1.

LINKING BRAIN AND BEHAVIOUR IN MOTOR SEQUENCE LEARNING TASKS

by

Claudia Cristina Gonzalez

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The candidate confirms that the work submitted is his/her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Appendix A:

This work is referenced throughout the thesis in the general introduction (chapter 1), in the introduction and discussion of chapters 4, 5 and 6.

Title: ***The Brain uses Efference Copy Information to Optimize Spatial Memory***

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This work was jointly submitted to the Journal of Experimental Brain Research by Claudia Gonzalez (PhD student) and Dr Melanie Burke (supervisor and co-author). Contents include voluntary control of eye movements and the use of efference copy. My contributions included data collection as well as data analysis and manuscript preparation.

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Abstract

Sequence learning is a fundamental brain function that allows for the acquisition of a wide range of skills. Unlearned movements become faster and more accurate with repetition, due to a process called prediction. Predictive behaviour observed in the eye and hand compensates for the inherent temporal delays in the sensorimotor system and allows for the generation of motor actions prior to visual guidance. We investigated predictive behaviour and the brain areas associated with this processing in (i) the oculomotor system (Eye Only (EO): saccade vs. pursuit) and (ii) during eye and hand coordination (EH). Participants were asked to track a continuous moving target in predictable or random sequence conditions. EO and EH experiments were divided into 1) EO behavioural and 2) EO fMRI findings, and 3) EH behavioural and 4) EH fMRI findings. Results provide new insights into how individuals predict when learning a sequence of target movements, which is not limited to short-term memory capacities and that forms a link between shorter and longer-term motor skill learning. Furthermore, brain imaging results revealed distinct levels of activation within and between brain areas for repeated and randomized sequences that reflect the distinct timing threshold and adaptation levels needed for the two oculomotor systems. EH results revealed similar predictive behaviour in the eye and the hand, but also demonstrated enhanced coupling between the two motor systems during sequence learning. EH brain imaging findings have provided novel insights into the brain areas involved in coordination, and those areas more associated with sequence learning. Results show evidence of common predictive networks used for the eye and hand during learning.

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Abbreviation list

ANOVA	Analysis of variance
Abs Err	Absolute error
ACC	Anterior cingulate cortex
ANT	Anterior
BA	Brodmann's area
BG	Basal ganglia
BOLD	Blood oxygenation level dependent
CBM	Cerebellum
CEF	Cingulate eye fields
CV	Coefficient of variability
deg	Degrees of visual angle
DLPFC	Dorsolateral prefrontal cortex
EH	Eye and hand
EO	Eye only
FEF	Frontal eye fields
fMRI	Functional magnetic resonance
FP	Frontopolar
IC	Insular cortex
IFG	Inferior frontal gyrus
IPL	Inferior parietal lobe
IPS	Intraparietal sulcus
L	Left
LIP	Lateral intraparietal
LTD	Long-term depression
M1	Primary motor cortex
MFG	Middle frontal gyrus
MOC	Middle occipital cortex
ms	Milliseconds
MST	Middle superior temporal
MT	Middle temporal
MTG	Superior temporal gyrus
MTL	Middle temporal lobe
MTL	Middle-term memory
OPN	Omnipause neurons
PAcc	Peak acceleration
PCC	Posterior cingulate cortex
PCG	Precentral gyrus
PEF	Parietal eye fields
PFC	Prefrontal cortex
PHC	Parahippocampal cortex
PJerk	Peak jerk
PMA	Premotor area
POS	Posterior
PPC	Posterior parietal cortex
PRD	Predictive
PRDp	Predictive pursuit
PRDs	Predictive saccade
PS	Predictive saccade
PUR	Pursuit task
PV	Peak velocity
R	Right
RND	Random
RNDp	Random pursuit
RNDs	Random saccade
RS	Reactive saccade
RT	Reaction time
s	Seconds
SAC	Saccade task
SC	Superior colliculus
sd	Standard deviation
SEF	Supplementary eye fields
SEQ	Sequence
SFG	Superior frontal gyrus
SMA	Supplementary motor area
SMG	Supramarginal gyrus
SPL	Superior parietal lobe
SRT	Serial reaction time
STG	Superior temporal gyrus

t_{COR}	Time at max correlation
TH	Thalamus
TMS	Transcranial magnetic stimulation
t_{PUR}	Pursuit latency
t_{SAC}	Saccade latency
TTPV	Time to peak velocity
V1	Visual area 1
V2	Visual area 2
V5	Visual area 5
VLPFC	Ventrolateral prefrontal cortex
WM	Working memory

Chapter 1

1 General introduction

1.1 Motor sequence learning

Much of our daily activities involve learning new sequences of movements as well as executing learned behaviour (Lee & Quessy, 2003). Learning motor sequences is a fundamental brain function. Through brain plasticity mechanisms, we are able to adapt to novel environmental demands and carry out novel and more complex movements. Knowledge surrounding motor learning is hindered by the fact that learning itself cannot be measured, but rather, it is inferred from improvements in performance (i.e., observed behaviour in a specific situation and time) such as making faster and more accurate movements (Seidler et al., 2005). A major challenge of motor performance resides in the inherent delays in the sensorimotor system due to sensory feedback. The ability to generate an appropriate motor action that compensates for these motor processing delays can be achieved through prediction mechanisms. Prediction and pre-planning of a motor action requires the selection of task-appropriate sensorimotor signals together with the copy of the motor command (efference copy) to enhance the estimation of the sensory consequences of the motor actions (Shadmehr, Smith, & Krakauer, 2010). Predictive behaviour may occur as a consequence of an adaptive process following the repetition of a stimulus (Barnes & Schmid, 2002; Miyake, Onishi, & Pöppel, 2004). Indeed, the improvements in motor performance that occur with learning are usually associated with decreases in motor delays as a

consequence of predictive behaviour. During the early phases of motor learning, movements are unskilled and depend highly on attention and feedback. With practice, movements can become faster, more accurate and less reliant on feedback (Halsband & Lange, 2006).

Adaptive modifications of movement parameters through learning may vary according to the motor system (e.g., hand or eyes) and are also dependent on task demands. Much of the knowledge surrounding motor learning has been acquired through the use of serial reaction time (SRT) tasks, which has been extensively used to investigate behaviour and the links between behaviour, cognition and the biological processes involved in learning and memory (Robertson, 2007). These previous studies have often incorporated a choice reaction time task containing repeated sequences, in which participants eventually learn and exhibit prediction to each element of the sequence (Robertson, 2007). Typically, participants are required to respond with simple finger-press or finger-tapping movement and decreases in finger reaction times (RT) are the taken as evidence of learning. Thus the SRT task involves a series of connected events (sequence), which elicits temporal processing behaviour, higher order associations and prediction of the upcoming event (Keele, Ivry, Mayr, Hazeltine, & Heuer, 2003).

Brain imaging techniques have been used in a variety of experiments to identify the areas associated with the acquisition of a motor sequence (Doyon et al., 2009; Hikosaka, Takikawa, & Kawagoe, 2000; Orban et al., 2011). Regarding the functional neuroanatomy of motor learning, changes in behaviour are also paralleled by changes in the involved neural circuitry (Halsband & Lange, 2006). For instance, some studies have found decreases in cerebellar activation as the

task is learned, paralleled by an increase in basal ganglia activation and motor cortex areas including the primary motor cortex (M1) and supplementary motor area (SMA) (Doyon et al., 2002; Penhune & Doyon, 2002; van Mier, Tempel, Perlmutter, Raichle, & Petersen, 1998). Indeed, many imaging studies have found brain activation related to motor-skill learning in the motor cortex, cerebellum and the basal ganglia, however, more recent findings also suggest that differing networks of cortical and subcortical regions are preferentially activated during the early or late stages of learning (Penhune & Doyon, 2002; Ungerleider, Doyon, & Karni, 2002; van Mier et al., 1998). Even though the SRT task is easy to implement to investigate both behavioural and the neural circuits supporting learning there are a number of misunderstandings and questions surrounding the measures of improvements found in the motor performance. During SRT tasks, participants may exhibit a general ability to perform the task; however a more specific measure of learning the sequences may be acquired by contrasting sequential response times to response times from randomised presentations, thus minimizing the influences of fatigue, motivation and high expectancy (Robertson, 2007). Furthermore, an individual may be unaware (implicit) or aware (explicit) of learning a sequence, for which differing brain networks have been observed (Orban et al., 2010). A challenge of the SRT task has been to identify if and when a participant becomes aware of learning. Some have suggested that tests of implicit knowledge are not specific or sensitive enough to detect awareness and in addition, this assumes that awareness occurs in a moment in time, but it could be gradually acquired (Robertson, 2007). Another discrepancy surrounding SRT tasks is that although it is considered a motor learning task, it remains unclear whether learning is constrained within the motor domain. This can have

