm, s.d. = 2.40mm). The data entered into the ANOVA was organised by increasing delay duration. For this factor a linear trend was found that was approaching significance ($F_{(1,11)} = 4.565$, $p = 0.056$). No significant delay x order ($F_{(3,33)} = 0.354$, N.S.), delay x saccade ($F_{(3,33)} = 1.072$, N.S.), or order x saccade ($F_{(1,11)} = 0.987$, N.S.) interactions were found. The delay x order x saccade interaction was however found to be approaching significance ($F_{(3,33)} = 2.611$, $p = 0.068$). The data from this analysis are shown below in Figures 4.6 and 4.7.

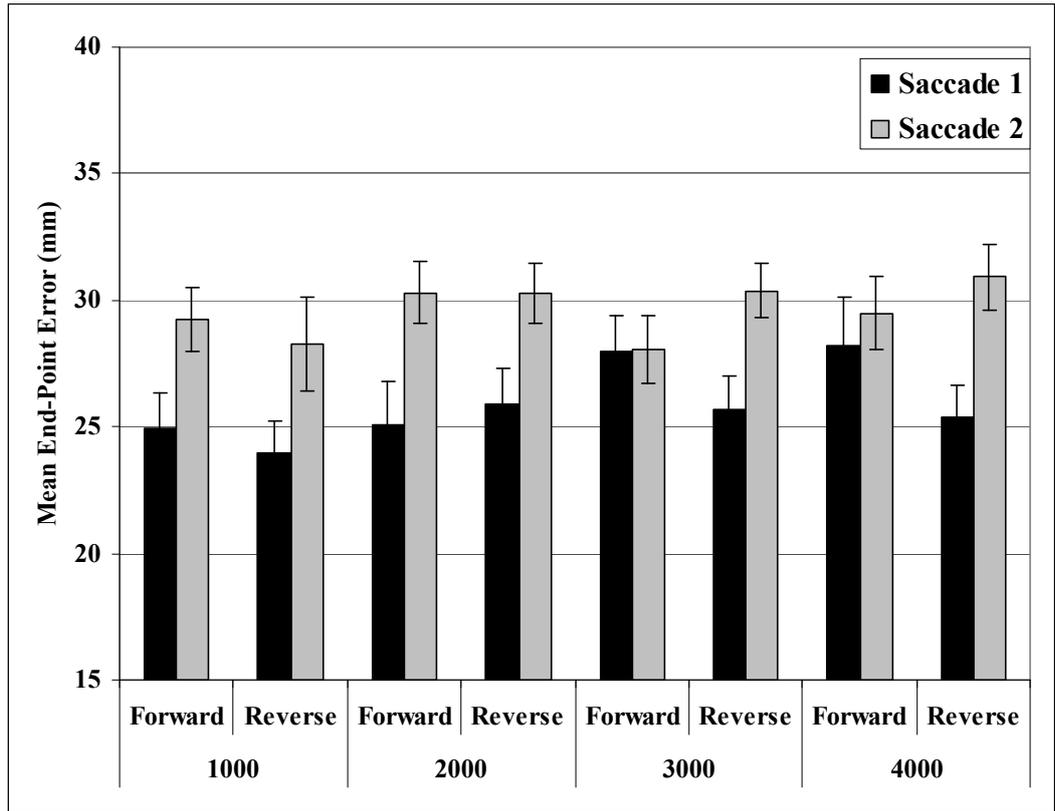


Figure 4.6: Group mean end-point error (in mm) for saccades 1 and 2 in the forward and reverse trials with each of the four delay durations. Error bars show standard errors.

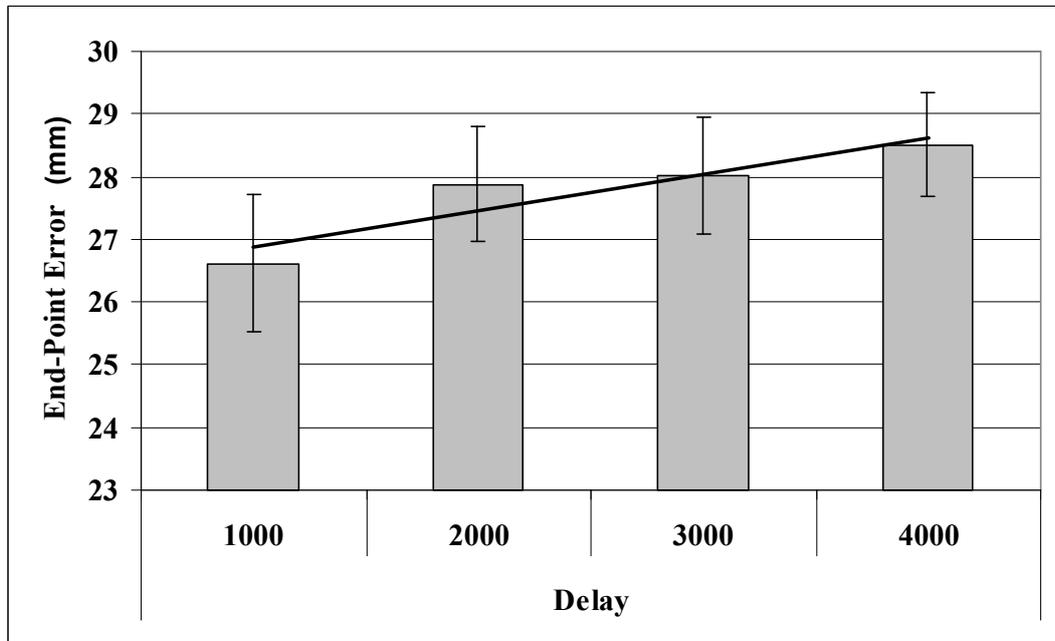


Figure 4.7: Linear trend in group mean end-point error (in mm) for each of the four delay durations. Error bars show standard errors.

Amplitude and Angular Compensation Measures: A 4 x 2 within-subjects ANOVA with the factors: delay (1000ms, 2000ms, 3000ms and 4000ms) and order (forward and reverse) was used to analyse the group mean compensatory amplitude gain scores. No main effect of delay was found ($F_{(3,33)} = 1.418$, N.S.), nor was a significant delay x order interaction ($F_{(3,33)} = 0.309$, N.S.). There was however a significant main effect of order presentation, with greater compensation in the reverse compared to the forward condition (forward: mean = 0.958, s.d = 0.063; reverse: mean = 0.995, s.d = 0.056; $F_{(1,11)} = 22.466$, $p < 0.001$). The data from this analysis are shown below in Figure 4.8.

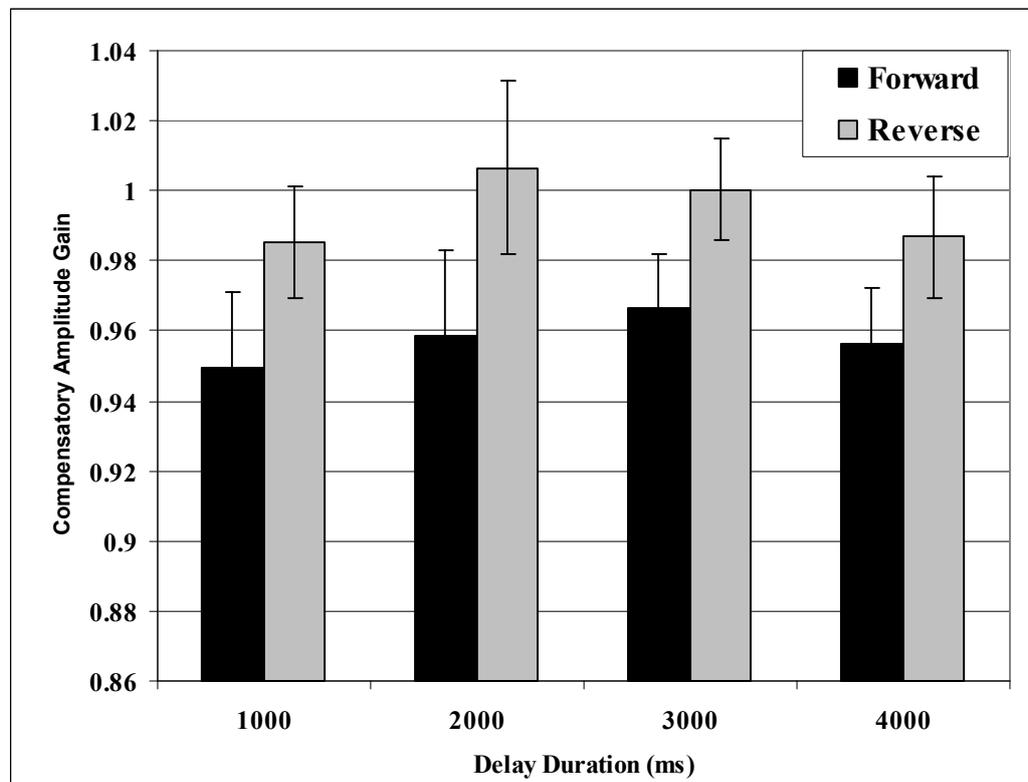


Figure 4.8: Group mean compensatory amplitude gain for forward and reverse trials for each of the delay durations. Error bars show standard errors.

The same analysis was then conducted on the group mean absolute angular difference values (between the angle of saccade 2 and the angle required given error in saccade 1). No significant main effects of delay ($F_{(3,33)} = 0.465$, N.S.) or order ($F_{(1,11)} = 1.666$, N.S.) were found, nor was a significant

delay x order interaction ($F_{(3,33)} = 0.506$, N.S.). The data from this analysis are shown below in Figure 4.9.

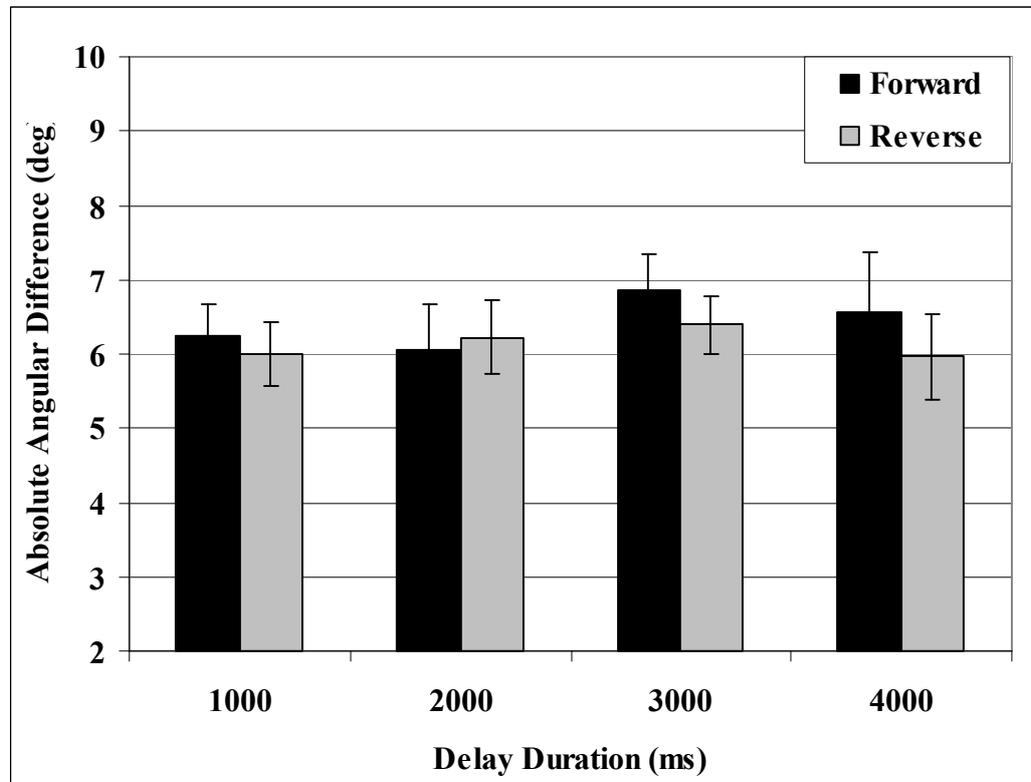


Figure 4.9: Group mean absolute angular difference (in deg, between the angle of saccade 2 and the angle required given error in saccade 1) for forward and reverse trials for each of the delay durations. Error bars show standard errors.

Discussion

Overall, the main effect of order on latency shows that the previously noted difference between forward and reverse order trials persists despite the increasing delays. It does not however appear to be present for all of the delay durations, and may therefore be affected to some extent by the fact that there is more time to complete the more complicated spatial computations necessary for the reverse order trials.

In terms of end-point error, accuracy was found to be generally worse for the second saccade compared to the first as observed in Experiment 7. At the

longer delays of 3000ms and 4000ms, there is some evidence from the results to suggest that there may be a benefit of seeing the target for saccade 1 second on the reverse order trials. For these delays there is a much smaller difference between the end-point error for saccades 1 and 2 on the forward order trials (compared to the 1000ms and 2000ms delays), but a large difference between the two saccades for the reverse order trials. Further to this, error for saccade 1 appears to be lower for the reverse compared to the forward order trials at these delay durations. This can probably be explained as a recency effect in terms of the target for the first saccade in the sequence, which becomes more pronounced with increasing temporal delays.

A difference between reverse and forward order trials that was not significant in Experiment 7, but that was seen in this study, is that of compensatory amplitude gain, where this is greater for the reverse target presentation order for all of the four delay durations. Although for the angular compensation measure, the effect of target presentation order is not significant, it does appear to be in the same direction, i.e. lower for the reverse order trials (reflecting greater compensation), for three out of the 4 delay durations at least. This therefore provides further evidence to suggest that saccade planning and spatial remapping of target locations may be affected by the nature of the task, such as the order of target presentation. When the targets are presented in reverse order, it may be harder to form a complete motor program from the first target location to the next.

There is no evidence from these data however to support the hypothesis that incrementing the temporal delay between the two targets leads to a progressive increase in the amount of compensation. Since the targets are viewed in succession in both the forward and reverse order trials, it is possible that they may still be encoded in relation to each other to some extent despite the increasing temporal separation. For example, a motor code could be made to target 1, and then target 2, and then the plan for the sequence might somehow be reversed.

One way of investigating this issue is through the introduction of a third target; this would also allow evaluation of compensation in target presentation orders where a simple reversal of the saccade plan is not possible, i.e. targets 1 and 2, or 2 and 3 are separated in the presentation order, and thus target

encoding might somehow be different. If task factors that make it harder to code the targets in relation to one another lead to greater levels of spatial remapping, it might be expected that this would be reflected in the values of the compensation measures for these saccades. This idea will be investigated in the next experiment.

4.4. Experiment 9: Investigating the Effects of Target Presentation Order on Spatial Remapping in a Triple-Step Saccade Paradigm

Given the difference in latency seen in Experiment 7 for the forward and reverse target presentation orders, it would be expected that for a triple-step version of the task similar results would be seen, i.e. processing time should be shorter when the targets are presented in a forward order compared to the others, given the lower level of spatial computation expected. The difference in latency in Experiment 7 might also be related to the position in the presentation order of the first target in the sequence, i.e. processing time is quicker when this is presented first compared to second. The difference in latency between the conditions when saccade-target 1 is shown first compared to last will thus be considered, with the prediction that seeing it first will lead to a faster reaction time. Similarly, therefore, a greater latency could be expected when the last saccade target in the sequence is shown first compared to being shown at the end of the presentation order.

In terms of end-point error, an overall effect of execution order might be expected with inaccuracy increasing linearly from saccade 1 to saccade 3 as a result of error accumulation (Bock et al., 1995). In the memory-guided triple-step saccade study carried out by Heide et al., (2001), an increase in error was seen for saccades 2 and 3 compared to saccade 1, although there was no evidence of a linear trend. It is not known whether target presentation order might also affect accuracy, as a result of the memory-related factors discussed in Experiment 7. In order to assess this, it would thus be useful to consider error for a particular saccade e.g. saccade 1 in relation to the presentation order of its corresponding target (i.e. 1st, 2nd or 3rd). This will therefore be done for all three of the saccades in the sequence.

The extent of amplitude and angular compensation will also be evaluated in relation to target presentation order for saccades 2 and 3 for the reasons discussed above. Particular consideration will be given to the comparison of those target presentation orders when the relevant targets are presented in succession, i.e. the targets for saccades 1 and 2, for saccade 2 and the targets for saccades 2 and 3 for saccade 3, compared to when they are at different ends of the presentation order. Overall, based on Experiment 8, compensation might also be expected to be higher when the targets are presented in ‘reverse’ order compared to a ‘forward’ order, particularly in terms of amplitude.

Methods

Participants

Twelve healthy participants (8 females) aged 22-27 (mean 24.8 years) participated in this task. Four of these had previously taken part in both Experiments 7 and 8. One participant had previously participated in just Experiment 7 and another in just Experiment 8. All participants had normal or corrected to normal vision.

Materials

Eye tracking and stimulus generation and presentation were carried out in the same way as for Experiment 7. In this study however three circular targets each 6mm (0.63deg) diameter were used, one red, one green and one blue.

Procedure

Oculomotor Task: This task was very similar to the double-step saccade task described above, except that participants were required to make three memory-guided saccades towards the remembered locations of the targets, instead of two.

At the start of each experimental session participants were instructed as to which of the six possible orders they should look towards the targets (either:

Red-Green-Blue (RGB), Red-Blue-Green (RBG), Green-Blue-Red (GBR), Green-Red-Blue (GRB), Blue-Green-red (BGR), or Blue-Red-Green (BRG)), regardless of the order of target presentation.

A black fixation cross on a grey background appeared on the screen, which signified the start of each trial. This remained on until the eye-tracker determined that the participant was correctly fixating the cross, i.e. the pupil was directed to a region of the screen 15mm (1.56deg) around the centre. The first target was then presented and remained on for one second, after which it was extinguished and the screen went blank for 500ms. The second target then appeared, also for a duration of 1000ms, followed by a blank display for a period of 500ms. This was followed by the third target, which was similarly displayed for 1000ms, followed by the blank screen for 500ms. Participants were instructed to remain fixating centrally throughout the target presentation and during the ensuing delay period. This had a variable duration, based on a normal distribution (mean = 500ms, s.d. = 125ms). This was followed by an auditory beep, which was the cue for participants to start the saccades; a variable delay was used to help minimise the number of anticipatory saccades, by making the time of the go-signal less predictable. The eye tracker continued to record for a further 4500ms to allow participants enough time to complete both saccades. The trial then ended and the fixation cross reappeared for the start of the next trial.

As previously, the screen was split into quadrants (top and bottom, left and right) and targets could appear at nine possible locations within each of these areas (see Experiment 3, Chapter 2 for details). On each trial an index of the nine possible target positions for the pre-specified quadrants for targets 1, 2 and 3 was shuffled, and target positions pseudo-randomly selected by the computer.

There were six possible orders of target presentation: RGB, RBG, GBR, GRB, BGR, and BRG. These were counterbalanced across trials so that participants saw an equal number of trials (24) of each target order. A sixth of the trials could therefore be described as forward, i.e. participants saw the targets in the exact order in which they had to look to them, whereas the other trials varied from this to different extents. The order of the quadrants that the targets appeared in was also counterbalanced across the trials. There were 24

possible combinations of quadrant order: 1,2,3; 1,2,4; 1,3,2; 1,3,4; 1,4,2; 1,4,3; 2,1,3; 2,3,1; 2,3,4; 2,4,3; 2,4,1; 2,1,4; 3,1,2; 3,1,4; 3,2,1; 3,2,4; 3,4,1; 3,4,2; 4,1,2; 4,1,3; 4,2,1; 4,2,3; 4,3,1; 4,3,2; where 1 = bottom-left quadrant, 2 = bottom-right quadrant, 3 = top-left quadrant and 4 = top-right quadrant. Within a trial targets could not appear in the same quadrant twice. Participants completed 144 trials in total, one of each of the possible trial-order combinations (6 target orders x 24 quadrant orders).

Data Analysis: As in the double-step saccade study, plots of eye movement traces using x and y coordinates from eye-position data recorded every 20ms were analysed. Trials in which participants started the eye movement before the go-signal, or looked to targets in the wrong order, were rejected. The fixation end-points, latencies and ISIs were determined using an algorithm, in the same way as for the double-step saccade experiment (see Experiment 3, Chapter 2 for details). Coordinates for x and y eye position obtained from the eye tracker were compared with the x and y coordinates for the target positions to calculate a measure of error, using the following equations:

$$\begin{aligned} \text{Saccade 1 Error} &= \sqrt{[(x(\text{target 1}) - x(\text{fixation 1}))^2 + (y(\text{target 1}) - (-y(\text{fixation 1})))^2]} \\ \text{Saccade 2 Error} &= \sqrt{[(x(\text{target 2}) - x(\text{fixation 2}))^2 + (y(\text{target 2}) - (-y(\text{fixation 2})))^2]} \\ \text{Saccade 3 Error} &= \sqrt{[(x(\text{target 3}) - x(\text{fixation 3}))^2 + (y(\text{target 3}) - (-y(\text{fixation 3})))^2]} \end{aligned}$$

From these error values in terms of distance (in mm) of the fixation locations from the target positions were obtained. Measures of compensation in terms of amplitude and angle of the second and third saccade were calculated using the same method as for Experiments 7 and 8, except this was done for both saccade 2 and saccade 3. An example trial can be seen in Figure 4.10, below.

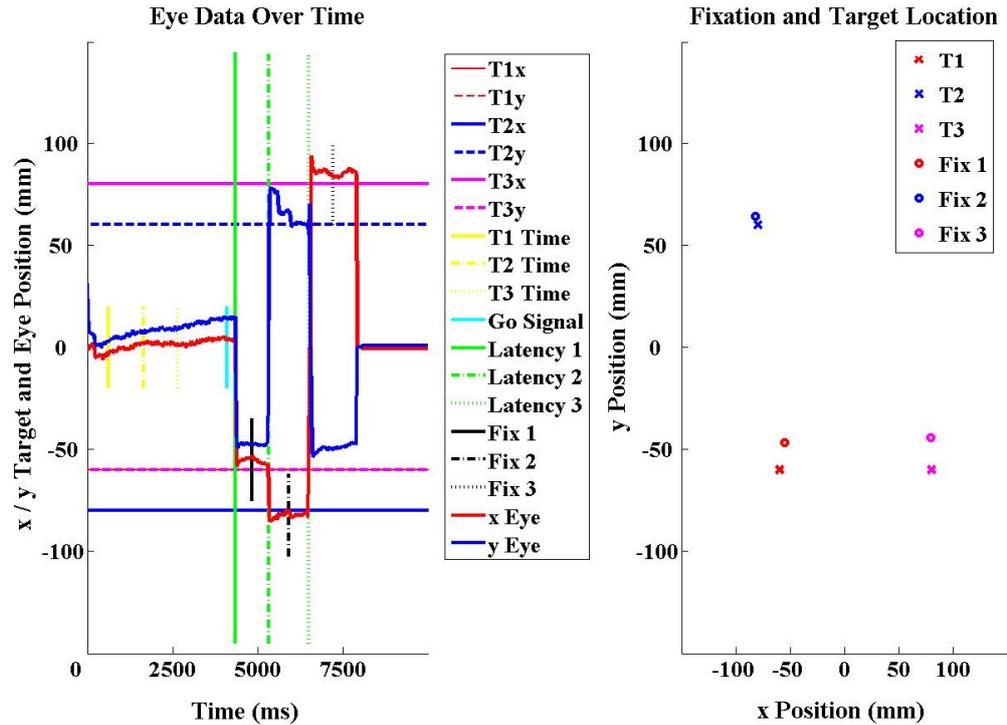


Figure 4.10: Plot of eye movement trace using x (red trace) and y (blue trace) coordinates from eye-position data for one trial (left plot), the participant can be seen to be fixating the centre of the screen (0 on y axis) until the go-signal (light blue vertical bar). The horizontal blue bars represent the x (solid) and y (dashed) location of target 2, and the horizontal pink bars represent the x (solid) and y (dashed) location of target 3 (the x and y locations of target 1 clash with those of target 2 and 3 and so can't be seen). The three saccades and fixations can be seen clearly following the go-signal, after which the participant looks back to the centre of the screen. The times of the three target presentations are shown as yellow vertical bars (solid, target 1; dashed, target 2; dotted, target 3). The green vertical bars show the latency of the 3 saccades, and the black horizontal bars show the end-point fixations (solid, saccade 1; dashed, saccade 2; dotted, saccade 3). The end-point of these saccades is also plotted in relation to the target positions on the screen (right plot).

Results

Latency: A 1 x 6 repeated measures ANOVA used to assess differences in latency for the six possible orders of target presentation did not reveal a significant main effect of order ($F_{(5,50)} = 2.043$, N.S.). The data entered in the ANOVA was organised in the following order: 123, 132, 213, 231, 312, 321, i.e. in categories according to which target appeared first, (saccade-target 1, 2

or 3). A significant linear trend was found to be present ($F_{(1,10)} = 6.561$, $p < 0.05$). Further to this, a one-tailed paired samples Student's t-Test was conducted to test the a-priori prediction that the mean latency would be significantly greater in the 'reverse' condition (i.e. the 321 target presentation order) compared to the completely 'forward' condition (i.e. the 123 target presentation order). A significant difference was found for this comparison in the direction expected (forward: mean = 268.27ms, s.d. = 86.77ms; reverse: mean = 310.10ms, s.d. = 91.02ms; $t_{(10)} = 2.814$, $p < 0.05$). The data used in these analyses are shown in Figure 4.11, below.

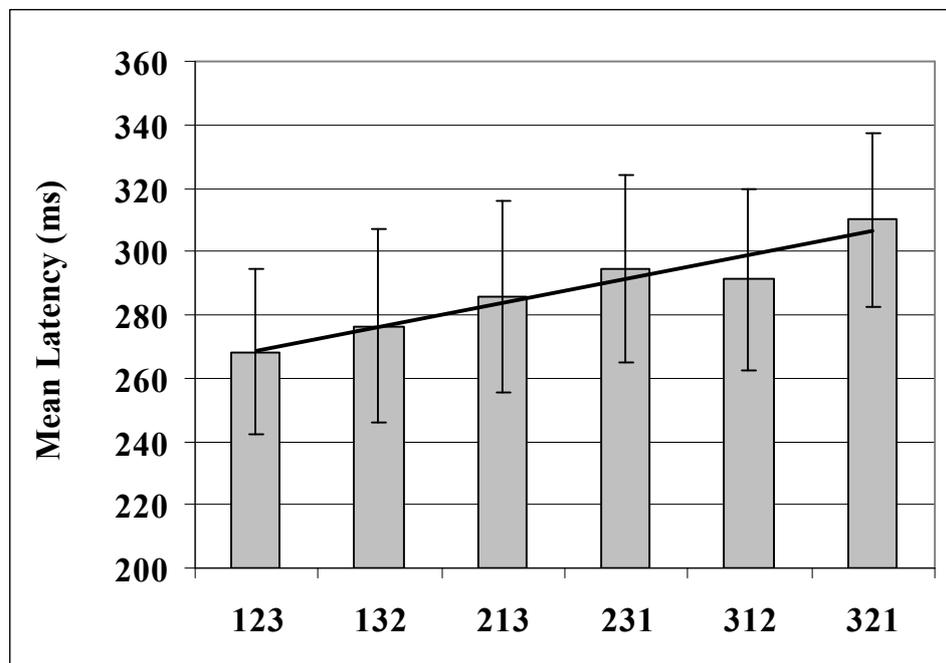


Figure 4.11: Effect of order of target presentation on latency (ms). Error bars show standard errors.

Three 1 x 3 repeated measures ANOVAs were conducted in order to assess separately the effect of target 1 position, target 2 position and target 3 position on latency. In each of these ANOVAs the factor, target position, always had 3 levels: 1st, 2nd and 3rd, which corresponded to where in the presentation order the targets for each saccade were displayed. Thus for target 1 position, the first saccade-target (i.e. the one that had to be looked to first)

could be presented 1st, 2nd or 3rd. This ANOVA showed a significant main effect of target 1 position ($F_{(2,20)} = 3.930, p < 0.05$); Mauchly's test of sphericity was non-significant, so sphericity could be assumed. The a-priori prediction that the mean latency would be significantly greater in the conditions when the target for saccade 1 appeared last (i.e. the 321 and 231 target presentation orders) compared to those when it was shown first (i.e. the 123 and 132 target presentation orders) was tested using a one-tailed paired-sample Student's t-Test. A significant difference was found for this comparison in the direction expected (target 1 shown first: mean = 272.32ms, s.d. = 92.78ms; target 1 shown last: mean = 302.31ms, s.d. = 93.03ms; $t_{(10)} = 3.019, p < 0.01$).

Post-hoc comparisons were conducted in the form of two-tailed paired-sample Student's t-Tests to compare differences between the remaining levels of the independent variable, a Bonferroni correction was applied and the significance level was therefore set at $0.05/2 = 0.025$. No significant differences were found either for the comparison of target 1 being shown first versus second (target 1 shown first: mean = 272.32ms, s.d. = 92.78ms; target 1 shown second: mean = 288.29ms, s.d. = 92.49ms; $t_{(10)} = 1.754, N.S.$) or second versus third (target 1 shown third: mean = 302.31ms, s.d. = 93.03ms; $t_{(10)} = 1.232, N.S.$). The latency data used in these analyses are shown in Figure 4.12, below.

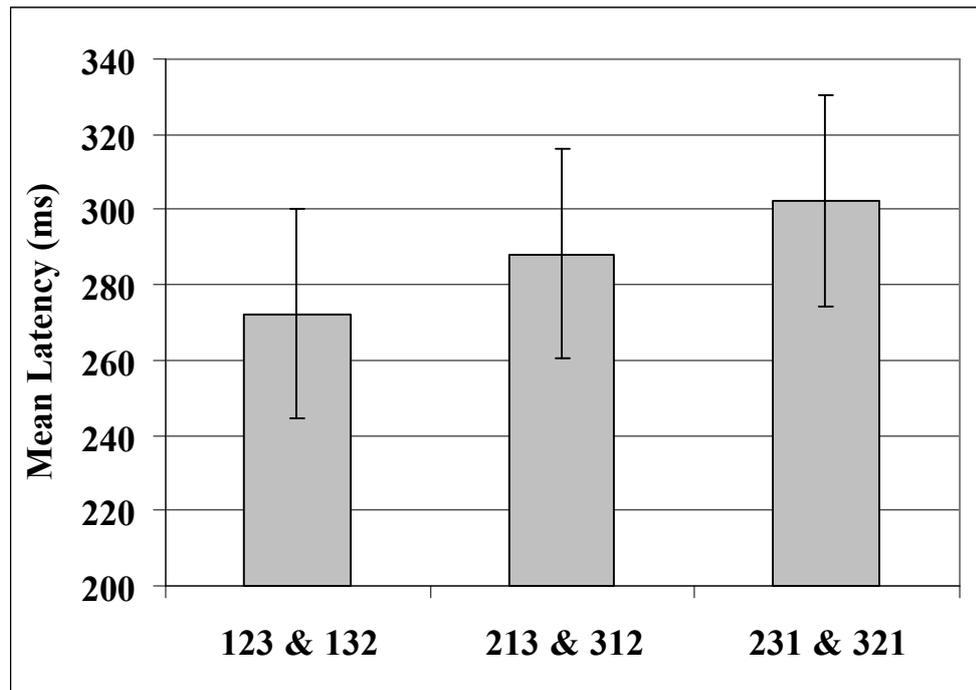


Figure 4.12: Effect of target 1 position on latency (ms). Error bars show standard errors.

No significant main effect of order was found for the ANOVA with the factor target 2 position ($F_{(2,20)} = 0.239$, N.S.). The latency data used in these analyses are shown in Figure 4.13, below.

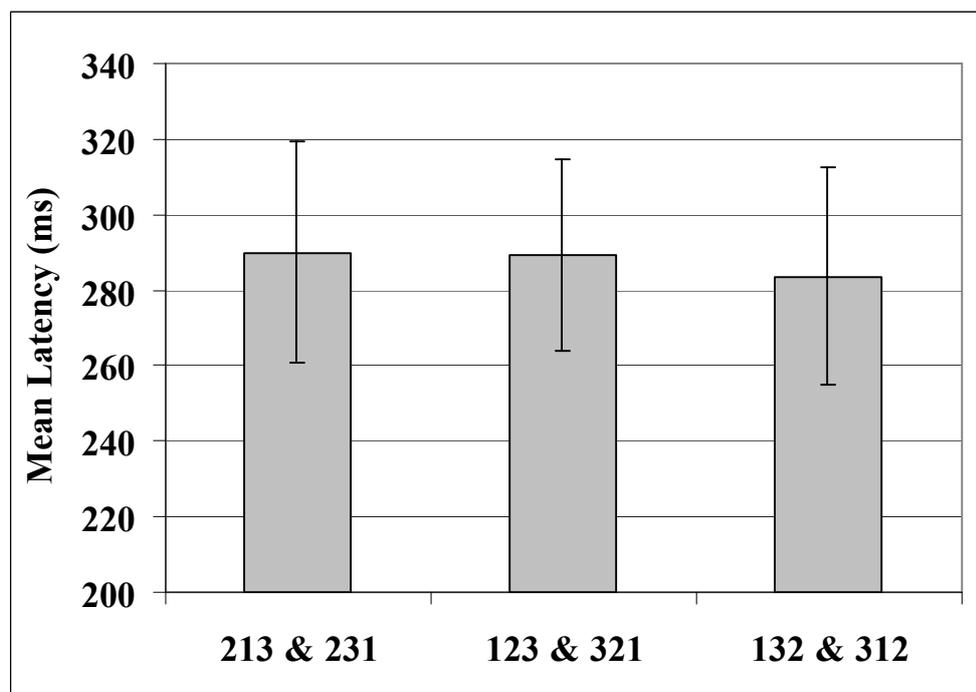


Figure 4.13: Effect of target 2 position on latency (ms). Error bars show standard errors.

For the ANOVA assessing the effect of order of presentation of saccade target 3 Mauchly's test of sphericity was found to be significant. The Greenhouse-Geisser estimate was 0.638, and since Girden (1972) recommends that when estimates of sphericity are less than 0.75, a Greenhouse-Geisser correction should be applied, this correction was chosen. With the adjusted degrees of freedom, no significant main effect of order was found for this analysis ($F_{(1.277,12.768)} = 2.977$, N.S.). The a-priori prediction that the mean latency would be significantly greater in the conditions when the target 3 appeared first (i.e. the 312 and 321 target presentation orders) compared to those when it was shown third (i.e. the 123 and 213 target presentation orders) was tested using a one-tailed paired samples Student's t-Test. A significant difference was found for this comparison in the direction expected (target 3 shown first: mean = 300.56ms, s.d. = 89.18ms; target 3 shown last: mean = 276.92ms, s.d. = 89.07ms; $t_{(10)} = 2.238$, $p < 0.05$). The latency data used in these analyses are shown in Figure 4.14, below.

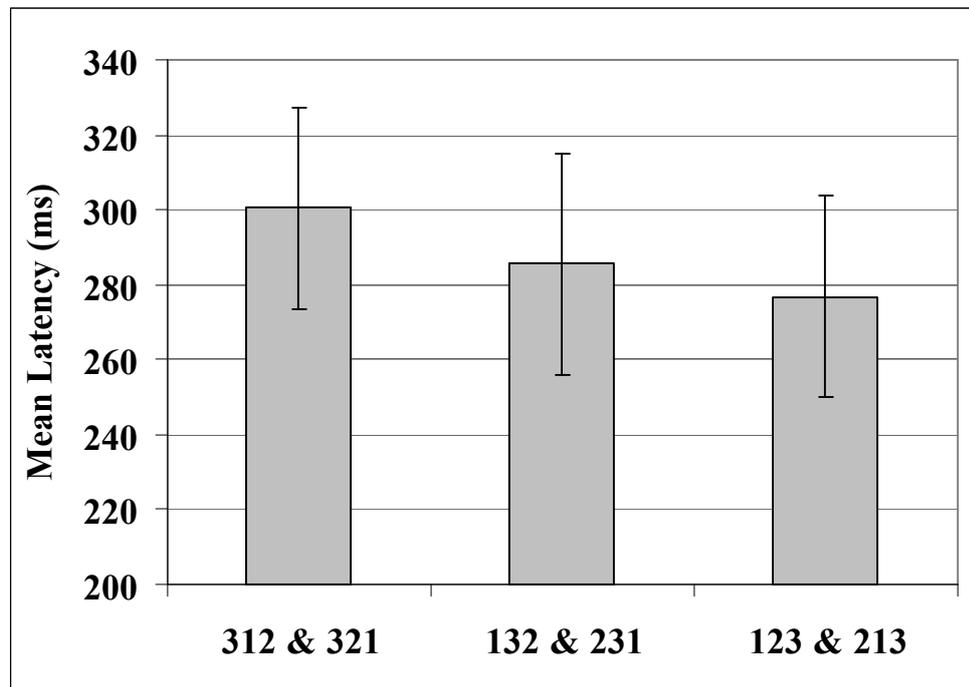


Figure 4.14: Effect of target 3 position on latency (ms). Error bars show standard errors.

Intersaccadic Interval: A 6 x 2 repeated measures ANOVA was conducted to assess the effects of order of target presentation on intersaccadic interval, with the factors: order, with 6 levels and ISI, with 2 levels. No significant main effects were found for order ($F_{(5,50)} = 0.236$, N.S.) or for ISI ($F_{(1,10)} = 2.931$, N.S.) and no significant interaction ($F_{(5,50)} = 1.171$, N.S.).

The data were next regrouped on the basis of saccade-target 1 position in the presentation order (as had been done for the latency data) and a 3 x 2 repeated measures ANOVA with the factors: target 1 position (1st, 2nd or 3rd) and ISI (1 and 2) was conducted. No main effect of target 1 position was found ($F_{(2,20)} = 0.348$, N.S.), no significant main effect of ISI ($F_{(1,10)} = 2.932$, N.S.) and no significant interaction between these two factors ($F_{(2,20)} = 0.426$, N.S.).

A second 3 x 2 repeated measures ANOVA in which the data were grouped on the basis of target 2 position in the target presentation order was also conducted. No significant main effect of target 2 position was found ($F_{(2,20)} = 0.125$, N.S.) no significant main effect of ISI ($F_{(1,20)} = 2.931$, N.S.) and no significant interaction between these two factors ($F_{(2,20)} = 1.058$, N.S.).

Finally a third 3 x 2 repeated measures ANOVA was conducted on the data, when they were grouped by the position in the presentation order of saccade target 3. No significant main effect of target 3 position was found ($F_{(2,20)} = 0.430$, N.S.), no significant main effect of ISI ($F_{(1,10)} = 2.932$, N.S.) and no significant interaction between these two factors ($F_{(2,20)} = 2.110$, N.S.).

A two-tailed paired-sample Student's t-Test showed that the overall difference between ISI 1 and ISI 2 was not significant (ISI 1: mean = 973.88ms, s.d. = 168.94ms; ISI 2: mean = 1018.74ms, s.d. = 190.19ms; $t_{(10)} = 1.712$, N.S.). The data from these analyses can be seen in Figure 4.15, below.

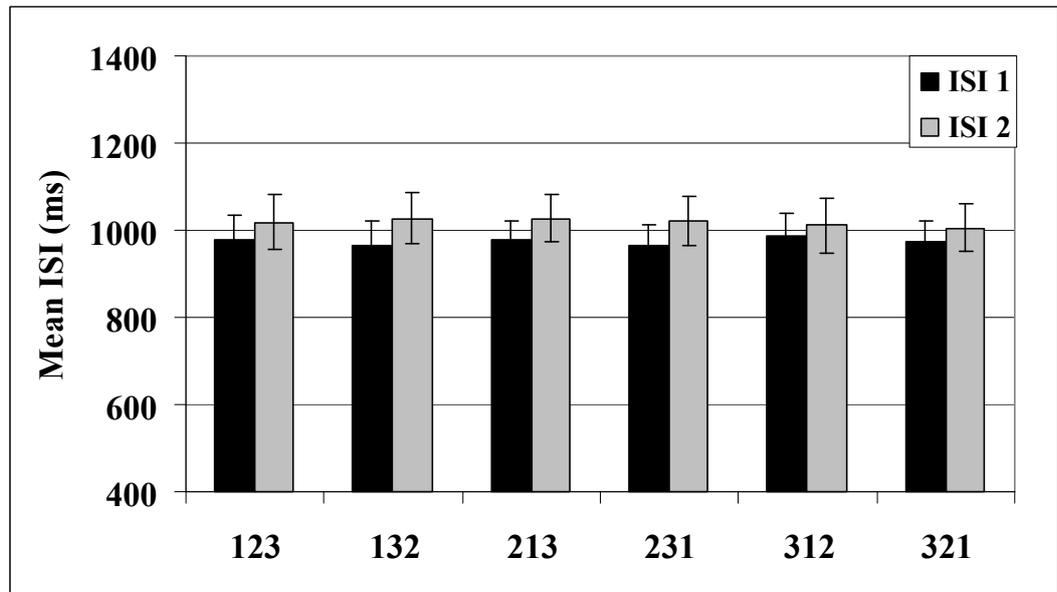


Figure 4.15: Effect of target presentation order on mean intersaccadic interval 1 (black bars) and 2 (grey bars). Error bars show standard errors.

End-Point Error: A 6 x 3 repeated measures ANOVA with the factors target presentation order (123, 132, 213, 231, 312, 321) and saccade (1, 2 and 3) was conducted and a significant main effect of saccade was found ($F_{(2,20)} = 45.080, p < 0.0001$). There was no significant main effect of order ($F_{(5,50)} = 0.786, N.S.$) or order x saccade interaction ($F_{(10,100)} = 1.620, N.S.$). Figure 4.16 below shows the end-point error data used in this analysis. From this graph it is first clear to see, that end-point error appears to increase from saccade 1, to saccade 2 and from saccade 2 to saccade 3. The order of execution therefore appears to influence saccadic error. Post-hoc two-tailed paired-sample Student's t-Tests were used to compare differences between the mean error scores for saccades 1, 2 and 3 (saccade 1: mean = 19.19mm, s.d. = 4.36mm; saccade 2: mean = 22.81mm, s.d. = 4.30mm; saccade 3: mean = 26.89mm, s.d. = 4.53mm). Significant differences were found between the error for saccades 1 and 2 ($t_{(10)} = 4.682, p < 0.001$, saccades 2 and 3 ($t_{(10)} = 4.298, p < 0.005$) and saccades 1 and 3 ($t_{(10)} = 11.921, p < 0.001$). These results are still significant after a Bonferroni correction ($\alpha = 0.05/3 = 0.017$) has been applied.

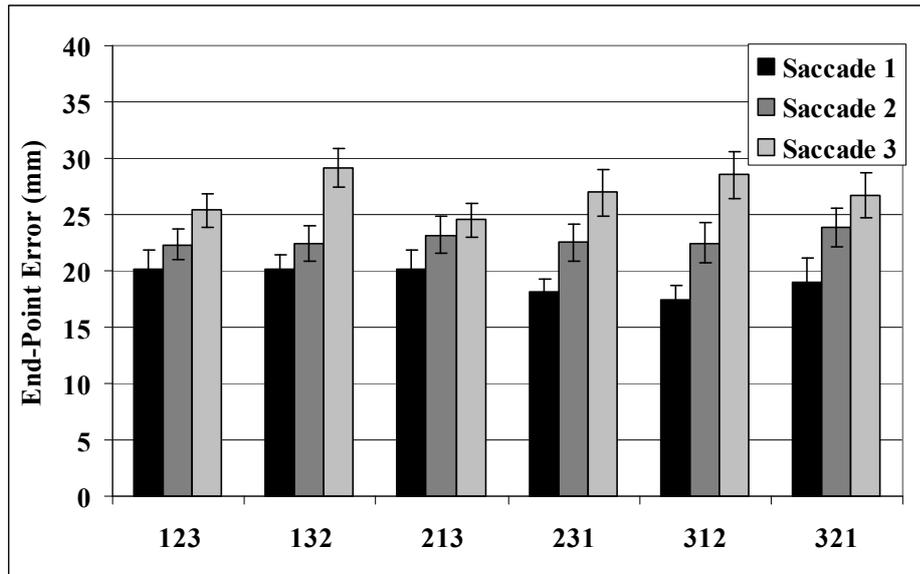


Figure 4.16: Mean end-point error (mm) for saccades 1, 2 and 3 for the six different target presentation orders. Error bars show standard errors.

As with the other independent variables, the data were also analysed on the basis of target 1, target 2 and target 3 position, to see which of these, if any most influenced end-point error.

A one-way repeated measures ANOVA for the factor target 1 position (three levels: 1st, 2nd or 3rd) was used to assess error on saccade 1. No main effect of target 1 position ($F_{(2,20)} = 1.863$, N.S.), was found. Figure 4.17 below illustrates the effect of target 1 position on mean end-point error for saccade 1.

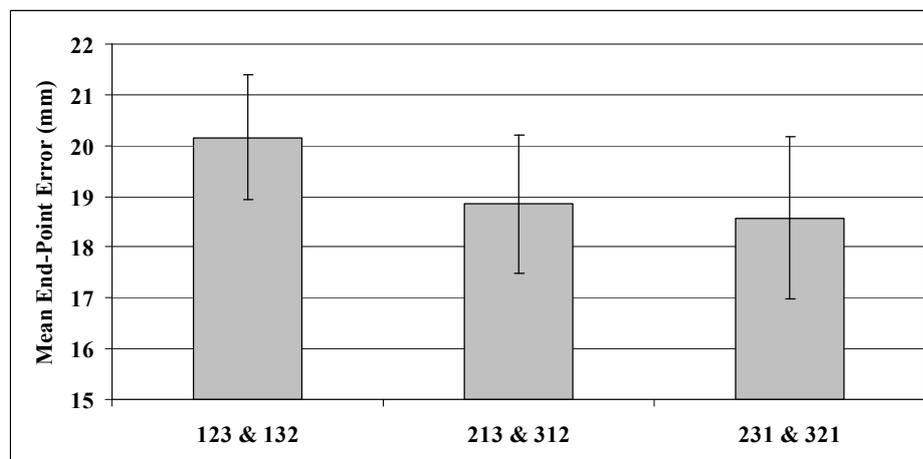


Figure 4.17: Effect of target 1 position in terms of target presentation order on mean end-point error (mm) for saccade 1. Error bars show standard errors.

A second one-way repeated measures ANOVA for the factor target 2 position (three levels: 1st, 2nd or 3rd) was used to assess error on saccade 2. No main effect of target 2 position ($F_{(2,20)} = 0.189$, N.S.) was found. Figure 4.18 below illustrates the effect of target 2 position on mean end-point error for saccade 2.

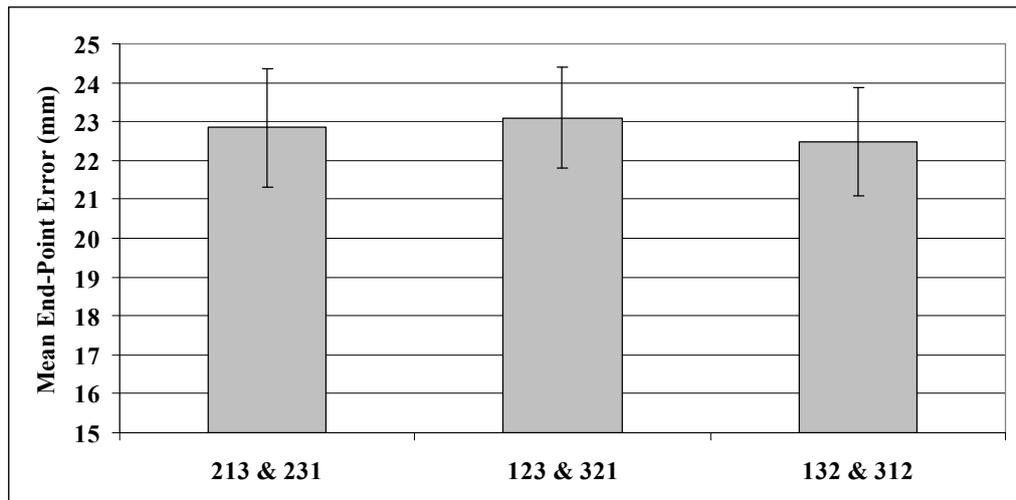


Figure 4.18: Effect of target 2 position in terms of target presentation order on mean end-point error (mm) for saccade 2. Error bars show standard errors

Finally a third one-way repeated measures ANOVA for the factor target 3 position (three levels: 1st, 2nd or 3rd) was used to assess error on saccade 3. No main effect of target 3 position ($F_{(2,20)} = 2.567$, N.S.), was found. Figure 4.19 below illustrates the effect of target 3 position on mean end-point error for saccade 3.

Mauchly's test of sphericity was non-significant for all end-point error analyses.

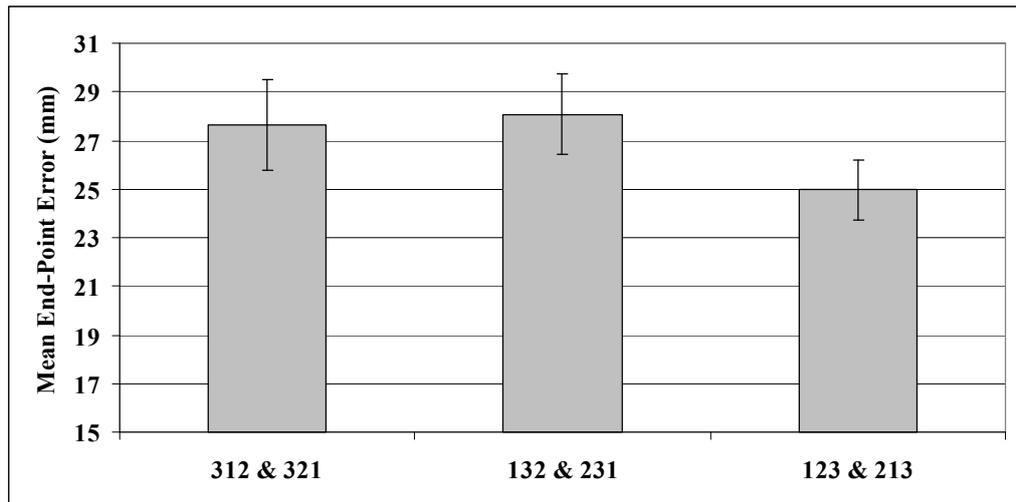


Figure 4.19: Effect of target 3 position in terms of target presentation order on mean end-point error (mm) for saccade 3. Error bars show standard errors.

Amplitude and Angular Compensation Measures: A 6 x 2 repeated measures ANOVA, with the factors order of target presentation (six levels) and saccade (2 and 3) was conducted using the compensatory amplitude gain data. A main effect of target presentation order ($F_{(5,50)} = 2.928$, $p < 0.05$) was found as was a significant order x saccade interaction ($F_{(5,50)} = 4.383$, $p < 0.005$). There was however no significant main effect of saccade ($F_{(1,10)} = 1.853$, N.S.). Mauchly's test of sphericity was not significant for these analyses. A one-tailed paired-sample Student's t-Test was used to test the prediction that compensatory amplitude gain would be higher for the reverse target presentation order (i.e. '321') compared to the forward order (i.e. '123'). This was found to be significant in the direction expected ('123': mean = 0.96, s.d. = 0.03; '321': mean = 1.00, s.d. = 0.06; $t_{(10)} = 3.662$, $p < 0.005$). Figure 4.20 below shows the data from these analyses.

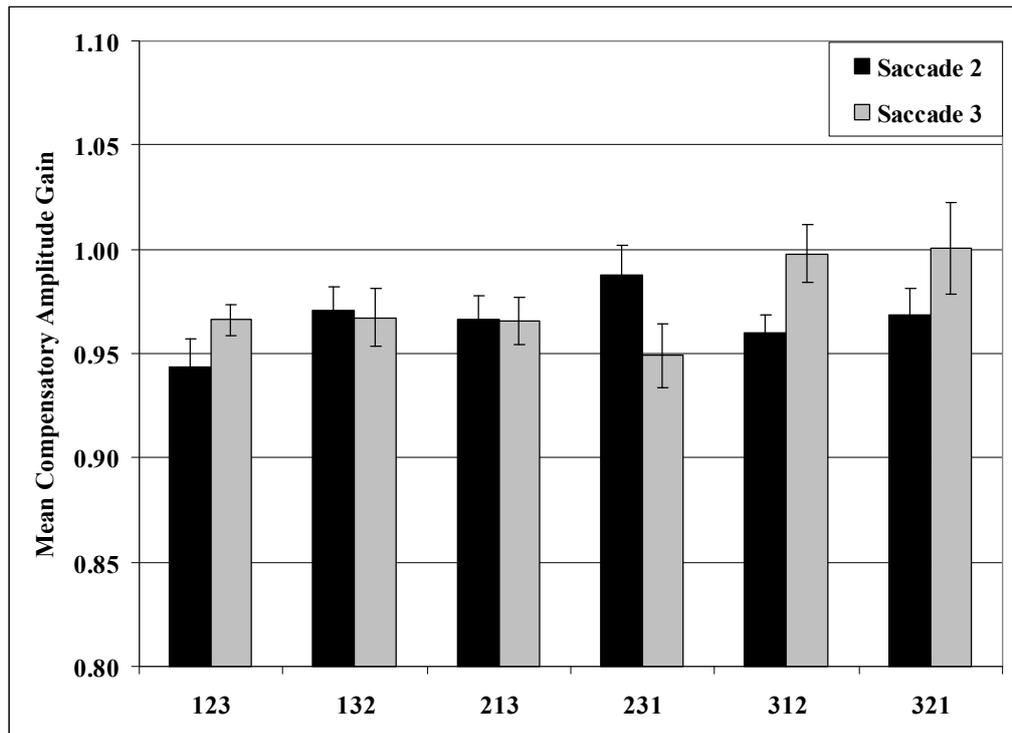


Figure 4.20: Effect of target presentation order on mean compensatory amplitude gain for saccades 2 and 3. Error bars show standard errors.

A 6 x 2 repeated measures ANOVA, with the factors order of target presentation (six levels) and saccade (2 and 3) was similarly conducted to compare the mean absolute difference between the angle of saccade 2 and the angle required given error in saccade 1, and between the angle of saccade 3 and the angle required given error in saccade 2, for the six target presentation orders. There was no significant main effect of order ($F_{(5,50)} = 0.339$, N. S.), however there was a significant main effect of saccade ($F_{(1,10)} = 11.848$, $p < 0.01$; saccade 2: mean = 4.80° , s.d. = 1.17° ; saccade 3: mean = 5.52° , s.d. = 1.02°), and a significant order x saccade interaction ($F_{(5,50)} = 2.691$, $p < 0.05$). (Mauchly's test of sphericity was non-significant). A one-tailed paired-sample Student's t-Test was used to test the prediction that the angular compensation measure would be lower for the reverse target presentation order (i.e. '321') compared to the forward order (i.e. '123'). This was not found to be significant ('123': mean = 5.17, s.d. = 1.61; '321': mean = 5.23, s.d. = 1.62; $t_{(10)} = 0.827$, N.S.). The data assessed in this analysis can be seen in Figure 4.21 below.

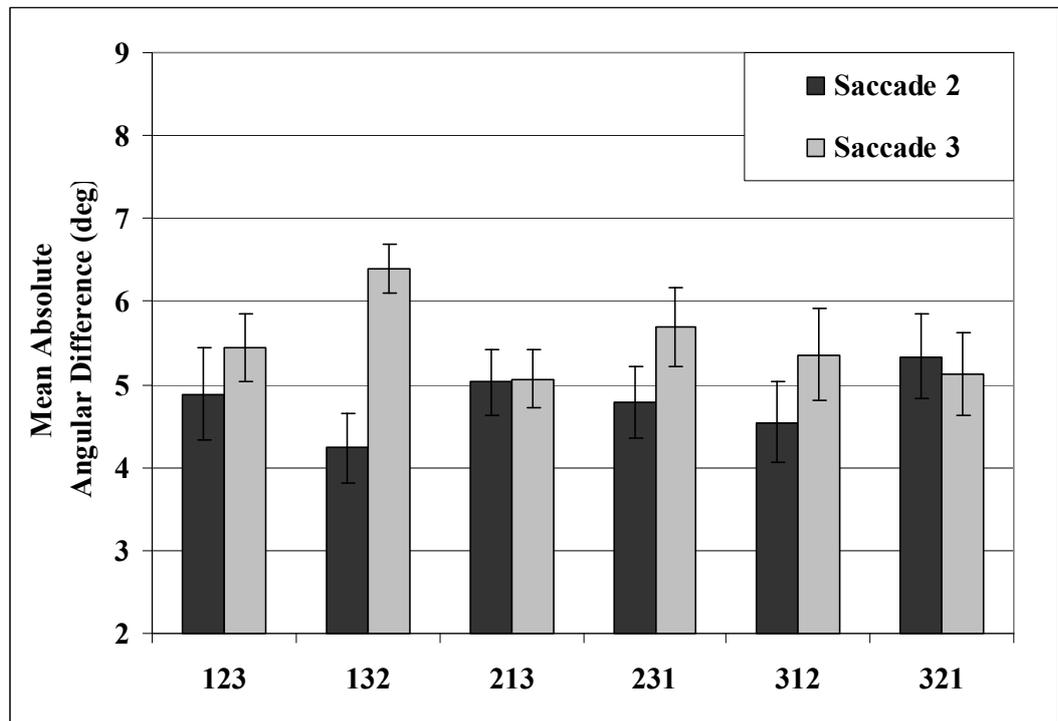


Figure 4.21: Mean absolute difference (in degrees) between the angle of saccade 2 and the angle required given error in saccade 1, and between the angle of saccade 3 and the angle required given error in saccade 2, for the six possible orders of target presentation. Error bars show standard errors.

These measures were next considered in terms of whether the relevant targets were presented in a successive order or not, i.e. for saccade 2, whether targets 1 and 2 were presented together i.e. 123, 213, 312 and 321, or apart, i.e. 132 and 231. For saccade 3 similarly, targets 2 and 3 could be presented together i.e. 123, 132, 231 and 321, or apart i.e. 213 and 312.

One-tailed paired-sample Student's t-Tests were used to compare mean compensatory amplitude gain for saccades 2 and 3 when the relevant targets were either next to one another in the presentation order, or apart. A significant difference was found in the direction expected for saccade 2, i.e. compensation was greater when targets 1 and 2 were presented apart ($t_{(10)} = 3.221, p < 0.01$). For saccade 3, the difference was found to be approaching significance, with the trend in the direction expected, i.e. greater compensation

when targets 2 and 3 were presented apart ($t_{(10)} = 2.051, p=0.07$). The data for these analyses are shown below in Figure 4.22.

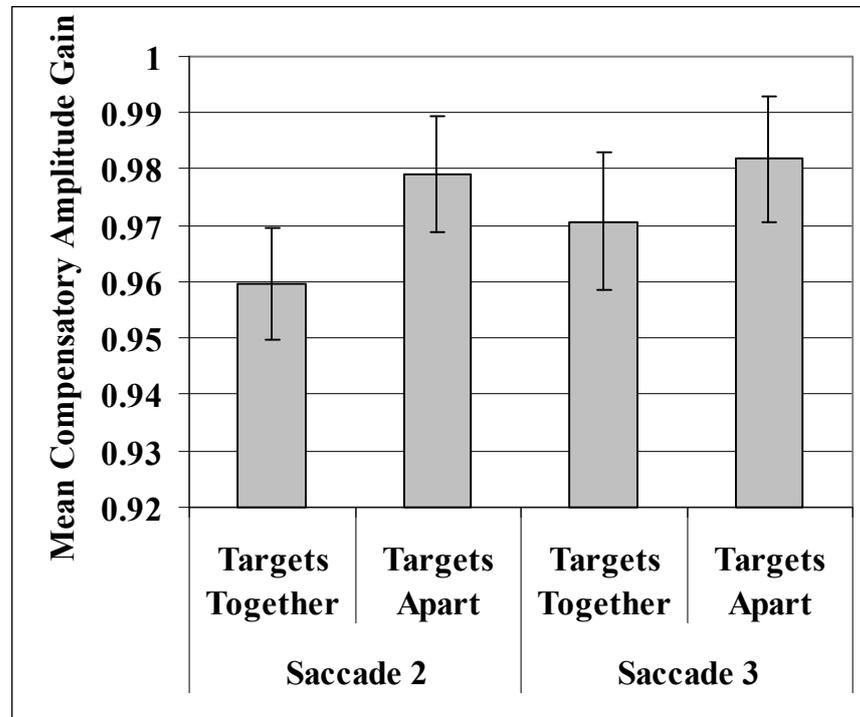


Figure 4.22: Mean compensatory amplitude gain for saccades 2 and 3, when the relevant targets are either presented together, or apart. Error bars show standard errors.

One-tailed paired-sample Student's t-Tests were similarly used to compare the mean absolute difference between the angle of saccade 2 and the angle required given error in saccade 1, and between the angle of saccade 3 and the angle required given error in saccade 2, when the relevant targets were either next to one another in the presentation order, or apart. For saccade 2, the difference was found to be approaching significance in the direction expected i.e. compensation was greater when targets 1 and 2 were presented apart ($t_{(10)} = 2.125, p = 0.06$). For saccade 3, the difference, although not significant, was found to be in the direction expected, i.e. greater compensation when targets 2 and 3 were presented apart ($t_{(10)} = 1.626, p = 0.13$). The data for these analyses are shown below in Figure 4.23.

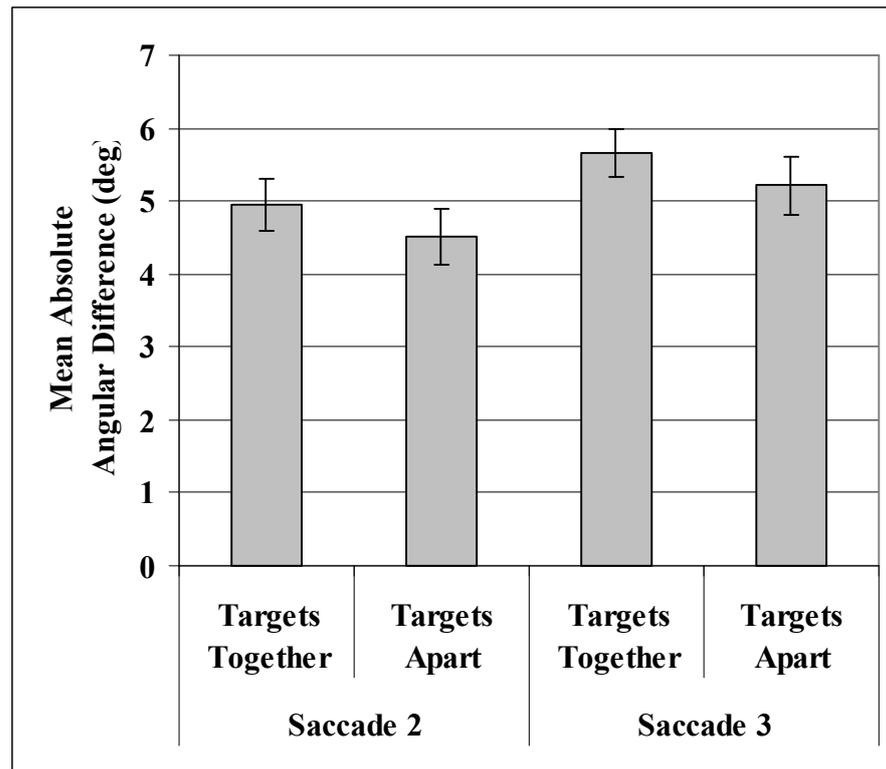


Figure 4.23: Mean absolute difference (in degrees) between the angle of saccade 2 and the angle required given error in saccade 1, and between the angle of saccade 3 and the angle required given error in saccade 2, when the relevant targets are either presented together, or apart. Error bars show standard errors.

Discussion

As expected, order of target presentation was shown to affect latency. On the bases of Experiment 7, it was suggested that this was due to the greater complexity of the reverse compared to forward order trials. Whilst the results still support this theory in that the ‘321’ order had a greater latency than the ‘123’ order, the data from this study also suggest that in particular, target 1 position has an effect on this variable. More specifically latency is shortest when the target for saccade 1 is seen first, longer when seen second and then greatest when seen last in the target presentation order. From this, it suggests that the difference in latency seen in Experiment 7 could be due to the position of the target for saccade 1, i.e. it is longer in the reverse condition when it is seen second compared to the forward condition when it is seen first.

The reasons for this however may still be related to the complexity of saccade planning and the spatial computations required, since in order to plan the saccade sequence, the location of the end-point of the first saccade needs to be known, so the sooner this is known the easier the planning should be. Although there were no significant effects for saccade target 2 and 3 position, this could be due to the redundancy inherent in this analysis, since the position of target 2 and 3 is clearly affected by the position of target 1 in the presentation order. From the graph showing the latencies for all six presentation orders (Figure 4.11) there is in fact some support for the idea that the position of the other targets also has an effect, since the two conditions where target 1 is seen first (or second or third) do not show the same latencies. From these data it seems that the position of target 2 might also have an effect, since in a comparison of the two conditions for each of the three saccade-target 1 positions in the presentation order, the one in which saccade-target 2 is seen earliest appears to have the smaller latency, e.g. '123' is quicker than '132', '213' is quicker than '312' and '231' is quicker than '321'. This would make sense theoretically, since this is related to the order that the saccades need to be executed, i.e. target 1 position has the most effect, followed by target 2 position.

A previous study by Zingale and Kowler (1987) investigating the planning of saccade sequences, noticed that latency increased with sequence length. They suggested that this reflected the fact that saccades are controlled by a provisional plan for the entire sequence, this would thus take longer when more saccades have to be planned. By comparing the 'forward' and 'reverse' order trials in Experiment 7 with those in the current study (i.e. '123' and '321'), it can be seen that the data here support their finding, i.e. longer latencies are seen for the triple-step compared to double-step sequences.

As in Experiment 7, ISI did not appear to be affected by order of target presentation and rather seemed fairly uniform across the six conditions. Zingale and Kowler had previously shown that this variable also appeared to increase with sequence length and the data here do not disagree with this since the ISI between saccades 1 and 2 appears to be higher in the triple-step compared to double-step version of the task. Zingale and Kowler argue that the length of the ISI is not therefore dependent on a need to correct position

errors from the previous saccade. Instead they refer to a model of motor planning proposed by Sternberg et al., (1978) in which the plans for a sequence of responses are stored in memory prior to execution and thus latency and inter-response intervals increase with the length of the sequence since they reflect the time taken for it to be retrieved, which itself rises as a function of the number of stored plans.

End-point error appears to demonstrate order of execution effects as in Experiment 7, i.e. accuracy decreases with the performance of more saccades in a sequence. As mentioned previously, this could be due to error accumulation (Bock et al., 1995). Order of target presentation, however, did not seem to have a great effect on this variable. From the graphs displaying error as a function of target position, however, there did seem to be some evidence to suggest that end-point error may to some extent be influenced by a recency effect. In all three cases (target 1, 2 and 3 position) accuracy appears to be best when the corresponding target is presented last, i.e. less time has passed since it was viewed. The linear trend seen in Experiment 8, where end-point error appears to increase with time since viewing the target for a saccade would back up the idea that this might be due to decay in working memory for the target locations over time.

The data from this study further support the idea that compensation is affected by target presentation order. Firstly, it was shown that amplitude compensation was greater for the 'reverse' (i.e. '321') order of presentation compared to the forward order ('123'), which agrees with the results found previously in Experiment 8. Secondly, and perhaps of more interest in terms of understanding this process, there appeared to be greater spatial remapping when targets are not encoded in relation to each other i.e. compensation (both amplitude and angular) is better when they are presented apart compared to together. This may be related to the frame of reference used, i.e. object-based versus retinotopic, or the fact that a motor code cannot be formed as easily for the whole sequence when the order of presentation differs from the order of execution. In such a case perhaps the initial saccade plan is less pre-formed prior to execution and thus more open to modification or elaboration as a result of visual error signals during execution (Zingale and Kowler, 1987). It could potentially be this modification or elaboration process in response to visual

signals (i.e. the change in target location) that was being disrupted by the TMS in Experiment 6 (Chapter 3).

4.5. General Discussion

4.5.1. Task-Dependent Nature of Spatial Remapping in Saccade Planning

Overall the experiments discussed in this Chapter have supported the idea put forward in Chapters 2 and 3 that the encoding of target locations and their spatial remapping in the planning of saccade sequences may be affected by certain aspects of the task. In particular the method of target presentation (i.e. simultaneous vs. sequential) appears to be important, with the order of target presentation also exerting an influence. In general it appears that factors related to task specifications that make it harder for the targets to be coded in relation to each another, or for a motor plan from one target location to the next to be programmed at the time of target presentation, lead to increased levels of spatial remapping.

What the experiments in this Chapter have not helped answer however, is the question addressed in Chapters 2 and 3, of where in the brain this spatial remapping process takes place. They have however raised the additional question of whether the purported differences in saccade planning discussed in the experiments so far, might also indicate differences in the areas or extent of cortical involvement dependent on the nature of the task.

4.6. Conclusions

The experiments discussed so far go some way towards helping us understand the spatial remapping of target locations for the planning of saccades, and have in particular provided insight into how this may be influenced by task-related factors. Ecologically this idea makes sense, since it would be useful for a behaviour such as this to be modifiable in response to the demands of the specific task in hand. Given this, therefore, it might be expected that the cortical areas involved in the performance of saccade tasks such as these discussed so far, might also vary according to the task

requirements and the way these influence how the saccade is to be planned. The next Chapter will therefore focus on investigating the cortical areas associated with spatial remapping in saccade sequences, through the use of functional magnetic resonance imaging.

Chapter 5: Investigation of the Cortical Areas involved in Encoding and Remapping Target Locations for the Planning of Memory-guided Saccades

5.1. Introduction

The remapping of visual information that occurs in advance of an eye movement has been used to help explain how our visual perception of the world remains stable and up-to-date despite the almost constant shifts of gaze that we perform. The intention to make a saccade appears to be sufficient to trigger this spatial updating process (Duhamel et al., 1992), which takes into account changes in the position of the eye that will be brought about by the movement. This is thought to be done through the use of eye position or eye displacement signals (Andersen and Buneo, 2002) such as an efference copy of the motor command or corollary discharge, a signal of intended movement (Schlag and Schlag-Rey, 2002).

The majority of previous studies investigating spatial updating of visual information, including both single-neuron studies in non-human primates (e.g. Duhamel et al., 1992; Snyder et al., 1997) and functional imaging studies in humans (e.g. Heide et al. 2001; Medendorp et al. 2003; 2006; Merriam et al. 2003; Sereno et al. 2001) have supported the idea that this process may have a parietal locus. A number of other studies that made use of a double-step saccade task to investigate saccade-related spatial updating have similarly concluded that the parietal cortex is essential to this process. These have included cortical inactivation in monkeys, through the use of muscimol injection (Li and Andersen 2001), an analysis of the performance of human patients with posterior parietal lesions (Heide et al., 1995) and the use of virtual lesions, brought about by transcranial magnetic stimulation (TMS), in healthy subjects (van Donkelaar and Müri 2002). One region of the posterior parietal cortex in particular has been identified in monkeys, a region known as the lateral intraparietal area (area LIP). The corresponding area in humans is similarly thought to be located on the banks of the intraparietal sulcus and has been termed ‘the parietal eye field’ (Andersen et al., 1992). Activity believed to correspond to this area was found in an fMRI study by Heide et al., (2001)

involving triple-step saccade sequences. This focus of this activity was located on the lateral bank of the IPS (IPL, BA 40, Talairach coordinates: 44, -48, 36).

Two main interpretations exist regarding the functional significance of neuronal activity in area LIP. Colby & Duhamel, (1996) for example, have argued that neurons in this area are responsive for attended spatial locations encoded in retinotopic coordinates. In contrast to this however, work by Snyder et al., (1997) among others, have led them to conclude that activity within this area is indicative of the intention to make a saccade towards a particular location. The matter of debate therefore lies in whether this predictive remapping response is sensory or motor in nature.

Some recent single-neuron studies have provided evidence that it may not just be visual neurons in posterior parietal areas such as LIP that exhibit remapping behaviour; areas thought previously to have a more purely visual function may also be involved in this process. The results of a study by Nakamura and Colby (2002), for example, suggested that signals for intended saccades lead to the updating of visual information in extrastriate cortex. It was proposed therefore that remapping may not be restricted to just the attentional and oculomotor areas seen previously, such as LIP, FEF, and the superior colliculus. Alternatively, this process might take place in parietal cortex initially but can then later be observed in extrastriate cortex as a result of back projections from LIP.

Further support for an involvement of occipital cortex in this process comes from a study by Supèr et al. (2004). Presaccadic activity for memory-guided saccades in the primary visual area, V1, was observed and on the basis of this it was proposed that neuronal responses in this area might reflect the use of eye displacement signals in saccade planning. The role of V1, it was suggested, might be to provide motor areas with the relevant visual information required for planning eye movements.

Few of the previous fMRI studies investigating spatial remapping have discussed the possibility of contributions to this process from occipital areas. The existence of activity in this cortical region has occasionally been mentioned, but most have it seems, on the basis of expectations from previous research, chosen to focus on activity within the parietal lobes (c.f. Medendorp et al., 2003). In the triple-step saccade study by Heide et al., (2001), for

example, images were only acquired from the dorsal part of the brain above the temporal and occipital poles, thus precluding any discussion of activity within the primary visual cortex during this task. In the current study, therefore, although the principal region of interest will similarly be the PPC, consideration will additionally be given to other areas of activity throughout the cortex, including the occipital lobes if appropriate.

The effect of manipulating order of target presentation on the planning and execution of double- and triple-step memory-guided saccade sequences was discussed in Chapter 4. These studies demonstrated, firstly, a difference in latency between the forward and reverse order conditions, when the delay between the target presentations was short (500ms), suggesting a difference in the complexity of the spatial computations required to plan the sequence. Secondly, and more consistently at the longer delay durations, a difference in the extent of amplitude compensation was seen; this effect revealed greater compensation on reverse compared to forward order trials in the double-step task and also greater compensation on the triple-step task when the relevant targets were presented apart as opposed to together in the presentation order. In terms of angular compensation, however, any differences seen were much less consistent across the three studies. These studies therefore proved useful in terms of increasing understanding of the experimental factors affecting spatial remapping, but could not provide any insight into the cortical areas involved in this process. It was therefore decided to investigate this issue through the use of an event-related fMRI version of the reverse double-step saccade task. Due to the nature of event-related fMRI, longer delays were required between the presentations of successive stimuli of interest than had been used previously in Experiments 7 and 8. An additional behavioural version of the task incorporating these longer delays was therefore conducted. By using the same participants in this and the fMRI task, this served the dual purpose of supplying further information on the effects of task-related factors such as delay on saccade metrics, whilst also providing a pre-scanning training session on the task.

5.2. Experiment 10: Extended Reverse Double-Step Saccade Study

Based on the findings of the behavioural studies discussed in Chapter 4, it was predicted that a difference in amplitude compensation would again be observed between the forward and reverse orders of target presentation. Specifically, it was expected that spatial remapping, quantified in terms of compensatory amplitude gain, would be greater on the reverse compared to the forward order trials. Since the results in terms of angular compensation had been less consistent in the previous behavioural studies, no specific predictions were made regarding this variable.

Given the results of Experiment 8, a difference in latency was not necessarily expected since extended delay durations were also being used in this task. There was also no reason on the basis of previous results to predict a difference in inter-saccadic interval (ISI), although this measure was still calculated for the sake of completeness.

End-point accuracy in the two conditions was also compared. Based on Experiments 7 and 8 it was expected that larger differences would be seen between the error for saccades 1 and 2 in the reverse compared to forward-order trials. Such a result would be expected due to the interacting influences of time since viewing the target and order of execution effects. For the same reason, it would be predicted that accuracy would overall be worse in this study than in Experiments 7 and 8 (Chapter 4) since the delay duration following the target presentations has again been increased.

Methods

Participants

Nineteen healthy participants (13 females); aged 21-48 (mean 24.8 years) took part in this task. All had normal or corrected to normal vision.

Materials

The experimental setup was identical to that described in Experiment 7 (Chapter 4).

Procedure

Oculomotor Task: This was essentially the same as that described in Experiment 7 (Chapter 4), the only differences being in relation to the timing of experimental events. The time between successive target presentations was increased to 5000ms in the present study. The delay period prior to the go-signal also had a slightly longer variable duration, based on a normal distribution (mean = 1000ms, s.d. = 250ms). Following this, the eye tracker continued to record for a further 9000ms to allow participants enough time to complete both saccades. Stimulus presentation and task instructions did not differ from those in Experiment 7 (Chapter 4).

Data Analysis: This was performed in the same way as that described previously in Experiment 7 (Chapter 4). Latency, ISI, end-point error and measures of amplitude and angular compensation were calculated as before. An example trial showing x and y eye position over time can be seen in Figure 5.1 below.

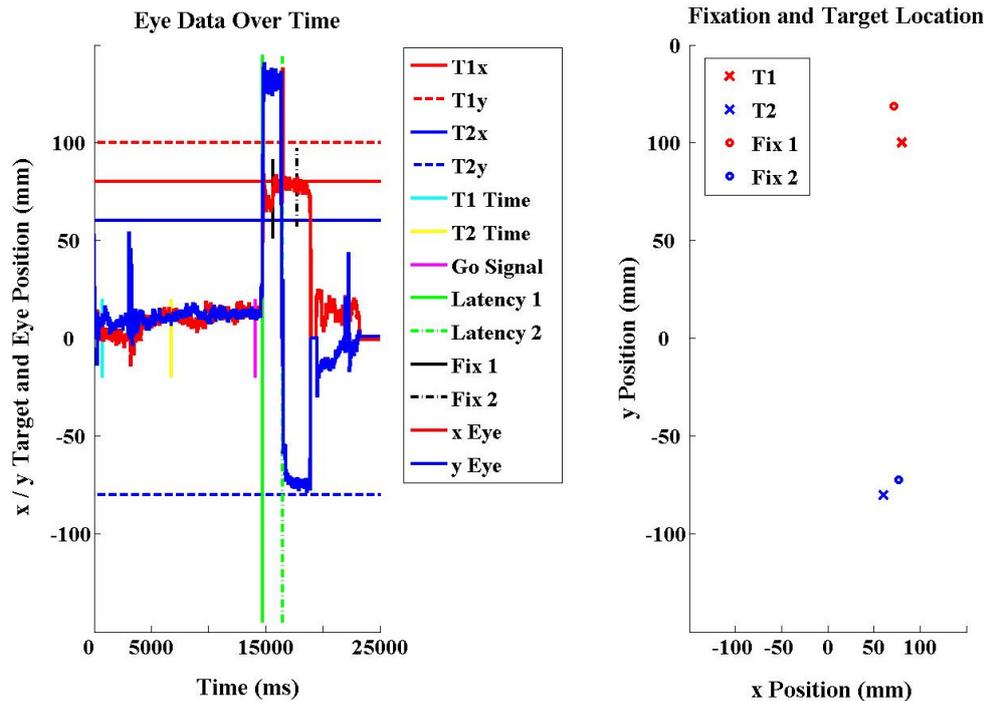


Figure 5.1: Plot of eye movement trace using x (red trace) and y (blue trace) coordinates from eye-position data for one trial (left plot). The participant can be seen to be fixating the centre of the screen (0 on y axis) until the go-signal (vertical pink bar). The horizontal red and blue bars show the x (solid) and y (dashed) locations of targets 1 and 2 respectively. The two saccades and fixations can be seen clearly following the go-signal, after which the participant looks back to the centre of the screen. The times of the two target presentations are shown as light blue (target 1) and yellow (target 2) vertical bars. The green vertical bars show the latency of the 2 saccades, and the black vertical bars show the end-point fixations (solid, saccade 1; dashed, saccade 2). The end point of these saccades is also plotted in relation to the target positions on the screen (right plot).

Results

Latency: A two-tailed paired sample Student's t-Test was used to assess for a difference in the group mean latency between the forward and reverse conditions. No significant difference was seen (forward: mean = 340.41ms, s.d. = 128.93ms; reverse: mean = 330.87ms, s.d. = 125.94ms; $t_{(18)} = 1.450$, N.S.).

Intersaccadic Interval: A two-tailed paired-sample Student's t-Test was used to assess for a difference in the group mean ISI between the forward and reverse conditions. The difference was found to be approaching significance (forward: mean = 1366.81ms, s.d. = 414.51ms; reverse: mean = 1328.70ms, s.d. = 413.73ms; $t_{(18)} = 2.049$, $p = 0.055$).

End-Point Error: A 2x2 within-subjects ANOVA with the factors order (forward and reverse) and saccade (saccades 1 and 2) was used to assess group mean end-point error in the two conditions. There was no main effect of order ($F_{(1,18)} = 2.835$, N.S.). There was however a significant main effect of saccade ($F_{(1,18)} = 11.602$, $p < 0.005$) and a significant order x saccade interaction ($F_{(1,18)} = 12.919$, $p < 0.005$). The data from these analyses are shown in Figure 5.2 below.

Amplitude and Angular Compensation Measures: A one-tailed paired-sample Student's t-Test was used to assess the a-priori prediction that the group mean compensatory amplitude gain would be greater in the reverse compared to the forward order condition. A significant difference was found in the direction expected (forward: mean = 0.96, s.d. = 0.06; reverse: mean = 0.98, s.d. = 0.05; $t_{(18)} = 2.398$, $p < 0.05$). For the angular compensation measure, a two-tailed paired-sample Student's t-Test was used to assess for a difference in the group mean values. A significant difference was found, with angular compensation being greater in the forward than the reverse condition (forward: mean = 5.56, s.d. = 1.77; reverse: mean = 7.01, s.d. = 1.50; $t_{(18)} = 3.461$, $p < 0.005$).

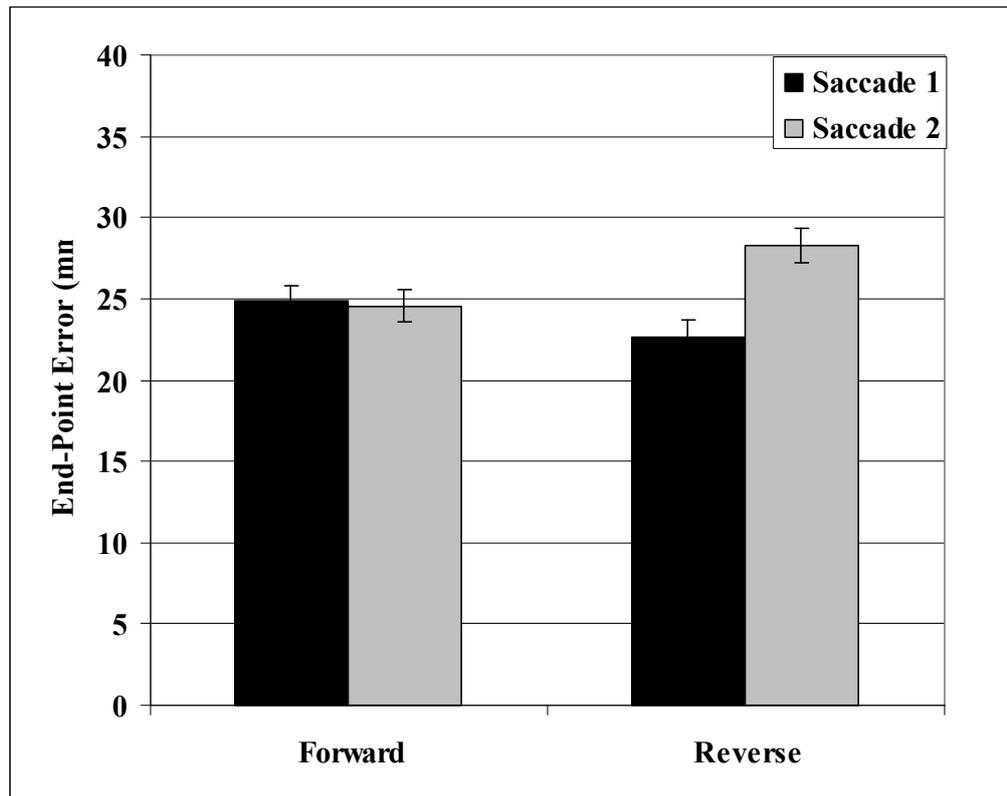


Figure 5.2: Mean end-point error for the first (black bars) and second saccade (grey bars) in both the forward and reverse order conditions. Error bars show standard errors.