implications about how a study's findings are compared across experiments as one type of SRT task may involve a different kind of learning (e.g., perceptual) (see Willingham, 1999) and therefore explain differences in neural activity associated with that learning. Indeed, discrepancies between studies in the anatomical areas associated with SRT sequence learning are often described as being task-related differences. For example, the involvement of the lateral prefrontal cortex and the cerebellum in sequence learning remains controversial (Halsband & Lange, 2006; Miall & Jenkinson, 2005; Seidler et al., 2005). A transcranial magnetic stimulation (TMS) study by Robertson et al (2001) suggested that lesions to the PFC prevent learning of SRT sequences. However, this was apparent only when learning was based on spatial information (Robertson, Tormos, Maeda, & Pascual-Leone, 2001; Seidler et al., 2005). Similarly, in contrasts to the findings reported above, learning-related increases in activation of the cerebellum have been previously reported (Halsband & Lange, 2006). However, Miall and Jenkinson (2005) and others have found that the cerebellum might not be involved in actual learning per se, but that cerebellar activation is possibly related to error correction (Orban et al., 2011; Seidler et al., 2002). A study by Boyd and Weinstein (2004) found that cerebellar patients had intact learning of spatial but not temporal features, suggesting that only tasks which require a temporal component may elicit cerebellar activation during sequence learning (Penhune & Doyon, 2005). Even though SRT task studies have provided valuable insights into the neural correlates involved in learning, discrepancies of the brain areas associated with this learning exist between studies possibly due to the nature of the learning process being either implicit or explicit and/or either spatial or temporal (Halsband & Lange, 2006). More

importantly, these tasks may involve more abstract learning and abstract representations (i.e., insensitive to the form of input such as digits, verbal numbers, auditory, etc.) of sequence elements rather than motor learning per se (Orban et al., 2011; Philip, Wu, Donoghue, & Sanes, 2008). SRT tasks may mainly involve a temporal measure rather than an accuracy (spatial) measure for the "learning" and are often force-choice, meaning that some guesswork may also play a role. Furthermore, learning-based performance improvements are usually measured without assessing the effects of visual information on the eye and/or hand and their interaction (Philip et al., 2008) and thereby ignoring the differential effects this may have on the brain.

1.2 The oculomotor system

Eye movements play an important role in the way we navigate within our environment and often play a key role in the coordination of motor sequences. The study of the oculomotor system not only provides information regarding the control of action but it also enables the investigation of cognitive control and behaviour. Eye movements have been of particular interest in past research because they provide valuable information not only about the basic execution of movements but also, their interaction with cognitive processes in the brain such as planning and predicting. Eye movements are easier to interpret than complicated multi-joint limb movements and provide a wide range of parameters to describe their dynamics and control (Luna, Velanova, & Geier, 2008). In addition, current and readily available technology allows the precise and detailed measurement of eye movements through non-invasive sensitive eye tracking techniques. Oculomotor tasks are relatively easy to perform and therefore ideal

for investigating the brain mechanisms and motor control in healthy populations and certain patient populations (Luna et al., 2008; Rommelse, van der Stigchel, & Sergeant, 2008). These tasks may involve simple reflexive movements, which show basic aspects of attention as well as more complex movements that require high cognitive demands used to examine the interactions between brain and behaviour (Luna et al., 2008). Thus, investigating the neural and behavioural mechanisms of the oculomotor system is also ideal for identifying normal versus abnormal motor control and learning.

Generally, humans use two main types of eye movements when visually examining their environment: saccades and pursuit. Saccades are rapid eye movements (velocities of up to ~500 °/s) performed to bring an object of interest into the line of sight (Becker, 1989; Collins, Semroud, Orriols, & Doré-Mazars, 2008; Jin & Reeves, 2009). While saccades are quick and discrete movements, smooth pursuit eye movements are slow (< 100 °/s) and continuous eye movements that allow the tracking of moving objects and, unlike saccades, mainly use visual feedback to guide the movement and match the object's velocity (e.g., tracking a moving ball). When the velocity of the moving object can't be matched due to high speed, lack of attention or impairment in the system, saccadic movements are introduced as a means to "catch-up" (Boman & Hotson, 1992; Rashbass, 1961; Rommelse et al., 2008). There has been extensive research into saccadic and pursuit behaviour as well as the anatomical pathways involved in these movements.

1.2.1 Saccadic eye movements

Motor actions can be described as reactive or voluntary. The later usually involves making movements that are internally generated, for example, to remembered or imagined locations. Reflexive saccades are automatic and generally guided by vision with minimal cognitive control whereas, voluntary saccades are cognitively controlled and goal-directed. The voluntary control of saccades can be determined through successfully inhibiting reflexive responses, the ability to retain and process on-line information, and through the ability to voluntarily shift attention (Miyake et al., 2000). Thus, voluntary saccades are ideal for investigating cognitive control in planned behaviour.

When a saccade is made, the velocity is determined by burst neurons in the brainstem and is not voluntarily controlled (Collins et al., 2008; Sparks, Rohrer, & Zhang, 2000). In the oculomotor system, an object of interest in the visual field must be detected in the periphery in order to generate a response to orient that image on the fovea. Once the information is integrated (from retinal and extraretinal sources) a pre-programmed saccadic motor command is generated based on this information. If needed, corrections are made once the first saccade is completed (Lewis, Gaymard, & Tamargo, 1998). Thus, a premotor plan estimates the velocity of a saccade and it is usually directly related to the size of the movement (e.g., larger movements are consistent with higher peak speed) (Chen, Lin, Chen, Tsai, & Shih, 2002). Likewise, as saccade amplitude increases, so does the duration of the saccade in a linear fashion, which is known as the main sequence (Carpenter, 1988). Saccade latencies (i.e., the interval between the appearance of a target to the initiation or onset of a response) vary between 200 and 250 ms (Yang, Bucci, & Kapoula, 2002). Across studies, saccadic latencies vary

in accordance to the characteristics of the task. For instance, different latencies of the eye may be observed for closer versus further targets or for random versus predictable targets. In addition, it has been suggested that saccade latencies are affected by the disengagement of fixation, the shifting of visual attention to a new location, and the estimation of premotor parameters (e.g., velocity and duration of movement) (Yang et al., 2002). Saccades abruptly stop at the target of interest and correctly position the image along the line of sight. Inaccurate saccades occur when the generated motor response is not the appropriate magnitude, and/or direction to reach the target (Optican & Robinson, 1980). Previous studies have reported that adults frequently make measurable errors; generally undershooting the target in visually guided tasks and that saccade accuracy can also be influenced by the characteristics of the stimulus (e.g., size) and the task (Binsted, Chua, Helsen, & Elliott, 2001; Cohen & Ross, 1978).

Saccades are considered ballistic open loop movements and can be initiated in the absence of a visual stimulus (Burke & Barnes, 2006; Joiner & Shelhamer, 2006). Oculomotor tasks used to elicit predictive saccadic behaviour typically involve a stimulus alternating between fixed positions and fixed timings (single frequency) (Joiner & Shelhamer, 2006; McDowell, Dyckman, Austin, & Clementz, 2008; Ross & Ross, 1987). In these tasks individuals usually perform anticipatory saccades (i.e., prior to visual feedback) to the subsequent visual target. These predictive tasks promote learning and adaptive behaviour probably influenced by the on-line memory trace of the stimulus and/or motor signals generated from previous trials (McDowell et al., 2008). Predictive saccades display reduced amplitudes, reduced latencies and reduced peak velocities and are less accurate compared to visually guided saccades (Bronstein & Kennard,

1987; Smit, Van Gisbergen, & Cools, 1987). The dynamic properties of saccadic eye movements are not under voluntary control and are relatively stereotyped, usually described by the (linear) relationship between duration and amplitude (i.e., the main sequence, Carpenter, 1988) and the (logarithmic or exponential) relationship between peak velocity and amplitude (i.e., skewness) (Collins et al., 2008). Disorders in the saccadic system usually described as too slow or too fast, fall outside the normal peak velocity-amplitude relationship.