Discussion

As predicted, the group mean compensatory amplitude gain was found to be greater in the reverse target presentation order compared to the forward-order condition. This effect, which suggests a difference in the amount of spatial updating that takes place for the two trial types, therefore appears to be quite robust since it is seen at all of the delay durations tested in Experiments 8 and 10 (1000-5000ms). This effect was not significant however in Experiment 7 with the shorter delay duration of 500ms. This could potentially be explained by the fact that the two targets are easier to code in relation to each other when the time period between successive target presentations is so brief.

What is less clear however is why angular compensation was found to be significantly greater in the forward compared to reverse condition in this study. This variable had however been less consistent in terms of any differences

between the two presentation orders in the studies discussed previously in Chapter 4. Exactly how a motor plan for a saccade in a sequence is updated in terms of angle and amplitude, in order to account for end-point error in a previous saccade, is not yet understood. It may be, as suggested in Chapter 3, that the two aspects of the saccade plan are coded separately and therefore the factors that affect their updating might also differ.

The absence of a significant difference in latency was not surprising, since as mentioned in the introduction to this Chapter and in relation to Experiment 8 (Chapter 4), the time available is most likely more than sufficient for all necessary processing to be completed in both conditions. The difference in ISI between the forward and reverse order trials was found to be approaching significance, with a longer ISI seen for the forward condition. No effects on this metric had previously been seen in the other behavioural versions of this task. This does not appear to be related to the time required to retrieve a memory of the target location for the upcoming saccade, since a greater amount of time had passed since saccade-target 2 was seen in the reverse compared to the forward-order trials. It can also not be explained by the amount of amplitude compensation that occurs to account for error in saccade 2, since this was greater in the reverse condition than the forward. One possibility, however, is that it is instead related to the amount of angular compensation that occurred, since this *was* found to be greater on forward order trials. Angular compensation might, for example, occur at the end of the saccade, during the ISI period, in response to visual error signals, whereas amplitude compensation on the other hand may occur in a more predictive fashion, i.e. during execution of the first saccade, perhaps by means of a comparison of efference copy of the motor command (intended end-point) with sensory reafference (predicted end-point). This idea is supported by the results of Experiment 3 (Chapter 2) in which TMS was delivered after saccade 1 (i.e. during the execution of the double-step sequence) and an effect was seen in terms of improved angular, but not amplitude, compensation. If this is the case however, it is not clear exactly what the task-related reasons for this being greater in the forward- than reverse-order trials might be.

The pattern of results seen for end-point error, can, as predicted, be explained in terms of a combination of effects related to the order of execution

and time since viewing the corresponding target. In the forward order trials, for example, the order of execution effect that can normally be seen on a double-step saccade has been cancelled out by the effects of the long delay periods since target presentation. Given that it is around twelve seconds since the target for saccade 1 was presented, compared to around 6 seconds for the target for saccade 2, it is not surprising that the accuracy of saccade 1 would be reduced as a result of decay. On the reverse order trials in contrast, the target for saccade 1 has been seen more recently than that for saccade 2, so the order of execution effects have been augmented, with a greater difference in accuracy seen between the two saccades.

5.3. Experiment 11: Investigation of the Cortical Areas Involved in the Reverse Double-Step Saccade Task Using fMRI

In order to investigate the cortical areas responsible for the different aspects of the task, it was decided that the trials should be considered in terms of the three separate subcomponents: target 1 presentation (T1), target 2 presentation (T2) and the go-signal (Go). By doing this, it was hoped that areas involved specifically in for example coding the first target could be identified and comparisons made on the basis of whether or not this was the target for the first upcoming saccade in the sequence.

On the basis of findings from previous research such as that by Curtis et al., (2004), greater parietal activity would be expected in the reverse condition at the time of target 1 presentation, when a spatial code for the target location must be used, compared to the forward condition when the prospective motor code required to saccade to this target can instead be formed. However, since Andersen and Buneo (2002) have suggested that default movement plans may be formed to a visual stimulus, it might instead be the case that no difference will be seen between the activity in the PPC for the forward and reverse conditions.

Since similar processes would be assumed to take place at the time of the second target presentation and the go-signal, it was difficult to predict whether differences would in fact be seen between the forward and reverse order trials at these time points. At target 2 presentation, the double-step saccade sequence

could be planned as a whole, since all the requisite information is now available, the only potential difference being therefore in terms of the complexity of the spatial computations required. It was not known whether a difference in complexity would be sufficient for a difference in terms of the level of functional activity within a common area to be observed. At the time of the go-signal, similarly, in both cases participants would be executing the pre-planned double-step saccade sequence. At least a certain amount of similarity between the areas and level of activity in both conditions would therefore seem likely.

The present study also compared blocks of both single- and double-saccade trials; Heide et al., (2001) had similarly used single-saccade trials as a control condition in their task involving triple-step saccade sequences. These are useful as a control, since, as they point out, they require a similar motor output but a different level of spatial computation in terms of planning, i.e. they can be coded and executed in terms of retinal coordinates, whereas a sequence of saccades cannot. Heide et al. did not however assess activity at the time of each target presentation as well as in the saccadic response period.

In the interests of gaining a more complete picture of the areas of activity involved, and in light of the recent non-human primate findings suggesting a role for visual areas in saccade-related remapping, the areas of activity considered in this study included both parietal and occipital cortices, alongside other oculomotor areas related to saccade planning for example the frontal and supplementary eye fields. It was expected that if presaccadic remapping was in fact a process carried out exclusively by posterior parietal areas then greater activity would be seen in these regions on the double-saccade trials, when the upcoming saccade could not be carried out using a retinal vector and would thus presumably require a greater level of spatial remapping than single-saccade trials. It was decided that any posterior parietal activity seen would be compared to that found by Heide et al. in order to assess whether it corresponded to the proposed location of the PEF. Furthermore, if this process were in fact also a function of the occipital cortices, then greater activity would similarly be expected in this region on the double-step compared to single-step trials, when parietal encoding of target locations, whether as a prospective motor code or a spatial code, would be insufficient to complete the task.

Methods

Participants

Eighteen adults (12 females; ages 21-48 years) with normal or corrected to normal vision participated in this task. All had previously taken part in Experiment 10.

Materials

The stimuli were displayed to the participants using in-scanner goggles (Silent Vision, Avotec, Inc.). These stimuli were generated using Eprime (Psychology Software Tools Inc), but were essentially identical to those described in Experiment 10.

Participants wore ear protection and lay in a supine position in the scanner. They were calibrated in the in-scanner goggles so that they could see the visual display clearly. Imaging was performed at the Magnetic Resonance Centre (University of Nottingham), using a Philips 3.0-Tesla scanner equipped with a multiple-element Sense® head-coil (sense factor = 2). 36 contiguous axial slices (20.8 cm FOV, 64 x 64 matrix, 3mm slice thickness, in-plane resolution = 3.25 x 3.25 x 3 mm³) parallel to the AC-PC plane, which covered the whole brain using a gradient-recalled EPI sequence (TR = 2.1sec, TE = 35ms). fMRI data were stored in 750 volume image files. (FOV = field of view; TR = time of repetition; TE = Echo Time; AC = Anterior Commissure; PC = Posterior Commissure; EPI = Echo Planar Imaging).

Procedure

Oculomotor Task: This main task was the same as that described above for the behavioural version (Experiment 10), the only distinction being the inclusion of the control condition. In this, two targets were shown but participants only had to look to one of them.

Control procedure: The experiment began with a block of 8 control trials, in which eight of the 16 possible trial types were displayed (four forward and

four reverse target presentation orders, half in a top-bottom order, half bottom-top, and half left-right, half right-left). Two targets (one black, one white) were presented as before; participants were informed in advance which colour target they had to plan a saccade to.

Main procedure: There were 3 blocks of 18 trials; for two out of the 18 trials only a single target was displayed as a further control condition. On these trials participants were instructed to remember the location of the single target, regardless of its colour, and to look to its previous location following the beep. After each block a rest screen was displayed for 20ms. Participants were instructed in advance as to which order they should look towards the remembered locations of the targets (i.e. black-white or white-black).

Data Preprocessing and Analysis: PAR format images (Philips Medical Systems) were transformed into ANALYZE format using the MRIcro software (Chris Rorden, www.mricro.com). Analyses of the fMRI data were carried out using the Matlab SPM2 (Statistical Parametric Mapping) toolbox. Data preprocessing began with realignment (motion correction) using rigid-body registration to the mean image, with a 4th degree B-spline interpolation method. This was followed by spatial normalisation to an EPI (Echo-Planar Imaging) template, after which the images had a resolution of 3 x 3 x 3 voxels. Spatial smoothing was also performed using a Gaussian kernel (8mm, full-width half-maximal). BOLD (blood oxygen level dependent) signal changes evoked by events within each trial were modelled using a canonical haemodynamic response function convolved with time derivatives.

A General linear model (GLM) was used in order to search for significantly activated voxels. A design matrix was defined comprising contrasts that tested for all the events of interest within each trial and for the different trial types (single- and double-saccade trials, with forward and reverse target presentation orders). t-contrast images were defined for each subject and the data were analysed at the group level using random effects analysis performed in SPM2, which pools the data across each condition for all participants. One-sample t-tests were conducted using the appropriate contrast images for each participant, to assess for greater activity at the time of one experimental event compared to another. Statistical significance was set to a

height threshold of $p < 0.005$ ($t > 2.9$) and the resulting statistic images were assessed for cluster-wise significance with a spatial extent threshold of at least 40 contiguous voxels (cluster-level corrected, $p < 0.05$).

MNI coordinates from SPM2 were converted to Talairach space (Talairach and Tournoux, 1988) using Matthew Brett's Matlab function 'mni2tal.m' (see: <http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml>), which provides estimated Talairach coordinates, for given points in the MNI brain. Talairach labelling was then performed based on a search for the nearest grey matter using Talairach Daemon Java Client (see: <http://ric.uthscsa.edu/projects/talairachdaemon.html>) an electronic database of neuroanatomical locations (Lancaster et al., 2000).

Functional MRI Results: Overall effects of Reverse vs. Forward-order

Target 1: Forward vs. Reverse Order

Differences in brain activation at the time of target 1 presentation were first assessed for the forward and reverse presentation order trials in the double-saccade block. In both cases, the location of this target must be remembered for future use in a saccade plan, but in the forward condition this is the target for the first saccade in the sequence and so theoretically a prospective motor code could be formed at this point. In the reverse condition, in contrast, a plan cannot yet be formed since this is the target for the second saccade in the sequence. No areas of greater activation were seen for this comparison, in either direction.

An assessment of the areas of activity for the two conditions alone (i.e. compared to baseline) revealed three clusters of significant activation for the Target 1 Forward condition; the foci for these clusters were centred in the left PPC, with foci in the IPL, SPL and precuneus, and the left and right temporal lobe. For the Target 1 Reverse condition, 4 clusters of significant activation were found, with foci centred in the left (SPL/IPL) and right PPC (IPL/SPL/Precuneus) and the left and right temporal lobe. The coordinates

suggested by Heide et al., as corresponding to the PEF (+/-44,-48,36) fell just at the base of these clusters of parietal activation (See Table 5.1).

Target 2: Forward vs. Reverse Order

At the time of the second target presentation, participants should be able to use the information on both target locations to plan the double-step saccade sequence. In the reverse trials this will involve making use of previously remembered spatial information on the location of the target for the second saccade. In the forward condition, in contrast, participants could use information on the end-point of the first saccade, which may already have been planned. No areas of greater activity were seen in either direction for this contrast.

An assessment of the areas of activity for the two conditions alone (i.e. compared to baseline) revealed two clusters of significant activation for the Target 2 Forward condition in the left (SPL/IPL) and right PPC (SPL/IPL/precuneus). Similarly two clusters of significant activation in the left and right PPC (both SPL/IPL) were found for the Target 2 Reverse condition. The coordinates suggested by Heide et al., as corresponding to the PEF (+/-44,-48,36) fell just at the base of the bilateral parietal clusters for the reverse-order condition and the right-hand cluster for the forward condition; the PPC cluster on the left for the forward condition was located slightly superior to these coordinates. Two additional clusters of activity were seen for this condition in the right and left frontal lobe, in the region of the FEFs. (See Table 5.1).

Go-Signal: Forward vs. Reverse Order

The go-signal is the time at which participants will execute the double-step sequences. An equivalent motor output will be required for both the forward and reverse order trials. For this contrast no areas of greater activity were seen for the forward compared to reverse-order trials, or for the opposite comparison.

The extent of activation at the go-signal for the forward and reverse-order conditions compared to baseline was much greater than that seen for the target 1 and target 2 presentation times. Therefore a higher threshold was set to

determine significance, in order that the areas of activation could meaningfully be considered. The threshold was set to $t > 4.71$ ($p < 0.0001$), with clusters of greater than 40 contiguous voxels, corrected for multiple comparisons at the cluster level ($p < 0.05$). For the forward order trials, an assessment of activity at the go-signal compared to baseline showed six clusters of significant activation. These clusters were centred in the occipital lobe, left frontal lobe, left parietal lobe (precuneus/postcentral gyrus), right frontal lobe in the region of the FEF (middle frontal/ precentral gyrus, BA 6) and right temporal lobe. There was also a subcortical cluster of activity in the lentiform nucleus/thalamus. For the reverse-order trial at the time of the go-signal, eight clusters of significant activation were seen, with foci in the occipital lobe, bilateral frontal (in the region of the premotor cortex) and temporal lobes and left PPC (precuneus/SPL). There were also two clusters of sub-lobar activation seen bilaterally in the lentiform nucleus. The large clusters of activity with peaks in visual cortex incorporated bilaterally the coordinates suggested by Heide et al., as corresponding to the PEF (+44,-48,36) for both the forward and reverse order conditions.

A complete list of the clusters and foci of significant activation for the conditions discussed above is shown below in Table 5.1. Talairach labelling has been performed based on a search for the nearest grey matter using the Talairach Daemon Java Client (see Procedure above for details). Statistical parametric t maps depicting this activity are also shown below in Figure 5.3.

Region	Left Hemisphere							Right Hemisphere						
	k	x	y	z	t	Location	BA	k	x	y	z	t	Location	BA
Target 1 Forward														
PPC	559	-27	-58	64	5.95	SPL	7							
		-36	-39	38	4.88	IPL	40							
		-24	-56	47	4.57	Prec.	7							
OTC	361	-45	-70	1	5.41	ITG	19 / 37	280	39	-59	-15	4.87	Declive	
		-42	-66	12	4.68	MTG	4		48	-73	1	4.6	ITG	18
		-45	-56	-10	4.38	FG	37		24	-67	-9	4.2	FG	19
Target 1 Reverse														
PPC	510	-30	-58	61	8.06	SPL	7	449	36	-39	38	4.56	IPL	12
		-36	-36	38	5.61	IPL	40		24	-59	50	4.35	Prec.	7
		-30	-56	50	5.39	SPL	7		15	-64	56	4.01	SPL	7
OTC								339	36	-62	-10	5.69	Declive	
									42	-56	-12	5.21	FG	37
									48	-75	9	4.89	MTG	39
Visual	500	-45	-70	1	5.71	ITG	19/37							
		-45	-81	10	5.48	MOG	19							
		-30	-73	-4	4.47	LG	18							
Target 2 Forward														
PPC	771	-27	-58	61	6.1	SPL	7	404	24	-58	61	4.28	SPL	7
		-39	-44	49	5.32	IPL	40		39	-44	46	4.2	IPL	40
		-45	-47	55	5.26	IPL	40		9	-59	47	4.05	Prec.	7
Target 2 Reverse														
PPC	779	-27	-58	61	6.67	SPL	7	621	24	-58	64	6.15	SPL	7
		-18	-64	58	6.09	SPL	7		15	-61	61	5.39	SPL	7
		-39	-47	49	6.07	IPL	40		42	-38	46	5.28	IPL	40
FEF	204	-30	-3	47	4.39	MFG	6	140	21	8	36	4.78	ACc	32
		-24	2	41	4.27	MFG	6		30	0	58	4.37	MFG	6
		-24	-3	64	3.89	SFG	6		39	-3	58	3.49	MFG	6
Go Forward														
Parietal	570	-18	-77	43	7.74	Prec.	7							
		-12	-55	64	7.71	PostG	7							
		-50	-30	37	7.11	PostG	2							
Visual	3232	-12	-85	-1	11.01	LG	17							
		12	-78	20	10.18	Cuneus	18							
		36	-78	18	10	MOG	19							
FEF	335	-36	12	-3	10.19	Insula	13							
		-33	-3	47	7.05	MFG	6							
		-45	-6	50	6.4	PreG	6							
PMC								974	56	6	11	7.97	PreG	44
									45	-1	41	7.44	PreG	6
									53	5	36	7.37	MFG	9
Temporal								56	39	0	-3	5.64	Insula	13
									50	8	-8	5.52	STG	17
Subcortical	70	-15	-9	0	9.23	LN	MGP							
		-9	-20	-1	6.2	Thalamus	MB							
		-9	-20	7	6.2	Thalamus	MDN							
Go Reverse														
PPC	569	-18	-76	45	8.77	Prec.	7							
		-27	-58	61	8.22	SPL	7							
		-18	-64	58	7.89	SPL	7							
Visual	3207	-15	-76	-1	10.95	LG								
		-21	-75	18	10.4	Cuneus	18							
		12	-92	16	10.2	MOG	18							
PMC	60	-56	2	30	6.77	PreG	6	878	56	10	30	8.5	IFG	9
		-59	9	5	5.35	PreG	44		-30	-6	47	7.34	MFG	6
									59	7	19	7.33	IFG	44
Temporal	130	-39	9	-3	8.99	Insula	13	56	59	-26	-1	7.30	MTG	21
		-50	11	-6	6.84	STG	22	64	59	-43	8	6.79	STG	22
		-24	-3	0	6.2	LN	Put.		62	-37	18	6.10	STG	23
Subcortical								42	18	-3	-2	6.52	LN	GP
									24	1	11	6.30	LN	Put.

Table 5.1: Clusters and foci of significant activation ($p_{\text{corrected}} < 0.05$) in the regions of interest for the three main experimental events in the forward and reverse-order double-saccade trials. (Adapted from Heide et al., 2001). For target 1 and target 2, $t > 2.9$ and for the go-signal $t > 4.71$.

(k, cluster size; x, y, z, Talairach coordinates of the voxels showing peak activation in each cluster; t, the corresponding t-value; BA, Brodmann areas involved. ACc, caudal anterior

cingulate; FEF, frontal eye fields; FG, fusiform gyrus; GP, globus pallidus; IFG, inferior frontal gyrus; IPL, inferior parietal lobe; LG, lingual gyrus; LN, lentiform nucleus; MFG, middle frontal gyrus; MB, mammillary body; MDN, medial dorsal nucleus; MGP, medial globus pallidus; MOG, middle occipital gyrus; MTG, middle temporal gyrus; PMC, premotor cortex; PostG, postcentral gyrus; PPC, posterior parietal cortex; Prec., precuneus; PreG, precentral gyrus; Put., putamen; SPL, superior parietal lobe; STG, superior temporal gyrus).

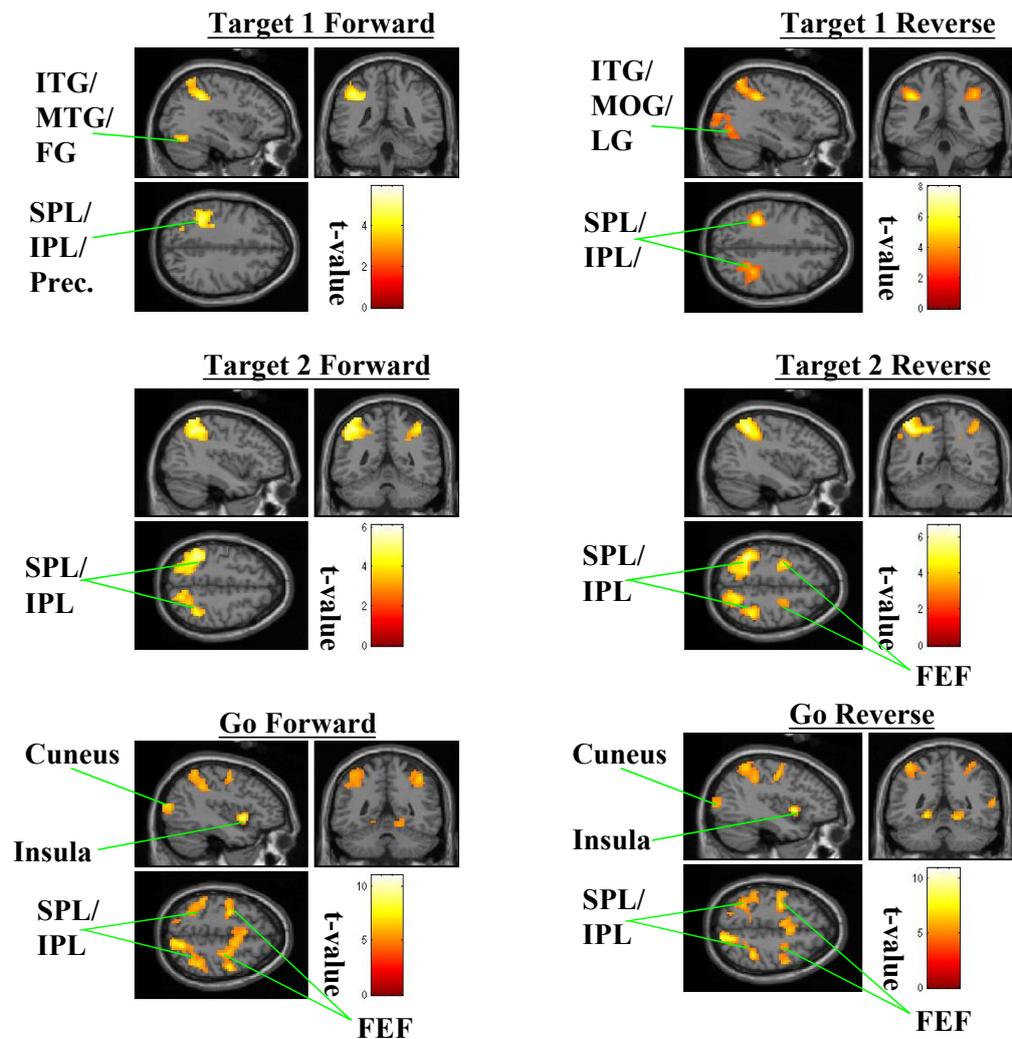


Figure 5.3: Clusters of significant activation ($p_{(\text{corrected})} < 0.05$) in the regions of interest for the double-saccade forward and reverse-order trials at target 1, target 2 and the go-signal. For target 1 and target 2, $t > 2.9$ and for the go-signal $t > 4.71$. Clusters of activity in the left and right superior parietal and inferior parietal lobes, and bilateral FEF activity have been labelled, as well as that in temporal and occipital cortex and sub-lobar activity in the insula.

Interim Discussion

The results discussed above indicate that although activity in the PPC, which appeared to correspond to the region of the PEF, was present as had been predicted, the same areas appeared to be activated for both the forward and reverse-order trials at the time of each of the three events, i.e. target 1, target 2 and the go-signal. These effects could be explained by the fact that similar processes were occurring for both forward and reverse order trials, or alternatively different processes were controlled by the same cortical area. It might also be possible that although the same areas were activated, the intensity of the activation was greater in one case compared to the other.

In order to focus more specifically on saccade-related and remapping processes, single vs. double-saccade comparisons were made. The single-saccade trials had an identical level of visual stimulation as the double-saccade trials, however the amount of planning required was lower (i.e. one saccade rather than two) and there was also no need to perform spatial computations on the location of the targets, since the saccades could be planned at the time of presentation simply on the basis of a retinal vector from the fixation point to the target.

Functional MRI Results: Single vs. Double-Saccade Contrasts

Not all of the possible single vs. double-saccade comparisons that can be made are actually theoretically meaningful, for example, comparing activity from single and double-saccade conditions at T1 for the reverse-order trials would reflect a comparison of a condition in which the participant is ignoring the target, in the single-saccade condition, with attending to the spatial location in the double-saccade condition. Such a contrast is not of particular interest to the current study and has thus been omitted. The comparisons deemed to be theoretically valid were as follows:

Single vs. Double at T1 for Forward-order condition

For both trial-types in this comparison participants must plan a single saccade to the first target at T1. The only difference being, in the single-saccade trials the complete motor plan can be formed at this point, whereas in the double-saccade trials, more information has yet to be added. For the single > double contrast, one cluster of greater activity was seen in the right occipital/temporal lobe. No areas of greater activity were seen for the opposite comparison, i.e. double > single-saccade. (See Table 5.2).

Single vs. Double at T2 for the Forward-order condition

This contrast compared the condition when participants are remembering a saccade plan or target location from the presentation at T1 and do not have to attend to this stimulus (single-saccade) to that when they are similarly remembering this information, but do have to attend to the second target presentation at T2 in order to plan a second saccade (double-saccade). No areas of greater activity were seen for the single > double-saccade contrast. For the double->single-saccade contrast, however, one cluster of greater activity was seen, centred in right occipital cortex.

Single vs. Double at T2 for Reverse-order condition

For this comparison, participants must plan a saccade in both cases, although for the single condition, they will have just ignored the target at T1, whereas for the double-saccade condition, they will have remembered the location of the target at T1 and will now have to plan saccade 1 of 2 to the target at T2, and saccade 2 of 2 from there to the remembered location of T1. No areas of greater activity were seen for the single compared to double-saccade trials, however there were four clusters of greater activity for the double- compared to single-saccade trials. These were centred in the right occipital cortex, anterior cingulate cortex and left and right frontal lobes. The frontal activity included one cluster in the DLPFC on the left and a second

much larger cluster with foci in the left and right hemispheres, in the region of the FEF (precentral and middle frontal gyri, BA4/6). (See Table 5.2).

Forward-Order Double vs. Reverse-Order Single at T2

This contrast compares activity at T2 when participants are either planning a single saccade to T2 (having ignored T1) in the reverse order trials, to that when they are planning saccade 2 of 2 to T2, having already planned saccade 1 at T1, in the forward order trials. In the reverse order trials therefore they are able to plan a saccade to the second target on the basis of a retinal vector, whereas in the forward order trials, in order to plan the saccade to T2, they must take into account the intervening saccade that will be made to T1 first. Two clusters of greater activity were seen for the forward-order double-saccade trials, these were in the left frontal (DLPFC) and right occipital lobes. Greater activity for the reverse-order single-saccade trials was also seen, with three clusters centred in the left and right temporal, and right occipital lobes. (See Table 5.2).

Single vs. Double at the Go-Signal for Forward-order trials

It was expected that greater parietal activity might be seen for the double-saccade compared to single-saccade trials, since these would be expected to be more complicated as a result of the additional saccade to be executed. In fact there was one cluster of greater activity for the double-saccade trials, centred in left occipital cortex. For the opposite comparison, i.e. single > double-saccade trials, no areas of greater activity were seen.

Single vs. Double at the Go-Signal for Reverse-order trials

For this comparison as for the forward-order contrast, greater activity was expected for the double-saccade trials than for the single-saccade ones, since executing two saccades would be expected to be more complicated. In addition to this, the reverse-order of target presentation would be expected to add to the level of complexity for saccade planning and thus perhaps increase the extent of posterior parietal activity. Greater bilateral parietal activity in the

precuneus was in fact seen for the double > single-saccade contrast, along with clusters in the right occipital and anterior cingulate cortex. (See Table 5.2).

Table 5.2, below, shows a complete list of the clusters and foci of significant activation for the single vs. double-saccade contrasts discussed above. The corresponding statistical parametric t maps are shown in Figure 5.4.

Left Hemisphere								Right Hemisphere							
Region	k	x	y	z	t	Location	BA	k	x	y	z	t	Location	BA	
Target 1 Forward: Single > Double															
OTC								163	56	-67	3	4.74	MOG	37	
								45	-65	-14	4.65		FG	37	
								59	-59	-10	4.21		ITG	37	
Target 2 Forward: Double > Single															
Visual								235	18	-90	7	4.4	Cuneus	17	
								30	-75	18	3.81		MOG	12	
								9	-90	10	3.72		Cuneus	17	
Target 2 Reverse: Double > Single															
Visual	3121	9	-98	13	9.91	Cuneus	18								
		-30	-92	-3	9.7	IOG	18								
		-27	-95	13	8.24	MOG	18								
DLPFC	220	-12	56	22	7.63	SFG	9								
		-21	48	25	5.29	SFG	10								
		6	65	11	3.9	MFG	10								
FEF								1433	27	-20	56	6.76	PreG	4	
								-6	-23	65	6.36		MFG	6	
								18	-29	62	5.44		PreG	4	
Cingulate	492	-15	38	-4	5.61	MFG	10								
		12	32	-7	5.23	ACc	32								
		-12	47	-2	5.18	ACc	32								
Target 2: Forward Double > Reverse Single															
DLPFC	312	-15	59	5	4.94	MFG	10								
		-9	47	-2	4.92	MFG	10								
		-3	27	7	4.75	ACr	24								
visual								124	24	-81	12	4.5	Cuneus	17	
								21	-87	7	4.32		Cuneus	17	
								27	-79	-4	4.19		LG	18	
Target 2: Reverse Single > Forward Double															
OTC	174	-42	-56	-10	6.19	FG	37	373	56	-64	-7	4.69	MOG	37	
		-50	-73	1	4.76	MOG	19	45	-71	-12	4.5		FG	19	
		-53	-52	-15	4.03	FG	37	39	-56	-20	4.37		Declve		
DLPFC								128	42	31	37	4.9	MFG	9	
								36	45	28	3.95		MFG	9	
								27	59	16	3.54		SFG	10	
Go Forward: Double > Single															
Visual	2527	-15	-50	3	7.17	PHG	30								
		0	-82	3	6.83	LG	18								
		-5	-74	3	6.58	LG	18								
Go Reverse: Double > Single															
Visual								3367	-9	-95	13	13.23	MOG	18	
								9	-99	0	8.92		Cuneus	17	
								27	-79	-11	8.7		LG	18	

Table 5.2: Clusters and foci of significant activation ($p_{\text{corrected}} < 0.05$) in the regions of interest for the single vs. double-saccade comparisons. (Adapted from Heide et al., 2001).

(k, cluster size; x, y, z, Talairach coordinates of the voxels showing peak activation in each cluster; t, the corresponding t-value; BA, Brodmann areas involved. ACc, caudal anterior cingulate; ACr, rostral anterior cingulate; DLPFC, dorsolateral prefrontal cortex; FEF, frontal

eye fields; FG, fusiform gyrus; IPL, inferior parietal lobe; ITG, inferior temporal gyrus; LG, lingual gyrus; MFG, middle frontal gyrus; MOG, middle occipital gyrus; OTC, occipito-temporal cortex; PHG, parahippocampal gyrus; PostG, postcentral gyrus; PPC, posterior parietal cortex; Prec., precuneus; PreG, precentral gyrus; SFG, superior frontal gyrus).

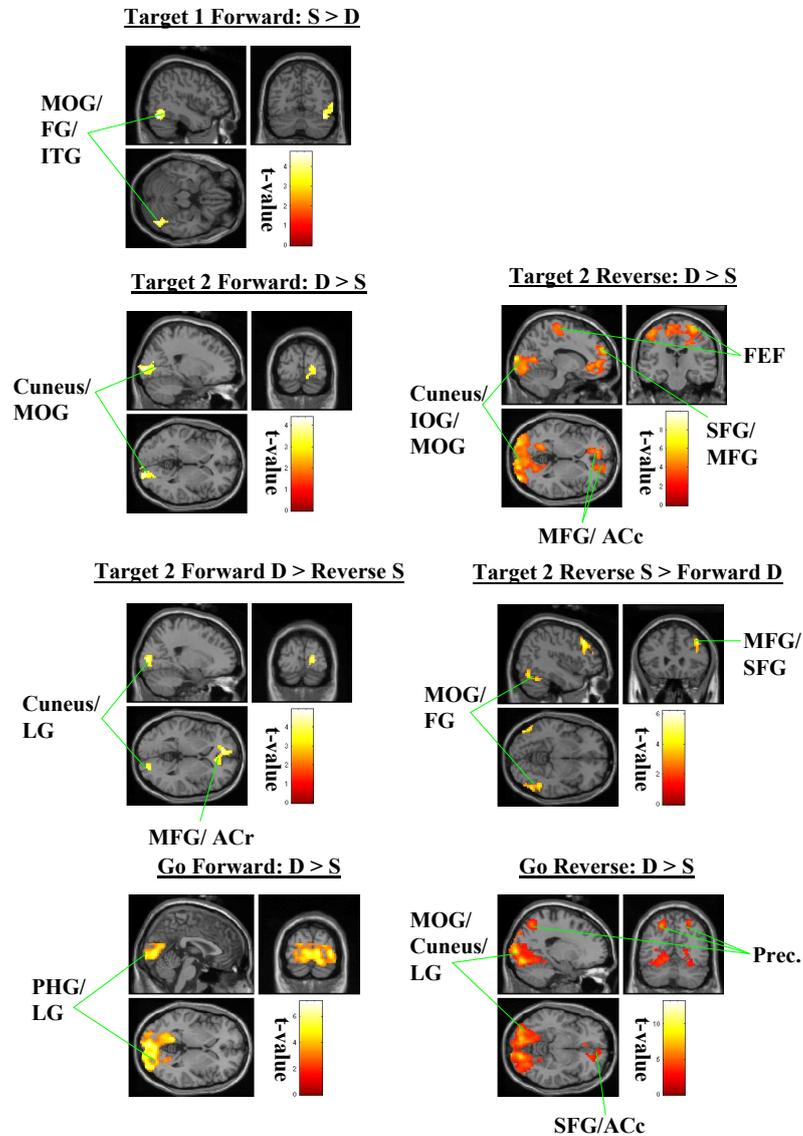


Figure 5.4: Clusters of significant activation ($t > 2.9$, $p_{(\text{corrected})} < 0.05$) in the regions of interest for the single vs. double-saccade forward and reverse-order contrasts at target 1, target 2 and the go-signal. Clusters of activity in frontal, temporal, occipital and cingulate cortex have been labelled.

Functional MRI Results: Exclusive Masking

In order to assess potential differences related to the order of target presentation, the double > single-saccade forward and reverse-order contrasts were masked against each other at T2 and Go. These contrasts show activity above that which is just related to the visual target presentation and planning a single-saccade, thus giving the additional activity that results from the planning of a second saccade. By masking the forward and reverse-order contrasts, it is thus possible to evaluate any greater activity that might be present as a result of the more complicated spatial computations that should be required on the reverse compared to forward-order trials.

T2 Reverse: Double>Single masked exclusively with T2 Forward: Double>Single

When the target 2 reverse-order double > single contrast was exclusively masked with the target 2 forward-order double > single contrast (p=0.05) four clusters of significant activity were seen, two of which were centred in the left occipital lobe, and the other two in the left and right frontal lobes in the region of the FEF (precentral and middle frontal gyri, BA 4/6). No areas of greater activity were seen when the target 2 forward-order double > single contrast was exclusively masked with the Target 2 reverse-order double > single contrast (p=0.05). (See Table 5.3).

<u>Left Hemisphere</u>								<u>Right Hemisphere</u>							
Region	k	x	y	z	t	Location	BA	k	x	y	z	t	Location	BA	
T2 Reverse: Double >Single masked exclusively with T2 Forward: Double > Single															
FEF	128	-45	-15	53	4.69	PostG	3	313	27	-21	54	5.47	PreG	4	
		-33	-26	57	3.26	PreG	4		24	5	36	4.42	MFG	6	
									30	-10	39	3.88	MFG	6	
Visual	184	-9	-99	10	5.17	Cuneus	18								
		-24	-58	6	4.56	LG	18								
		0	-87	13	4.34	Cuneus	18								
Visual	121	-27	-85	-13	4.14	IOG	18								
		-15	-96	-5	3.88	LG	17								
		-33	-93	-3	3.83	IOG	18								
Go Reverse: Double >Single masked exclusively with Go Forward: Double > Single															
Parietal	120	-33	-33	49	4.4	PostG	3								
		-30	-44	55	4.33	PostG	5								
		-24	-58	55	3.93	SPL	7								

Table 5.3: Clusters and foci of significant activation ($p_{\text{corrected}} < 0.05$) in the regions of interest for the exclusive-masking comparisons. (Adapted from Heide et al., 2001). This activity is also depicted as statistical parametric t maps in Figure 5.5 below.

(k, cluster size; x, y, z, Talairach coordinates of the voxels showing peak activation in each cluster; t, the corresponding t-value; BA, Brodmann areas involved. IOG, inferior occipital gyrus; LG, lingual gyrus; LN, lentiform nucleus; MFG, middle frontal gyrus; PostG, postcentral gyrus; PreG, precentral gyrus; SPL, superior parietal lobe).

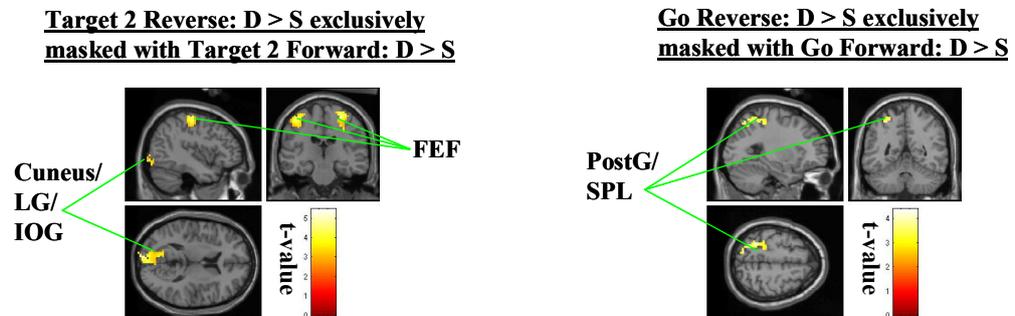


Figure 5.5: Clusters of significant activation ($t > 2.9$, $p_{\text{corrected}} < 0.05$) in the regions of interest for the exclusive masking contrasts (reverse: double > single exclusively masked by forward: double > single) at target 2 and the go-signal. Clusters of activity in occipital and parietal cortex have been labelled.

Go Reverse: Double>Single masked exclusively with Go Forward:

Double>Single

When the go-signal reverse-order double > single contrast was exclusively masked with the go-signal forward-order double > single contrast ($p=0.05$), one cluster of significant activity was seen, which was centred in the left parietal lobe, with foci in the postcentral gyrus (BA3/5) and SPL (BA 7). This did not therefore appear to correspond to the proposed location of the PEF within the IPS (IPL, BA 40). No areas of greater activity were seen when the go-signal forward-order double > single contrast was exclusively masked with the go-signal reverse-order double > single contrast ($p=0.05$). (See Table 5.3).

Table 5.3 shows the clusters and foci of significant activation for the exclusive-masking contrasts discussed above.

Discussion

A variety of areas that have been associated with saccadic activity in previous fMRI studies were found to be activated in the current experiment,

e.g. the PPC (Heide et al., 2001). Other areas also activated may have been related to the task in hand as a result of attention, working memory and other aspects of movement control. The activations found for the various contrasts are discussed below in terms of the cortical areas of activity and the particular behaviours required at these times.

Posterior Parietal Cortex Activity

Significant clusters of activity were seen in the posterior parietal cortex for all six of the main experimental conditions, i.e. forward and reverse-order double-saccade trials at the time of T1, T2 and Go.

Target 1: For the target 1 forward condition, the cluster of PPC activity was located in the left hemisphere and had 3 main foci, in the IPL, SPL and precuneus. The same parietal regions were also activated in the target 1 reverse condition, although the SPL and IPL activity was seen bilaterally, with right hemisphere precuneus activity. The contrast between these two conditions did not however reveal any areas of greater PPC activity in either direction; there does not therefore appear to be any evidence for suggesting lateralisation effects for the two conditions. It seems more likely that right hemisphere PPC activity was simply sub-threshold in the T1 forward condition. These regions of activity appear to correspond with those suggested by Heide et al., (2001) as the location of the PEF. These authors point out that similar foci have previously been found to be activated by covert shifts of visuospatial attention (e.g. Nobre et al., 1997; Corbetta et al., 1998; Gitelman et al., 1999). It is also possible, as mentioned in the introduction, that in the reverse condition, in the absence of any specific saccade plan, a default plan may instead be formed towards the location of the target until it is replaced by an alternative plan (Andersen and Buneo, 2002). This could thus also potentially account for the lack of differences between these two conditions.

Target 2: At the time of target 2 presentation, there were similarly bilateral areas of posterior parietal activation for both the forward and reverse-order trials. These clusters were located in a region spanning the SPL and IPL, and seemed to incorporate the purported location of the PEF bilaterally for the reverse-order trials and on the right, but just superior to this location on the left,

for the forward-order trials (Heide et al., 2001). A direct contrast of the two conditions, as at target 1 presentation, failed to reveal any significantly different areas of parietal activation. As in the target 1 contrasts, it is again hard to determine whether this is because the processes involved at this time are very similar, i.e. in planning saccade 2 of 2 (while taking into account the intervening saccade to the remembered location of target 1) in the forward-order condition versus planning 1 of 2 to target 2 as it is presented, and then saccade 2 of 2 from this target location to the remembered location of target 1. Alternatively, the processes involved might differ, i.e. greater spatial computations might in fact be required in the reverse-order condition, however the cortical areas associated with this processes might overlap, with the result that it is hard to distinguish any separate areas involved in these processes.

Go-signal: Although not listed as a separate cluster of activity, bilateral posterior parietal activity in the region of the SPL/IPL was also present for both forward and reverse-order trials at the time of the go-signal. The activity here was in fact part of a much larger cluster also incorporating regions in visual cortex, although in the reverse order condition, a distinct cluster of activity in the left SPL was also seen. This large cluster of activity also included bilaterally the coordinates suggested by Heide et al., as corresponding to the PEF (see Table 5.1).

As for the target 1 and target 2 presentation times, the contrasts between the forward and reverse-order trials at the time of the go-signal also failed to show any differences. This could again be due to the fact that similar processes would be expected to be occurring at this time, since in both target presentation orders participants would at this time be required to execute the pre-planned series of double-step saccades. Since there was no significant difference in latency seen for the behavioural version of this extended double-saccade task (i.e. with the longer delay periods), it might be that any differences inherent in the planning of the sequences might already have been completed by the time of the go-signal, since a sufficient amount of time would have elapsed.

Overall, therefore, the areas of PPC activity seen for all three experimental time periods appear to correspond closely with those activated in

the similar fMRI task conducted by Heide et al., (2001) involving triple-step saccades, that were believed to correspond to the PEF. The activity seen in this study did not however exhibit the same right-ward bias as found by Heide et al., with bilateral activity of the parietal lobe in fact being observed. The failure to find, as predicted, a difference between the forward and reverse-order trials might be explained due to a difficulty in distinguishing activity related to intention and attention (for the target 1 time) or due to similarities in the nature of the processes to be carried out by the participants (at target 2 and the go-signal). It is possible however, that although the same areas were activated for forward and reverse-order trials at each of the three time periods, that there were in fact differences in the intensity of the activation, although it is not possible to judge this from the current analyses.

Go Reverse: Double > Single: In terms of contrasts, the only comparison to reveal a significant region of posterior parietal cortex activity was when double-saccade trials were compared to single-saccade trials for the reverse target presentation order. Greater activity was seen for the double-saccade trials, with one cluster in the right hemisphere over the IPL (BA 40), precuneus (BA 7) and postcentral gyrus (BA2) and one cluster on the left in the precuneus (BA 7). This result concurs with Heide et al., who similarly found greater PPC activity in the right precuneus for the comparison of triple-step saccades with single memory-guided saccades in their study. However, no significant activity was seen in this region for the corresponding comparison with forward-order trials, which would have been expected based on their result. To ensure that this was not an artefact of the threshold used, an exclusive mask was applied to find areas significant for the reverse but not the forward-order trials. This confirmed greater parietal activity for the reverse-order trials, with one left-hemisphere cluster in the postcentral gyrus (BA 3/5) and SPL (BA 7).

This therefore supports the prediction that greater PPC activity would be seen for reverse-order double-saccade trials at the time of the go-signal since they are more complicated in terms of planning than the forward-order double-saccade trials.

Primary Visual Cortex Activity

T2: Double > Single: Both the forward and reverse-order comparisons of double vs. single-saccade trials at T2 exhibited greater occipital cortex activity in primary visual areas, including the cuneus (BA 17) and middle occipital gyrus (BA 12) for the forward-order contrasts and the right cuneus, left inferior and middle occipital gyri (all BA 18) for the reverse-order contrast. Through the use of exclusive masking, it was possible to determine visual cortex activity that was present in the reverse but not the forward-order contrast. This comprised two left hemisphere clusters centred in the cuneus (BA 17), lingual gyrus (BA 17/18) and inferior occipital gyrus (BA 18).

Go Forward: Double > Single: For this contrast, one cluster of greater activity was seen for double-saccade trials in left visual cortex, over the parahippocampal (BA 30) and lingual gyri (BA 18).

Go Reverse: Double > Single: Similarly for this contrast, greater visual cortex activity was seen for the double-saccade trials, although this time the focus was centred more in the right hemisphere, over the middle occipital and lingual gyri (both BA 18) and cuneus (BA 17).

One possible explanation for this comes from single-unit recording studies in monkeys that have demonstrated saccade-related activity in visual and extrastriate cortex thought to reflect remapping processes. Nakamura and Colby (2002), for example, suggested that the updating of visual signals in extrastriate cortex may be brought about via back projections from LIP through which information on intended movements would pass. Presaccadic activity in V1 for memory-guided saccades was also observed by Supér et al., (2004), who suggested that the role of V1 may be to provide motor areas with the relevant visual information required for the planning of eye movements. These theories fit with the idea in the current study of greater activity related to remapping of visual signals on the double-saccade compared to single-saccade trials.

Temporal Cortex Activity

Activity in the temporal cortex was seen in a region that appeared to correspond with what other functional neuroimaging studies have defined as

the ventral visual stream (e.g. Carpenter et al., 1999; Passingham and Toni, 2001). The contributions of the so-called dorsal and ventral visual streams have previously been defined in terms of the processing of form information for the identification of objects (ventral), also known as the ‘what’ stream, and the processing of spatial information in order to assess spatial relations (dorsal) a.k.a. the ‘where’ stream (Ungerleider & Mishkin, 1982; Mishkin et al., 1983). It has alternatively been suggested by Milner & Goodale (1995) that spatial information may in fact be relayed to the dorsal stream, whereas form information is relayed to both the dorsal and the ventral streams, albeit for different purposes. They suggested that this information was used by the ventral stream for object identification, and by the dorsal stream for determining action. They did however also note that there must be some integration between the streams, and that the selection of ‘goal objects’ and also the action to be performed on them might also rely on contributions from the ventral stream. Support for this comes from a study by Passingham & Toni (2001) in which participants were first required to identify *what* stimulus was present, in order to perform the correct action on each trial. The action therefore varied according to the nature of the visual stimulus presented. Activity was identified for this task in ventral temporal and ventral prefrontal cortex.

This leads to the possibility therefore that the activation seen in the ‘ventral visual’ areas in the current study might reflect the identification of the target and the consequent decision regarding the action to perform on it. The contrasts in which activity was seen in this region included the single > double-saccade comparison at target 1, on the forward order trials. At this point the participant has to first consider the colour of the target, since they had been instructed in the single-saccade block that they only had to execute one saccade towards either the black or the white target. They then decide either to plan a saccade towards the target or ignore it. In the double-saccade condition in contrast, both the black and the white targets were behaviourally relevant.

A second contrast showing activity in this region, was the reverse-order single-saccade > forward-order double-saccade at target 2. In this case participants again had to determine that the target presented was in fact the

appropriate one to plan a saccade towards, whereas in the double-saccade condition both targets were important to the task.

The final contrast to show a cluster of activity in a temporal region was also a single > double-saccade one, for forward and reverse-order trials combined at the time of the go-signal. This however does not fit as well with the interpretation outlined above, since there is at this point no visual presentation from which participants must select an appropriate action. This area of activity might however be distinct from the temporal activity seen in the previous two contrasts; whereas these were in BA 37, this activity was in BA 22, in the middle and superior temporal sulci. Toni et al., (2001) noted preparatory activity in this area and suggested it might reflect an ‘anatomical bridge’ between inferotemporal visuoperceptual areas involved in object vision and frontoparietal visuomotor areas involved in spatial vision. They suggested that posterior temporal cortex along the superior temporal sulcus, might play a role in the extraction of contextual and intentional cues during goal-directed behaviour. This activity might therefore be related to the latency period just prior to saccade execution, when participants must decide, on the single-saccade trials, which of the two targets to make a saccade towards.

In the fMRI study by Carpenter et al., (1999), a mental rotation paradigm was employed, and activation was seen both in the region of the dorsal stream, in the left and right intraparietal sulci, as well as in the region of the ventral stream, in the fusiform gyrus and inferior temporal areas. The authors suggested that these systems may interact, with the additional involvement of a third set of systems, i.e. motor systems in the precentral gyrus, posterior middle frontal gyrus and interhemispheric fissure, necessary for the computation of head and eye movements. They concluded that the specialisation of such systems is only partial, and that each most likely contributes to the computations of the other systems in addition to performing its own ‘preferred’ computation.

An alternative explanation for the activity in temporal cortex therefore, is that areas in the ventral visual stream contribute to this visuomotor task alongside the activity of dorsal stream frontoparietal areas more generally accepted to be involved in tasks that require knowledge of the spatial location of visually presented objects. However, the reasons why such activity would

be seen particularly for the single > double-saccade comparisons discussed, is not clear in such an explanation.

In a visual working memory task, Pessoa et al., (02) found stronger responses in extrastriate visual areas in a dorsal occipital region in the MOG (BA 18/19) and an inferior temporal region (BA 37) during encoding (perceptual processing of stimulus) for correct compared to incorrect trials. In the current study it was not possible to compare saccadic accuracy against BOLD signal at the time of target encoding, this might however have proved interesting to consider.

Frontal Cortex Activity

A number of areas of frontal activity were seen for both the main experimental conditions in this task and the contrasts. Activity in the region of the FEF (precentral/ middle frontal gyrus, BA 6) was, for example, seen bilaterally for both the forward and reverse-order trials at the time of the go-signal, and for reverse-order trials at target 2 presentation. The FEF activation seen in the study by Heide et al., (2001) was said to be related to the execution of saccades in general, although predominantly for the control of internally generated intentional saccades, with additional involvement in generating sequences of memory-guided saccades. This therefore fits with the activity seen at the go-signal in the current study, although does not explain why activity was also seen here at target 2 specifically for the reverse-order trials.

Activation in this area was however also seen in a PET study by Petit et al., (1996), who suggested that as well as reflecting the triggering of prelearned saccade sequences, it could also be indicative of spatial computations needed for spatial accuracy. This was based on results from single-unit recording studies in macaques, in which it was claimed that the saccade-related efference copy signal was represented by FEF as well as LIP neurons (Goldberg & Bruce, 1990; Umeno & Goldberg, 1997; Tian et al., 2000).

An important role for the FEF in planning, maintaining and triggering memory-guided saccades was also concluded by Curtis et al., (2004); they found activity in this area was greater for trials in which participants were able to maintain a motor code for the forthcoming saccade compared to when they

could not. No activity in this region was seen to be greater for the forward-order trials compared with the reverse, either at the time of target presentation or at the go-signal in this study. However for the opposite comparison, i.e. reverse > forward-order, greater FEF activity was seen bilaterally at target 2 presentation. An exclusive mask confirmed that this activity was unique to the reverse-order double > single comparison at this time, and not present in the equivalent forward-order contrast.

More anterior regions of frontal activity were also seen in BA 9/10 (predominantly left hemisphere) for the target 2 reverse-order: double > single-saccade and target 2: forward-order double-saccade > reverse-order single-saccade contrasts, and in the right hemisphere for the opposite comparison, i.e. target 2: reverse-order single-saccade > forward-order double-saccade.

Frontopolar activity in left BA 10 and 11 (medial frontal and rectal gyri) was also seen for Go (forward and reverse-order combined): double > single-saccade. This activity might correspond to the dorsolateral prefrontal cortex (DLPFC); evidence that this area is important for the accuracy of memory-guided saccades is discussed in a review by Leigh and Kennard (2004). Neurons in this area have for example been shown to hold memory-specific visuospatial coordinates in a topographical memory map (Sawaguchi & Ibl, 2001), and TMS studies have implicated bilateral DLPFC during the memorization stage, following target presentation (Pierrot-Deseilligny et al., 2002).

The prefrontal cortex is also involved more generally in memory retrieval and executive functions such as planning (Courtney et al., 1998). Its activation in this task, when memories of target locations / pre-planned saccade sequences must be retrieved and decisions have to be made about the exact eye movement to be executed, is thus not surprising.

Premotor Cortex Activity

Activity in the ventrolateral premotor cortex (PMC) was noted by Heide et al., (2001) for all saccade tasks in their study. Activity in similar areas (BA 6/9/44) was also seen in the current study for both forward and reverse-order trials at the time of the go-signal. It has previously been suggested that this

area might be generally involved in saccade-related and attentional processes, forming part of a parieto-premotor network for visuomotor control (Gitelman et al., 1999; Nobre et al., 2000). This network is thought to be involved in the transformation of visuospatial target location information into motor commands (Heide et al., 2001); its activation in the current study at the time of the go-signal would thus make sense in this context.

Cingulate Cortex Activity

Two distinct areas of anterior cingulate activation could be distinguished in the current study. One of which, seen in the double > single-saccade contrast at target 2 on the reverse-order trials, appeared to correspond with what Heide et al., (2001) termed the ‘cingulate eye fields’. This region was in BA 32, and was suggested by the authors to be important for the control of intentional saccades; it was seen in their study for the contrast between self-paced saccade sequences and triple-step memory-guided saccade sequences. A second area, located more anterior and ventral to this one was also identified by Heide et al., and seemed to correspond with the activity seen in BA 24 in the double > single-saccade contrast for the forward and reverse-order trials combined at the time of the go-signal, and also at the time of target 2 for the forward-order double-saccade > reverse-order single-saccade contrast. The activity in this area was proposed by Heide et al. to reflect sustained attention and online monitoring of performance, and was seen by these authors in the contrast between triple-step memory-guided and visually-guided saccades. These two areas were labelled the caudal anterior cingulate (ACc) and rostral anterior cingulate (ACr) respectively (Heide et al., 2001).

In general, the activity seen in the cingulate cortex seemed to result from contrasting double- and single-saccade trials. It seems therefore that performing a sequence of two saccades evokes more activity in this region than performing single memory-guided eye movements.

5.4. General Discussion

The studies in this Chapter aimed to investigate potential differences in the encoding of target locations under varying task circumstances, i.e. when a future saccade could and could not be planned at the time of target presentation. It was initially predicted that there might be differences in the cortical activity associated with encoding the target location for each of these two situations. In particular, it was predicted that greater posterior parietal activity might be seen when the spatial location of the target had to be remembered, but could not simply be encoded in the form of a motor plan for the upcoming saccade. In the current experiment this situation is seen at target 1 presentation, however no differences in activity were found for the comparison between forward-order trials when the saccade could be planned and reverse-order trials when it couldn't.

Another aspect of this study was the investigation of the cortical areas associated with the remapping of previously encoded target locations held in memory. Given the behavioural differences demonstrated in the experiments in Chapter 4 in terms of saccade metrics (latency and amplitude compensation) when the order of target presentation was manipulated, it was predicted that greater posterior parietal activity would be seen for the reverse target presentation order due to the greater level of complexity assumed. Since no such difference in latency was seen in Experiment 11 of the current Chapter, probably as a consequence of the extended time available, it was proposed that any differences related to preparatory spatial remapping might instead occur at the time of target 2 presentation. All the visual information required to complete any spatial transformations necessary for the saccade plan would become available at this point. Potential differences at the time of the go-signal were also considered however, since the failure to find a difference in latency at this time period might not necessarily reflect identical cortical activity. Further to this, the behavioural effect of improved amplitude compensation for reverse compared to forward-order trials had been found to persist for the time-scale used in the current study. It was thought that this might be attributable to the manner in which the targets were encoded. As at target 1 presentation however, no differences in cortical activation were seen

between the forward and reverse-order trials either at the time of target 2 presentation or at the go-signal.

There are a number of potential explanations that might help to account for the lack of differences seen in the comparisons made between the two target presentation orders. Firstly it seems possible that although different processes may be occurring, for example in terms of encoding the target location at T1, common areas might in fact be recruited for these functions. The area of the PPC activated in both of the forward and reverse-order condition might be responsible both for formulating and holding a motor plan in memory, and also for maintaining a memory of the spatial location of the target. Alternatively the processes occurring might genuinely be the same, for example if a 'default plan' was formed at T1 in the reverse-order trials. This idea is supported by Andersen & Buneo (2002) who suggest that in the absence of any alternative plans, default plans are formed to behaviourally significant stimuli, but are deleted if alternative plans are later made; a parietal locus was suggested for this process.

At target 2 and the go-signal, it seems plausible that the absence of differences might be due the occurrence of similar processes in each condition at these times. Since in both cases the task requires either the planning or execution of a double-step saccade sequence, common areas are likely to be activated. Any differences between them might thus be expected to be in terms of the intensity of the signal in a common area, rather than specific areas involved, although this was not shown to be significant for current contrasts.

Comparisons of double-saccade trials to the single-saccade control trials did however yield some interesting findings. The use of single-saccade trials as a comparison controls for activity related to a) viewing two visual targets successively, b) planning a single-saccade to one of the two targets and c) activity generally associated with the execution of a memory-guided saccade. Thus greater activity seen for double-saccade trials should reflect additional processing specifically associated with planning, remembering and executing a sequence of two saccades rather than one. Further to this, an alternative comparison of the differences between forward and reverse-order trials can be made, by masking the two double vs. single-saccade contrasts against each other. By doing this, areas in visual cortex and the frontal eye fields at target 2

presentation and posterior parietal cortex at the go-signal were revealed to be more active for the reverse-order trials. No areas of greater activity were found when the mask was applied in the opposite direction, i.e. greater for the forward-order double > single-saccade contrast. This suggested that the parietal activity at the go-signal might reflect the greater complexity of spatial transformations that would be expected in the reverse-order condition.

Greater visual cortex activity was seen for all of the double > single-saccade contrasts conducted, whether forward or reverse-order, and for both target 2 presentation and at the go-signal. This thus supports the idea based on single-unit recording studies in non-human primates that this area might participate in oculomotor behaviour (Supèr et al., 2004), possibly through spatial remapping of remembered visual locations (Nakamura and Colby, 2002).

5.5. Conclusions

The findings from the functional imaging study discussed in this chapter were not entirely as predicted on the basis of behavioural results from Chapter 4. However, potential explanations for this have been discussed alongside interpretations for the more unexpected findings that were seen. This study has been useful in terms of providing insight into the cortical areas involved in both planning and executing a double-step saccade, including those seen at the time of encoding. By making use of a single-saccade control condition it has also been possible to gain a better understanding of the cortical areas involved in spatial remapping of a previously encoded target location. It will thus be interesting to further test the idea, as suggested by the current findings, that the activity seen in visual cortex is indicative of spatial remapping processes occurring in this region. Alongside this some further questions related to the formulation of default saccade plans at the time of visual target presentation have also arisen and it would thus be interesting to further investigate this process, and the ways in which these might be replaced or updated in response to the formation of a newer plan. The experiments discussed in the subsequent Chapter will attempt to address these issues through the use of a saccade paradigm that manipulates the necessity of spatially remapping a target

location or updating any default plan that has been formed, in order to accurately execute a single-memory guided saccade.

Chapter 6: Investigating the cortical areas associated with spatial remapping of a remembered target location by means of a double-intervening-saccade task

6.1. Introduction

On the basis of findings in the previous Chapter it was proposed that, in the absence of a specific plan for an upcoming saccade, a default plan may be formed at the time of target presentation in a memory-guided saccade task. Andersen & Buneo (2002) suggested that this default plan would then be erased should an alternative saccade plan be formed, for example in response to the presentation of a subsequent target towards which a saccade must be planned. Bracewell et al., (1996) investigated changes in motor plans on a single-saccade task. In this study monkeys were trained to perform memory-guided saccades to the location of the most recently presented visual target. The monkey had no way of predicting how many targets (one, two or three) would be presented on a particular trial and thus had to form a plan to each one in turn, and replace or update this should a later target be displayed. Such a process was found to result in alteration in activity in neurons in LIP, believed to reflect the changes to motor intention.

An intervening saccade occurring between the presentation of a visual target and a memory-guided saccade towards that location would similarly require any default plan made at the time of target presentation to be replaced or updated, by means of spatially remapping the remembered target location. This process was investigated in an event-related fMRI study by Medendorp et al., (2003); they used an intervening saccade task in which participants were first presented with a 'goal target' to the left or right of the screen whilst fixating centrally, subsequently a 'refixation target' (for the first saccade) was displayed. Following a six second delay during which visual distractors were presented, participants made a saccade to the location of the refixation target. A further delay of twelve seconds then followed, after which a saccade was made to the location of the goal target, and then back to the centre. The authors demonstrated that the intervening saccade resulted in spatial updating, in the parietal cortex, of the location of the goal target relative to the centre of gaze. Interestingly, in light of the findings in Experiment 11 (Chapter 5),

activity was also observed in occipital and frontal areas, although unfortunately only the posterior parietal activity was discussed.

The present study also makes use of an intervening saccade paradigm in order to investigate spatial remapping; it differs from that used by Medendorp et al., (2003) however in that two intervening saccades occur between visual target presentation and the corresponding memory-guided saccade. By using a double rather than single intervening saccade, it is possible to compare a situation when the second of these saccades returns the eye to the original fixation location (i.e. the centre of gaze at the time of target encoding), to that when it moves the eye to a new location. For the situation when the second intervening saccade moves the eye to a new fixation location prior to saccade execution, the remembered spatial location of the target must be updated and a new saccade plan formed as described by Medendorp et al., (2003). In contrast, when the second intervening saccade instead returns the eye to the original fixation location two scenarios are theoretically possible. Firstly, the saccade plan formed towards the target at the time of encoding (i.e. the 'default' plan) may be automatically updated in response to each of the intervening saccades in an identical way to that which would be expected in the new fixation condition. Alternatively, the plan formed at the time of target encoding may not be completely erased or replaced, but may still be available. This task thus manipulates whether or not spatial remapping of the target location is *necessary* in order to accurately perform the task. For the 'new' fixation location it is, but with the 'original' fixation location it might not be essential, but could occur anyway.

These saccadic conditions will also be compared to a control task, in which the visual stimulation is identical, but participants do not perform the intervening saccades, i.e. their eyes remain stable up until the time to execute the memory-guided saccade. In this condition therefore there is no need to update the default saccade plan.

The task was first performed behaviourally to compare saccadic metrics for the new and original fixation conditions in both the saccade and fixation tasks. This was followed by a functional imaging version of the task, to assess potential differences in cortical activity related to the various experimental conditions.

6.2. Experiment 12: Double-Intervening-Saccade Study

As described above, this experiment was used to assess for any behavioural differences between the ‘new’ and ‘original’ fixation conditions in both the saccade and fixation tasks. Karn et al., (1997) had previously investigated the effect of intervening eye movements on saccade metrics. Specifically they investigated the effect on error of varying the number of saccades made during the memory delay period. They reasoned that if the target location was encoded (and updated) retinotopically, then error associated with updating the remembered target location in response to each of the intervening saccades should be cumulative, and vary with the number of saccades performed. If alternatively, a head-centred frame of reference was employed to encode the target location, then only intervening head, and not eye movements, should influence saccadic error. They found that error did in fact increase with additional intervening saccades, although the effect was only slight. They concluded that the updating process does not rely solely on retinotopic coordinates, but also makes use of information about eye position in relation to the head. They also observed that the presence of visual landmarks that allow exocentric coding of target locations reduced updating-related error but did not completely abolish it.

In light of this, greater end-point error would be expected in the saccade task when participants performed intervening saccades, compared to the fixation task when they did not. The number of intervening saccades in both conditions of the saccade task is identical, and thus if this is the only variable influencing accuracy then no difference would be expected between the two conditions. Alternatively, if the position of the eye at the time of target encoding is important, then a difference might be observed. It was hypothesised that accuracy might be better for the ‘original’ trials in which the memory-guided saccade to be executed was the same as that which could be formed at the time of visual encoding, i.e. when the target could be said to be ‘visually available’ at the time of saccade planning. In the new condition in contrast, the target location would have to be updated in response to the intervening saccades and so greater error might be expected.

If the presence of intervening saccades in both conditions of the saccade task led to remapping of the target location, then no differences (in terms of latency) would be expected between the two conditions. If however, in the original condition, participants were in fact able to make use of a default saccade plan made in response to the presentation of the visual target, and did not necessarily have to spatially remap the target location in order to plan and execute the memory-guided saccade, then the processing time (and therefore latency) for this condition should be shorter than for the new condition when spatial remapping would be required.

Methods

Participants

Ten healthy participants (eight females); aged 22-30 (mean 25.1 years) took part in this task. All had normal vision.

Materials

A pupil and dual first Purkinje image Video Eyetracker (Cambridge Research Systems) was used with a sampling frequency of 250Hz and an accuracy of 0.125-0.25°. The calibration involved a built-in procedure in which 20 small black dots (0.25 deg arc) on a grey background appeared on the screen one at a time at positions around a 5 x 4 grid scaled to 70% of the display size. The dots remained on for 500ms each and the accuracy of the participant in looking to each region of the screen was then assessed, this procedure was repeated if necessary until the participant had accurately foveated all of the positions on the grid. During the experimental session a video image of the eye could be seen by the experimenter on a separate computer screen, this made it possible to monitor the participants' position in the eye-tracker throughout the progress of the experiment. Participants viewed the stimuli binocularly, although only the right eye was tracked. An EyeLock headrest (Cambridge Research Systems) attached to the eye tracker was used to

keep participants' heads in position, and this was placed on a Vision Science height-adjustable workbench (Cambridge Research Systems).

The eye tracker was set in front of a 17in Elo Touchscreen monitor with a spatial resolution of 640 x 480 pixels at a frame rate of 60Hz, on which visual stimuli were displayed, at a viewing distance of 46cm. Stimuli were generated using the MATLAB (The MathWorks) CRS (Cambridge Research Systems) Toolbox. These stimuli consisted of a black fixation cross (Arial font, size 18) and a circular black target of 8mm (1deg) diameter. A speaker was used to play auditory beeps, and the study was carried out in a darkened room.

Procedure

Oculomotor Task: Participants were required to make a single memory-guided saccade towards the remembered location of a visually presented target. This saccade was performed either after a series of two intervening saccades that followed the target presentation, or alternatively during a period of fixation; the visual stimulation was identical in both of these two behavioural tasks.

In the double-intervening-saccade version of the task, a black fixation cross on a grey background appeared 8cm (9.87deg) from the centre on either the left or the right side of the screen. This signified the start of each trial, and remained on until the eye-tracker determined that the participant was correctly fixating the cross, i.e. the pupil was directed to a region of the screen 20mm (2.49deg) around the cross. Once this had been established the circular target was presented and remained on for 1000ms, after which it was extinguished and the fixation cross was shown for a further 500ms. During this time participants were told to continue fixating on the cross. The fixation cross was then displayed at the centre of the screen for 1000ms, and participants were told to follow this 'jump' and the one that followed, in which the fixation cross was displayed either on the opposite side of the screen, or back at its original location for 1000ms. The screen then went blank (grey background) for a variable duration (mean = 500ms, s.d. = 125ms) after which participants heard an auditory beep (duration = 150ms). This was the go-signal, i.e. the cue for participants to make a saccade towards the remembered location of the visual

target. After making this saccade, participants remained fixating at the saccade end-point until the next trial started, which was signified by the appearance of the fixation cross, either on the left or the right-side of the screen. The eye tracker continued to track following the go-signal for a period of 2000ms. (See Figure 6.1 below).

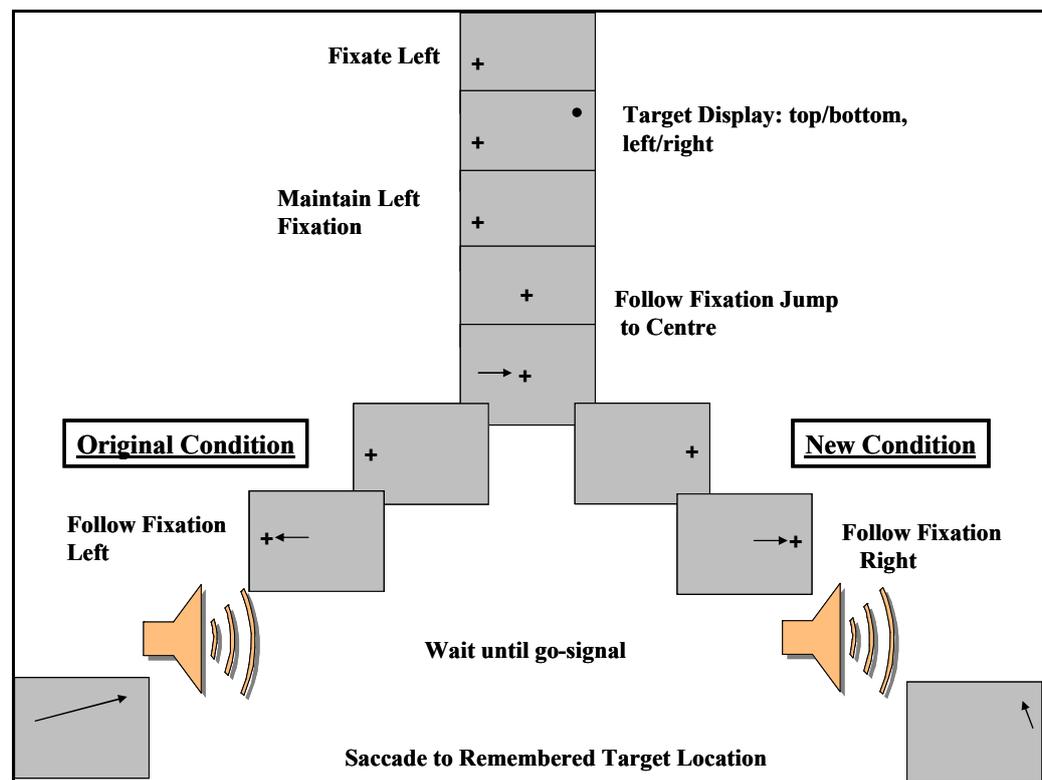


Figure 6.1: Comparison of original and new conditions, for a trial when the initial fixation cross is displayed on the left.

The fixation version of the task was identical in terms of the visual display, however in this task, participants were required to maintain fixation at the location where the fixation cross was initially displayed throughout the trial up until the go-signal.

The screen was split into quadrants (top and bottom, left and right) and targets could appear at nine possible locations within each of these areas in a 16cm^2 3×3 grid centred 8cm (9.87deg) to the left or right of the centre of the screen and 8cm (9.87deg) above or below it. On each trial an index of the 9 possible target positions for the pre-specified quadrant for the target was shuffled, and pseudo-randomly selected by the computer. The quadrant in

which the target appeared was counterbalanced across the trials, so half the time it appeared on the same side as the original fixation cross (e.g. both left or both right), and half the time on the opposite side (i.e. one left, one right). This meant that for half of the trials the saccade required was short (6-12cm, 0.75-1.49deg), and for half it was long (15.5-20.5cm, 1.93-2.55deg). For half of these trials the target was in an upper quadrant and for half in a lower quadrant. On half of the trials the fixation cross returned to its original location and on the other half it jumped to a new location, these conditions will thus be referred to as 'original' and 'new' trials. There were therefore 16 possible trial types (4 target quadrants x 2 initial fixation location x 2 final fixation location). Participants performed 160 trials in total; they were also given the opportunity for a break every 20 trials (the experiment continued when they made a key press on the keyboard). The two tasks were performed in an A-B-B-A design, with half the participants performing the saccade task first (i.e. 40 saccade trials, 80 fixation trials and then 40 more saccade trials) whilst the other half did the fixation task first.

Data Analysis: Plots of eye movement traces using x and y coordinates from eye-position data recorded every 4ms were analysed (see Figure 6.1). Trials in which participants made eye movements at the time of target presentation, or did not performed the task correctly, i.e. followed the fixation cross jump when they were supposed to remain fixating, or vice-versa, were rejected. The latency of each saccade was determined using an algorithm that calculated the absolute change in eye position for every sample recorded by the eye tracker ($\sqrt{(\text{latest}(x))^2 + \text{latest}(y)^2} - \sqrt{(\text{previous}(x))^2 + \text{previous}(y)^2}$); a saccade was defined a change between two successive samples that exceeded a threshold of 5mm.). Coordinates for x and y eye position obtained from the eye tracker were compared with the x and y coordinates for the target locations to calculate a measure of end-point error, using the following equation:

$$\text{Saccade Error} = \sqrt{[(x(\text{target } 1) - x(\text{fixation } 1)))^2 + (y(\text{target } 1) - (-y(\text{fixation } 1)))^2]}$$

This gives an error value in terms of distance (in mm) of the fixation location from the target position.

An example trial showing the x and y position of the eye over time can be seen in Figure 6.2, below.

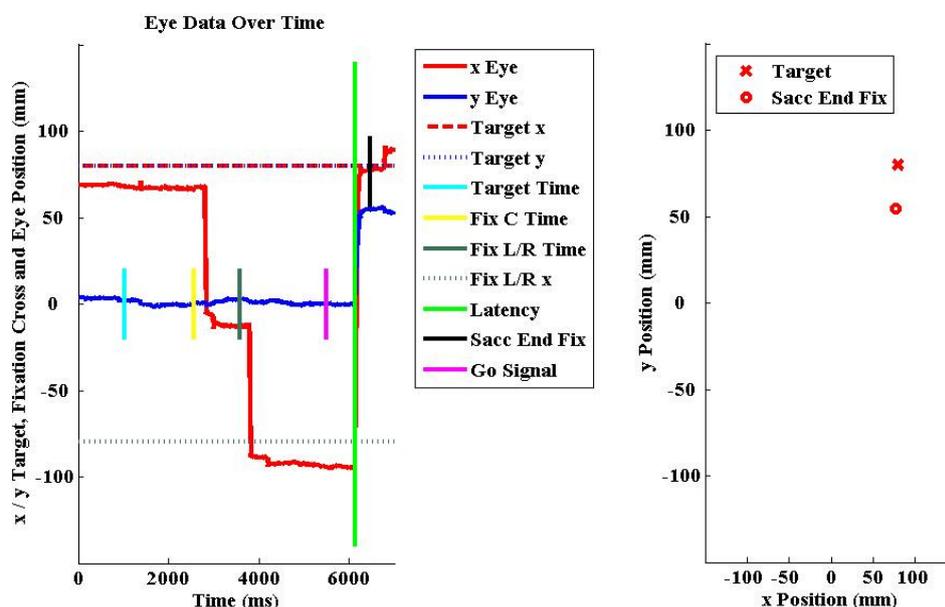


Figure 6.2: Plot of eye movement trace using x (red trace) and y (blue trace) coordinates from eye-position data for one trial (left plot). The participant can be seen to be fixating the right of the screen (~70 on y axis) at the start of the trial. The time of the target presentation is shown as a light blue vertical bar. The yellow vertical bar represents the first fixation jump to the centre of the screen (Fix C Time) and the dark green vertical bar represents the second fixation cross jump to the left of the screen (Fix L/R Time). The dark green dashed horizontal bar indicates the x location of the second fixation cross jump (Fix L/R x). The two saccades towards each of the fixation jumps can be seen clearly. The pink vertical bar shows the go signal, the long light green vertical bars show the latency of the memory-guided saccade, and the black vertical bar shows the end-point fixation. The end point of the saccade is also plotted in relation to the target location on the screen (right plot).

Results

Latency: A one-tailed paired-sample Student's t-Test was used to test the prediction that latency would be longer in the 'new saccade' compared to the 'original saccade' condition. A significant difference was found, in the

direction predicted between the group means for these conditions ($t_{(9)} = 2.651$, $p < 0.05$).

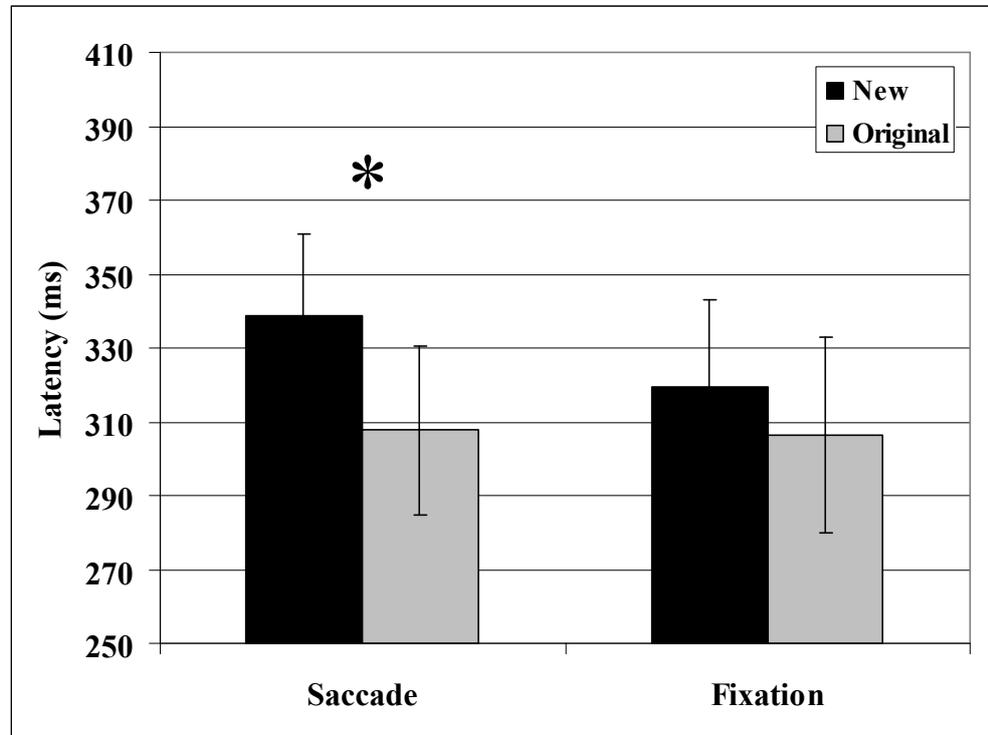


Figure 6.3: Group mean latency (ms) for new and original trials in both the saccade and fixation tasks. Error bars show standard errors. * = significant at $p < 0.05$.

A two-tailed paired-sample Student's t-Test was used to compare the group mean latency data for the 'new' and 'original' trials in the fixation task. No significant difference was found between the means for these conditions ($t_{(9)} = 1.070$, N.S.). The data from these analyses are shown above in Figure 6.3.

End-Point Error: A one-tailed paired-sample Student's t-Test was used to test the prediction that end-point error would be greater in the 'new saccade' compared to the 'original saccade' condition. A significant difference was found, in the direction predicted between the group means for these conditions ($t_{(9)} = 3.200$, $p < 0.01$).

A two-tailed paired-sample Student's t-Test was used to compare the group mean end-point error data for the 'new' and 'original' trials in the

fixation task. No significant difference was found between the means for these conditions ($t_{(9)} = 0.133$, N.S.). The data from these analyses are shown below in Figure 6.4.

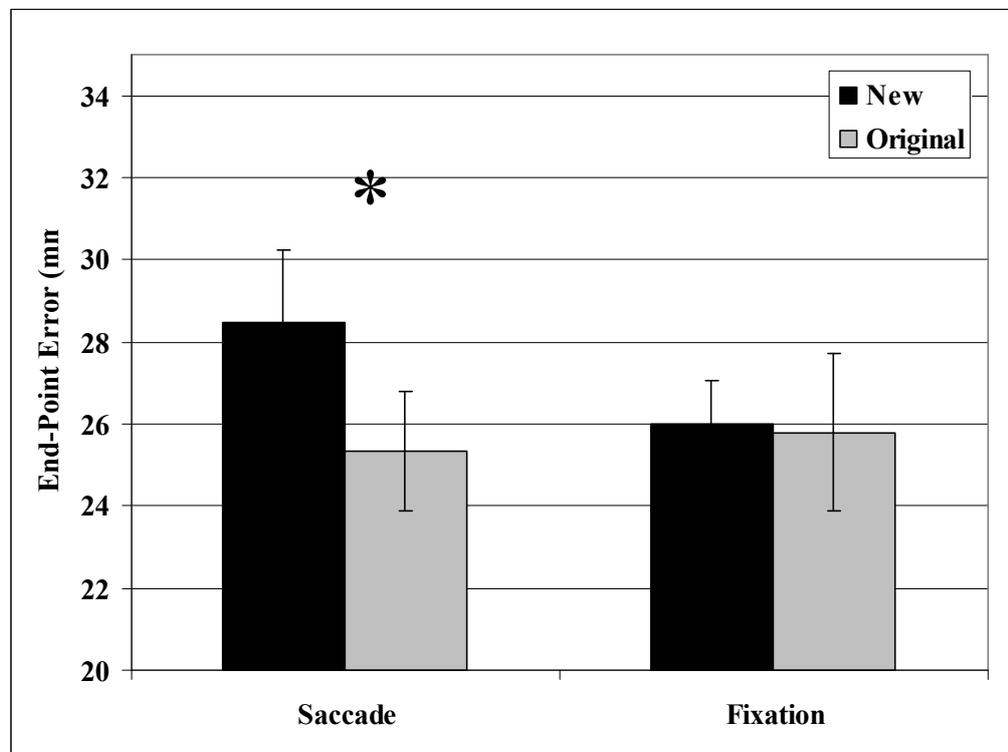


Figure 6.4: Group mean end-point error (mm) for new and original trials in both the saccade and fixation tasks. Error bars show standard errors. * = significant at $p < 0.01$

Discussion

The results of the double-intervening-saccade task therefore appear to confirm the prediction that latency would be greater for single memory-guided saccades when a spatial computation is required in order to calculate the angle and amplitude of the saccade to the targets' remembered location compared to when it is possible to make use of a retinal vector from the time of target encoding. In the fixation task, participants did not move their eyes in the time between target presentation and the go-signal. There was therefore no need for spatial remapping of the target location in response to a change in eye position, and no need to update the saccade plan based on the retinal vector from the

original location of the fixation cross to the target. There was no difference in latency between the two trial types on this task, i.e. when the fixation cross ended at a new location, compared to returning to the original; this is as expected since the saccade could be planned in the same way in both cases. Further to this the latency for the original trials in the saccade task is very close to both latencies on the fixation task, which supports the idea that participants were making use of a remembered retinal vector to plan the saccade rather than recalculating in response to the intervening saccades.

In terms of end-point error, the pattern of results is very similar to the latency data. There is no difference between the original and new trials on the fixation task, and in addition, the end-point error on the original trials in the saccade task were similar in value to those in the fixation task. Accuracy was however found to be significantly worse for new trials on the saccade task compared to original trials, corresponding to the only condition when spatial computations were actually essential in order to plan and execute the saccade. In the original trials of the saccade task, participants might have performed spatial computations in order to re-calculate the saccade plan to account for the intervening saccades, or alternatively might have relied on a remembered retinal vector. The data appear to support the second of these possible strategies.

If this is the case, this would suggest that in terms of cortical involvement, it is only the new trials on the saccade task that should result in activity related to spatial remapping, whereas the other conditions would be expected to instead reflect activity of areas involved in maintaining a memory of the target location, possibly as a retinal vector from the original fixation point. If these two behaviours are carried out by distinct cortical areas, then differences in terms of the associated activations would be expected.

6.3. Experiment 13: Double-Intervening-Saccade fMRI Study

Posterior parietal activity was seen for the double-saccade trials at the time of the go-signal in the previous fMRI study (Experiment 11, Chapter 5). This activity was not shown to be greater for the double compared to single-saccade forward-order trials suggesting it was similarly present in both cases.