1.2.2 Pursuit eye movements

Smooth pursuit eye movements are made to stabilize a moving visual object onto the retina. Ideally, pursuit eye velocity will match the moving object's velocity with a pursuit gain (i.e., ratio between pursuit velocity and target velocity) of 1.0 and depends on whether the stimulus' movements are predictable or unpredictable (Lencer & Trillenberg, 2008; Tavassoli & Ringach, 2009). Humans can track targets up to 100°/s (Meyer, Lasker, & Robinson, 1985), however, optimal pursuit has been observed for target speeds between 15°/s and 30°/s (Lencer & Trillenberg, 2008). The pursuit system is said to be configured as a negative-feedback loop that attempts to minimize the differences between the desired velocity (i.e., target velocity) and actual eye velocity (error signal) (Tavassoli & Ringach, 2009). In addition, due to the pursuit system's dynamic properties, if the object of interest is stabilized on the fovea, the retinal stimulus signal (retinal-slip) that evokes a pursuit response is erased and thus, the object becomes fixated (Lencer & Trillenberg, 2008). There is evidence to suggest independence between the fixation and pursuit systems; however, the high degree of interaction needed for fixating moving targets suggests that these are

overlapping oculomotor systems with the goal of foveal image stabilization (Lencer & Trillenberg, 2008). Errors in pursuit such as positional errors due to decreased eye velocity are quickly corrected by catch-up saccades (Orban de Xivry, Bennett, Lefèvre, & Barnes, 2006). This also suggests close interactions between the pursuit and saccadic system, which humans typically use a combination of these oculomotor systems to obtain detailed information of their environment.

The parameters of smooth pursuit are not stereotypical as in saccades and are usually measured based on initiation and maintenance. Smooth pursuit in humans to a novel moving visual target has been found to take between 100 and 300 ms to initiate (Tavassoli & Ringach, 2009). From this point, the next 50 to 100 ms of pursuit initiation is not volitionally controlled but rather, driven by visual feedback input and therefore, pursuit gain initiation better reflects the use of visual motion information at this early time (Lencer & Trillenberg, 2008; Orban de Xivry et al., 2006). Maintenance of pursuit gain is therefore affected by pursuit initiation (Lencer & Trillenberg, 2008) and it is driven by a combination of visual feedback and the prediction of the target velocity (Barnes & Asselman, 1991; Bennett & Barnes, 2003). In addition, the maintenance of pursuit (once feedback is available) involves internal representations of the target's motion to update and enhance performance. After initiation of pursuit, eye movement reaches peak velocity (close to target velocity) and either oscillates or slightly declines. It is difficult to estimate the precise timing of pursuit initiation, however, it is usually measured using eye velocity traces and estimated as a point between target onset and peak velocity (Lencer & Trillenberg, 2008).

An important feature of the pursuit system is the processing delays between the onset of the retinal image and pursuit onset (feedback delays). To overcome this delay and avoid positional and velocity errors (i.e., decrease error signal) it is then necessary to predict the moving target's velocity. Unlike saccades, pursuit eye movements normally require visual feedback of the moving stimuli, which is why this system is suggested to rely on feedback to minimize the retina error signal (Kveraga, Fendrich, & Hughes, 2001). However, Kowler et al (1989) showed that high expectancy provided by symbolic cues of a salient moving target could elicit pursuit initiation prior to the onset of movement. Additional research has also shown that high expectancy and highly predictable stimuli can elicit pursuit eye movements prior to obtaining visual feedback (Barnes & Schmid, 2002). Predictive smooth pursuit has been investigated using sinusoidal oscillating targets and repeated constant velocity moving targets (see Barnes & Asselman, 1991; Barnes & Donelan, 1999). Predictive responses to repeated stimuli show the pursuit system's capabilities to exert some voluntary (internally generated) control, based on the stored information from previous experience (Barnes & Asselman, 1991). For example, it has been shown that when a moving target temporally disappears, eye velocity can be sustained for a short period (up to ~ 4 s) and velocity gain is again dependent on the expectancy of the target's reappearance (Bennett & Barnes, 2004, 2005; Collins & Barnes, 2005). The extraretinal guidance of pursuit (during the removal of retinal input) is thought to be driven by the internal storage of velocity information (Bennett & Barnes, 2004; Collins & Barnes, 2005). During repeated presentations of identical motion stimuli, predictive responses are initiated before target motion onset in which eye velocity during this early period is scaled to the upcoming target velocity (Barnes

& Asselman, 1991; Collins & Barnes, 2005; Kao & Morrow, 1994). This suggests that velocity information from prior stimulation was stored and subsequently used as an estimate of the upcoming motor response (Collins & Barnes, 2005; Ohashi & Barnes, 1996). The pursuit system is ideal for investigating motor learning since it is not normally possible to self-initiate pursuit, but prediction can be observed following the repetition of a stimulus (Barnes & Marsden, 2002).

There is still controversy underlying the contributions of reflexive and voluntary mechanisms for the control of pursuit (Bennett & Barnes, 2006). Burke and Barnes (2006) showed that pursuit latencies during random and predictive (repeated) stimuli presentations overlapped and suggested similar processing in the generation of pursuit under these conditions. A recent study by Bennett et al. (2010) examining the predictive mechanisms of ocular pursuit during occluded stimuli, suggested that predictive pursuit is controlled by both memorized and online predictions of target motion. This was shown by the participant's ability to use between trial and within trial information to adapt to unexpected target parameter changes. These findings suggest that the pursuit system is capable of quickly adapting to accommodate and use motion visual feedback to monitor and enhance performance (Burke & Barnes, 2006). This behaviour is different from saccadic eye movements since, once a saccade is made, it cannot be stopped at will (ballistic) and usually corrections are made after the movement is terminated (i.e., corrective saccades). Thus, for saccades, in contrast to pursuit, there are clear limitations in making on-line corrections during the movement.

Although the dynamics of saccadic and pursuit eye movements seem to differ, there has been increasing evidence to suggest that they share common

neural pathways (Erkelens, 2006; Leigh & Zee, 1991; Missal & Keller, 2002). In particular, there is increasing evidence showing the two oculomotor systems share common mechanisms to drive reactive and predictive behaviour (Burke & Barnes, 2006; Krauzlis, 2005; Nyffeler, Rivaud-Pechoux, Wattiez, & Gaymard, 2008). Burke and Barnes (2006 and 2008) investigated quantitative differences and anatomical areas involved in pursuit and saccade initiation using directly comparable predictable and random paradigms. They found that pursuit and saccades had similar temporal advantages and showed identical patterns of response to velocity/displacement changes during predictable trajectories compared to random conditions. Joiner and Shelhamer (2006) also implemented analogous manipulations of a stimulus to elicit predictive and reactive saccadic and pursuit eye movements. They suggested that saccadic and pursuit movement preparations are influenced by the same factors as the phase transition from reactive to predictive responses is similar for both eye movement types.

1.2.3 Brain areas involved in the voluntary control of eye movements

Past studies have focused their attention on the neurophysiological and behavioural differences between saccades and pursuit, thus inferring that these two types of eye movements were controlled through distinct neural mechanism (for review see Leigh & Zee, 1991). However, there is growing evidence about the existence of shared brain regions by the saccadic and pursuit system (Erkelens, 2006; Krauzlis, 2005). Indeed, the neural pathways for the control of pursuit and saccades are complex and involve a wide network of cortical and subcortical areas (Keller & Heinen, 1991; Leigh & Zee, 1991). Recent neurophysiological studies

have found overlapping pathways between initiation (Krauzlis & Miles, 1998), inhibition (Missal & Keller, 2002), coordination (Erkelens, 2006) and prediction (Burke & Barnes, 2006; Nyffeler et al., 2008). Missal and Krauzlis (2002) found that a group of brain stem neurons called omnipause neurons (OPN), considered to be a part of the saccadic system only, also decreased their firing during the onset of smooth pursuit. There is also evidence that releases from fixation are mediated by mechanisms that have shared inputs (Krauzlis & Miles, 1998). Neurons in the rostral superior colliculus (SC) modulate their activity during pursuit, saccades and during fixation (Krauzlis, 2003). A transcranial magnetic stimulation study by Nyffeler and colleagues (2008) found that stimulation over the supplementary eye field (SEF) affected the latency of predictive saccades during predictable pursuit. They also suggested that this SEF area was a higher order structure needed to process complex mechanisms such as prediction that were independent of the oculomotor system (Nyffeler et al., 2008). These previous studies have shown evidence of common neural structures between the saccadic and the pursuit systems, and also provide new perspectives in the functional organization of the oculomotor system. However, behavioural studies don't often reflect this shared processing due to the inherent differences in performance (e.g., latency differences). A recent study by Burke and Barnes (2008) investigated the brain areas involved in pursuit and saccadic tasks with equivalent temporal and spatial characteristics and found evidence that these shared brain regions between the saccadic and pursuit systems are utilized to varying degrees, depending on the task demands. In particular, they found differing regions in the prefrontal cortex strictly involved in position-dependent (saccade) responses and motion-dependent (pursuit) responses. Prior to this,

Petit et al. (1997) found dissociation of saccade-related and pursuit-related activation in the human frontal eye fields (FEF) and suggested the existence of subregions within FEF involved in the execution of mainly saccadic and pursuit eye movements. Together, these findings suggest that there is wide structural overlap between saccadic and pursuit eye movements with distinct neural populations for the control of the two subsystems. Indeed for saccades, cortical areas evaluate and update potential target locations and provide motor commands for the generation of an appropriate response and these include the lateral intraparietal (LIP), frontal eye fields (FEF) and the supplementary eye fields (SEF) (Krauzlis, 2005). In pursuit, cortical areas process visual motion information and other control signals (e.g., extraretinal) and involve the middle temporal (MT), middle superior temporal (MST), LIP, FEF and SEF (Krauzlis, 2005). Thus, many cortical areas are involved in the control of saccades and pursuit (Figure 1.1). In addition, many functional imaging studies support the existence of parallel but distinct cortical pathways (Krauzlis, 2005). Similar to the OPN in brain stem and neurons of the superior colliculus, which have shown to modulate their firing during saccadic and pursuit eye movements, there are areas in the cortical regions involved in the control of voluntary eye (saccade and pursuit) movements such as predictive responses.

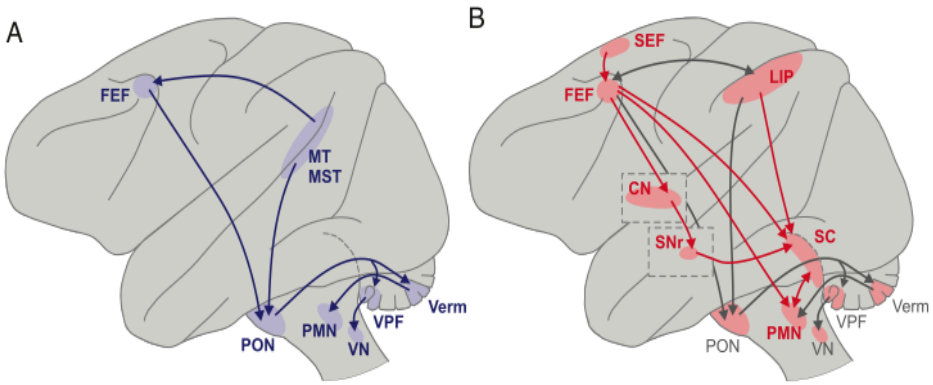

Figure 1.1 Anatomical areas (shaded areas) and their connections (arrows) for pursuit (A) and saccadic eye movements (B) from a non-human primate's brain (taken from Krauzlis 2004). Regions in dashed lines are deeper structures. CN= caudate nucleus, basal ganglia; FEF= frontal eye fields, SEF= supplementary eye fields, LIP=lateral intraparietal, MT=middle temporal, MST= middle superior temporal, PMN= brainstem premotor nuclei, SC= superior colliculus, PON= pontine nuclei (brain stem), VN=vestibular nuclei, VPN= ventral paraflocculus (cerebellum), Verm= oculomotor vermis (cerebellum).

Basal ganglia (BG)

The idea of parallel but distinct saccadic and pursuit pathways extends to circuits in the basal ganglia and thalamus (Krauzlis, 2004). The connections between FEF, caudate nucleus in the BG, and superior colliculus has been shown to modulate the triggering of saccades (Krauzlis, 2004). It has also been demonstrated that the caudate receives input from non-overlapping saccade-related and pursuit-related FEF pathways of equal strength (Cui, Yan, & Lynch, 2003; Krauzlis, 2004). The role of the caudate in saccades has been extensively studied with neurons showing activity that is time locked to voluntary (Cui et al., 2003) and memory guided saccades (Hikosaka et al., 2000). Cui et al (2003) found that the caudate nucleus may play an important role in the control of pursuit via feedback loops involving other areas of the basal ganglia and the thalamus. However, little is known about the caudate function during pursuit and hence this area warrants further investigation.

Cerebellum (CBM)

Krauzlis and Miles (1998) showed that electrical stimulation of the cerebellar vermis can produce either saccadic or pursuit eye movements, depending on the stimulation frequency and eye velocity. In particular, the cerebellum has been shown to be involved in the accuracy and adaptation of eye movements (Krauzlis, 2005; Robinson & Fuchs, 2001). Lesions of the cerebellum do not abolish eye movements but render them slow, variable and inaccurate (Robinson & Fuchs, 2001). Neurons in the cerebellum modulate their activity to reflect a push-pull arrangement that provides acceleration and braking signals (Krauzlis, 2005). In addition, the timing and size of the neural activation changes after the adaptations in eye movements occur. It is therefore clear that the cerebellum plays a key role in providing precise motor commands for both amplitude and timing adjustments in motor responses (Krauzlis, 2000).

In saccades, the brain must specify a motor command before the movement starts. Cerebellar activation has been shown to modify this command to maintain saccades as fast and accurate movements (Robinson & Fuchs, 2001). Lesions of the posterior medial cerebellum seem to abolish saccade adaptation and lesions of the oculomotor vermis have been shown to cause saccade dysmetrias (eye overshoot) (Robinson & Fuchs, 2001). The lateral nucleus of the cerebellum projects, via the thalamus, onto the saccade-related frontal eye fields (see Petit, Clark, Ingeholm, & Haxby, 1997) suggesting that this area may also be involved in the generation of saccades (Robinson & Fuchs, 2001). In pursuit, the cerebellum has specifically been implicated in the adaptation of gain to reduce visual slip (eye vs. target velocity error) (Robinson & Fuchs, 2001). Lesions to the

ventral paraflocculus have been shown to decrease steady-state (i.e., pursuit adaptation) smooth pursuit gain (Robinson & Fuchs, 2001).