Activity in this area would thus be expected for the fixation task, which similarly requires the planning and execution of a single memory-guided saccade without the requirement of spatial remapping to account for intervening saccades.

In the previous fMRI study (Experiment 11, Chapter 5) greater activity was seen in the visual cortex for the double compared to single-saccade trials. It was suggested that this might reflect spatial remapping processes occurring in this area. If this were the case, activity in visual cortex would also be expected in the current study on the trials in which spatial remapping of the target location occurred. This would therefore be expected at least for the new condition of the saccade task, and perhaps also the original condition as a result of the intervening saccades performed. The behavioural data suggest that in the original condition of the saccade task, participants may be relying on a previously formed saccade plan rather than calculating a new one to account for the intervening saccade, as reflected by the lower latency and end-point error seen in the original compared to the new condition. This does not however rule out the possibility that some spatial remapping of the target location may take place in this trial type, rather it suggests that this information is not necessarily made use of in the saccade plan executed.

Activity in this study was time-locked to the go-signal, since in the previous fMRI study (Experiment 11, Chapter 5) interesting differences in activity were seen at this time. Since the current task did not incorporate long delay periods, as the previous one had, it would have been difficult to separate activity related to different sub-components of the task. Since a difference in latency between the new and original trials had previously been seen for the behavioural version of this task (Experiment 12), there was also reason to believe that differences might be present in terms of the complexity of processing occurring for these trials at the time of the go-signal.

By incorporating a rest period during which participants' behaviour was controlled (all closed their eyes) the activity present in all four conditions could be assessed in relation to this common baseline.

Corresponding conditions in the saccade and fixation tasks could also be contrasted, thus accounting for any activity brought about by factors such as target presentation or planning and executing memory-guided saccades in

general. Activity more specifically associated to spatial remapping in response to intervening saccades could thus be better evaluated.

As in Experiment 11, this will also be supplemented by the use of exclusive masking in order to evaluate potential differences between the new and original saccade vs. fixation contrasts. Based on the reverse vs. forward-order double-step comparisons in Experiment 11 (Chapter 5), it was believed that this approach might also prove more effective than would a direct contrast of new vs. original saccade trials. As in the previous fMRI study fairly similar processes would again be expected in both cases.

Methods

Participants

Fifteen healthy participants (9 females); aged 19-36 (mean age 25.7) took part in this task. One of the participants was left-handed, and all had normal or corrected to normal vision.

Materials

The participants wore in-scanner goggles (Silent Vision, Avotec, Inc.) through which the experimental stimuli were created using Powerpoint (Microsoft) and presented as bitmap images using Eprime (Psychology Software Tools Inc), but were essentially the same as those described above in Experiment 12.

Participants wore ear protection and lay in a supine position in the scanner. They were calibrated in the in-scanner goggles so that they could see the visual display clearly. Imaging was performed at the Magnetic Resonance Centre (University of Nottingham), using a Philips 3.0-Tesla scanner equipped with a multiple-element Sense® head-coil (sense factor = 2). 34 contiguous axial slices (19.2 cm FOV, 64 x 64 matrix, 3mm slice thickness, in-plane resolution = 3 x 3 x 3 mm³) parallel to the AC-PC plane, which covered the whole brain using a gradient-recalled EPI sequence (TR = 2.1 sec, TE = 40ms).

fMRI data were stored in 625 volume image files. (FOV = field of view; TR = time of repetition; TE = Echo Time; AC = Anterior Commissure; PC = Posterior Commissure; EPI = Echo Planar Imaging).

Procedure

Oculomotor Task: This was the same as that described in Experiment 12; all participants performed practice trials (16 of the saccade task, and 16 of the fixation task) outside the scanner prior to the fMRI study to ensure that they understood the task. In the scanner, participants performed 128 trials in total in an A-B-B-A pattern (i.e. 32 of the saccade task, 64 of the fixation task and then 32 more of the saccade task, or vice-versa). Half the participants performed the saccade task first, and the other half started with the fixation task. An instruction screen was shown for 20s at the end of each block to inform participants which task to do in the next block. A rest screen was displayed for 20s after every 16 trials and participants were instructed to shut their eyes; the end of this period was signified by an auditory beep, after which they opened their eyes for the start of the next trial.

Data Preprocessing and Analysis: This was performed in exactly the same way as described in Experiment 11 (Chapter 5). PAR format images (Philips Medical Systems) were transformed into ANALYZE format using the MRIcro software (Chris Rorden, www.mricro.com). Analyses of the fMRI data was carried out using the Matlab SPM2 (Statistical Parametric Mapping) toolbox. Data preprocessing began with realignment (motion correction) using rigid-body registration to the mean image, with a 4th degree B-spline interpolation method. This was followed by spatial normalisation to an EPI (Echo-Planar Imaging) template, after which the images had a resolution of 3 x 3 x 3 voxels. Spatial smoothing was also performed using a Gaussian kernel (8mm, full-width half-maximal). BOLD (blood oxygen level dependent) signal changes evoked by events within each trial were modelled using a canonical haemodynamic response function convolved with time derivatives.

A General linear model (GLM) was used in order to search for significantly activated voxels. A design matrix was defined comprising

contrasts that tested for the different tasks (saccade and fixation) and trial types (new and original fixation locations) and the rest condition. t-contrast images were defined for each subject and the data was analysed at the group level using random effects analysis performed in SPM2, which pools the data across each condition for all subjects. One-sample t-tests were conducted using the appropriate contrast images for each participant, to assess for greater activity at the time of one experimental event compared to another. Statistical significance was set to a height threshold of $p < 0.005$ ($t > 2.98$), and the resulting t-statistic images were assessed for cluster-wise significance ($p < 0.05$, corrected) with a spatial extent threshold of at least 100 contiguous voxels (clusters smaller than this did not meet the cluster-level corrected significance threshold).

MNI coordinates from SPM2 were converted to Talairach space (Talairach and Tournoux, 1988) using Matthew Brett's Matlab function 'mni2tal.m' (see: <http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml>), which provides estimated Talairach coordinates, for given points in the MNI brain. Talairach labelling was then performed based on a search for the nearest grey matter using Talairach Daemon Java Client, an electronic database of neuroanatomical locations (see: <http://ric.uthscsa.edu/projects/talairachdaemon.html> Lancaster et al., 2000).

Functional MRI Results: Comparisons with Rest

Saccade New > Rest

Brain activation at the time of the go-signal for the new fixation location trials in the saccade task was first compared to that during the rest condition. Five clusters of significant activity were seen, three of these were in the right hemisphere in posterior parietal (SPL/IPL/precuneus), frontal and occipito-temporal regions. In the left hemisphere there was one large cluster extending over both posterior parietal (SPL) and occipital regions and a second cluster in frontal cortex. The bilateral frontal activity appeared to be in the region of the

FEF (precentral and middle frontal gyri, BA 6). The coordinates suggested by Heide et al., (2001) as corresponding to the location of the PEF were just inferior to the base of both the left and right hemisphere clusters.

Saccade Original > Rest

At the time of the go-signal for the original fixation location trials in the saccade task compared to the rest condition, six clusters of significant activation were seen. Four of these were in the right hemisphere, centred in posterior parietal (SPL/IPL/Precuneus), occipital, occipito-temporal and frontal regions. The frontal activity appeared to be in the region of the FEF (precentral and middle frontal gyri, BA 6). The two left hemisphere clusters were centred in occipital and posterior parietal cortex (SPL/precuneus). As in the previous contrast, the base of both the left and right hemisphere parietal clusters of activity was located just superior to the coordinates suggested by Heide et al., as corresponding to the location of the PEF.

Fixation New > Rest

Four significant clusters of activity were seen in the fixation task for the trials in which the fixation cross jumped to a new location compared to rest. Three of these were in the right hemisphere, centred in frontal, posterior parietal (SPL) and occipital cortex. The frontal activity appeared to be in the region of the FEF (precentral gyrus, BA 6). The fourth cluster was also centred in occipital cortex, with foci in both the left and right hemispheres.

Fixation Original > Rest

Similarly for the comparison of activity in the fixation task on trials in which the fixation cross returned to its original location with the rest condition, four significant clusters of activation were seen. As in the fixation new > rest contrast, three of these were in the right hemisphere in frontal, posterior parietal (precuneus/IPL/SPL) and occipital regions, whilst the fourth area was in occipital cortex with foci in both the left and right hemispheres. The frontal activity appeared to be in the region of the FEF (precentral and middle frontal

gyri, BA 6). The coordinates suggested by Heide et al. as corresponding to the location of the PEF fell just below the cluster of parietal activity.

A complete list of the clusters and foci of significant activation for the conditions discussed above is shown below in Table 6.1. Talairach labelling has been performed based on a search for the nearest grey matter using the Talairach Daemon Java Client (see Procedure above for details). Statistical parametric t maps depicting this activity are also shown below in Figure 6.5.

Region	Left Hemisphere						Right Hemisphere							
	k	x	y	z	t	Location	BA	k	x	y	z	t	Location	BA
Saccade New > Rest														
PPC/ Visual	549	-30	-53	52	5.16	SPL	7	428	36	-44	46	6.71	IPL	40
		-24	-83	21	4.85	Cuneus	18		27	-64	56	6.4	SPL	7
		-6	-84	2	4.52	LG	17		21	-65	50	5.73	Prec.	7
FEF	115	-30	-3	47	4.84	MFG	6	210	27	-3	53	5.57	Sub-Gyral	6
		-45	-1	47	3.62	PreG.	6		39	6	55	5.33	MFG	6
		-21	0	50	3.62	Sub-Gyral	6		48	2	50	3.67	PreG.	6
Sup. OTC								240	21	-80	32	5.37	Cuneus	7
									36	-72	9	4.16	MTG	39
									27	-84	18	4.11	MOG	19
Saccade Original > Rest														
PPC	139	-27	-50	49	5.05	Prec.	7	412	30	-64	47	6.54	SPL	7
		-21	-61	58	4.31	SPL	7		36	-46	42	6.12	IPL	40
		-18	-70	53	3.56	Prec.	7		21	-66	44	5.69	Prec.	7
Sup. OTC								241	21	-80	32	5.81	Cuneus	7
									36	-72	9	4.25	MTG	39
									27	-84	18	4.2	MOG	19
FEF								213	27	-3	53	5.6	Sub-Gyral	6
									39	6	55	5.59	MFG	6
									48	2	47	3.68	PreG.	6
Visual	146	-24	-83	21	5.12	Cuneus	18	171	12	-88	-6	4.59	IOG	17
		-24	-83	32	4.35	Cuneus	19		-9	-84	2	4.44	LG	17
Fixation New > Rest														
FEF								163	24	-3	53	6.52	Sub-Gyral	6
									48	2	47	4.28	PreG.	6
Visual								336	15	-90	-1	5.37	LG	17
									-9	-85	-1	4.84	LG	17
									-24	-84	18	4.55	MOG	18
PPC								157	21	-64	53	4.89	SPL	7
									21	-61	61	4.7	SPL	7
									30	-50	58	3.62	SPL	7
Sup. OTC								129	24	-80	23	4.49	Cuneus	31
									21	-80	32	4.34	Cuneus	7
Fixation Original > Rest														
FEF								184	24	-3	53	7.1	Sub-Gyral	6
									48	2	47	5.35	PreG.	6
									39	-3	53	4.12	MFG	6
Visual								503	15	-88	-6	6.29	IOG	17
									-24	-84	18	5.32	MOG	18
									-9	-82	-1	4.49	LG	18
PPC								250	18	-67	50	5.05	Prec.	7
									33	-39	38	5.03	IPL	40
									27	-53	47	4.79	SPL	7
Sup. OTC								168	24	-80	23	4.86	Cuneus	31
									33	-78	20	4.08	MOG	19

Table 6.1: Clusters and foci of significant activation ($p_{\text{corrected}} < 0.05$) in the regions of interest for the new and original conditions of the saccade and fixation tasks. (Adapted from Heide et al., 2001).

(k, cluster size; x, y, z, Talairach coordinates of the voxels showing peak activation in each cluster; t, the corresponding t-value; BA, Brodmann area. FEF, frontal eye fields; IOG, inferior occipital gyrus; IPL, inferior parietal lobe; LG, lingual gyrus; MFG, middle frontal gyrus; MOG, middle occipital gyrus; MTG, middle temporal gyrus; PPC, posterior parietal cortex; Prec., precuneus; PreG, precentral gyrus; SPL, superior parietal lobe; Sup. OTC, superior occipito-temporal cortex).

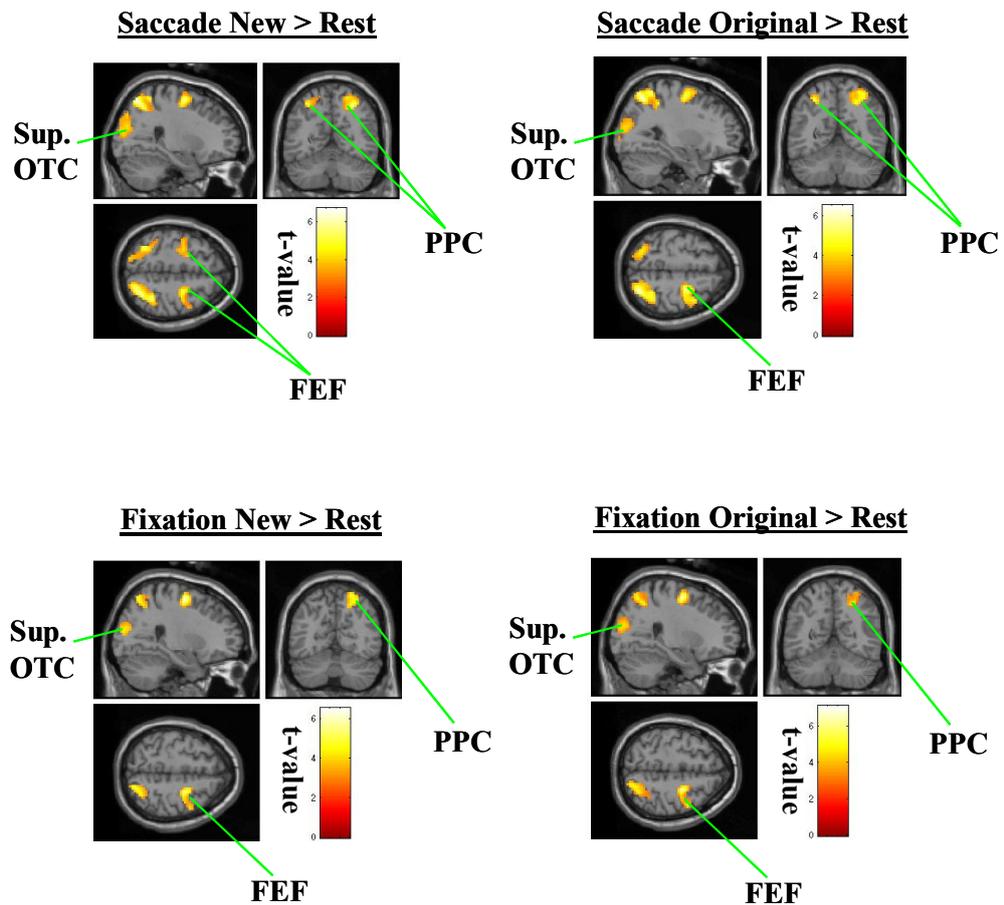


Figure 6.5: Clusters of significant activation ($t > 2.98$, $p_{\text{corrected}} < 0.05$) in the regions of interest for new and original conditions of the saccade and fixation tasks compared to rest. Clusters of activity in frontal, temporal, occipital and parietal cortex have been labelled.

Functional MRI Results: New vs. Original Trial Contrasts

When the new and original trials of the saccade task were compared, no areas of significantly greater activity were seen in either direction. Similarly for the fixation task, no significant activations were seen to be greater for either

the trials in which the fixation cross jumped to a new location or that when it returned to its original location.

Functional MRI Results: Saccade vs. Fixation Task Contrasts

New: Saccade vs. Fixation

When the trials in which the fixation cross jumped to a new location in the saccade task were compared to those in the fixation task, seven clusters of significantly greater activation were seen for the saccade task. Three of these were centred in the right hemisphere, one in temporal cortex, another extended over frontal and posterior parietal regions (precentral and postcentral gyrus and the SPL, BA4/5) and the third located subcortically in the region of the thalamus. In the left hemisphere, there were two clusters of activity centred in temporal and prefrontal cortex (middle and superior frontal gyri, BA8/10). The other two clusters had foci in both hemispheres; both were in the region of anterior cingulate cortex. No significant activity was seen for the opposite comparison, i.e. greater for new fixation compared to saccade trials.

Original: Saccade vs. Fixation

For the trials in which the fixation cross returned to the original location, greater activity was found for the saccade compared to the fixation task. There were six significant clusters, one of which was located in the right hemisphere in temporal cortex. The other five were all located in the left hemisphere, one of which was centred in temporal cortex, two in the frontal lobe, in the prefrontal and primary motor cortex, and two sub-cortically although with foci in temporal and anterior cingulate cortex. No areas of significantly greater activity were seen for the opposite comparison, i.e. greater for original fixation compared to saccade trials.

Left Hemisphere								Right Hemisphere							
Region	k	x	y	z	t	Location	BA	k	x	y	z	t	Location	BA	
New: Saccade > Fixation															
Sub-Lobar	484	-27	-15	1	6.11	LN	LGP	114	15	3	5	5.63	LN	LGP	
		-53	-9	-5	4.89	STG	22		-6	18	18	4.95	AC	33	
		3	14	-3	4.71	AC	25		6	18	18	3.85	AC	33	
Post. STG								449	50	-55	6	5.91	STG	39	
									59	-30	-9	5.6	MTG	21	
									65	-38	-8	5.17	MTG	21	
Parietal								525	33	-26	65	5.63	PreG.	4	
									21	-38	63	5.28	SPL	5	
									33	-43	63	4.93	PostG.	5	
Sup. OTC	98	-53	-66	25	5.39	MTG	39								
		-42	-80	23	5.05	MTG	19								
		-48	-72	23	4.66	MTG	39								
PFC	130	-30	48	20	5.38	MFG	10								
		-15	49	39	5.21	SFG	8								
		-33	41	12	3.85	MFG	10								
Subcortical								148	3	-8	14	4.77	Thalamus		
									24	-15	-2	4.48	LN	LGP	
									3	-17	12	4.09	Thalamus	MDN	
Original: Saccade > Fixation															
Sup. OTC	303	-45	-51	25	6.84	STG	39								
		-53	-72	15	6.37	MTG	19								
		-56	-63	22	4.91	STG	39								
Post. STG								538	53	-6	-2	6.61	STG	22	
									59	-31	15	5.57	STG	42	
									59	-32	-8	5.04	STG	21	
Sub-Lobar	245	-33	-18	-2	5.77	LN	Put.								
		-53	-9	-5	5.63	STG	22								
		-36	-9	3	5.58	Clastrum									
	156	-9	26	-1	5.24	AC	24								
		-30	9	0	4.72	Clastrum									
		-15	9	8	4.63	Caudate	CB								
PFC	292	-27	51	28	5.13	SFG	9								
		-3	54	36	4.48	MFG	9								
		-15	57	30	4.45	SFG	9								
M1	109	-12	-32	60	4.19	PostG.	4								
		-21	-26	68	4.18	PostG.	3								
		-21	-21	48	3.8	PreG.	4								

Table 6.2: Clusters and foci of significant activation ($p_{(\text{corrected})} < 0.05$) in the regions of interest for the new and original saccade > fixation contrasts. (Adapted from Heide et al., 2001). (k, cluster size; x, y, z, Talairach coordinates of the voxels showing peak activation in each cluster; t, the corresponding t-value; AC, anterior cingulate; BA, Brodmann area; CB, caudate body; LGP, lateral globus pallidum; LN, lateral nucleus; M1, primary motor cortex; MDN, mediodorsal nucleus; MFG, middle frontal gyrus; MTG, middle temporal gyrus; Post. STG, posterior superior temporal cortex; PreG, precentral gyrus; Prec., precuneus; Put., putamen; SFG, superior frontal gyrus; SPL, superior parietal lobe; Sup. OTC, superior occipito-temporal cortex).

A complete list of the clusters and foci of significant activation for the conditions discussed above is shown in Table 6.2. Talairach labelling has been performed based on a search for the nearest grey matter using the Talairach Daemon Java Client (see Procedure above for details). Statistical parametric t maps depicting this activity are also shown below in Figure 6.6.

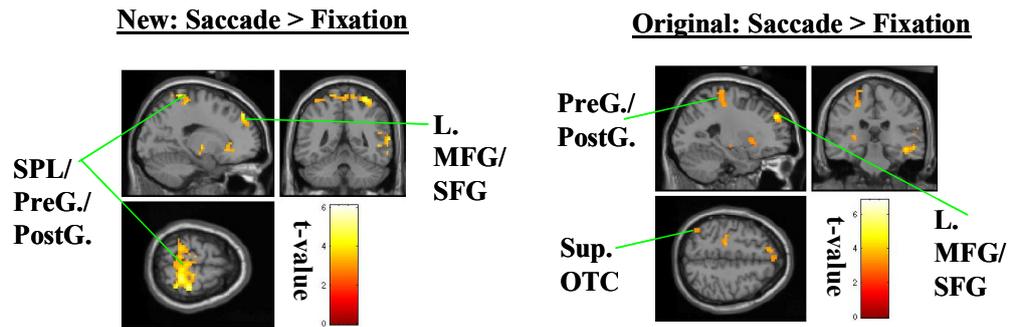


Figure 6.6: Clusters of significant activation ($t > 2.98$, $p_{(\text{corrected})} < 0.05$) in the regions of interest for new and original saccade > fixation contrasts. Clusters of activity in frontal, occipito-temporal, and parietal cortex have been labelled.

Functional MRI Results: Exclusive Masking

For the exclusive masking contrasts, a lower threshold was used to evaluate differences in activity between the contrasts discussed above since these areas did not survive using the previous level of stringency. Clusters with a height threshold of $t > 2.47$ ($p < 0.01$) and a spatial extent of more than 40 contiguous voxels ($p < 0.05$, uncorrected at the cluster-level) are considered below.

New: Saccade > Fixation exclusively masked with Original: Saccade > Fixation

By applying an exclusive mask (at $p = 0.05$), it was possible to determine three clusters of activity that were greater for the new: saccade > fixation contrast than for the original: saccade > fixation contrast. One of these clusters was in the right hemisphere, with foci in parietal (IPL, BA 40) and frontal (precentral and postcentral gyri, BA 3/4) cortex; this was located superior to the coordinates suggested by Heide et al., (2001) as corresponding to the location of the PEF. The second was centred in the left posterior parietal cortex (precuneus, BA 7/19). The third had both left and right hemisphere foci, in right posterior parietal (SPL, BA 7) and bilateral regions of the paracentral lobule.

Original: Saccade > Fixation exclusively masked with New: Saccade >

Fixation

Activity that was greater for the original: saccade > fixation contrast than for the new: saccade > fixation contrast was similarly evaluated using exclusive masking (at $p=0.05$). Four clusters were seen, two in the left hemisphere, in temporal/parietal (superior temporal and angular gyri, BA 39) and prefrontal (superior and middle frontal gyri, BA 8/9) cortex, and two with foci in both hemispheres, in prefrontal (middle frontal gyrus, BA 9/10) and parietal/posterior cingulate cortex (both BA 31).

<u>Left Hemisphere</u>								<u>Right Hemisphere</u>							
Region	k	x	y	z	t	Location	BA	k	x	y	z	t	Location	BA	
New: Saccade > Fixation exclusively masked with Original: Saccade > Fixation															
Paracentral								90	3	-41	63	3.8	ParaL.	5	
								30	-49	66	3.32	SPL	7		
								0	-35	49	3.05	ParaL.	5		
PPC	75	-12	-76	48	3.21	Prec.	7	73	45	-35	54	3.04	IPL	40	
		-21	-67	50	3.12	Prec.	7	36	-20	59	2.93	PreG.	4		
		-24	-80	40	2.96	Prec.	19	42	-18	53	2.85	PostG.	3		
Original: Saccade > Fixation exclusively masked with New: Saccade > Fixation															
Sup. OTC	132	-42	-51	27	5.38	STG	39								
		-56	-60	28	3.64	STG	39								
		-53	-65	34	3.2	AngG.	39								
PFC	50	-12	40	48	4.18	SFG	8								
		-6	43	39	3.31	MFG	8								
		0	45	34	2.9	MFG	9								
	58	-6	47	6	3.82	MFG	10								
		12	42	20	3.47	MFG	9								
		6	50	11	2.88	MFG	10								
PCC								65	9	-51	27	3.23	pCing.	31	
									-3	-51	30	2.91	Prec.	31	

Table 6.3: Clusters and foci of significant activation ($p_{\text{(uncorrected)}} < 0.05$) in the regions of interest for the exclusive masking contrasts. (Adapted from Heide et al., 2001).

(k, cluster size; x, y, z, Talairach coordinates of the voxels showing peak activation in each cluster; t, the corresponding t-value; AngG, angular gyrus; BA, Brodmann area; IPL, inferior parietal lobe; MFG, middle frontal gyrus; ParaL, paracentral lobule; PCC, posterior cingulate cortex; pCing, posterior cingulate; PreG, precentral gyrus; Prec., precuneus; PostG, postcentral gyrus; SFG, superior frontal gyrus; STG, superior temporal gyrus; SPL, superior parietal lobe; Sup. OTC, superior occipito-temporal cortex).

A complete list of the clusters and foci of significant activation for the conditions discussed above is shown in Table 6.3. Talairach labelling has been performed based on a search for the nearest grey matter using the Talairach

Daemon Java Client (see Procedure above for details). Statistical parametric t maps depicting this activity are also shown below in Figure 6.7.

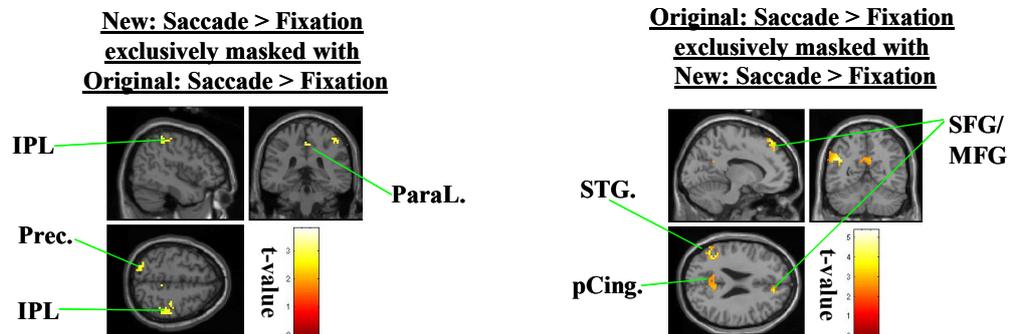


Figure 6.7: Clusters of significant activation ($t > 2.47$, $p_{\text{(uncorrected)}} < 0.05$) in the regions of interest for the exclusive masking contrasts. Clusters of activity in frontal, temporal, parietal and posterior cingulate cortex and the paracentral lobule have been labelled.

Discussion

Parietal Cortex Activity

Bilateral PPC activity was seen for both of the saccade trials (new and original) when compared to the rest condition, in the SPL (BA 7) on the left and SPL/IPL (BA 7/40) on the right. Posterior parietal activity was also seen for the fixation conditions (new and original) compared to rest, although only in the right hemisphere. For ‘fixation new’ the cluster was in the SPL (BA 7), whereas for ‘fixation original’ it had foci in the IPL (BA 40), SPL and Precuneus (both BA 7).

For these comparisons with the rest condition, the activity seen in the PPC appeared to be located a little superior to the coordinates suggested by Heide et al., as corresponding to the PEF. It was however located in the region of the IPS (with foci in SPL and IPL) and thus may still have corresponded to this area, except that the exact coordinates for the activity seen varied slightly from those reported by Heide et al. From the fact that this activity is seen bilaterally for conditions that involve accounting for a change in eye position (i.e. the saccade trials), whereas only right-hemisphere parietal activity is seen

for those that do not, it could be tempting to conclude that the spatial remapping requires bilateral involvement, whereas remembering a target location or saccade plan is dependent on the right PPC specifically.

However, when the saccade conditions are contrasted against the fixation conditions, the only area of greater parietal activity seen is actually on the right, with foci in the precentral gyrus (BA 4), SPL and postcentral gyrus (both BA 5), which appears to be too anterior to correspond the PEF. This is seen for new: saccade > fixation, for original: saccade > fixation, no parietal activity is seen. This suggests therefore that for the fixation > rest conditions, there was probably additional sub-threshold activity in the left PPC.

To determine whether PPC activity seen in new: saccade > fixation really reflected a difference from original: saccade > fixation, and was not similarly due to the threshold used to determine significance, the two conditions were exclusively masked against one another. Bilateral PPC activity was seen for the new: saccade > fixation contrast, but not original: saccade > fixation one. In the left hemisphere this was in the precuneus (BA 7/19), whereas in the right it was in the IPL (BA 40), precentral gyrus (BA 4) and postcentral gyrus (BA 3). There was also a separate cluster of activity in the right hemisphere which included a focus in the SPL (BA 7).

This pattern of greater activity seen in parietal cortex for the new: saccade > fixation than the original: saccade > fixation contrast could perhaps indicate that, as predicted from the behavioural data, greater processing occurs on trials when a new saccade plan must be formed to that when a previous one could in theory be made use of.

Primary Visual Cortex Activity

For the saccade new > rest comparison, the parietal activity seen on the left was part of a large cluster that also extended into occipital cortex, in the cuneus (BA 18) and the lingual gyrus (BA 17). Separate clusters of visual cortex activity were also seen for saccade original > rest, on the left in the cuneus (BA 18/19) and on the right in the inferior occipital gyrus and lingual gyrus (both BA 17).

From this it might be thought that it could reflect the same saccade-related remapping activity seen in Experiment 11 (Chapter 5). Importantly however, activity in visual cortex was not only seen for saccade > rest contrasts, but also for the fixation > rest ones. As with parietal activity, this was only in the left hemisphere, in the lingual gyrus (BA 17) and middle occipital gyrus (BA 18) for fixation new, and the inferior occipital gyrus (BA 17), middle occipital gyrus and lingual gyrus (both BA 18) for fixation original.

This could therefore reflect a remapping process that is not dependent on the future saccade plan, i.e. visual cortex may update a change in location of a visual stimulus of interest, even if the saccade plan itself remains unchanged.

Frontal Cortex Activity

Activity in the region of the frontal eye fields (precentral/middle frontal gyri, BA 6) was seen for all saccade and fixation conditions compared to rest. This was bilateral for saccade new and on the right for the other contrasts. It was not however greater for saccade than fixation trials. Heide et al., (2001) previously found the FEF to be active for both triple-step and single memory-guided saccades, and suggested that its primary role is in controlling internally generated intentional saccades. They also concluded, based on evidence from previous studies, that unlike the PPC, the FEF was not essential for spatial updating of saccade goals, since lesions in this area did not disrupt the ability to compensate for presaccadic eye displacements. The FEF activity seen in the current task is thus unlikely to be specific to this particular task, and is probably related to the execution of saccades in general.

Frontal activity that was greater for saccade than fixation trials was however seen more anteriorly, in the prefrontal cortex. Exclusive masking revealed that this was actually higher for the original: saccade > fixation contrast than new: saccade > fixation. This might be related to the well-documented role of the PFC in working memory (see Courtney et al., 1998 for a review), since in this condition subjects might make use of a previously formed plan still held in memory, rather than calculating a new one.

Petrides (2000) showed that the dorsolateral prefrontal cortex may be involved in the monitoring of visual working memory rather than maintenance, this might account for the fact that it is greater on the original saccade, rather than fixation trials, since in the fixation trials spatial information would need to be maintained but not monitored in the same way.

A later fMRI study by Glahn et al., (2002) suggested that the DLPFC may be involved in the manipulation of spatial information held in working memory, and that different regions of the PFC in the superior frontal sulcus may be involved in maintenance of spatial information. PFC activity in the region of the superior frontal gyrus was in fact seen for both the new and original saccade > fixation contrasts.

Temporal Cortex Activity

The activity seen in the temporal lobes in this study was not in the same region as that seen in Experiment 11 (Chapter 5). Whereas that was near inferior occipital cortex, the temporal activity in the current study was located more superior to this in the middle temporal gyrus (BA 39) and close to middle occipital gyrus (BA 19) and the dorsal part of the cuneus (BA 7/31). It was seen in the right hemisphere for all of the contrasts comparing the saccade and fixation conditions to rest, and was additionally greater for both the new and original saccade than fixation trials in the left hemisphere. Exclusive masking also showed a cluster of activity in the superior temporal and angular gyri (BA 39) that was greater for the original: saccade > fixation contrast than for the new.

Distinct clusters of temporal cortex activity in the posterior superior temporal gyrus (BAs 21, 22 and 42) were also seen to be greater for saccade than fixation trials in the right hemisphere for both new and original trials.

Activity in similar regions of temporo-occipital cortex were previously noted by Gitelman et al., (1999) in a spatial attention task. This activity was in the posterior parts of the superior and middle temporal gyri, and was suggested by them to be part of a distributed network for covert spatial attention. Activation in this region in the current task might thus be a result of the participant attending to the remembered location of the target. Gitelman et al.,

also suggested that this region might provide a ‘synaptic bridge’ between the dorsal and ventral streams of visual processing.

Primary Motor Cortex Activity

Greater primary motor cortex activity was seen in the left hemisphere for the original: saccade > fixation contrast. However, through exclusive masking it was not shown to be greater than in the new: saccade > fixation contrast, suggesting it may additionally have been present in this area for this comparison albeit at a sub-threshold level. It seems that this activity might most likely reflect the additional motor output required on saccade compared to fixation trials (prior to the onset of the memory-guided saccade). Although the BOLD activity in this study was time-locked to the go-signal, this was presented only a very short time after the intervening saccades would have occurred. Since event-related activity peaks around six seconds later, activity in this region might not yet have returned to baseline (Heeger & Rees, 2002).

Cingulate Cortex Activity

Through the use of exclusive masking, activity in cingulate cortex was revealed to be greater for the original: saccade > fixation contrast than new: saccade > fixation. This region was different from the activity in the cingulate cortex seen in Experiment 11 (Chapter 5). Whereas that had been in the anterior cingulate, this cluster of activity was in the dorsal posterior cingulate. A role for the posterior cingulate cortex in visual orienting and attention has been proposed by Dean et al., (2004) on the basis of a single-unit recording study in macaques. A saccade task was used, and the results suggested that neurons in this area may signal salient visual and oculomotor events. The reason why activity should be greater in this area for saccades returning to a previous fixation location rather than moving to a new one can not be concluded definitively within the context of the current task. However, Dean et al., did notice a reduction in neuronal responsiveness with divided attention, and it could be therefore that on new trials a greater number of spatial locations are attended to simultaneously (original fixation location, new fixation location

and target location) than in the original trials (original fixation location and target location), thus resulting in a lower level of activation for the new trials.

6.5. General Discussion

The finding of PPC activity in the fixation task when there was no need to perform spatial updating supports the idea that the posterior parietal cortex may be responsible for maintaining information on the spatial location of targets for use in future saccades. The findings have additionally supported the role of this area in saccade-related remapping; greater activity was seen in this region when the new: saccade > original contrast was exclusively masked with the original: saccade > fixation contrast. The new condition was expected to require a greater amount of remapping since an accurate saccade to the remembered target location could only be performed through the formation of a new saccade plan. In the original condition, in contrast, it was postulated, on the basis of behavioural data that demonstrated reduced latency for these trials, that participants may instead be able to make use of a previously formed plan. Given the shorter latency and lower PPC activity on the original saccade trials, this also leads to the suggestion that any default plans formed to the presentation of a visual target, may not necessarily be overwritten as a result of eye displacement, but could potentially be stored alongside any new plans formed when spatial updating occurs. Further behavioural studies using saccadic latency as an indicator of the extent of spatial computations required might prove useful in evaluating this idea.

Interestingly a number cortical areas were, in contrast to the PPC, actually found to be more greatly activated by the original saccade condition. These included regions in the PFC, superior OTC and posterior cingulate cortex. Activity in these areas might thus be associated with recognizing this location as the original fixation location and recalling the previous ‘default’ saccade plan assumed to be formulated at the time of target presentation.

When a visual object of interest changes position, but this change in spatial location is not in itself behaviourally relevant (for example the fixation cross jump in the trials of the fixation task), the pattern of activity seen seems to suggest that visual cortex remaps this. It thus appears that remapping in

visual cortex may occur in response to a change in eye position, and therefore more for a double than a single saccade (as in Experiment 11), but also as the result of a change in the location of the object, i.e. the behaviourally-irrelevant fixation cross jumps in the fixation task of the current experiment.

Further investigations could consider variations on the fixation and saccade tasks used here in which changes in activity from one hemisphere to the other would be expected as a result of spatial remapping, similar to that investigated by Medendorp et al., (2003). They made use of the fact that neurons in PPC respond preferentially to remembered stimuli in the contralateral visual hemifield, and demonstrated gaze-centred updating of visual space in this area in response to eye displacement. That is, when a target was presented in the right visual field during central fixation, and then a saccade was subsequently made to a more eccentric right-ward location, activity was seen initially in the left hemisphere, and then postsaccadically in the right hemisphere (since the remembered target location was now to the left of gaze). In such a study, based on the current experiment, updating would be expected in visual cortex for trials in which the fixation cross jumps but gaze remains constant. For saccade trials in contrast when a displacement of the eye causes the location of visual stimuli to change in relation to the centre of gaze, corresponding changes in the activity of each hemisphere would be expected in both the PPC and visual cortex. A study such as this would thus require the use of extended delays between the various sub-components of the trials, such as target presentation and the intervening saccades, similar to that used in Experiment 11 (Chapter 5).

6.6. Conclusions

The experiments presented in this Chapter have further investigated the involvement of the posterior parietal cortex in particular, alongside other cortical areas, in the planning and execution of memory-guided saccades. By making use of a double-intervening saccade task it was possible to demonstrate, behavioural differences in latency that were assumed to reflect the extent of spatial remapping required for the various saccade and fixation conditions of this task. Secondly, through the use of functional imaging, it was

also possible to investigate potential differences in cortical activity associated with saccade planning in these different task conditions. From this further insight was gained into the roles that might be played by different cortical areas in the spatial remapping of visual stimuli.

Chapter 7: Summary and Conclusions

The overall aim of this thesis was to investigate processes related to eye movement planning, with a particular focus on spatial remapping and the encoding and updating of target locations and/or saccade plans. Particular consideration was given to the role of the posterior parietal cortex in these processes, on the basis of previous research, which has strongly implicated this area in such saccade-related behaviours. A previous TMS study by van Donkelaar and Müri (2002) for example claimed a disruption to the encoding and updating of a target location in a particular reference frame following the delivery of TMS to the PPC. This study made use of a double-step saccade paradigm and its findings were consistent with those from other researchers who also found evidence to support the importance of the posterior parietal cortex in the accurate performance of this task. These have included neuropsychological investigations of task performance in patients with parietal lesions (Duhamel et al., 1992b; Heide et al., 1995), single-unit recording studies in monkeys (Mazzoni et al., 1996) and functional imaging studies in healthy humans on an extended triple-step saccade task (Heide et al., 2001). The double-step saccade task has thus been established as a useful means for investigating spatial remapping (Kusunoki and Goldberg, 2003; Schlag and Schlag-Rey, 2002) and as such many of the experiments discussed in this thesis have been variations and extensions to this idea.

Further to this, the experiments in this thesis have, as a side issue to the main theme, also allowed some investigation into how the more basic aspects of a saccade plan might be coded. This has included for example an evaluation of whether different saccade metrics, such as amplitude and direction might be planned independently and whether the same cortical areas are involved. Issues such as these are clearly pertinent when considering both how target locations are encoded, for example, if targets are coded as a spatial location relative to the centre of gaze, a retinal vector would include information on both the angle and distance of the target. Alternatively, target encoding in terms of a motor plan required to acquire a particular visual target could be very precise in terms of specifying both direction and amplitude or might be more loosely programmed to allow some room for modification.

The possibility that the processes involved in such tasks may not be rigid across different situations was also considered, and the influence of task-related factors was thus evaluated as well as potential variations in the cortical areas involved as a result of the specific nature of the task in hand. These have included considerations of whether remapping of a target location in response to an eye movement differs from remapping in response to a presaccadic target jump and whether motor intention affects target encoding. The storage in memory of target locations for use in future saccades was also debated, including the potential nature of this e.g. as motor plans, spatial locations or both, and if motor plans were used, could more than one of these be concurrently maintained in memory.

Such issues were also considered in relation to current debate in the literature over the nature of saccade-related neuronal activity in PPC. Whilst some have argued for an interpretation in terms of the encoding of salient target locations (e.g. Colby & Duhamel, 1996), others have conversely proposed an explanation based on motor intention, i.e. a plan to make a saccade towards this location (e.g. Andersen and Buneo, 2002; Snyder et al., 1997). The usefulness of the findings from the studies presented in this thesis in providing further insight into this issue is thus also discussed as are potential future studies that could be used to continue to advance our knowledge in this area.

7.1. How are saccade-related processes such as target encoding and spatial remapping affected by the specific nature of the task?

As mentioned above, previous studies involving single-unit recording in monkeys and neuropsychological investigations and neuroimaging in humans have implicated posterior parietal cortex in the process of saccade-related spatial remapping. In particular, in a study by van Donkelaar and Müri (2002) it was claimed that a disruption to this process in a double-step saccade task had been demonstrated through the use of TMS. More specifically, it was suggested that the craniotopic encoding of targets that is normally apparent in this task was affected by the TMS, thus leading participants to make use of a more object-based frame of reference, which in turn affected task performance.

Experiment 3 attempted to replicate the findings of van Donkelaar and Müri using a similar experimental setup, with a few important modifications. These included the use of a wider range of eye movement directions whereas their study had only incorporated horizontal saccades. It was thought that this would provide a more naturalistic setup, and additionally test the robustness of their finding, since spatial remapping should presumably occur for all possible saccade directions. Further to this, the method of visual presentation also varied; whereas they had used sequential target presentation as is usual in the double-step task, the target presentation in Experiment 3 was instead simultaneous. Based on previous studies there was no a-priori expectation that the remapping process would be affected by this factor.