Frontal Eye Fields (FEF)

The frontal eye filed area is a main cortical region involved in the execution of saccadic and pursuit eye movements. Neurons in FEF exhibit properties for determining voluntary oculomotor control (Krauzlis, 2005). In humans, the FEF is located at the junction of the precentral sulcus and the superior frontal sulcus (posterior part of Brodmann's area 8) (Müri & Nyffeler, 2008; Petit et al., 1997). Imaging studies, single cell recordings, lesion studies and microstimulation studies all have confirmed that there are saccade-related and pursuit-related subregions within the FEF (Petit et al., 1997; Rosano et al., 2002). Petit and colleagues (1997) found that the mean location of pursuit-related FEF in the human was inferior and lateral to the saccade-related FEF area. They also found smaller FEF activation in pursuit compared to saccade tasks. The primary inputs of the FEF involved in oculomotor control come from the MST area (in pursuit), the lateral intraparietal (LIP) (saccades), the supplementary eye field and principal sulcus region (Krauzlis, 2004). The FEF also projects to the deep layers of the superior colliculus (SC) and to neurons located in the brain stem (Pierrot-Deseilligny, Rivaud, Gaymard, Müri, & Vermersch, 1995). Lesions to the pursuit-related FEF area reduce pursuit acceleration and steady-state velocity and also seem to abolish predictive pursuit eye movements during periodic trajectory stimuli (Krauzlis, 2004; 2005). In saccades, latency variability has been related to FEF neuron firing rates suggesting that the FEF can regulate when and if a saccade is triggered (Krauzlis, 2005). However, the FEF has also been shown

to discriminate visual targets in the absence of a saccade or when a saccade is made to an alternate direction (e.g., anti-saccade task), suggesting that the FEF is more involved in the allocation of attention, rather that the preparation of a motor response (Krauzlis, 2005) . Indeed, FEF activation is involved in the disengagement of fixation and also plays a role in triggering voluntary saccades (Müri & Nyffeler, 2008).

Lateral Intraparietal (LIP)

The lateral intraparietal areas has also been associated with the control of both saccadic and pursuit eye movements. The LIP receives direct connections from the FEF, which suggest that the LIP also contains subregions dedicated to saccades and pursuit (Krauzlis, 2004). Indeed, stimulation to this area can evoke both types of eye movements (Krauzlis, 2004). In addition, neurons in the LIP exhibit activations that are directionally selective during pursuit and also show memory related activity in the absence of a stimulus (Krauzlis, 2004). Together with evidence of LIP neurons modulated by eye position and extraretinal signals, this suggests that this area may be involved in spatial representations (Krauzlis, 2004). Supporting this, studies have shown that the posterior parietal cortex (PPC) is an area that converges signals to generate spatial representations and coordinates for the generation of an appropriate motor response (e.g., of the head or hand), which are in turn affected by online attention and feedback (Colby & Goldberg, 1999). The non-human primate LIP area is suggested to be equivalent to the human parietal eye fields in the intraparietal sulcus (IPS) (Chao et al., 2011; Pierrot-Deseilligny, Müri, Rivaud-Pechoux, Gaymard, & Ploner, 2002). Lesion studies have shown that this area appears to be involved mainly in visually guided

saccades, with saccades exhibiting increased latencies (Pierrot-Deseilligny et al., 2002). Other studies have suggested that the posterior parietal cortex (PPC) plays an important role in spatial mapping, updating and shifting spatial attention and that the predictability of the stimulus can modulate the neural activity in this area (Chao et al., 2011). These findings may suggest that subregions of the parietal cortex may show distinct task-related specializations and these divergences have yet to be fully resolved.

Visual area 5 complex (V5+)

V5+ plays a key role in providing visual motion information that is necessary to guide pursuit eye movements and for adjusting saccadic eye movements to moving targets (Krauzlis, 2005). The primary visual cortex or striate cortex (V1) responds to signals from moving objects, but has a limited capacity in regards to its receptive field size (Lencer & Trillenberg, 2008). V1 projects onto the extrastriate V5+, which can be further divided into middle temporal (MT) and superior middle temporal (MST) areas according to non-human primate studies (Lencer & Trillenberg, 2008). In humans, these areas are found in the superior temporal sulcus (Brodmann's area 19) and in the occipito-temporal-parietal junction (Lencer & Trillenberg, 2008). Specifically, MT neurons are sensitive to the speed, acceleration and motion direction of a moving stimulus (Albright, 1984; Maunsell & van Essen, 1983). MT shows modulation based on attention and perception and exhibit selective winner-takes-all behaviour during competing stimuli (for review see Krauzlis, 2005). Neurons in this area are preferentially active during pursuit at a velocity of 30°/s (Lencer & Trillenberg, 2008). MST neurons are active when visually pursuing small targets and receive

vestibular input, thus they are able to encode moving stimuli in head-centred frames of reference (Lencer & Trillenberg, 2008). From V5, signals are sent to the frontal cortex, in particular, to the FEF where the motor command for pursuit initiation, maintenance and prediction is generated (Fukushima, Yamanobe, Shinmei, & Fukushima, 2002; Lencer & Trillenberg, 2008). In summary V5+ plays a key role not only in motion perception, but also in the control and maintenance of smooth pursuit.

Supplementary eye fields (SEF)

The supplementary eye fields are important for the control of eye movements. They have strong and reciprocal connections with the FEF and (in humans) are located in the posterior medial part of the superior frontal gyrus (Gaymard, Pierrot-Deseilligny, & Rivaud, 1990). Contrary to the FEF, the role of the SEF appears to be associated with internally driven actions, rather than external stimuli (Missal & Heinen, 2001). This is in accordance with human lesion and non-human primate neurophysiology findings indicating that the SEF is more important when more complex responses are required, specifically, when motor sequences need to be remembered and when a stimulus is predictable (McDowell et al., 2008). Magnetic stimulation studies have shown that the SEF appears to play a role during the learning phase of a sequence and before the generation of the sequence of saccades (Pierrot-Deseilligny et al., 2002). The SEF have also been shown to play a crucial role in supporting the generation of a saccade in the absence of a visual target (e.g., memory guided saccades), supporting the notion of the SEF's involvement in higher-order oculomotor processes involved in memory and learning (Missal & Heinen, 2004; Rosenthal, Hodgson, Husain, &

Kennard, 2008). During pursuit, SEF shows noticeable changes in activation during a target's directional changes, especially when those changes are predictable (Heinen & Liu, 1997). Indeed lesions to SEF have reported to increase latency to directional reversals in periodic pursuit (Gagnon, Paus, Grosbras, Pike, & O'Driscoll, 2006). In addition, microstimulation of SEF has been shown to increase pursuit acceleration during initiation and facilitates anticipatory pursuit (Missal & Heinen, 2001). Drew and van Donkelaar (2007) found that SEF mostly contributes to predictable changes in on-going pursuit, but that the FEF is more involved in maintaining pursuit or initiating pursuit to either predictable or unpredictable stimuli. Past research has focused on the role of the SEF as a higher order structure of saccadic and pursuit prediction (McDowell et al., 2008), however the role of the supplementary motor regions in motor sequence learning is still unclear.

Anterior Cingulate Cortex (ACC)

Oculomotor learning involves procedural changes from external or sensory driven to more internal volitionally driven circuitry (Müri & Nyffeler, 2008). Besides SEF other areas have been observed involved in the learning of saccadic sequences including the posterior parietal cortex (LIP and precuneus), frontal eye fields and the anterior cingulate cortex (ACC) (Heide et al., 2001; McDowell et al., 2008). The ACC has been implicated in self-initiation of saccades (without visual feedback), and this is supported by lesion studies (Gaymard et al., 1998). In addition, it was further observed that the rostral part of the ACC was more involved in sustained attention and on-line monitoring of performance during a saccadic sequence task (Heide et al., 2001). Similarly, studies

investigating prediction and learning in pursuit have found activation in SEF, FEF, ACC and parietal cortex (Ding, Powell, & Jiang, 2009; Lindner, Haarmeier, Erb, Grodd, & Thier, 2006; Schmid, Rees, Frith, & Barnes, 2001).

Dorsolateral Prefrontal Cortex (DLPFC)

Burke and Barnes (2008) analogous predictive and random saccadic and pursuit task was able to identify parallel neural activation responsible for predictive behaviour such as the SEF and the dorsolateral prefrontal cortex (DLPFC). The dorsolateral prefrontal cortex, located in the central sulcus, is not an ocular motor region per se, however it has been shown to play a critical part in cognitive control and it also generates actions based on internal goals (Miller & Cohen, 2001). It has been suggested that almost all intended behaviour is learnt and requires higher order processing to achieve a specific goal (Miller, 2000). The prefrontal cortex seems to be the centre for this processing (Miller, 2000). Notably, the DLPFC has been shown to play a key role in learning since its activation is linked to sensorimotor association (association between visual and motor commands) and working memory (Halsband & Lange, 2006).

Predictive pursuit responses decrease inherent delays in the system and minimize retinal slip, thus, these adaptations are a result of a learning process involving WM. Indeed, studies have found activation in the DLPFC associated with prediction and learning of pursuit eye movements (see Burke & Barnes, 2008; Schmid et al., 2001). Lesions to the DLPFC do not hinder pursuit eye movements, however, lesions to the right DLPFC have shown to impair pursuit of sinusoidal stimuli (Lekwuwa & Barnes, 1996). The DLPFC is said to be involved in saccadic control for spatial short-term memory and for inhibiting inappropriate reflexive

saccades (Müri & Nyffeler, 2008). Spatial working memory refers to those processes that support on-line visual-spatial information, for example, encoding the spatial location of a stimulus to later (after a delay) make an internally generated response to that stored location while the stimulus is no longer visible (Luna et al., 2008). Stimulation of the DLPFC has resulted in abnormalities in memory-saccade amplitudes during short (3s) and longer (30s) delays (Nyffeler et al., 2002). Another study found that stimulation of the right DLPFC prior to target onset has been shown to significantly increase anti-saccade task errors (Müri & Nyffeler, 2008). Inhibition of inappropriate saccades suggest that the DLPFC plays an important role in attention and also, activation has also been found during the preparation of a predictive saccade and in directional decision making of subsequent saccade movements (Burke & Barnes, 2011; Müri & Nyffeler, 2008; Pierrot-Deseilligny, Müri, Nyffeler, & Milea, 2005). Taken together, this area seems to be a higher-order structure for oculomotor learning associated with attention, decision-making and the use of short-term memory (range in seconds). Supporting this is the activation of DLPFC during SRT tasks and that this area plays a critical role in the short-term retention and manipulation of spatial information that is needed to learn a predictable sequence of actions (Robertson et al., 2001). Indeed a large body of electrophysiological and human neuroimaging studies have provided insight into the brain structures associated with learning a motor skill (Doyon et al., 2002; Hikosaka et al., 2002; Orban et al., 2010). However, it remains controversial whether these cerebral networks actually code for motor learning per se and truly reflect practice-driven modifications in brain representations during predictive sequence presentations (Orban et al., 2010). Another failing in previous research is that SRT tasks implement eye and hand

movements, but seldom investigate the interactions between the two motor systems and their individual contributions to learning.

1.3 Eye and hand coordination

Many skilled behaviours require coordination between the eye and the hand. Interactions between the eye and the hand have been extensively studied through visually guided pointing and reaching and usually involve saccadic eye movements or fixating a target of interest prior to making a simple limb movement tasks (see Binsted et al., 2001; Lewis et al., 1998; Wilmut, Wann, & Brown, 2006). Performing simple aiming tasks require accurate visual acquisition of the target, integration of proprioceptive signals, and the generation of a motor command to drive the hand towards the desired location. It is suggested that during these processes, internal representations of the limb, eye and visual target are generated and transformed into appropriate coordinate frames to accurately guide the response (Lewis et al., 1998; McIntyre, Stratta, Droulez, & Lacquaniti, 2000).

There is evidence that performance improves when the eye and the hand move together during coordinated movements (Maioli, Falciati, & Gianesini, 2007). Specifically, pursuit eye movements are more accurate and exhibit fewer catch-up saccades when accompanied by manual tracking of a sinusoidal moving target (Koken & Erkelens, 1992). In turn, hand movements have been found to be more accurate when eye movements follow the same spatial trajectory, suggesting that the oculomotor system assists in manual tracking (Miall & Jenkinson, 2005). It has also been found that making eye movements towards a target instead of maintaining fixation enhances motor performance of the hand

(Burke & Barnes, 2008). In particular improvements in hand accuracy have been found as a result of; 1) the eyes moving ahead, and guiding the limb to the target location (i.e. feedback), 2) the eye movement generating an efferent copy of the motor signal that is used as a calibration for subsequent trials (i.e. feedforward); and/or 3) increased attention to maintain the eye fixated on the target, which therefore impairs the generation of a hand response (Wilmut et al., 2006). As Goodale et al. (2006) suggested, these theories imply tight coupling between the hand and the eye. Previous studies have also looked at the effects of optical illusions on aiming tasks and found that the eye, but not the hand, was affected by the visual illusion with greater errors to the target location observed in the eye only (Binsted et al., 2001). Dissociations between the eye and hand may reflect the systems acting in an independent fashion (Binsted et al., 2001; Jin & Reeves, 2009). Indeed coupling between the eye and the hand has been shown to be task-dependent, but it is apparent that the oculomotor system contributes to optimal feedforward and/or feedback information to guide hand responses and that there are close interactions between the two systems. Supporting this, a recent study by Gonzalez and Burke (2012) investigating eye and hand coordination during memory guided GoGo and NoGo saccade/touch tasks found further evidence that making an eye movement to a target, rather than fixating, provided a motor advantage during the memory guided response (for full manuscript see Appendix A). In addition, a study by van Donkelaar and Staub (2000) investigating eye and hand coordination to visual and remembered targets showed that the timing of the hand relative to the onset of a saccade changed between conditions (closer onsets in memory guided tasks) and suggested that task-related effects have different processing effects on the timings of the coordinated movement. They

also suggested that certain aspects of coordination may be independently controlled, possibly by the cerebellum and basal ganglia, which are both involved in timing and sequencing and possibly interfere in the timing of coordinated eye movements.

Changes in the interactions between eye and hand during learning have been previously described and include findings of tight coupling between the eye and the hand, with strategies of looking ahead at a subsequent target prior to the hand response (Sailer, Flanagan, & Johansson, 2005). Taken together, these findings show that the signals made from eye movements are integrated into the planning of a hand response, and due to the tight coupling could possibly use a common neural network (van Donkelaar & Staub, 2000).

The coordination of the oculomotor and manual system has been extensively studied, however, the neurophysiological aspects of eye and hand coordination during motor sequence learning has not yet been fully described. The use of sophisticated eye-trackers and motion tracking devices mean we can record eye and hand movements simultaneously. Combing techniques in this manner has extended understanding of how we control and coordinate the eye and hand (Philip et al., 2008). A study by Engel and colleagues (2000) found that smooth pursuit and manual tracking were modulated in a similar manner, with each modality displaying similar reductions in speed during target directional changes. Likewise, Barnes and Marsden (2002) found predictive responses in the eye and hand when performing oculomanual tracking of a constant velocity moving target and suggested similar anticipatory mechanisms that control both. These arrays of behavioural findings suggest common neuronal networks are

used for both the eye and hand during prediction and learning, however support from neurophysiological and imaging studies is yet to be established.

1.3.1 Brain areas involved in eye and hand sequence learning

Eye and hand coordination is described as a skilful (learnt) integrated use of the eyes with the hand for generating precise movements (Boisseau, Scherzer, & Cohen, 2002). Cortical control of visually guided hand movements is believed to be distributed over the parietal and frontal brain regions. Preparatory activity for generating movements have been found to be controlled by a number of brain networks that include; the primary motor cortex (M1), premotor area (PMA), supplementary motor area (SMA) and the basal ganglia (Boisseau et al., 2002). Due to the brain's plasticity it is possible for an individual to acquire an unlimited number of complex motor actions. It is therefore clear that not only behavioural changes are observed with learning (faster and more accurate movements), but also alterations in the level and connectivity between brain networks in learning-related areas. Although there is no clear unanimity as to the areas involved in sequence learning, studies have found common areas associated with these processes and include; the primary cortex (M1), premotor and supplementary motor areas (PMA and SMA respectively), the putamen and the inferior parietal cortex (Seidler et al., 2005).

Dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC)

As previously mentioned, the prefrontal cortex is commonly reported during the initial stages of learning, when decision making, selection of movements and attention demands are required (Halsband & Lange, 2006). In

particular the DLPFC has been shown to be part of retrieval (right) and encoding (left) of stimulus/stimuli information (Halsband & Lange, 2006; Sakai et al., 1998). Activation of the cingulate cortex has also been observed together with prefrontal activation. Evidence suggests that the ACC receives input from the prefrontal cortex and plays a role in response selection and error processing in motor learning (Halsband & Lange, 2006).