This experiment therefore aimed to test the idea that the PPC is crucially involved in the remapping of target locations. Support for this idea would be expected by a finding of decreased remapping following the application of TMS to this cortical area at a critical point during task performance. However, in contrast to this expectation, improved remapping, at least in terms of a measure of angular compensation, was instead demonstrated on TMS trials.

A potential explanation for this in terms of both task specifications and the proposed additional role of the PPC in storing remembered target locations was thus put forward. Since in the study by van Donkelaar and Müri it had been suggested that TMS was disrupting craniotopic encoding of target locations, i.e. the reference frame that would be believed to be dominant in their version of this task, it might be that TMS in this study was also disrupting the dominant coordinate frame used to code the remembered target locations. As a result of the method of target presentation used however (simultaneous), this might have been more object-based, i.e. targets were coded in relation to each other, so that TMS resulted in the use of an alternative frame of reference, and thus a somewhat paradoxical improvement in the extent of spatial remapping.

This theme was explored further using behavioural eye-tracking methods in Experiments 7-10. Experiment 7 used sequential target presentation and compared trials that were equivalent to a traditional double-step saccade task as well as those with a reversed target presentation order, to assess the effect of this on target encoding. A difference in latency was seen between the two

conditions, with a significantly longer reaction time seen in the reverse target presentation order. This was attributed to an increase in the extent of processing required to plan the two saccades in this condition compared to the forward order, presumably since the spatial computations required would be more complex.

The idea that task-related factors such as presentation order could influence processes related to saccade planning was further examined in Experiments 8 and 10. From a comparison of the results of Experiments 3 and 7 in terms of compensation measures, there was some evidence to suggest that an increase in the temporal gap between target presentations (from 0ms to 500ms) had affected the extent of remapping. Based on this, it was reasoned that maybe further increasing this gap would continue to reduce the degree to which the two targets were coded in relation to each other. If this was the case it would be expected to be demonstrated in terms of a linear improvement in the compensation measures with increasing gap (delay) length. Although the results failed to reveal such a linear trend, one finding of particular interest was an effect of the order of target presentation on compensation. This was found to be significantly greater for the reverse order trials in terms of amplitude compensation. Although the results for angular compensation were not significant, visual inspection of the data appeared to provide some suggestion that this might, in contrast, be slightly better for the forward order trials.

These findings thus inspired a further investigation of the effects of target presentation order on saccade sequences, through the use of a triple-step paradigm. The inclusion of an additional target naturally increased the number of possible presentation orders, thus providing the potential to examine its effect on remapping in more detail. The results of this study further supported the conclusion drawn from Experiments 3, 7 and 8. Firstly they suggested that the finding of greater amplitude compensation for the reverse compared to the forward order of target presentation was a robust one. Secondly, and perhaps of more interest in terms of understanding this process, compensation (both amplitude and angular) appeared to be greater the further apart the corresponding targets were displayed within the presentation order. This was interpreted in terms of the extent to which the targets could be encoded in relation to each other, i.e. spatial remapping is better when the spatial

relationship between them is less obvious. This may be related to the frame of reference used, i.e. object-based versus retinotopic, or the fact that a motor code for the entire sequence cannot so easily be preformed when the orders of target presentation and saccade execution are in conflict.

In conclusion, target encoding and spatial remapping do appear to be influenced by task specifications. Certain tasks may inadvertently encourage encoding of targets in a particular reference frame (or encourage one reference frame to be dominant among multiple ones), and this may in turn affect the extent of spatial remapping that occurs to account for previously executed saccades as indexed by measures of compensation.

7.2. Are different saccade metrics controlled independently?

The suggestion that angular and amplitude compensation may be separately controlled was raised in Experiment 3, since effects of TMS were found for the former but not the latter. Experiment 2 in the same Chapter was an attempt to use a functional localisation procedure to systematically determine the location of the PEF based on the effect of TMS on saccade latency. Since stimulation at the sites defined based on this (i.e. test and control) did not show the expected pattern of results, i.e. an effect of TMS at the control site was seen rather than at the test site as expected. To try and account for this, it was suggested that the sites chosen may have had additional effects on other saccade metrics such as error that were not considered in this study but which may also have been important.

This issue was addressed in Experiment 5 by comparing the effects of TMS over a grid of parietal sites on not only latency but also amplitude and angular error. No single site that affected all three variables could easily be determined within individuals, further supporting the idea that these factors might be programmed independently. There was also evidence to suggest substantial individual differences in the effects of TMS to a specific parietal site. (See Ryan et al., 2006, included in Appendix 3, for further discussion of this idea).

The idea that different control mechanisms may be employed for updating in response to amplitude and angular perturbations was brought up in

light of the results from Experiment 6, in which an effect of TMS to the right hemisphere test site was found for amplitude but not angular perturbations. This was followed by the suggestion, based on the results from Experiment 10, that the process of angular compensation in saccade sequences may occur during the intersaccadic interval, whereas amplitude compensation may be more predictive in nature, perhaps occurring online during saccade execution.

The combination of findings from a number of the experiments discussed in this thesis therefore seems to point towards the idea that the coding and updating of amplitude and angular information specifying the goals for future saccades may be independently controlled. On the basis of TMS results in Experiment 5 there was some indication that stimulation to the same cortical area may not affect both of these saccade metrics in the same way. In light of the findings from Experiments 6 and 10, it seems that there may also be differences in terms of the time at which amplitude and angular compensation occurs during saccade execution. Further to this, the effects of manipulating the order of target presentation appear to differ for angular and amplitude compensation measures, providing additional support to the idea they may be independently controlled.

7.3. How are targets for future saccades represented in the posterior parietal cortex and is this affected by task-related factors?

Experiments 2, 5 and 6 all required participants to perform single reflexive saccades to the presentation of a visual target. In these three experiments therefore, there was no way to differentiate between attended spatial locations and locations as goals for the upcoming saccade, since the target could always be simply encoded relative to the centre of gaze.

In Experiment 3, in contrast, whereas the first target could similarly be coded in such a retinotopic manner, the second target could not. Thus while participants must remember the spatial location for this target, the exact plan executed must also take into account the intervening saccade to target 1, and any errors made in the execution of this. From this study it was concluded that parietal TMS may disrupt the dominant spatial representation of the target locations, which for this particular task might be object-based, i.e. the targets

are coded in relation to one another. As a result of the TMS, it was suggested, participants were forced to rely on an alternative reference frame for the target location. Previous studies have concluded that spatial locations for targets may be coded in multiple frames of reference within the PPC, and a previous TMS study by van Donkelaar and Müri (2002) had similarly argued for a specific disruption from parietal TMS to the dominant reference frame in their particular task.

In Experiment 11, a reverse double-step saccade task was used to assess whether differences in cortical activity would be seen for target encoding as a result of differences in motor intention, i.e. whether you intend to make a saccade directly to the target from the fixation location or not. In the forward target presentation order, as on a reflexive saccade task, the target for the first saccade can be encoded as a motor plan at the time of target presentation. For the first target on the reverse presentation order trials, however, the target cannot usefully be encoded in such a way since an as yet undefined intervening saccade must be executed before the saccade to this target is initiated. In other words, the start point of the saccade to this target is not yet known. It was suggested therefore that this target might instead be encoded as a spatial location rather than in terms of a motor plan, or retinal vector.

The results from this study, however, failed to find any difference between the two conditions, with activity in the region of the PEF seen in both cases. One explanation for this could be that this parietal activity reflects attention to the spatial location of the target. Alternatively, and in support of previous studies by Andersen and Buneo (2002), it could reflect the formation of default motor plans to a behaviourally relevant visual target. These plans could then be updated in response to the presentation of the second target, after which a plan for the entire sequence could be formulated.

Experiment 12 investigated whether the formation of an updated saccade plan, or remapping in response to saccades executed after target presentation, causes a previous plan to be overwritten. In this task a visual target was presented, but before a memory-guided saccade could be made towards its remembered location, two intervening saccades had to be performed. There were two contrasting experimental conditions, which differed in terms of whether the intervening saccades brought the eye back to its original fixation

location (as it was at the time of target presentation) or instead took it to a new fixation location. In the latter of these, a new saccade plan would be required to account for the eye displacement, thus necessitating spatial remapping of the target location. In the former, however, it seemed there were two possible solutions: participants might, as in the other condition update the saccade plan to account for the intervening saccades. Alternatively, it was also a possibility that they might somehow be able to make use of the plan formed previously at the time of target presentation. If the second of these two options was true it would be expected to be demonstrated by a reduced processing time, i.e. a shorter latency, and also perhaps decreased error since there would be less potential for inaccuracies caused by spatial remapping. Support for this idea came from the behavioural findings for both of these saccadic measures. This thus raises the possibility that the parietal cortex might in fact be capable of storing multiple saccade plans concurrently and that previously formed plans are not necessarily overwritten as a result of spatial updating.

7.4. Role of the posterior parietal cortex in saccade planning

The aim of the experiments discussed in this thesis was to extend our understanding of the role played by parietal cortex in the planning of saccades. Previous studies in this research area have suggested that one of the primary functions of the PPC lies in representing target locations for future saccades either as a map of space (Serenio et al., 2001) or as a map of motor plans (Andersen and Buneo, 2002). The results of the studies discussed here support this idea of a representation of saccade goals within parietal cortex, for example in the fixation task in Experiment 13 activity was seen in the PPC suggesting that it may be responsible for maintaining information on the spatial location of targets for use in future saccades. These studies also implicate parietal areas in the updating of target representations following changes in the spatial relationship between the centre of gaze and target location, whether these changes occur as a result of a movement of the eye or the target.

These findings do not however exclude a role for other cortical areas in the planning of saccades, since for example the role of frontal areas in these processes was not greatly considered in the experiments discussed in this

thesis. On the basis of previous studies, however, it seems probable that the parietal lobe operates in conjunction with frontal cortex, as part of a network of areas involved in saccade control (Gaymard et al., 1998; Pierrot-Deseilligny et al., 2003).

In addition to this it seems that posterior parietal cortex may not be the only area involved in spatial remapping. In particular, the results from Experiments 11 and 13 seemed to suggest that the visual cortex might also play a role in this process, albeit one that differs from that of the parietal lobes. It seemed that updating of visual representations occurred in this area in response to an eye movement as in the double-step saccade task and also in response to a change in the location of an object of interest, regardless of whether this was relevant to the task. This was based on the finding of occipital activity on the fixation trials in the intervening saccade task in Experiment 13, when the fixation cross moved but no saccades were required in response to this.

In terms of the attention vs. intention debate regarding the functional significance of saccade-related activity in this area, it has been hard on the basis of the findings to conclusively rule out either of these alternatives. Parietal activity was for example seen in the region of the PEF at the time of target 1 display in the double-step saccade task in Experiment 11, regardless of whether it made sense for a saccade plan to be formed or not. There was no real way of deciding whether this was due to the formation of default plans towards the visual target, as would be proposed by the motor intention argument, or whether the lack of difference between the reverse and forward order trials was because attention was similarly directed to the target location in both cases. The behavioural results in Experiment 12 go some way towards supporting the idea that target locations may be specified in terms of motor plans, since less time was required to perform a single memory-guided saccade when the start point corresponded to the location of the eye at the time of target presentation, compared to when the formation of a new plan to account for the intervening saccades was essential to the task. Findings from this study also suggested however, that while parietal cortex may remap behaviourally relevant spatial information, such as goals for future saccades, remapping in the visual cortex may occur for all visual stimuli regardless of whether they a movement is planned or not. This suggests that activity in parietal cortex to a

greater extent reflects a map of motor plans, whereas that in visual cortex represents spatial locations for visual stimuli.

Extensions to the fixation and saccade tasks discussed in this thesis could be used to further investigate these issues. For example, based on the findings from Experiment 13 (Chapter 6), a study incorporating trials in which a fixation cross jumps but gaze remains constant, would be expected to show updating in visual cortex. In contrast to this, for trials in which the relationship between the location of visual stimuli and the centre of gaze is altered by a change in eye position, changes in activity would be expected in both the PPC and visual cortex. Since it has been shown that activity within a subregion of PPC possibly corresponding to the human homologue of area LIP, is directionally selective for the memory of a target location presented in the contralateral hemifield (Medendorp et al., 2006; Sereno et al., 2001), changes in cortical activity might best be seen when the movement of the fixation cross caused the location of this relative to the centre of gaze to move from one hemifield to the other. A study such as this would thus require the use of extended delays between the various sub-components of the trials, such as target presentation and the intervening saccades, similar to that used in Experiment 11 (Chapter 5). This might also be useful for providing further insight into potential hemispheric specialisation within these cortical areas, something that has not been given a great deal of consideration within this thesis, but has been a focus for previous studies in this area investigating cortical involvement in similar tasks using TMS (e.g. Müri et al., 1996; 2000; Oyachi and Ohtsuka, 1995).

This study might not however be most useful for furthering understanding in relation to the intention/attention debate, since both of these would be changed by a movement of the eye or the fixation cross. Additionally, in the fixation condition, it could be argued that the main attended location would be where they planned to look, rather than the new location of the fixation cross. Attention and intention would therefore remain difficult to dissociate in this task. One way to separate them might be to attempt a delayed single-saccade task, based on the reflexive saccade task used in Experiment 6, where the location of the target jumps presaccadically, but in this case participants must ignore this and instead make an eye movement

towards its previously presented location. Thus although the location of this behaviourally-relevant visual stimulus will have changed, motor intention should remain the same. Of course it could similarly be argued for this that although the stimulus moves, the participant will continue to attend at its original location. Activity could perhaps be contrasted against a condition in which the stimulus moves and the participant does update their saccade plan to incorporate this. The use of retinotopic mapping, such as that employed by Sereno et al., (2001) to consider cortical activity in parietal and occipital regions might be informative in determining exactly where in the visual field the greatest activity in PPC corresponded to. Activity in frontal areas in the region of the FEF might also be worth assessing, since in a recent imaging study by Curtis and D'Esposito (2006) involving a delayed saccade task, it was argued that activity in this area might reflect movement planning signals whilst that in parietal cortex is more related to the processing and storage of visual signals.

7.5. Conclusions

In this thesis I have presented a number of experiments investigating processes related to saccade planning, which have provided further insight into the mechanisms of how goals for future saccades are encoded and updated within the PPC.

Firstly, by making use of various eye-tracking paradigms, these experiments have demonstrated an apparent flexibility in these processes. They do not appear to be rigid across all saccade tasks, but are in fact influenced by at least some task-related factors, including both the method (simultaneous/sequential) and order of visual target presentation. The influence that method of target presentation has on target encoding may be related to the frame of reference that it encourages, which may in turn have an impact on the extent of spatial remapping.

A second finding from these experiments relates to how target locations may be specified in terms of angle and amplitude of the saccade plan required to acquire them. Since the discussed effects of target presentation order did not seem to similarly affect compensation measures in terms of saccade amplitude

and angle, there was some evidence to suggest that these may be independently controlled. This was also supported by the results of TMS studies showing apparent separate disruptions to measures of either amplitude or angular compensation through stimulation to the PPC during saccade tasks requiring spatial updating.

A further important finding was the failure to reveal differences in cortical activity within the PPC to the presentation of a visual target solely on the basis of whether it was possible to plan the future saccade directly towards it or not. This could however equally be explained in terms of attention, since the target was behaviourally relevant and its spatial location had to be remembered, or intention, i.e. the formation of a default plan towards it since no alternative plan could be formed at that point. On the basis of these results these alternative explanations were not readily dissociable.

A later finding however provided some support for the idea that multiple saccade plans may be concurrently maintained in memory. A saccade which could have been previously programmed, possibly as a default plan made at the time of target presentation, was found to be more quickly executed than a saccade for which this would not have been possible. This suggested that the spatial remapping of a target location in response to a saccade may not necessarily overwrite previous plans and that they may instead remain available for use.

The findings from these experiments therefore support the idea that the PPC is important for representing saccade goals and updating these following a change in the spatial relationship between the centre of gaze and the target location for a future saccade, both whether this is due to a movement of the eye or of the target.

Other cortical areas may however also be involved in this remapping process, for example the results of the neuroimaging studies discussed here appear to additionally implicate the visual cortex in this process. In these studies the role of other cortical areas that may also have been important, such as frontal oculomotor areas was not greatly considered and thus a potential role for these areas has not been excluded.

In conclusion, the results seemed to suggest that the posterior parietal cortex may maintain a map of motor plans for saccade goals rather than a

representation of sensory information concerning the visual environment, which might instead be a function of visual cortex. This theory will however require further testing and sophisticated future studies will be required to determine whether it is in fact possible to dissociate intention and attention and thus draw a definitive conclusion regarding the functional significance of neural activity within the posterior parietal cortex.

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Appendix 1: Figures showing Effects of TMS from Experiment 2, Chapter 2

Right-Hemisphere Stimulation

Participant 1: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.1). The mean latency for the real TMS trials at site number 7 was found to be significantly greater than for sham TMS trials ($t_{(29)} = 2.076$, $p < 0.05$); this site was therefore defined as the test site. Site number 2 was chosen as the control site since the difference between real and sham TMS for this site was not significantly different ($t_{(19)} = 0.262$, N.S.).

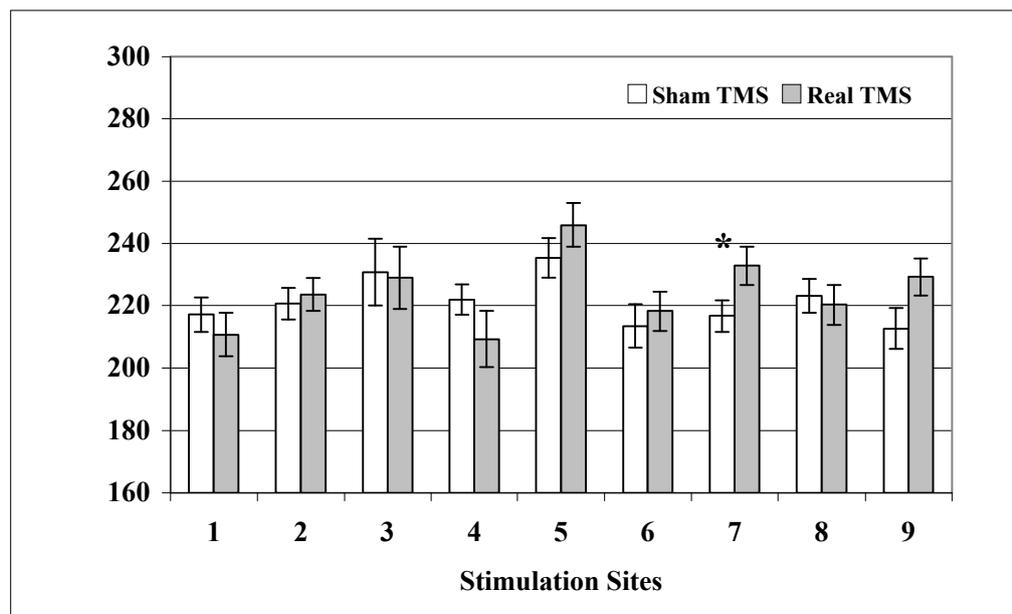


Figure A1.1: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 1. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.05$.

Participant 2: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.2). The mean latency for the real TMS trials at site number 9 was found to be significantly greater than for sham TMS trials ($t_{(24)} = 3.217$, $p < 0.005$); this site

was therefore defined as the test site. Site number 5 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(23)} = 0.389$, N.S.).

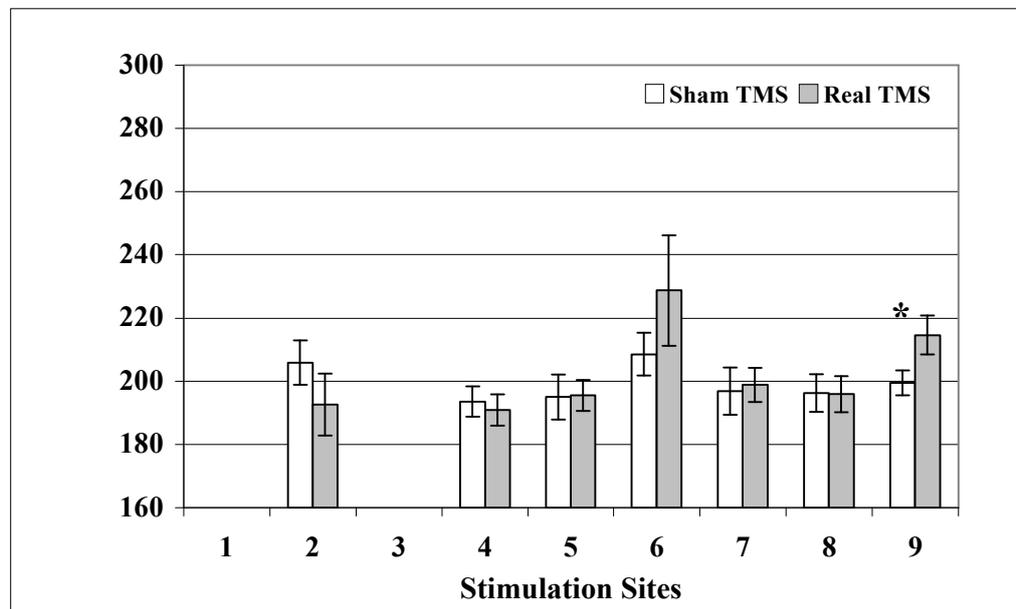


Figure A1.2: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 2. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.005$.

Participant 3: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.3). The mean latency for the real TMS trials at site number 7 was found to be significantly greater than for sham TMS trials ($t_{(29)} = 4.006$, $p < 0.0005$); this site was therefore defined as the test site. Site number 8 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(15)} = 0.954$, N.S.).

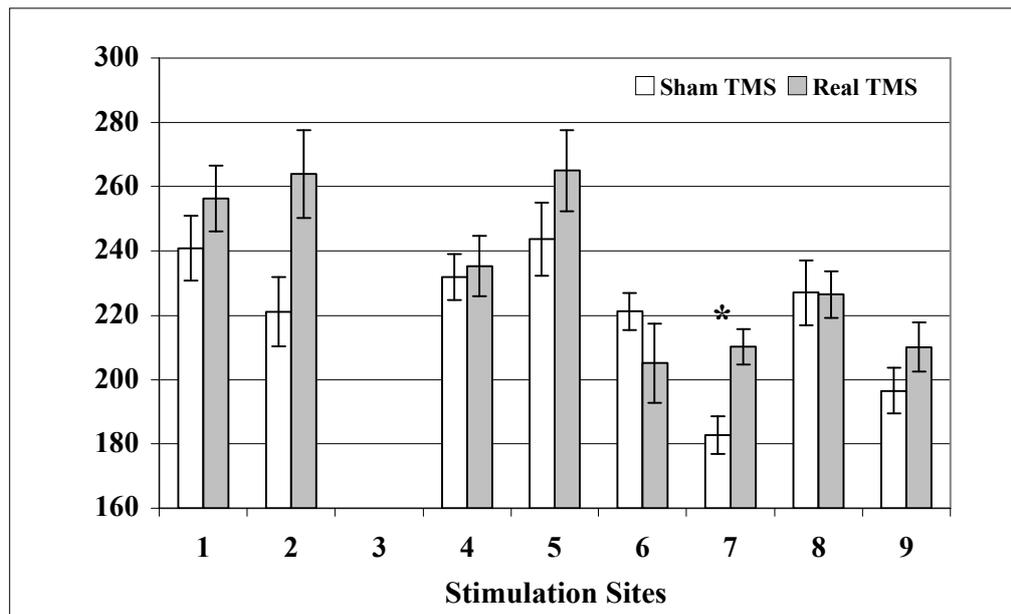


Figure A1.3: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 3. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.0005$.

Participant 4: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.4). The mean latency for the real TMS trials at site number 9 was found to be significantly greater than for sham TMS trials ($t(22) = 5.625, p < 0.00005$); this site was therefore defined as the test site. Site number 2 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t(23) = 0.991, N.S.$).

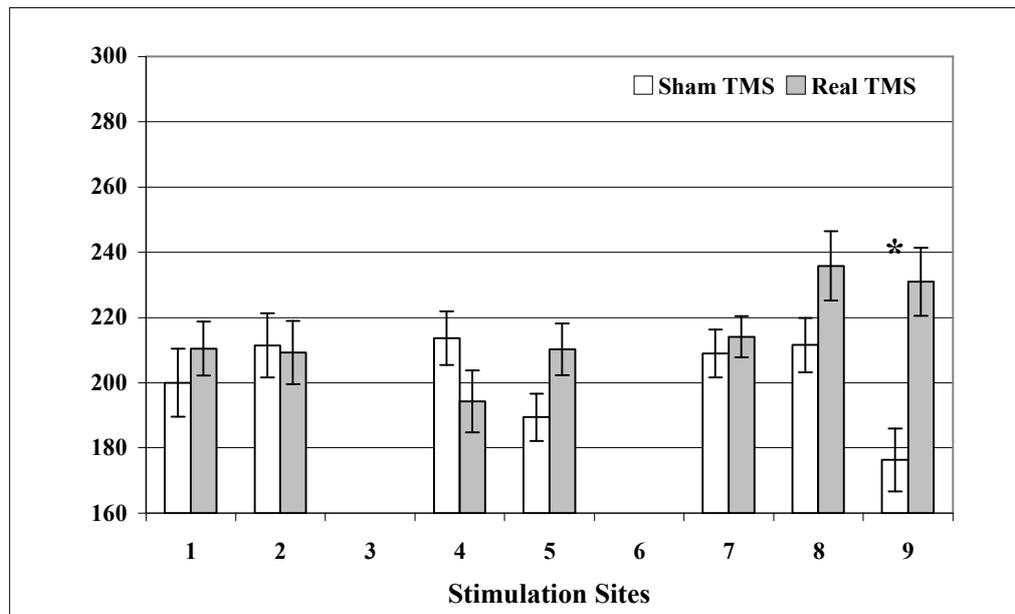


Figure A1.4: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 4. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.00005$.

Participant 5: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.5). None of the stimulation sites were found to have a greater mean latency for the real TMS trials than for sham TMS trials. The site with the largest difference, site number 1, was therefore chosen as the test site ($t_{(25)} = 1.316$, $p = 0.20$). Site number 7 was chosen as the control site since this had the smallest difference between real and sham TMS ($t_{(27)} = 0.741$, N.S.).

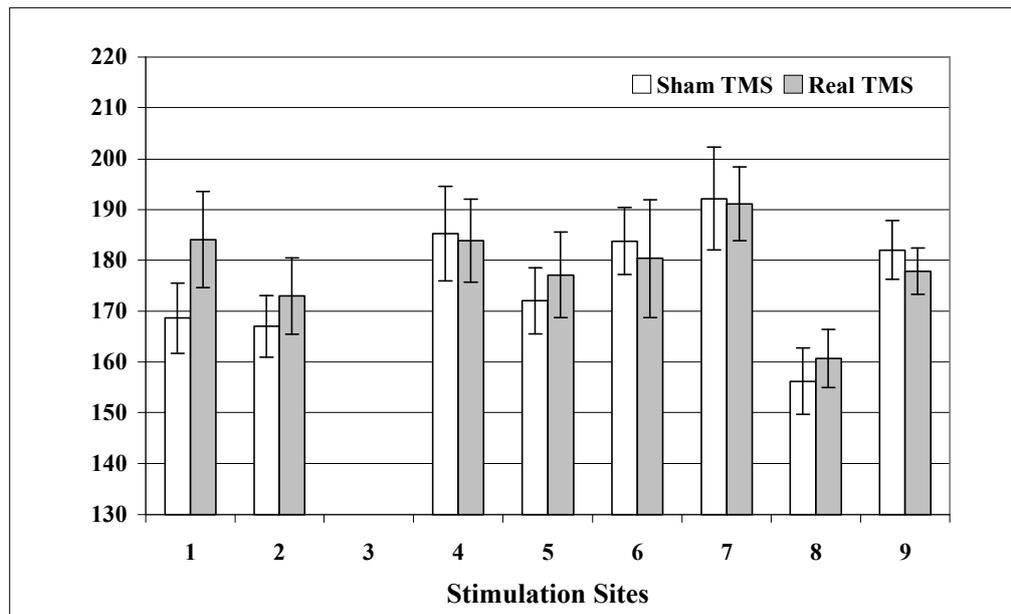


Figure A1.5: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 5. Error bars show standard errors.

Participant 6: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.6). The mean latency for the real TMS trials at site number 9 was found to be significantly greater than for sham TMS trials ($t_{(16)} = 2.771$, $p < 0.05$); this site was therefore defined as the test site. Site number 1 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(23)} = 0.947$, N.S.).

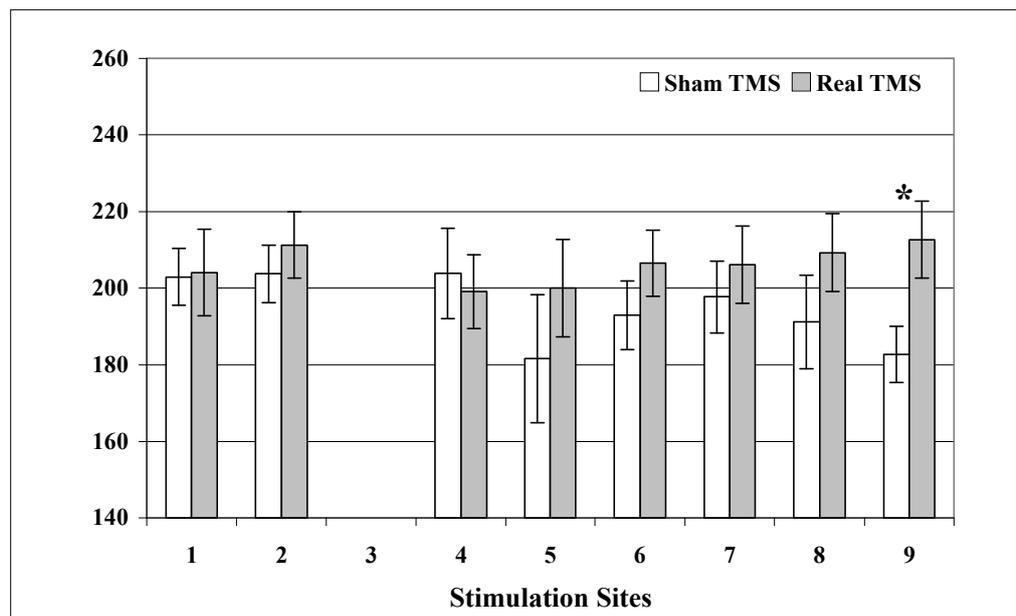


Figure A1.6: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 6. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.05$.

Participant 7: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.7). None of the stimulation sites were found to have a greater mean latency for the real TMS trials than for sham TMS trials. One of the sites, number 7, was however approaching significance and was therefore chosen as the test site ($t_{(26)} = 1.951, p = 0.06$). Site number 6 was chosen as the control site since this had the smallest difference between real and sham TMS ($t_{(26)} = 0.953, N.S.$).

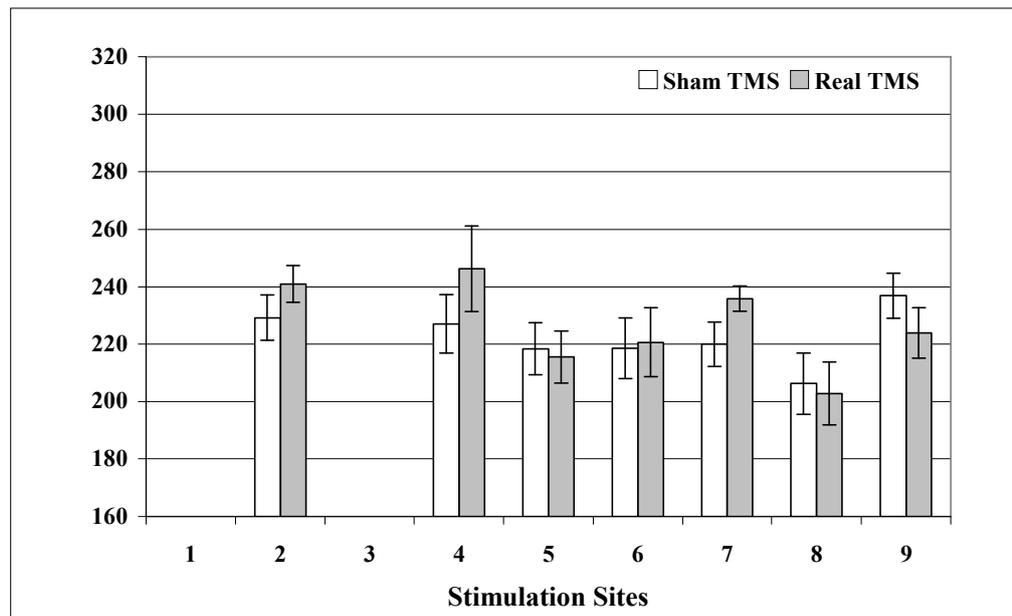


Figure A1.7: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 7. Error bars show standard errors.

Participant 8: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.8). The mean latency for the real TMS trials at site number 7 was found to be significantly greater than for sham TMS trials ($t_{(27)} = 3.898$, $p < 0.001$); this site was therefore defined as the test site. Site number 5 was chosen as the control site, the difference between real and sham TMS for this site was not found to be significantly different ($t_{(26)} = 1.300$, N.S.).

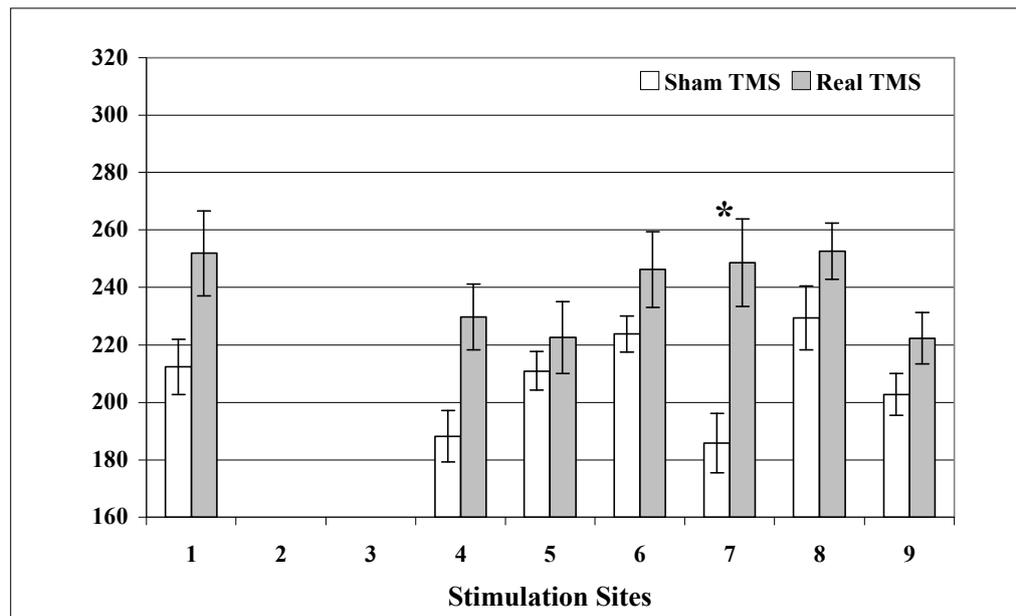


Figure A1.8: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 8. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.001$.

Participant 9: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.9). The mean latency for the real TMS trials at site number 2 was found to be significantly greater than for sham TMS trials ($t_{(27)} = 2.395$, $p < 0.05$); this site was therefore defined as the test site. Site number 4 was chosen as the control site, the difference between real and sham TMS for this site was not found to be significantly different ($t_{(26)} = 1.031$, N.S.).

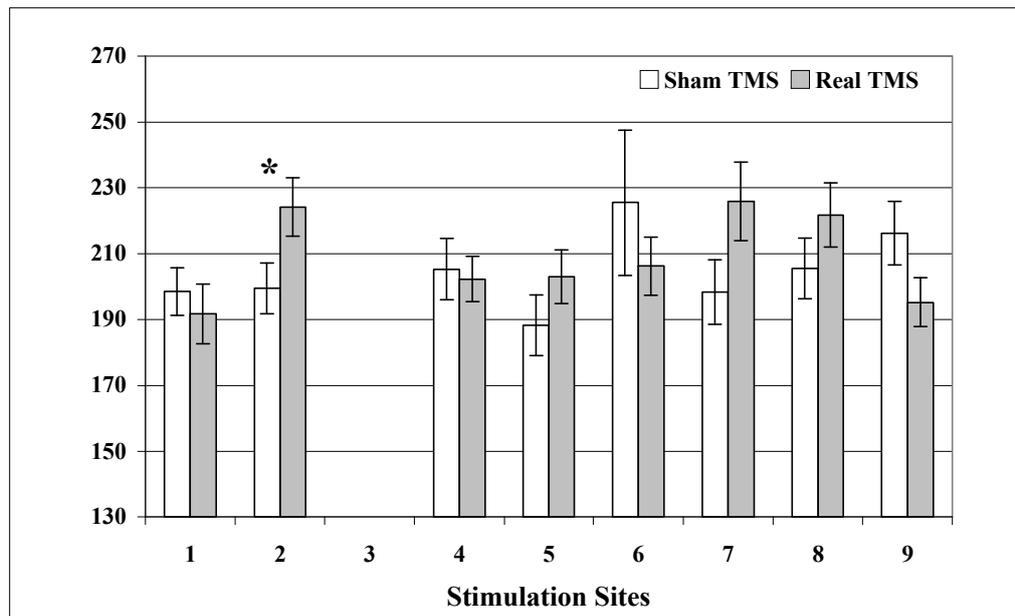


Figure A1.9: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 9. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.05$.

Participant 10: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.10). The mean latency for the real TMS trials at site number 1 was found to be significantly greater than for sham TMS trials ($t_{(27)} = 3.236$, $p < 0.005$); this site was therefore defined as the test site. Site number 6 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(26)} = 0.872$, N.S.).

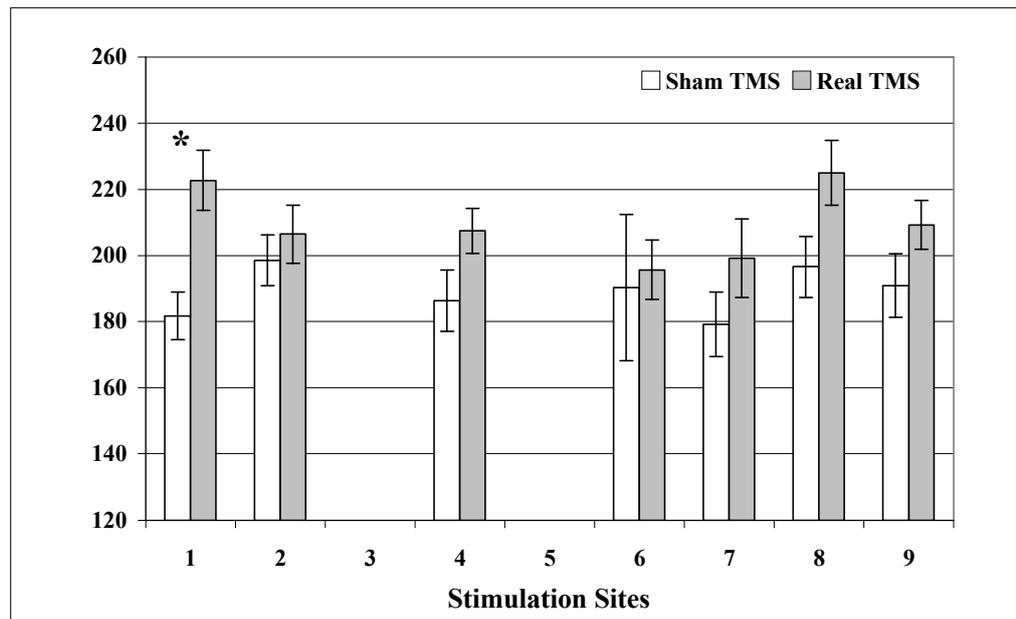


Figure A1.10: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 10. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.005$.

Left-Hemisphere Stimulation

Participants 1 and 2: Neither participant 1 nor participant 2 in the left-hemisphere TMS condition of the localiser task showed any sites for which the latency on real TMS trials was significantly greater than on sham TMS trials. Since no suitable sites could be determined as test sites, these participants did not take part in the memory-guided double-saccade task that followed. Mean and standard error latencies for real and sham TMS at each of the stimulation sites for these participants are shown in the graphs below (Figures A.11 and A.12).

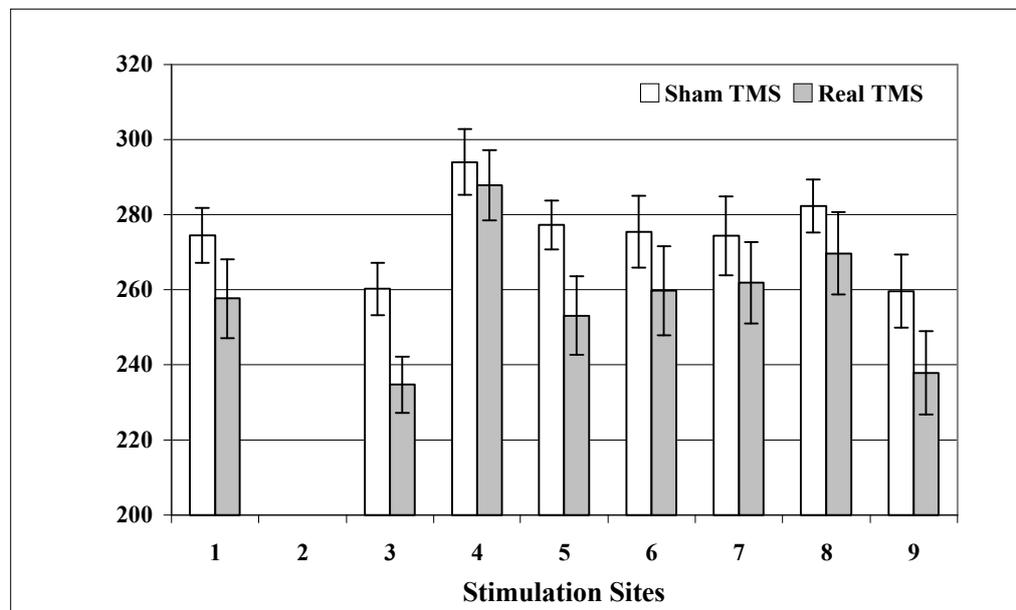


Figure A1.11: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 1. Error bars show standard errors.

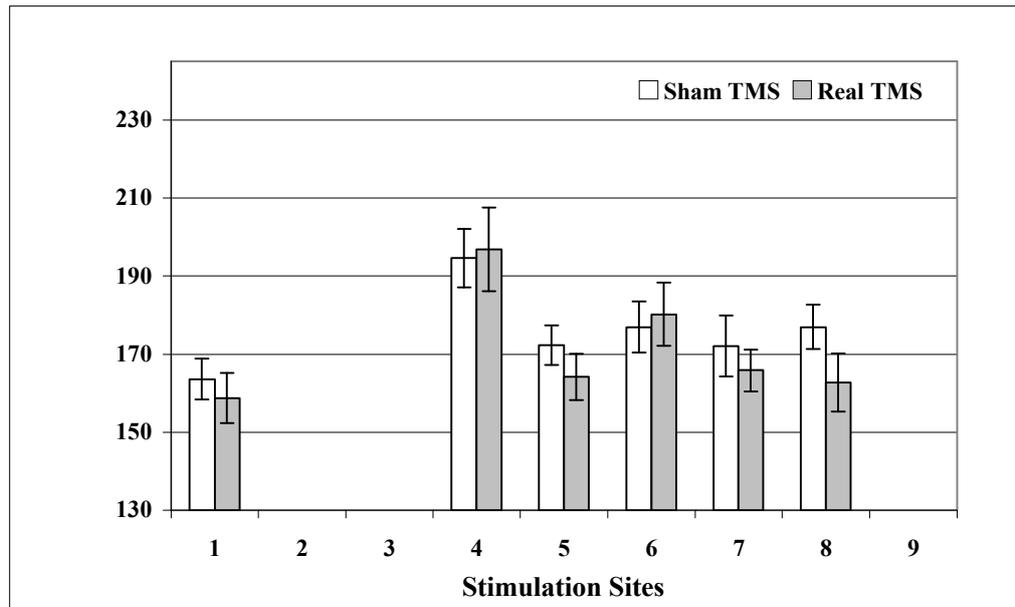


Figure A1.12: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 2. Error bars show standard errors.

Participant 3: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.13). The mean latency for the real TMS trials at site number 3 was found to be significantly greater than for sham TMS trials ($t_{(27)} = 2.458$, $p < 0.05$); this site was therefore defined as the test site. Site number 1 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(24)} = 0.872$, N.S.). N.B. Site number 7 also showed a significantly greater latency for real than sham TMS ($t_{(8)} = 3.078$, $p < 0.05$), but since a large proportion of real TMS trials at this site had to be discarded (21 out of 30), site number 3 was chosen instead.

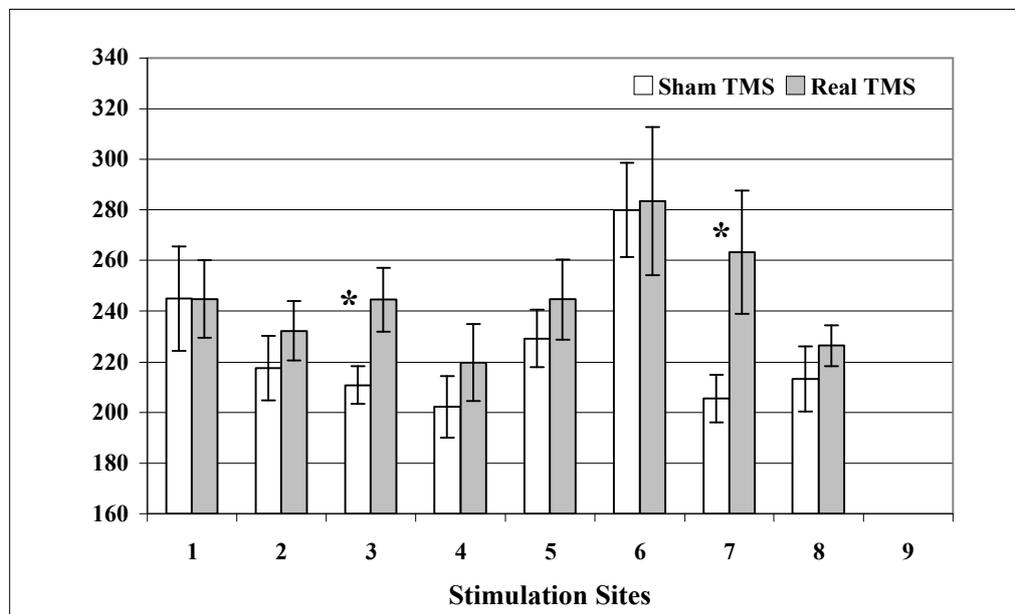


Figure A1.13: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 3. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.05$.

Participant 4: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.14). The mean latency for the real TMS trials at site number 4 was found to be significantly greater than for sham TMS trials ($t_{(28)} = 4.011$, $p < 0.0005$); this site was therefore defined as the test site. Site number 1 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(27)} = 1.009$, N.S.).

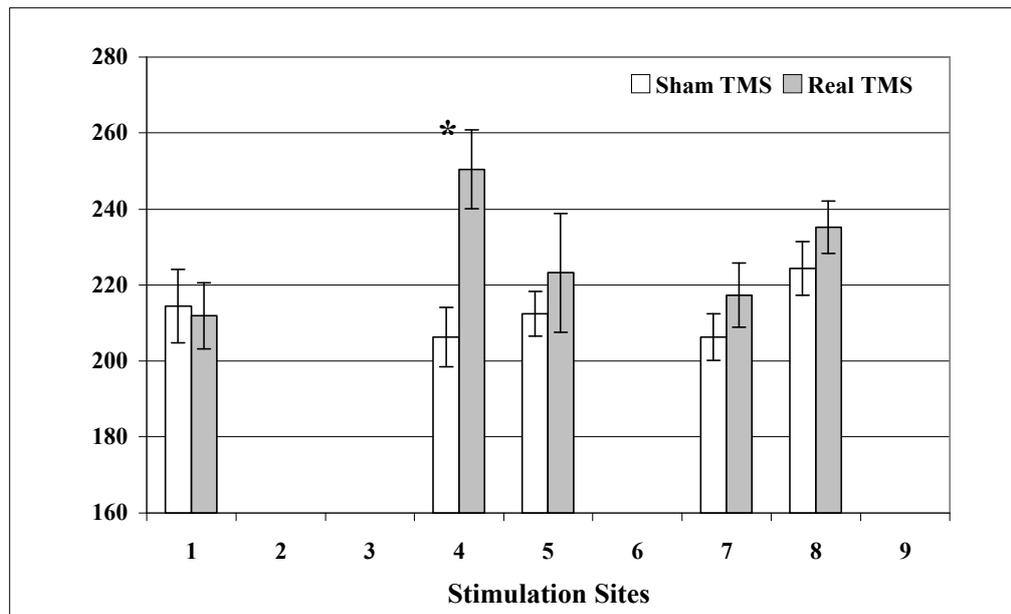


Figure A1.14: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 4. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.0005$.

Participant 5: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.15). The mean latency for the real TMS trials at site number 2 was found to be significantly greater than for sham TMS trials ($t_{(27)} = 2.927$, $p < 0.01$); this site was therefore defined as the test site. Site number 6 also showed a significantly greater mean latency for real TMS compared to sham TMS, although to a lesser extent ($t_{(24)} = 2.479$, $p < 0.05$). Site number 8 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(26)} = 0.755$, N.S.).

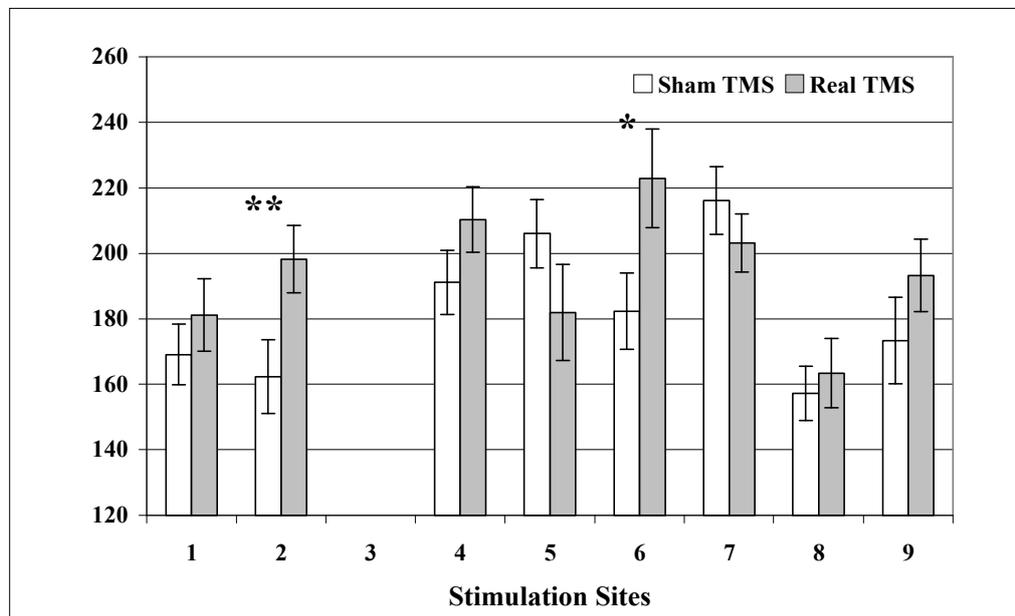


Figure A1.15: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 5. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.05$; ** = Difference between sham and real TMS is significant at $p < 0.01$.

Participant 6: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.16). The mean latency for the real TMS trials at site number 2 was found to be significantly greater than for sham TMS trials ($t_{(23)} = 2.024$, $p=0.05$); this site was therefore defined as the test site. Site number 6 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(23)} = 1.223$, N.S.).

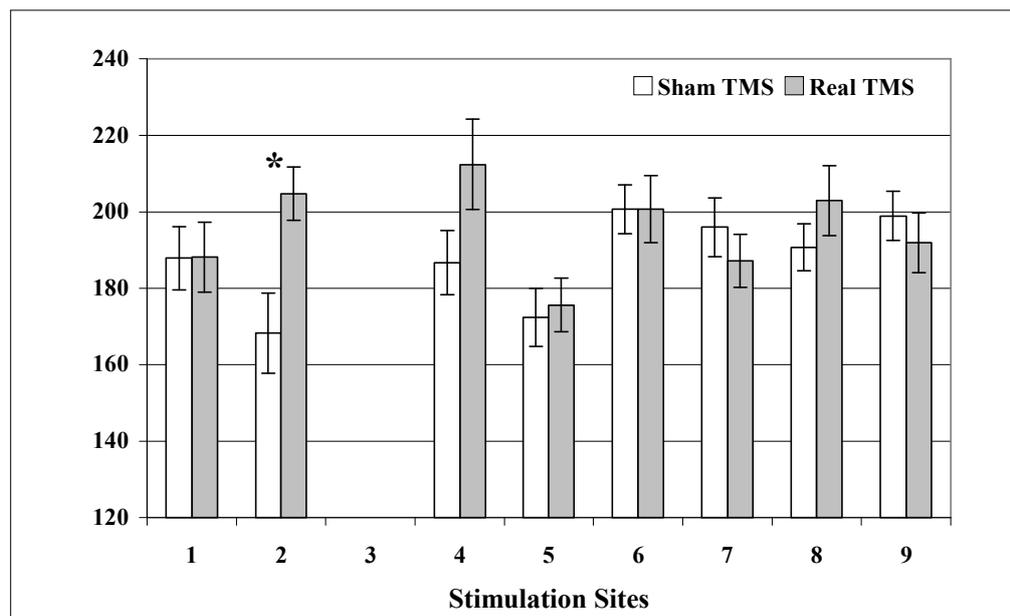


Figure A1.16: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 6. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p=0.05$

Participant 7: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.17). The mean latency for the real TMS trials at site number 2 was found to be significantly greater than for sham TMS trials ($t_{(26)} = 2.167$, $p < 0.05$); this site was therefore defined as the test site. Site number 1 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(20)} = 1.119$, N.S.).

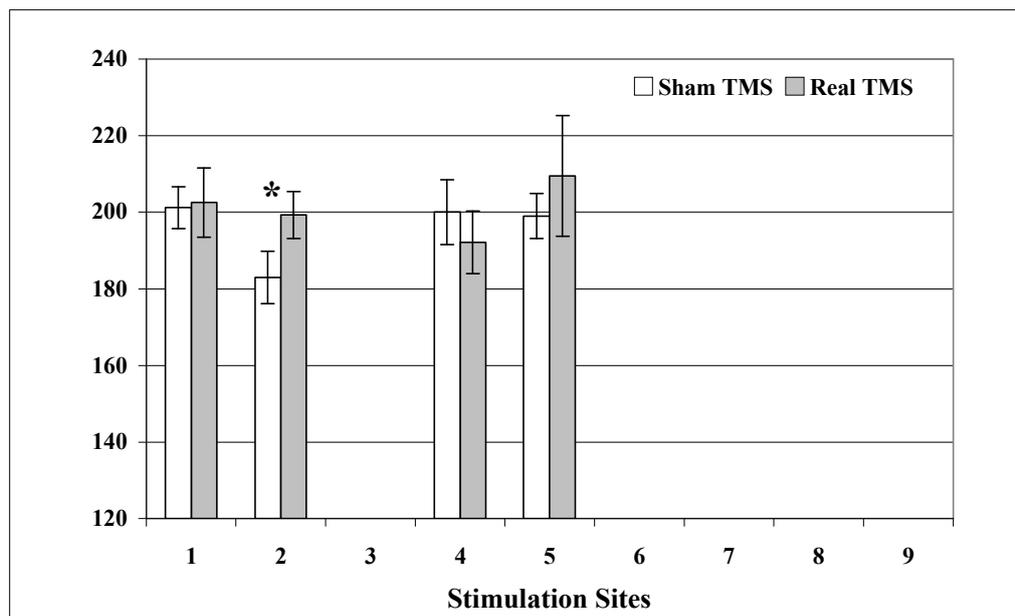


Figure A1.17: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 7. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.05$.

Participant 8: Mean and standard error latencies for real and sham TMS at each of the stimulation sites are shown in the graph below (Figure A1.18). The mean latency for the real TMS trials at site number 7 was found to be significantly greater than for sham TMS trials ($t_{(16)} = 2.563$, $p < 0.05$); this site was therefore defined as the test site. Site number 8 was chosen as the control site since the difference between real and sham TMS for this site was not found to be significantly different ($t_{(24)} = 0.804$, N.S.).

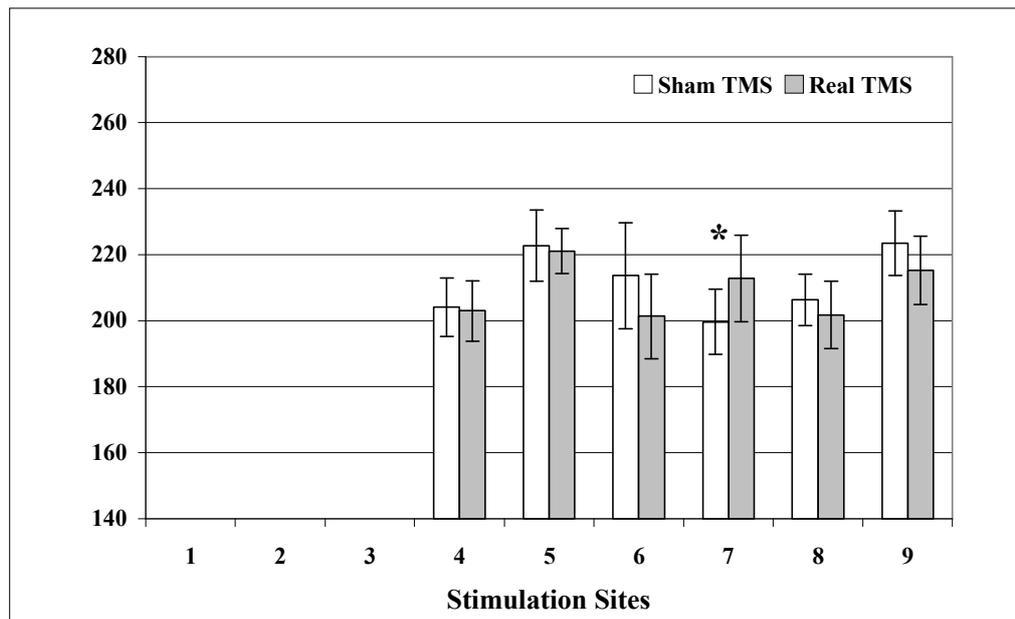


Figure A1.18: Mean latencies for real and sham TMS trials at each of the stimulation sites for participant 8. Error bars show standard errors. * = Difference between sham and real TMS is significant at $p < 0.05$.

Appendix 2: Figures showing Effects of TMS from Experiment 5, Chapter 3

Participant 1: Right Hemisphere

The graph below (Figure A2.1) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 1. Site number 8 was chosen as the test site, since the difference between real and sham TMS was found to be significant for two of the 3 saccade metrics: latency and angular error. Site number 5 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

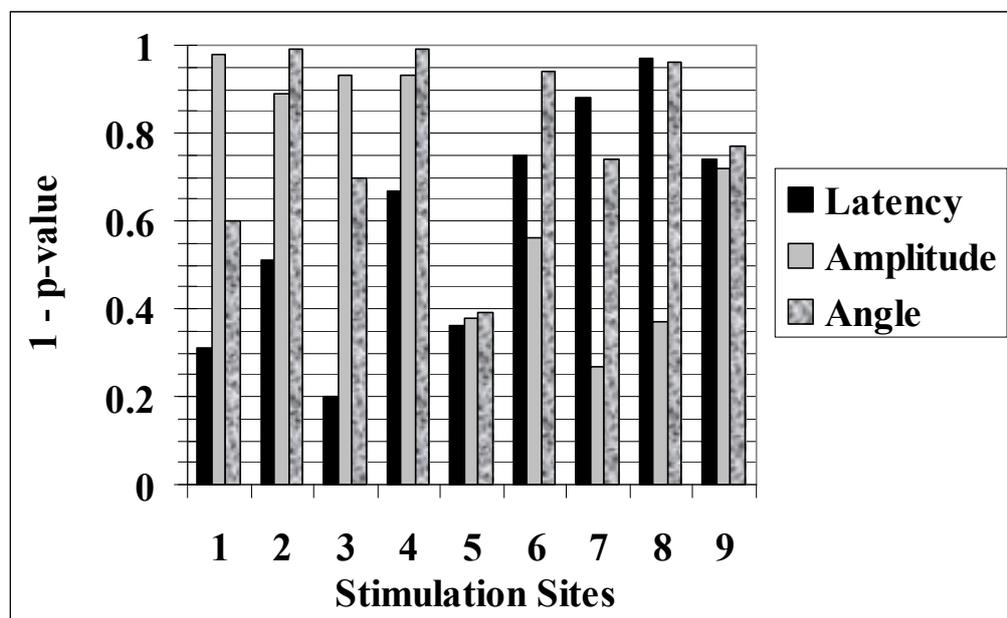


Figure A2.1: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 1. Black bars show latency, uniform grey bars show amplitude error, and mottled grey bars show angular error. The effects of TMS are displayed in terms of significant differences (1 - p-value) for the comparison of the median latency or error for real and sham TMS trials at that site, therefore a higher value on the y-axis indicates a more significant effect of TMS.

Participant 1: Left Hemisphere

The graph below (Figure A2.2) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine

stimulation sites in participant 1. Site number 7 was chosen as the test site, since the difference between real and sham TMS was found to be significant for two of the 3 saccade metrics: amplitude and angular error. Site number 4 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

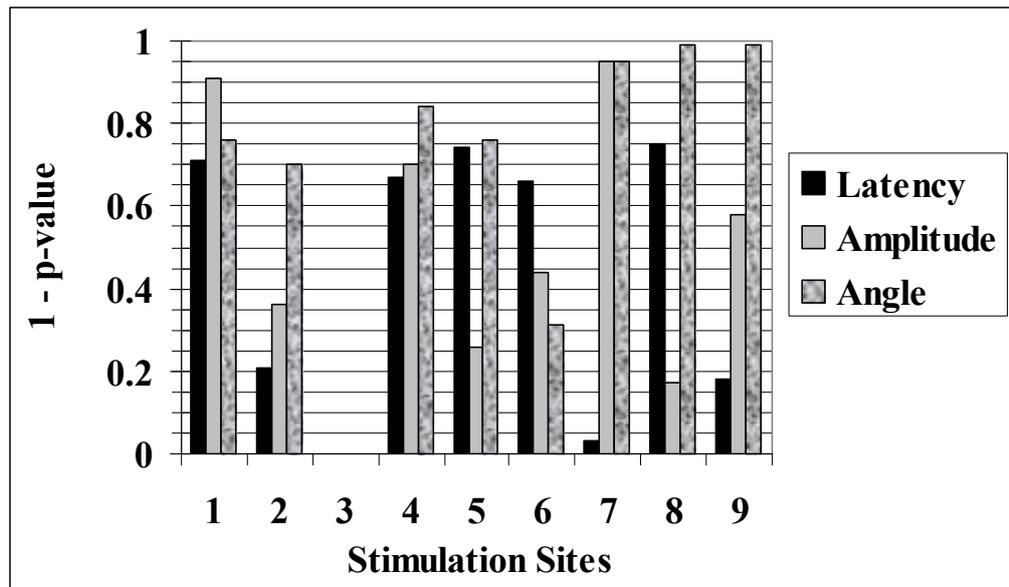


Figure A2.2: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 1.

Participant 2: Right Hemisphere

The graph below (Figure A2.3) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 2. Site number 8 was chosen as the test site, since the difference between real and sham TMS was found to be significant for latency. Site number 7 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

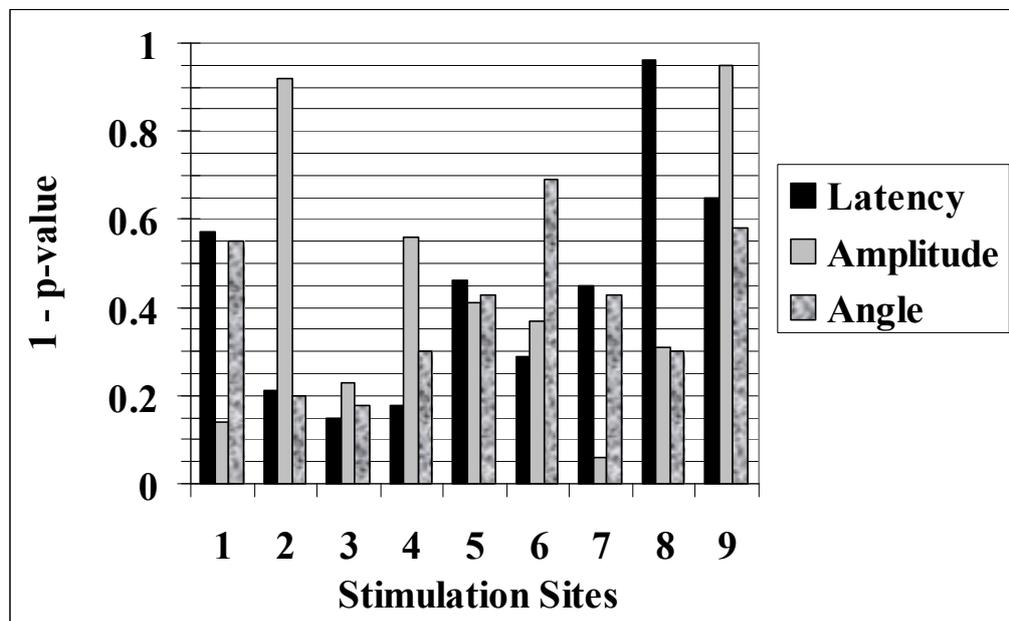


Figure A2.3: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 2.

Participant 2: Left Hemisphere

The graph below (Figure A2.4) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 2. Site number 6 was chosen as the test site, since the difference between real and sham TMS was found to be significant for amplitude error. Site number 4 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

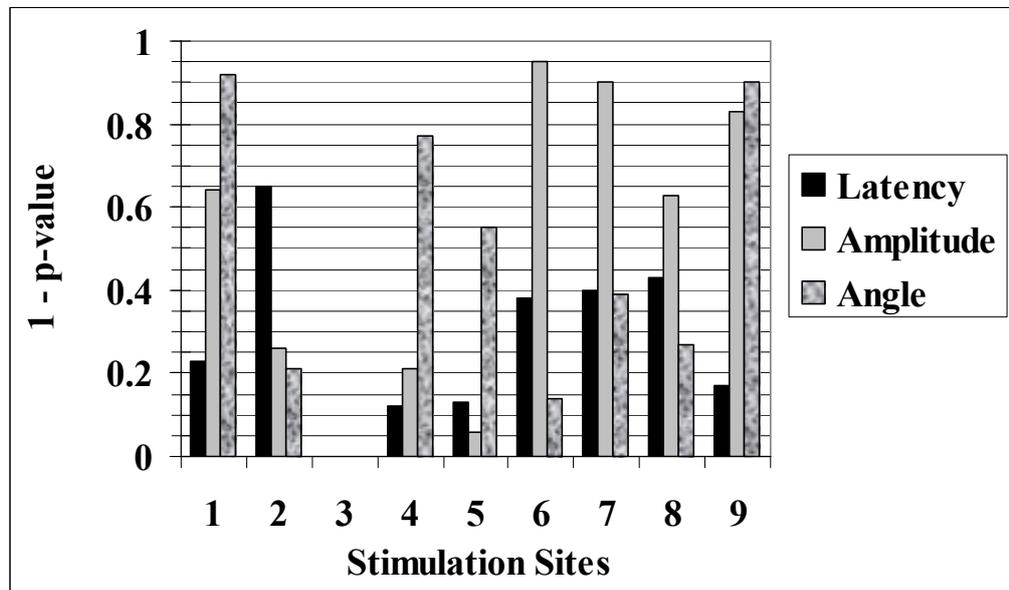


Figure A2.4: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 2.

Participant 3: Right Hemisphere

The graph below (Figure A2.5) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 3. Site number 1 was chosen as the test site, since the difference between real and sham TMS was found to be significant for latency. Site number 8 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

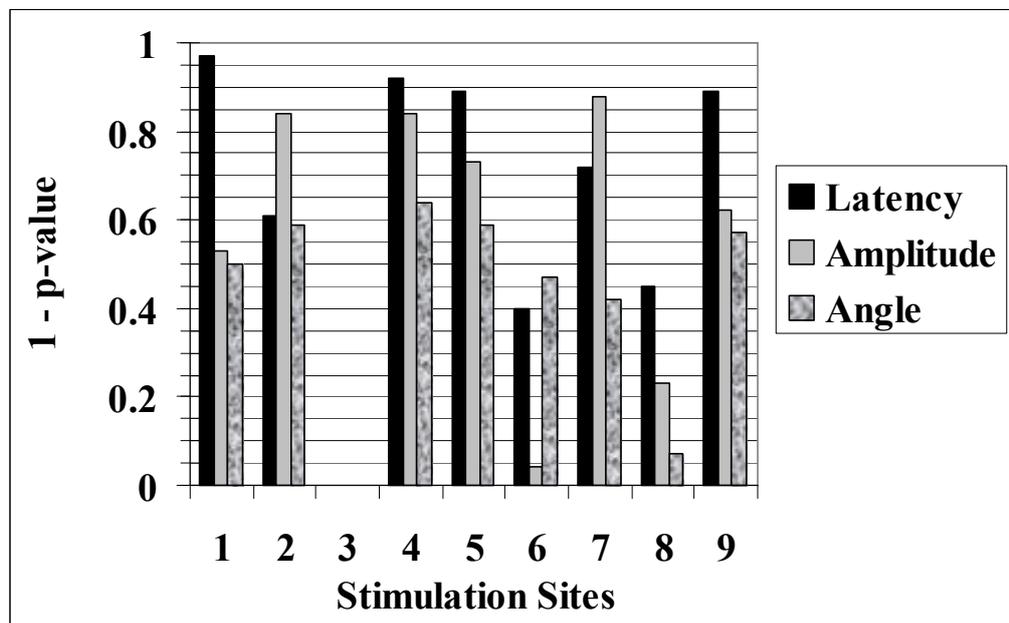


Figure A2.5: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 3.

Participant 3: Left Hemisphere

The graph below (Figure A2.6) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 3. Site number 1 was chosen as the test site, since the difference between real and sham TMS was found to be approaching significance for angular error ($p = 0.08$). Site number 9 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

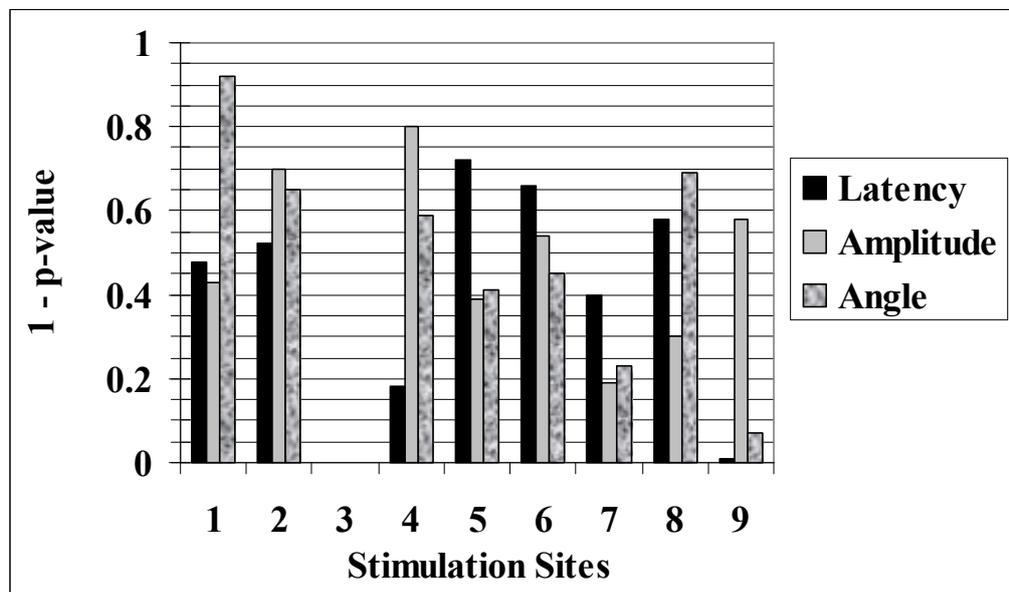


Figure A2.6: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 3.

Participant 4: Right Hemisphere

The graph below (Figure A2.7) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 4. Site number 3 was chosen as the test site, since the difference between real and sham TMS was found to be significant for latency. Site number 1 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

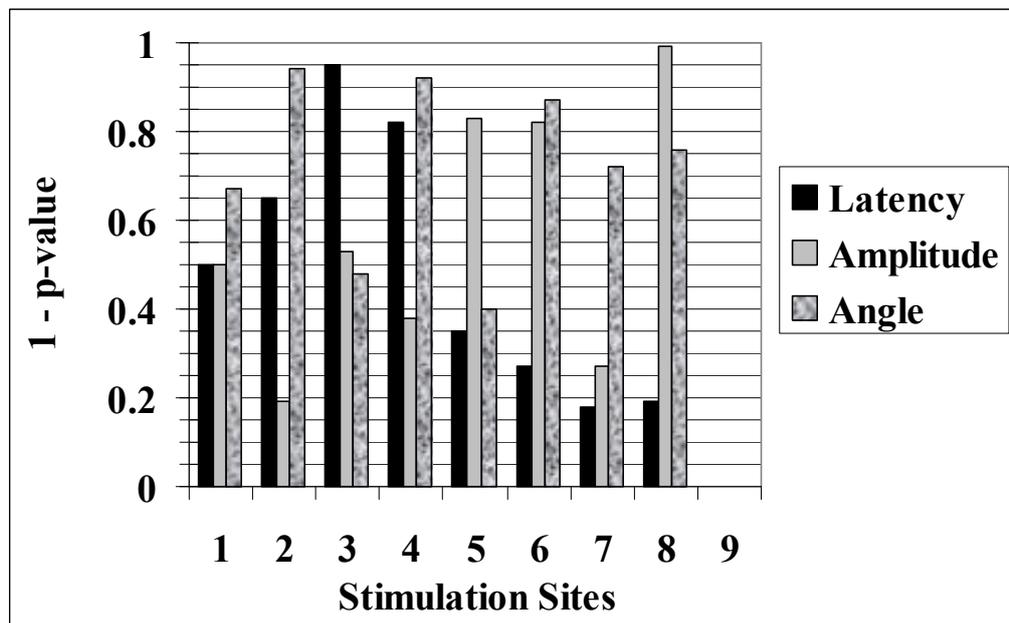


Figure A2.7: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 4.

Participant 4: Left Hemisphere

The graph below (Figure A2.8) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 4. Site number 3 was chosen as the test site, since the difference between real and sham TMS was found to be the closest to significance for angular error at this site ($p=0.098$). Site number 4 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

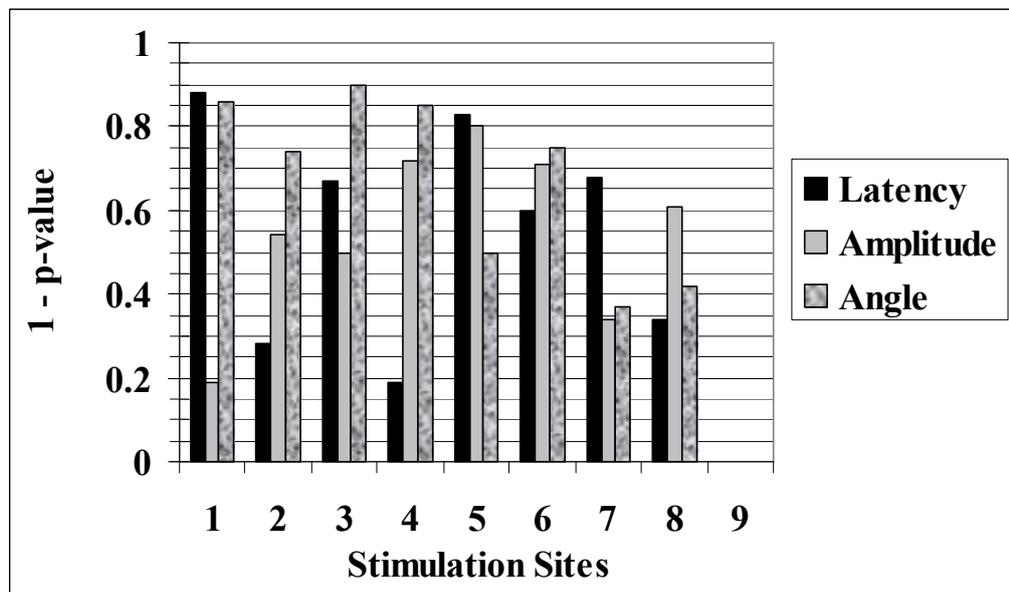


Figure A2.8: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 4.

Participant 5: Right Hemisphere

The graph below (Figure A2.9) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 5. Site number 5 was chosen as the test site, since the difference between real and sham TMS was found to be significant for two of the 3 saccade metrics: latency and amplitude error. Site number 4 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

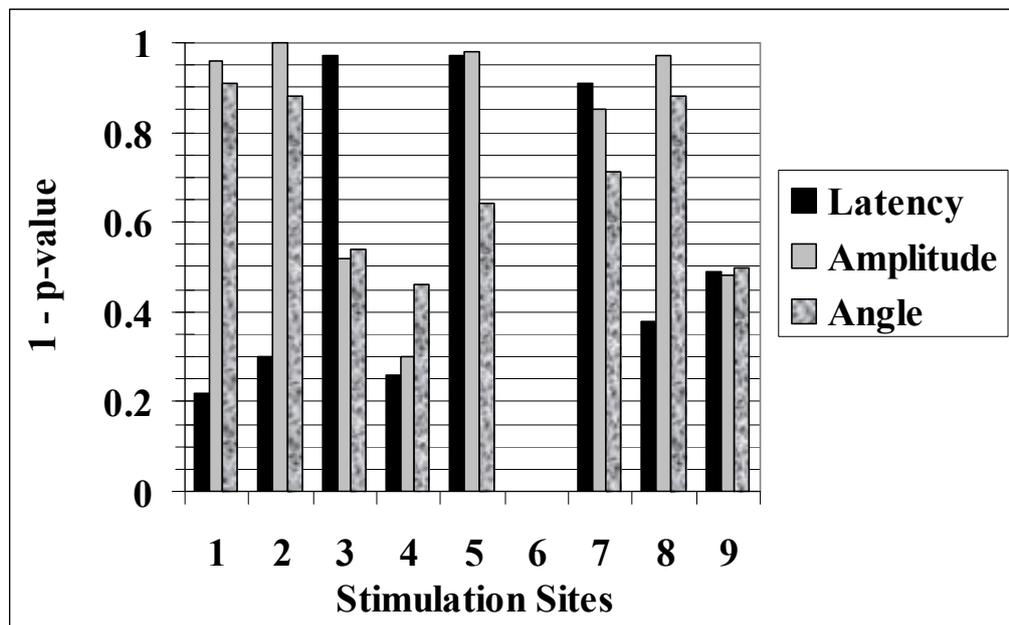


Figure A2.9: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 5.

Participant 5: Left Hemisphere

The graph below (Figure A2.10) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 5. Site number 1 was chosen as the test site, since the difference between real and sham TMS was found to be significant for two of the 3 saccade metrics: amplitude and angular error. Site number 5 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

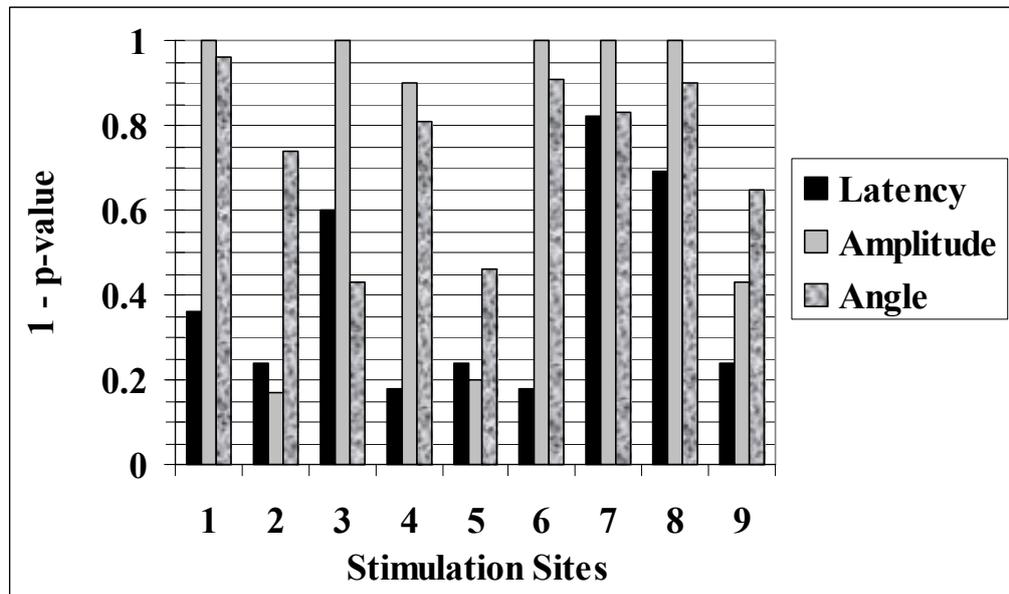


Figure A2.10: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 5.

Participant 6: Right Hemisphere

The graph below (Figure A2.11) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 6. Site number 1 was chosen as the test site, since the difference between real and sham TMS was found to be significant for amplitude error. Site number 6 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

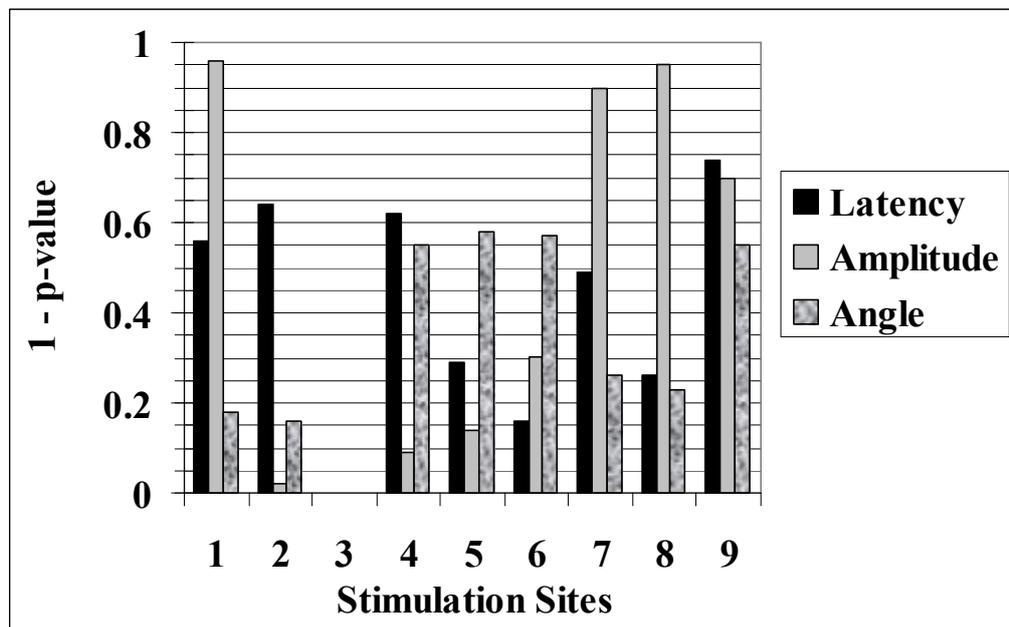


Figure A.211: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 6.