Supplementary motor area (SMA) and premotor area (PMA)

Imaging studies as well as single cell recordings have shown that the SMA, located in the medial part of Brodmann's area 6, anterior to the primary motor cortex, plays an important role in the voluntary initiation of movements, sequence learning and bimanual coordination (Lee & Quessy, 2003). However, its role in learning sequential movements is not well understood. SMA activation has been observed in some studies in association with previously learnt sequences of movements when compared to new sequences, but this activation has not been consistent across studies (Lee & Quessy, 2003). These differences may be attributed to task-related differences and issues surrounding the SRT tasks (e.g., if the task involves implicit vs. explicit learning). However, lesion studies have supported the notion that the SMA is involved in tasks that require actions of a sequential nature, and may also be involved in the storage of these sequential movements that require precise timing (Halsband & Lange, 2006; van Mier et al., 1998). In addition to the SMA, the right premotor area has also been linked with the early acquisition of spatial information whilst the left PMA has been associated with the later stages of motor learning and the storage of the acquired skill (Halsband & Lange, 2006). Findings suggest that the premotor area is

bilaterally organized for the sensory cueing of movement and for motor learning (Halsband & Lange, 2006).

Posterior parietal cortex (PPC)

A number of studies support the increase of activation in the parietal cortex associated with motor learning, but findings indicate that distinct areas of the parietal cortex code for different functions (Halsband & Lange, 2006; Pammi et al., 2012; Sakai et al., 1998). These previous studies demonstrate the role of the inferior parietal cortex during motor learning can be attributed to the integration of sensory information from multiple modalities (e.g., visual or auditory) and feedback processing (Halsband & Lange, 2006). Activation in the IPS (Brodmann's area 40) is observed during performance monitoring when re-tracing a motor action is compared to the generation of a free trace, also indicating a role in feedback (Jueptner & Weiller, 1998). Conversely, the role of the superior parietal lobe (SPL) is related to spatial processing as seen by the increase of activation during spatially coded stimuli when compared to features such as colour (Halsband & Lange, 2006). The SPL is part of the dorsal visual system, carrying spatial information in the posterior part of the IPS, and is associated with saccadic eye movements and possibly related to the processing of eye and hand coordination (Sakai et al., 1998). Overall, parietal areas seem to be important for coding visuospatial information and for the translations of this information into limb-related information during motor learning.

Cerebellum

The cerebellum has also been implicated in sequence learning; however, recent research has suggested that the cerebellum participates in error correction and the formation of internal models to predict the sensory consequences of a motor action (Miall & Jenkinson, 2005). Additionally, it plays a key role in the coordination of eye and hand movements (Miall & Jenkinson, 2005; Penhune & Steele, 2012). It has been shown that cerebellar patients have difficulty using visual information to control guide their hand or arm and visually guided movements are therefore disrupted (Miall, Imamizu, & Miyauchi, 2000).

1.4 Purpose of study

The relative simplicity of recording behavioural outcomes of the oculomotor system makes it ideal for investigating the processes involved in motor learning, while at the same time adding to the knowledge of the neural substrates involved in oculomotor sequence learning. A further aim of investigation in this project is to identify areas that are specific or overlapping between the saccadic and pursuit oculomotor systems during learning. This will help our understanding of whether a single mechanism is the key to all visuomotor learning in the brain. For this purpose, we investigated activation in the brain and also recorded behaviour during analogous saccade and pursuit tasks that aimed to provide valuable information on how the two systems differ and/or interact. It has been shown that both oculomotor systems exhibit predictive behaviour to a known target, based on prior experience. However whether these eye movements exhibit similar learning during analogous tasks has not yet been fully determined. In addition, whether saccades and pursuit share

common predictive processing compared to reactive eye movements remains controversial and warrants further investigation.

Sequence learning is the outcome of a series of predictions made by the brain to each upcoming target when a set of stimuli is repeated and often requires the interaction of multiple motor systems. Simultaneous recordings of the eye and the hand during sequence learning tasks provide further knowledge of the behaviour and the brain areas involved in the coordination of eye and hand and how this interaction is affected with learning.

Another component of motor learning that has yet not been fully studied is the effect of cognitive load on the oculomotor system. Oculomotor studies have shown that this system has limitations and further repetitions of targets were needed to reach steady-state in pursuit (Burke & Barnes, 2007; Collins & Barnes, 2005). However, the cortical control of the oculomotor system during sequence learning and the limitations of the short-term storage process require further examination.

The primary aims of this PhD were to:

i) determine oculomotor adaptive behaviour (prediction) as well as identify brain areas involved in saccade and pursuit sequence learning implementing an analogous sequence task for each eye movement type (eye only experiment: EO),

ii) determine eye and hand coordination during sequence tasks, as well as characterize learning-related behaviours and the brain areas associated with this processing (eye and hand experiment: EH).

The secondary aims of this PhD were to:

iii) investigate limitations in the oculomotor and manual short-term memory by comparing sequence learning behaviour and brain areas involved during high versus low cognitive load, through the addition of components to the motor sequence tasks (in each eye only and eye and hand experiments).

1.5 Description of experiments

To achieve this, we performed 4 main experiments, which consisted of the analysis of 1) the behaviour and 2) brain areas involved in oculomotor sequence learning (EO); and 3) the behaviour and 4) brain areas involved in eye and hand sequence learning (EH). Similar to SRT tasks, our learning task consisted of predictable, repeated sequences in which performance improvements (e.g., decreased latency and better accuracy) were interpreted as evidence of learning a sequence, however, our task elicited both eye and hand movements. Also, unlike other previous studies using uni or bidirectional double-step and repeated discrete ramps (see Burke and Barnes, 2006; Collins and Barnes, 2005), we used a novel sequence task in which each element of a sequence was connected in a continuous movement using 4 possible directions, making this a more ecologically relevant situation of every day life such as sequences of movements to catch a ball or when driving a car. In addition, we implemented this task using equivalent stimuli for eliciting saccadic and pursuit eye movements as well as eye (pursuit) and hand coordinated responses. Given that much attention has been given to saccadic eye movements when studying eye and hand coordination (in visually guided reaching and pointing tasks), a pursuit sequence task was used for our eye and hand coordination experiments since in pursuit, a build up of predictive

behaviour needs to occur to show learning unlike saccades and hand movements, which can be initiated without visual feedback and without training. Improvements seen in the eye and hand experiment would then reflect pursuit learning and also skilled coordination between the eye and the hand. Overall, we presented our sequences in predictable (repeated) and random conditions to determine learning-related changes in behaviour and also compared short versus long sequences to investigate the limits in short-term memory. We also implemented these tasks using a fMRI compatible eye-tracker and an optical fibre joystick for eye and hand tracking to investigate brain activation (blood oxygen level dependent data) during our sequence tasks and identify brain areas related to sequence learning. The eye only and eye and hand experiments also describe brain activation associated with learning in saccades and pursuit and during eye and hand coordination.

1.6 Hypothesis

From previous evidence we hypothesized that:

i) in eye only experiments, participants would exhibit eye (saccades and pursuit) predictive responses as evidence of motor sequence learning during predictable stimuli presentations (PRD) compared to the random conditions (RND); and that if saccades and pursuit share a common predictive drive, then results would reveal similar learning and activation in parallel brain areas between the two types of eye movements,

similar to the network of DLPFC→ACC→FEF→SEF→IPL→BG. Finally we expected that eye movements would show predictive responses during short sequences, but would exhibit deterioration in prediction during the longer sequences, demonstrating a limit in the storage of sequence elements.

ii) in eye and hand experiments participants would also exhibit predictive eye and hand responses in predictive (PRD) sequence presentations compared to random (RND) presentations and that coupling of the eye and the hand would show differences between conditions. Specifically, we expected that during random sequences, hand movements would include visual feedback and show temporal delays compared to predictive responses. We expected that learning-related changes in the activation of brain areas would highlight areas such as the DLPFC→ACC→PPC→PMA→SMA→BG→ cerebellum. Finally, we expected a decrease in predictive eye and hand responses during the longer sequences compared to the shorter sequences.