Participant 6: Left Hemisphere

The graph below (Figure A2.12) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 6. Site number 1 was chosen as the test site, since the difference between real and sham TMS was found to be the closest to significance for angular error at this site ($p = 0.13$). Site number 6 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

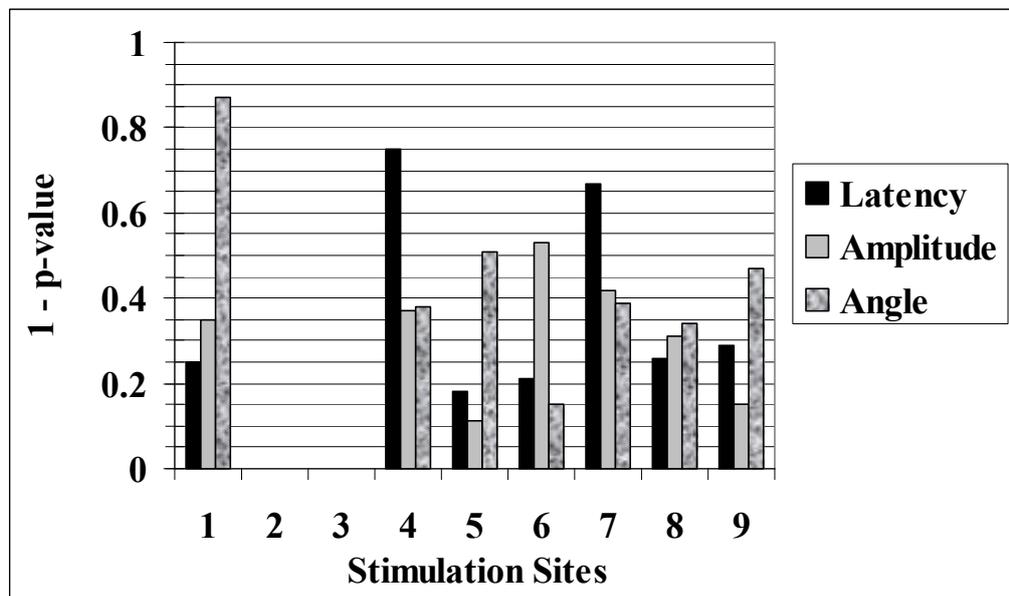


Figure A2.12: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 6.

Participant 7: Right Hemisphere

The graph below (Figure A2.13) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 7. Site number 7 was chosen as the test site, since the difference between real and sham TMS was found to be significant for two of the 3 saccade metrics: latency and amplitude error. Site number 6 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

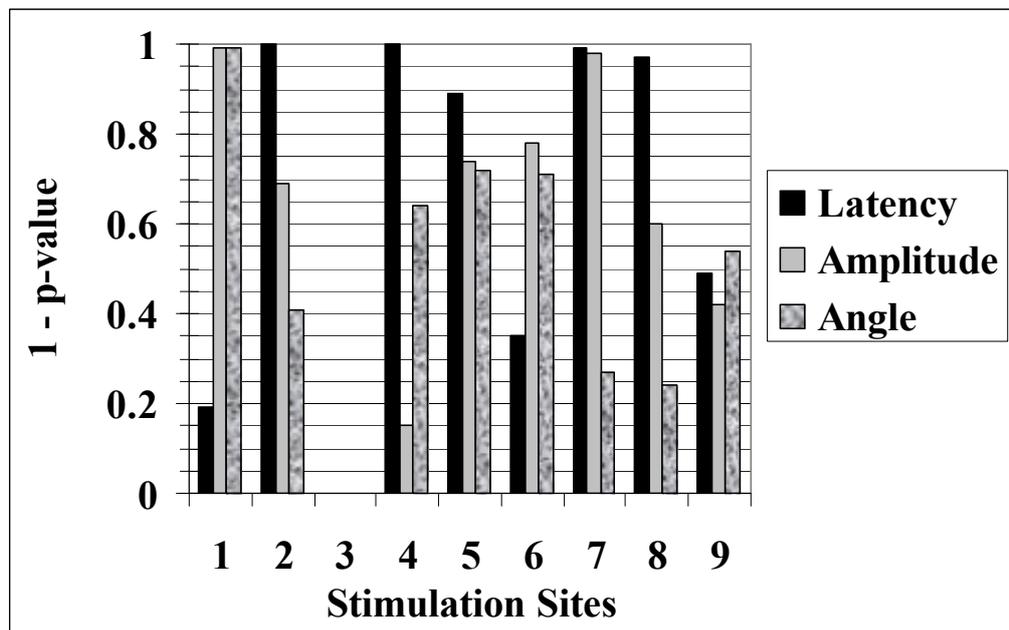


Figure A2.13: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 7.

Participant 7: Left Hemisphere

The graph below (Figure A2.14) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 7. Site number 1 was chosen as the test site, since the difference between real and sham TMS was found to be significant for latency. Site number 6 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

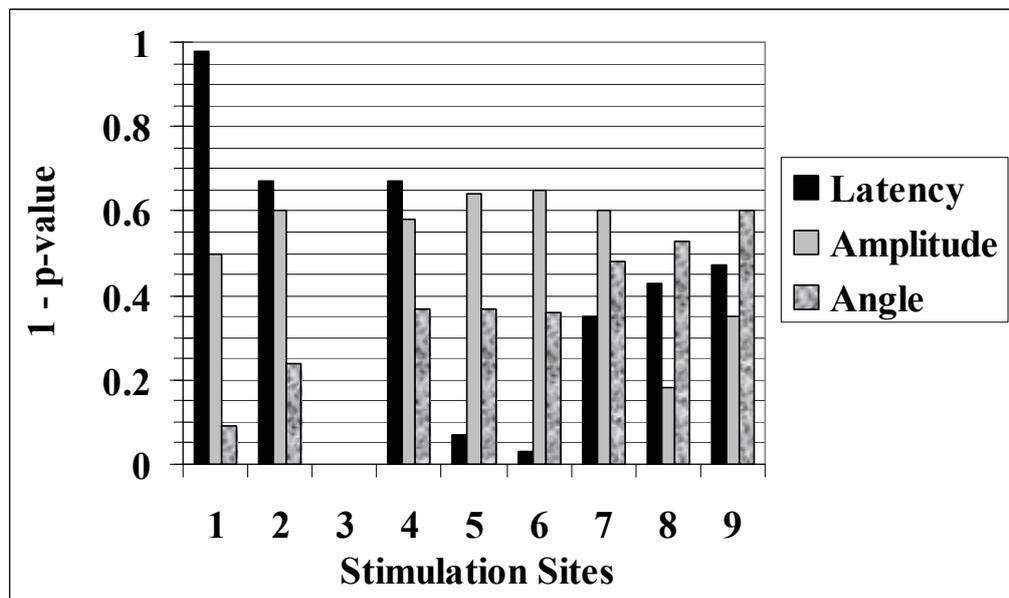


Figure A2.14: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 7.

Participant 8: Right Hemisphere

The graph below (Figure A2.15) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 8. Site number 7 was chosen as the test site, since the difference between real and sham TMS was found to be the closest to significance for amplitude error at this site ($p=0.13$). Site number 5 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

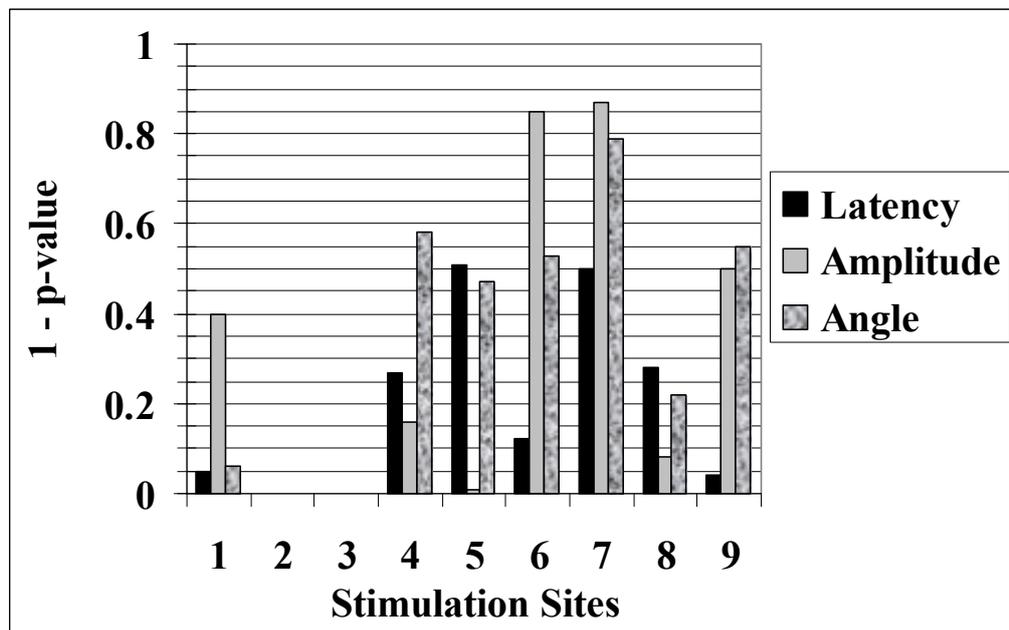


Figure A2.15: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 8.

Participant 8: Left Hemisphere

The graph below (Figure A2.16) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 8. Site number 9 was chosen as the test site, since the difference between real and sham TMS was found to be closest to significance for latency at this site ($p=0.1$). Site number 2 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

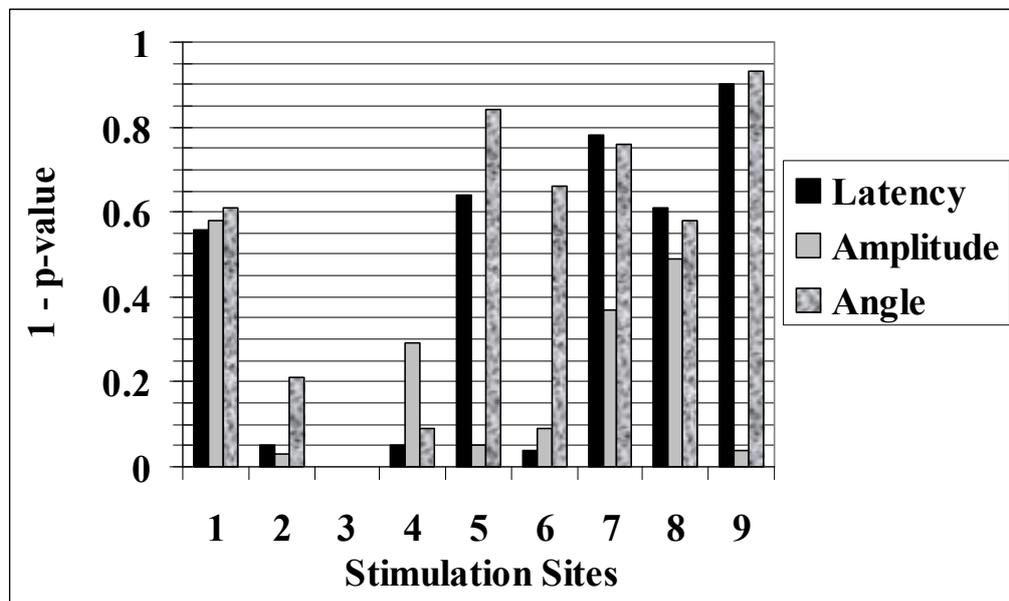


Figure A2.16: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 8.

Participant 9: Right Hemisphere

The graph below (Figure A2.17) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 9. Site number 1 was chosen as the test site, since the difference between real and sham TMS was found to be significant for amplitude error. Site number 5 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

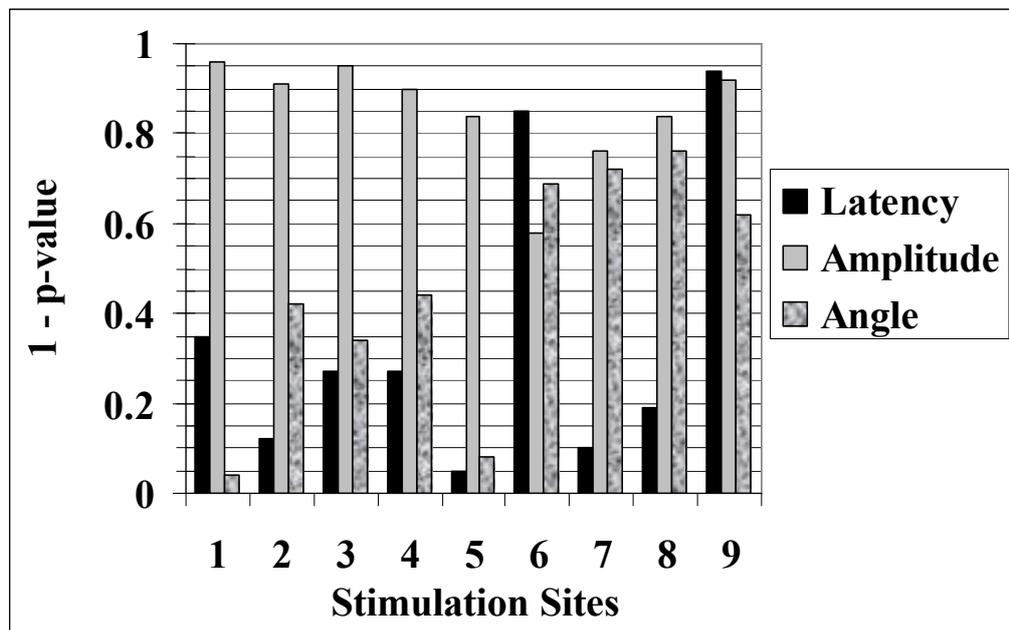


Figure A2.17: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 9.

Participant 9: Left Hemisphere

The graph below (Figure A2.18) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 9. Site number 2 was chosen as the test site, since the difference between real and sham TMS was found to be significant for amplitude error. Site number 6 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

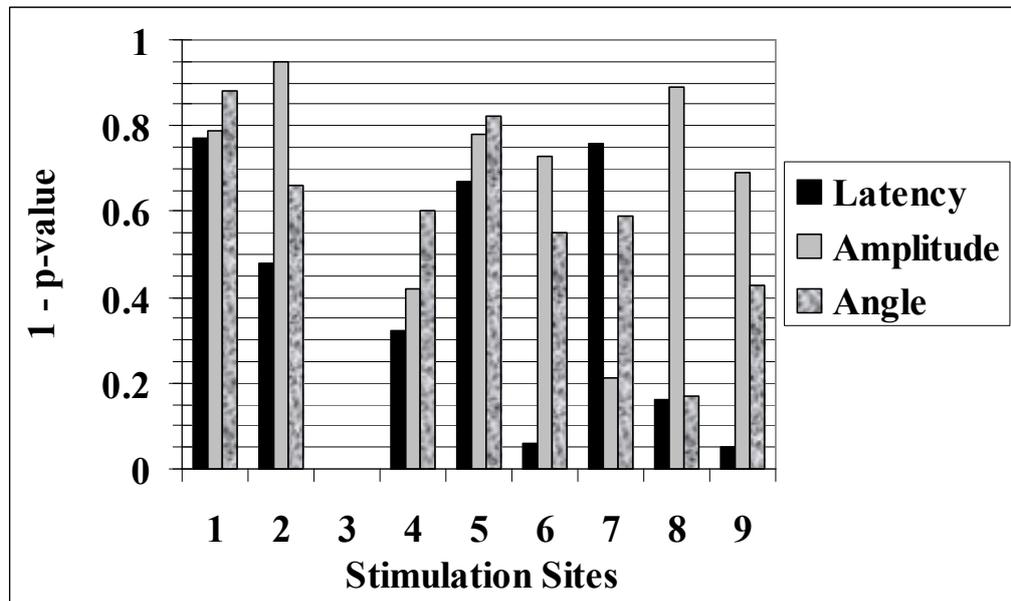


Figure A2.18: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 9.

Participant 10: Right Hemisphere

The graph below (Figure A2.19) illustrates the effects of right-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 10. Site number 4 was chosen as the test site, since the difference between real and sham TMS was found to be significant for amplitude error. Site number 9 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

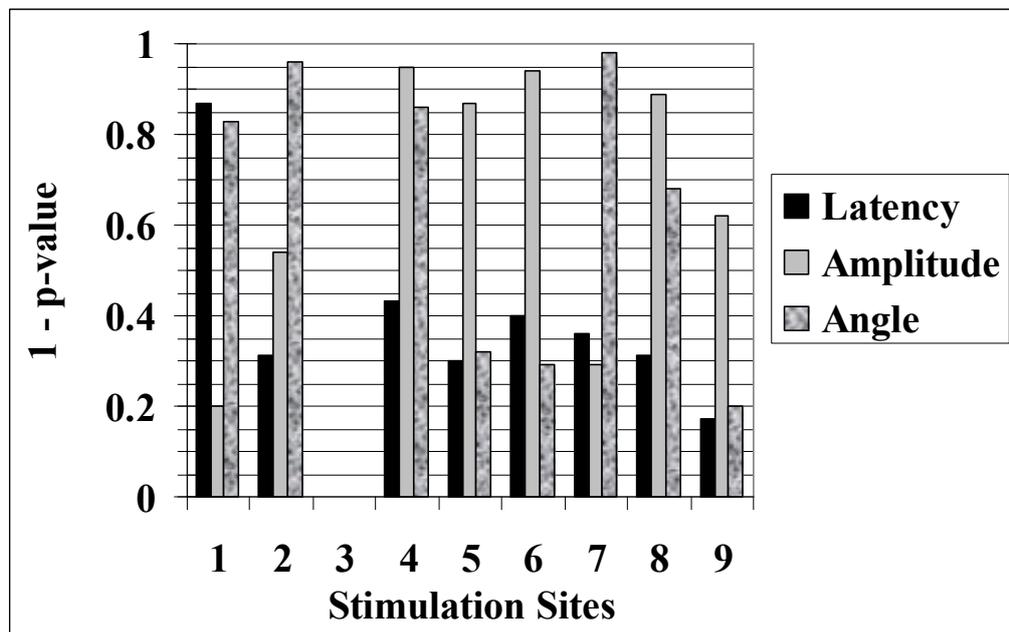


Figure A2.19: Effects of right hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 10.

Participant 10: Left Hemisphere

The graph below (Figure A2.20) illustrates the effects of left-hemisphere TMS on latency, amplitude error and angular error for each of the nine stimulation sites in participant 10. Site number 2 was chosen as the test site, since the difference between real and sham TMS was found to be significant for amplitude error. Site number 1 was chosen as the control site, since no significant differences were found between real and sham TMS for any of the saccade metrics at this site.

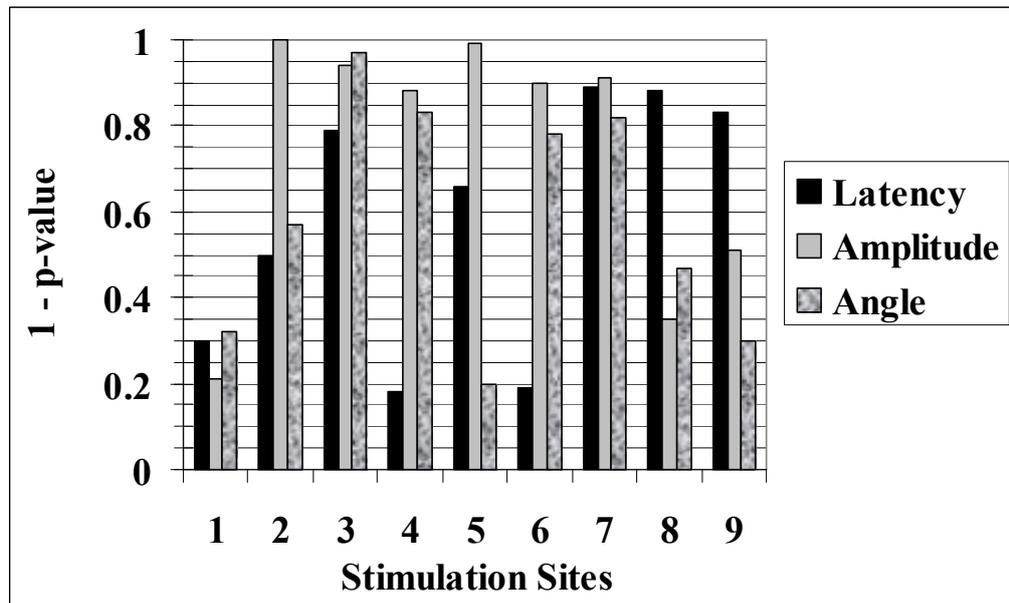


Figure A2.20: Effects of left hemisphere TMS to each of the nine stimulation sites on the metrics of a single reflexive saccade for participant 10.

**Individual variation in the location of the parietal eye fields: A
TMS study**

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Keywords: posterior parietal cortex; transcranial magnetic stimulation [TMS];
individual variability; saccadic eye movements

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Abstract

Transcranial magnetic stimulation (TMS) is a popular technique that can be used to investigate the functional role of specific cortical areas with reference to a particular behavioural task. Single-cell recording studies performed in non-human primates have demonstrated that a region of the parietal lobe known as the lateral intraparietal area (LIP) is specialized in the planning and control of saccadic eye movements. The homologue of this area in humans is termed the parietal eye fields (PEF) and its role in relation to saccades has previously been examined using TMS. In this paper individual variability in the functional effect of parietal TMS on the latency, amplitude and angular direction of visually-guided saccades has been assessed.

By examining individual variability in the spatial distribution of scalp-based localization and brain surface anatomy and stereotaxic localizations of the PEF it was shown that the distances between the sites determined by these three methods were not negligible, which raises problems regarding the most reliable anatomical localization technique to use. An assessment of the effect of TMS on saccade metrics (latency, amplitude error and angular error) at a grid of locations over parietal cortex demonstrated a large amount of intra-individual variability in the site where TMS had most affected saccades leading to the conclusion that there is individual variability in the functional effects of parietal TMS on saccade planning and execution. This study confirms the idea that it may be problematic to use a fixed scalp location for every participant in a study. It may in fact be more appropriate to determine TMS sites functionally on an individual basis if possible. This finding may guide further studies using TMS and saccade planning in order to optimize their capability to investigate this area and to draw meaningful biological conclusions.

Introduction

The posterior parietal cortex (PPC) is thought to play an important role in the representation of corporeal and peripersonal space and in the sensorimotor transformations associated with goal-directed movements (Andersen et al. 1997; Rizzolatti et al. 1997; Jackson 2001). Moreover, it has

also been implicated in the allocation of visuo-spatial attention, patients with posterior parietal lesions have for example been shown to be disrupted in their ability to shift attention (Posner et al. 1984). Another function performed by the PPC is the integration of information from multiple sensory modalities in order to build up a multimodal representation of the relationship between our body and the world around us. This is necessary for the accurate planning of oculomotor movements, a process also thought to involve this area (Andersen et al. 1997; Colby and Goldberg 1999). This multimodal spatial representation is continually updated to take account of such oculomotor movements, thereby maintaining spatial constancy despite the constant shifts of gaze that we perform (Ross et al. 2001)

Within a specific region of the PPC, termed the ‘parietal eye field’ (PEF) salient stimuli have been shown to be coded in coordinate frames relative to the centre of gaze (Colby and Duhamel 1996). In non-human primates, this area is located in the inferior parietal lobe (IPL), on the lateral bank of the intraparietal sulcus (IPS) and is thought to be specialised for the spatial processing essential to the planning of saccadic eye movements (Andersen and Gnadt 1989; Andersen et al. 1992; Andersen et al. 1997). Evidence to support this can be drawn from single-cell recording studies such as that by Duhamel, Colby and Goldberg (1992a) who showed that an eye movement that brings a previously flashed visual stimulus into the receptive field of an LIP (lateral intraparietal area) neuron, will cause this neuron to fire even though the stimulus is no longer present at the end of the eye movement. Inactivation studies in monkeys provide additional support for the importance of LIP in planning saccades in eye-centred coordinates (Snyder et al. 1997). Li, Mazzoni and Andersen (1999), for example used muscimol injections to investigate the effects of a reversible inactivation of this area in macaques. An increased latency to targets in contralesional space was found for both visual and memory-guided saccades. Memory-guided saccades to contralesional space were also found to be hypometric, whereas for the visually guided saccades this metric was not affected.

The identification of a potential homologue of this area in humans has been attempted through the use of neuroimaging techniques, in particular fMRI (e.g. Heide et al. 2001; Sereno et al. 2001; e.g. Medendorp et al. 2003;

Merriam et al. 2003). Transcranial magnetic stimulation (TMS) has also been used to functionally investigate the existence of a human homologue of area LIP in humans. For instance there have now been a number of studies that have evaluated the effects of applying TMS to the PPC during the planning or execution of saccadic eye movements. Furthermore the majority of these studies have made use of analogous tasks to those used previously in monkey electrophysiology research to study the functional properties of area LIP (e.g. Merriam et al. 2003). The following studies have demonstrated an impairment of saccadic latency following parietal TMS (Elkington et al. 1992; Terao et al. 1998; Muri et al. 2000; Kapoula et al. 2001; Yang and Kapoula 2004) similarly studies by Oyachi and Ohtsuka (1995), Müri et al (1996) and van Donkelaar and Müri (2002) have shown that saccade accuracy can also be affected by parietal TMS using memory-guided saccade tasks, reflexive saccades and anti-saccades.

While such studies have proven interesting in terms of furthering our understanding of parietal involvement in saccade planning and control, there are a number of problems associated with the use of TMS to investigate this function. For instance, a number of TMS studies have centred TMS stimulation at the P3 and P4 sites of the international 10-20 electrode system, (e.g. Elkington et al. 1992; Müri et al. 1996; Muri et al. 2000; Kapoula et al. 2001; van Donkelaar and Müri 2002; Yang and Kapoula 2004). The locations of P3 and P4 can be determined in relation to landmarks on the scalp such as the vertex (e.g. van Donkelaar and Müri 2002), which is itself found using the nasion-inion line and the line between the preauricular points. Coil placement made on the basis of such bony landmarks may lead to problems in terms of the brain region targeted by TMS (Pascual-Leone et al. 1999) and does not allow for potential intraparticipant variability in either the anatomical location of the IPS in relation to the scalp, or, in the functionally effective site of stimulation. The use of digital co-registration to aid coil-positioning allows for individual differences in brain size and anatomy by employing each participant's magnetic resonance imaging (MRI) scan to determine scalp location. Nevertheless, this technique still fails to take into account the functional significance of a cortical area in relation to task demands (Pascual-Leone et al. 1999).

An alternative to using a small number of fixed scalp locations, e.g. P3 and P4, is to systematically sample across a number of parietal locations. For example, Oyachi & Ohtsuka (1995) were able to identify, using a grid of stimulation sites and coregistration with 3D MRI, the most effective site of stimulation for a memory-guided saccade task. This site was taken as the one that produced the greatest decrease in saccadic accuracy; however, the existence of individual differences in the location of this site were not reported. Likewise Ashbridge, Walsh & Cowey (1997) also used a ‘hunting’ paradigm for determining coil position on a visual search task. The behavioural effects of TMS to an initial scalp location are assessed, and this is then repeated as necessary at adjacent locations until either a ‘hot spot’ is determined, or a certain threshold number of trials is reached without a site being found for that participant. However, these authors also fail to discuss the existence or extent of individual variability observed using this technique.

In order to evaluate potential individual variability the current study assesses both the spatial distribution of sites determined using three different TMS localization procedures: EEG scalp locations, brain surface anatomy and stereotaxic coordinates, and also the potential existence of functional variability between participants. This is done through the use of a grid of stimulation sites, covering both left and right parietal cortices, similar to those used by Oyachi and Ohtsuka (1995) and Terao et al (1998), and the effect of TMS on three saccade metrics: latency, amplitude and angular accuracy, are considered.

Methods

Study 1: Comparing Localization Techniques

Participants

9 healthy adults (6 females, mean age: 25.44 years) underwent a procedure to compare sites determined by different localization techniques.

Procedure

This study compared within participant variability in the spatial distribution of scalp locations on the right hemisphere corresponding to the PEF as determined by three alternative localization procedures. The scalp location of the right hemisphere EEG site (P4 in the 10-20 international electrode system), based on that used in previous studies was defined as the spot 3cm lateral and 3cm posterior to the vertex. This corresponds to parietal cortex, and was compared against sites found using two alternative procedures as follows. First, functional imaging studies (Luna et al. 1998; Heide et al. 2001; Sereno et al. 2001; Konen et al. 2004) suggest that the human homologue of PEF is located within or near to the intraparietal sulcus (IPS), although its precise location is still a matter of discussion. Therefore to examine individual variability in the spatial coordinates of the IPS, T1-weighted MRI scans were obtained, and the location of the IPS was defined visually for each participant based upon a comparison of the scan using MRICro (www.mricro.com) with a neuroanatomical atlas showing the outer surface of the cerebral hemisphere (Fig. 517 in Gray, 1918). The corresponding scalp location was then found using digital co-registration using MRIreg (<http://people.cas.sc.edu/rorden/MRIreg.html>) and Minibird (Ascension Technology Corporation) (Figure 1 a, blue circle). FMRI studies have been used to locate the likely position of the IPS; such studies provide Talairach coordinates for the location of the right IPS (Figure 1 a, orange circle) based on group data of the most active voxels in tasks believed to involve the PEF. Therefore Talairach coordinates were obtained from a recent article examining the function of the right IPS (Mort et al. 2003): X= 36, Y= -58, Z= 58. The scalp location associated with these coordinates was then found in individual participants by performing digital co-registration as above. The distances between the scalp locations in each participant based on these three techniques were then measured.

Results

The mean distance between the visually-defined location of the IPS and the Talairach coordinates was 10.1mm, between the visually-defined location

and P4 it was 22.4mm and between the Talairach coordinates and P4 this was 24.6mm.

Study 2: Functional Localization of PEF using Transcranial Magnetic Stimulation

Participants

Ten healthy, right-handed adults (6 females, mean age: 21.2) participated in the transcranial magnetic stimulation and eye-tracking task.

Experimental Procedure

Two grids of stimulation sites were marked on surgical hoods worn by the participants. The nasion, inion and pre-auricular points were first marked on the hoods, and lines were then drawn through these to locate the vertex. The grids were 4cm², and made up of 4 x 2cm² squares, with a centre at P3 (on the left) and P4 (on the right), i.e. 3cm lateral and 3cm posterior to the vertex (Figure 1 b). Nine points on each grid were used as stimulation sites, i.e. 3 on each row of the grid, each spaced 2cm apart.

A Magstim Rapid TMS machine (The Magstim Company Ltd) with a double 70mm coil was used to deliver TMS. During real stimulation the coil was placed flat and tangential to the scalp surface at each of the grid points; during sham TMS trials the coil was held perpendicular to the scalp with one end of the coil positioned at the centre of the grid on the hemisphere being tested. Thus although a magnetic field was no longer induced in the cortex the participants still heard the clicking sounds accompanying the magnetic pulse, and still felt the coil against their head. This procedure controls for the accessory cues provided by sensory inputs accompanying TMS, such as the click sounds, which may themselves affect saccadic reaction time (Terao et al. 1998); the contraction of muscles in the scalp, however, would not be felt during sham TMS. The wand was always held with the handle at the back of the head, so that the current would flow in a postero-anterior direction, which has been shown to be most effective for a Magstim Rapid coil (Kammer et al. 2001). Stimulation was set to 120% of the motor threshold determined for

each participant. The order in which participants received left and right hemisphere stimulation was counterbalanced across individuals. Blocks of sham TMS were completed at the start and end of each session. Experimental trials involving stimulation at each of the 9 sites within a hemisphere took place between the sham blocks. The order of stimulation for these sites was pseudo-randomly determined by computer. There were 18 blocks of real TMS, each consisting of 15 trials. Each sham block also contained 15 trials. Participants completed 300 trials in total. In all cases TMS was delivered before eye movement onset (see below).

Visual Display

The stimuli were displayed using a 20in Dell Trinitron Monitor with a spatial resolution of 800 x 600 pixels at a frame rate of 100Hz and a viewing distance of 55cm. Stimuli were generated using the MATLAB (The MathWorks) Psychophysics Toolbox (Brainard 1997; Pelli 1997). The stimuli consisted of a black central fixation cross and a single black target (3mm diameter), that could appear on the screen at a variable orientation between 0° and 360°, pseudo-randomly determined by computer, at an amplitude of around 90mm (based on a normal distribution with mean = 90mm and standard deviation = 5mm).

Oculomotor Task

Participants were required to make a single visually-guided reflexive eye movement towards the target. A beep was used to signify the start of each trial, at which point a black fixation cross appeared on the screen against a grey background. This remained on until the eye-tracker determined that the participant was correctly focusing on the fixation cross, i.e. the pupil was directed to a region of the screen within 15mm of its centre. Once this had been established a single black peripheral target was presented. Participants were instructed to execute a saccade to the peripheral target as soon as it was detected. 100ms after the appearance of the target a double-pulse of 25Hz TMS was delivered. The target remained on the screen for a total of 200ms, after which the screen went blank and the eye tracker continued to record for a

further 2 seconds. The trial then ended and the fixation cross reappeared for the start of the next trial.

Eye-Movement Recording

A pupil and dual first Purkinje image Video Eyetracker (Cambridge Research Systems) was used with a sampling frequency of 50Hz and an accuracy of 0.5-0.25 degrees of visual angle. The calibration involved using a built-in procedure in which 20 small white dots (0.25 deg arc) appeared on the screen one at a time at positions around a 5x4 grid scaled to 90% of the display size. The dots remained on for 500ms each and the accuracy of the participant in looking to each region of the screen was then assessed, this procedure was repeated if necessary until the participant had accurately foveated all of the positions on the grid. During the experimental session a video image of the eye could be seen by the experimenter on a separate computer screen, this made it possible to monitor the participants' position in the eye-tracker throughout the progress of the experiment. Participants viewed the stimuli binocularly, although only the left eye was tracked. An EyeLock headrest (Cambridge Research Systems) attached to the eye tracker was used to keep participants' heads in position, and this was placed on a Vision Science height-adjustable workbench (Cambridge Research Systems).

Data Analysis

Plots of eye movement traces using x and y coordinates from eye-position data recorded every 20ms were analysed. Trials showing artefacts in the eye movement trace, such as blinks were rejected. Three dependent variables were collected from the eye-movement data: latency, amplitude error, and angular error. The latency was defined as the time at which the absolute change in eye position from the start position (calculated as: $\sqrt{(\text{latest}(x)^2 + \text{latest}(y)^2)} - \sqrt{(\text{previous}(x)^2 + \text{previous}(y)^2)}$) exceeded a threshold of 25mm. The end-point of the saccade was determined using a similar algorithm; the participant was taken to be fixating when the change in eye position over two samples remained stable (i.e. <25mm). Coordinates for x and y eye position obtained from the eye tracker were converted to obtain the amplitude

and orientation of the end point and this was compared to the target position to obtain error data for these measures.

A bootstrapping resampling method with 5000 iterations was used to statistically assess the probability that the difference between the median for the sham condition and the medians for the TMS conditions at each of the sites were due to chance. This was done separately for the latency, amplitude error and angular error data.

Results

TMS

The frequency of sites on the left and the right hemisphere that showed a significant effect of TMS on each of three saccade metrics is shown in Figure 2. In total across all 10 participants statistical analyses revealed a significant effect when TMS was applied at 14 sites for latency (2 left hemisphere, 12 right hemisphere) (Figure 2 a), 23 sites for amplitude error (8 left, 15 right) (Figure 2 b) and 14 sites for angular error (6 left, 8 right) (Figure 2 c).

Figure 3 illustrates the differing effects of TMS compared to sham TMS at each of the 18 grid locations for a single participant. These plots are based on p-values. For latency (Figure 3 a) the positive p-values are represented by the lighter end of the scale, indicating a longer latency than for sham TMS. For amplitude error (Figure 3 b), the scale is the same with positive p-values indicating a longer, more hypermetric movement than for sham TMS. The angular errors of the saccades (Figure 3 c) were instead considered in terms of the absolute difference from the angle of the target, as it does not make theoretical sense to predict that TMS would result in errors that are specifically clockwise or anti-clockwise in direction; the difference in angular error for the TMS and sham TMS conditions increases as the scale progresses from dark to light.

Overall, therefore, a large number of the 18 TMS sites showed significant effects for each of the saccade metrics. However, specifically at which site TMS was found to most disrupt eye movements was not uniform across

participants. In fact, a large amount of individual variability in the effects of TMS at each site was apparent. Within individual participants no one site on the left or right hemisphere was consistently found to disrupt both latency and error (amplitude or angular).

Discussion

Previous research into the role of the parietal lobe in the planning and control of saccades supports the existence of a human homologue of area LIP, the primate 'parietal eye field'. By examining individual variability between the spatial distribution of scalp morphometric locations, brain surface anatomy and stereotaxic coordinates regarding the PEF it was shown that the distance between the sites determined by these three localization techniques had a maximum mean of around 2.5cm. The grid of sites used in this study covered a large area around P3 and P4 and thus the sites determined by these three procedures would be expected to have been covered by the functionally effective area of the TMS grid. This distance is however not negligible and raises problems regarding the most reliable anatomical localization technique to use.

An assessment of the effect of TMS on saccade metrics (latency, amplitude error and angular error) at a grid of locations over parietal cortex demonstrated a large amount of intra-individual variability in the site where TMS had most affected saccades.

Interestingly, no one parietal site stood out across participants as consistently demonstrating a significant effect of TMS on any of the saccade metrics. Within participants it was also not possible to select a single site that affected all three measures of saccade metrics.

In some participants no significant effects of TMS compared to sham were found at any site for one or more of the saccade metrics; a number of possible reasons could account for this. Firstly within the grid there were 2cm gaps between the stimulation sites used; although similar sized grids have been used by previous studies (e.g. Terao et al. 1998) there is some evidence to

suggest that the spatial resolution of TMS may be more focal than this, possibly as low as 0.5-1cm (Brasil-Neto et al. 1992). Using a grid with smaller distances between stimulation sites could potentially have revealed a site at which TMS was effective.

This study confirms the idea that it may be problematic to use a fixed scalp location for every participant in a study e.g. based on bony landmarks as with an EEG site. Given the individual variability demonstrated, using a set site based on bony landmarks for every participant in a study is unlikely to be the most effective method of determining a suitable TMS site. It may in fact be more appropriate to determine TMS sites functionally on an individual basis if possible.

Another important issue to consider when using TMS is the difficulty in knowing the exact area of cortex being targeted; the exact pathway taken by the current following cortical stimulation is not yet fully known. The activation induced by TMS in terms of neuroanatomy may vary across both the area stimulated as well as across participants (Pascual-Leone et al. 1999). The results of the current study demonstrate variability in the effect of TMS across participants when delivered to the parietal lobes. It is possible that TMS to other areas of association cortex, such as the prefrontal cortex would show a similar pattern of results; this could offer a potential explanation for inconsistent results in terms of the effectiveness of frontal TMS used clinically to treat depression (See e.g. Couturier 2005 for a review of such studies). The combination of neuropsychological tools such as functional imaging and TMS (e.g. Bestmann et al. 2004) may provide further insight into the resultant spread of activation and its associated cortical effects. This may eventually lead to a more clearly defined account of the function-anatomy relationship in this technique and prove useful in terms of optimal coil placement for investigating the functional significance of an area of cortex for a particular task.

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Figure Legends

1. a. Visually-defined ROI around the IPS (blue circle), and Talairach location (orange circle). b. Diagram of grid location on the scalp for the right hemisphere. c. Spatial location of grid on the brain in relation to the visually-defined IPS (blue circle) and Talairach coordinates for the IPS (orange circle).
2. Frequency of significant TMS sites for a. latency, b. amplitude and c. angular error collapsed across participants.
3. Effect of TMS over the two grids for 1 participant. a. latency, b. amplitude error c. angular error, *Top colour key* shows effect of TMS for latency (a) and amplitude error (b): positive p-values are represented by the *lighter end of the scale*, indicating a longer latency, or a more hypermetric movement, than for sham TMS. *Bottom colour key* shows effect of TMS for angular error (c): *lighter areas* show largest difference between TMS and sham TMS.

Figure 1.

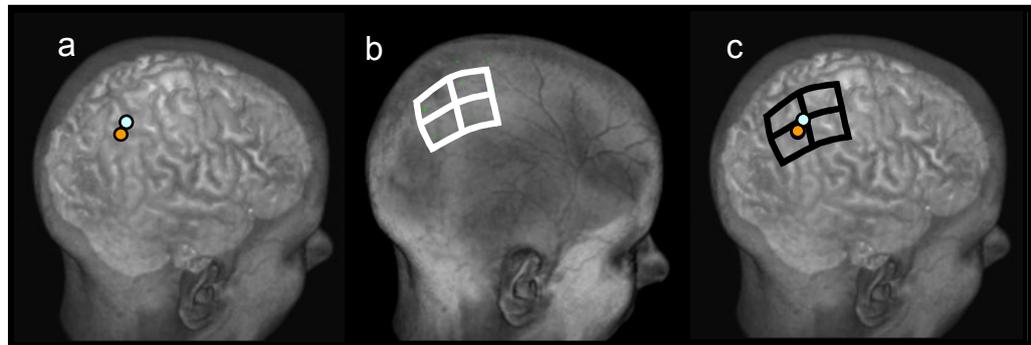


Figure 2.

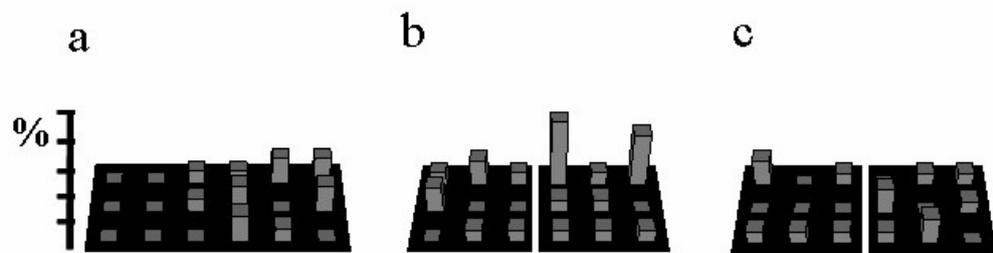


Figure 3.