Chapter 2

2 Sequence learning: Eye only behavioural experiment

2.1 Introduction

The preparation and/or execution of a motor command can be stored, and subsequently provide a behavioural advantage by avoiding processing time delays in the motor system. This predictive behaviour is a basic aspect of motor learning as it shows evidence of the ability to store information, such as the velocity and position of a known stimulus, and use this information to generate an appropriate response. Predictive behaviour is observed during motor learning and typically, serial reaction time tasks have shown procedural decreases in hand (finger-tap or finger-press) reaction times as a sequence is learnt (Robertson, 2007). Thus this predictive behaviour has been shown to be indicative of learning all the elements in a sequence (Visser, Raijmakers, & Molenaar, 2000).

Predictive oculomotor behaviour has also been shown to occur as a consequence of an adaptive process following the repetition of a stimulus (Barnes & Schmid, 2002; Miyake et al., 2004). There is evidence for predictive adaptations in the two main types of eye movements, saccades and pursuit, which can be initiated in the absence of a visual stimulus (Barnes & Donelan, 1999; Becker & Fuchs, 1985; Ross & Ross, 1987). When presented with targets that appear at known locations and frequencies, saccades occur prior to a target's appearance after only a few repetitions of the stimulus (Isotalo, Lasker, & Zee, 2005; Ross & Ross, 1987). Saccades of a predictive nature have shown to display reduced amplitudes and peak velocities and are less accurate than saccades made to

targets that are already present (Bronstein & Kennard, 1987; Smit et al., 1987). Studies have also found that smooth pursuit eye movements can be initiated prior to obtaining sensory feedback of a moving target after having actively or passively pursued the moving stimulus (Barnes & Donelan, 1999; Burke & Barnes, 2008; Kveraga et al., 2001; Ohashi & Barnes, 1996; Poliakoff, Collins, & Barnes, 2005). Pursuit responses initiated prior to obtaining stimulus motion feedback display a slow velocity build up that is often scaled to the velocity of a previously viewed moving target (Barnes et al., 2000).

Barnes and Donelan (1999) suggested that predictive pursuit eye movements to identical constant velocity ramps are controlled by stored timing and velocity information and that these processes are carried out by separate mechanisms. Similarly, Joiner and Shelhamer (2006) also suggested that predictive saccades to alternating targets within a predictive frequency range are accurately mediated by an internal timing reference. Both oculomotor subsystems exhibit similar adaptive behaviour when following a stimulus with a predictable trajectory, which suggests that these adaptations may be mediated by a common predictive process between the two subsystems (see Nyffeler et al., 2008), however, they also exhibit important differences. Pursuit eye movements normally rely on error correction obtained from continuous stimulus feedback (Barnes & Asselman, 1991), whilst saccades are considered ballistic open loop movements and can be initiated in the absence of a visual stimulus (Burke & Barnes, 2006; Joiner & Shelhamer, 2006).

Evidence from electrophysiological and fMRI studies show the involvement of higher level predictive processing in the frontal lobe regions during both saccadic and pursuit eye movements (Fukushima et al., 2002; Müri & Nyffeler, 2008), but

also suggest that within these, separate sub-regions exist for saccade and pursuit eye movements (Burke & Barnes, 2006). Burke and Barnes (2006) investigated quantitative differences in pursuit and saccade initiation using comparable predictable and random double step and double step-ramp paradigms. Their results showed that both types of eye movements had similar temporal advantages during predictable trajectories compared to random conditions, however they exhibited differences in timing, with saccades initiated earlier in predictive conditions compared to pursuit responses. Burke and Barnes (2006) argued that the differences in motor delays between saccades and pursuit are a result of different threshold criteria of frontal eye fields (FEF) or additional memory requirements in dorsolateral prefrontal cortex (DLPFC) for each subsystem. Joiner and Shelhamer (2006) also implemented analogous manipulations of a stimulus to elicit predictive and reactive saccadic and pursuit eye movements using an alternating target. However, they suggested that saccadic and pursuit movement preparations are influenced by the same factors as the phase transition from reactive to predictive responses is similar for both eye movement types. Whether saccades and pursuit eye movements share a common predictive temporal shift compared to reactive eye movements and/or show similar adaptation levels remains controversial and warrants further investigation through analogous paradigms for saccadic and pursuit eye movements.

Barnes and Schmid (2002) developed a paradigm where participants had to perform pursuit eye movements to repeated 2 and 4 ramp motion sequences. Their results revealed that the sequence stimuli were quickly learned since predictive responses were observed to each ramp component after only a couple

of presentations. Collins and Barnes (2005), further investigated the ability to learn more complex sequences of 4 or 6 ramps. The experimental trials consisted of either uni-directional or bi-directional discrete ramp sequences along the horizontal axis and the stimulus could move at one of four speeds. Collins and Barnes (2005) found that motor learning and prediction were a function of the complexity of the sequences, and again, only a few repetitions were required to achieve motor predictive adaptation when pursuing these simple unidirectional sequences. However, increasing the number of directions and possible speeds resulted in participants' learning at a slower rate. Hence, increasing the cognitive demands of a sequence learning task has also shown decreases in predictive measures which may suggest possible limitations on short-term memory storage (Burke & Barnes, 2007). In summary, regardless of the complexity of the task, pursuit performance often reaches a plateau where prediction occurs quickly after prior experience, and then performance is maintained (Burke & Barnes, 2007).

Extending from previous research, the present study formulated a sequence learning paradigm, which requires the storage of target information to perform each elements in a sequence, similar to that implemented for pursuit eye movements previously (Barnes & Schmid, 2002; Burke & Barnes, 2007; Collins & Barnes, 2005). This stimulus information has been suggested to be stored in DLPFC, FEF and supplementary eye fields (SEF) and fed forward to trigger the initiation of pursuit for a short period until visual feedback of the moving target is acquired (Burke & Barnes, 2008). We investigated the effects of predictable conditions versus reactive conditions when tracking a continuously moving

stimulus along two dimensions using saccadic and pursuit eye movements. Similar to Burke and Barnes (2006) the experimental paradigm for saccadic and pursuit eye movements used directly comparable stimuli to assess if the same mechanism drives both types of eye movements' behaviour and/or show equivalent adaptation. However, our experiment consisted of sequences of continuous motion stimuli that were shown in either predictable conditions (i.e., same sequence repeated 4 consecutive times) or in random conditions (i.e., single presentation of new sequences). This task allowed us to investigate oculomotor learning adaptations to continuous sequences of movements and the ability to store a series of connected events, which better reflect real life motion tracking. In addition, this study aims to address the effects of learning adaptations when increasing the number of components in a sequence while keeping the number of repetitions and the target velocity constant.

From previous findings, we hypothesized i) that participants would exhibit predictive behaviour during repeated presentations of a sequence compared to random presentations; ii) that if saccades and pursuit share a common network in sequence learning then results would reveal similar adaptation levels between the two types of eye movements; and iii) that pursuit responses would exhibit prediction during shorter 4-ramp sequences but would find difficulty in doing so for the longer 8-ramp sequences and that participants would exhibit evidence of requiring more repetitions to learn the longer sequences.

2.2 Methods

2.2.1 Participants

Thirteen participants 22 to 34 years of age (26.1 ± 3.83 yrs, 9 females) with normal or corrected eyesight and no known neurological conditions took part in the study. All participants gave informed consent prior to experimental sessions. This study was approved by The University of Leeds ethical committee and conducted in accordance with the standards laid out in the 1964 Declaration of Helsinki.

2.2.2 Experimental set up

Participants were tested in a dark room while seated, and their heads positioned in a forehead-and-chin rest of an eye tracker (Eyelink 1000, SR research, Canada). Participants were located 57 cm in front of a computer monitor (17 in CRT colour monitor, 1024 by 768 pixel resolution, 75Hz) where the experiment was presented. Experimental trials were designed using custom-made programs (COGENT, Psychtoolbox, MatLab, Mathworks, USA). Eye movement data was recorded using a video-based eye-tracker (EyeLink, SR Research Ltd, Canada) sampled at 1000 Hz and was stored for subsequent offline analysis. Eye movement calibrations took place prior to each of the 6 experimental blocks and rest breaks were given between each block, in which the lights were turned back on in order to avoid dark adaptation and fatigue. The experimental session lasted for approximately 60 minutes.

2.2.3 Experimental design

Experiments were designed to elicit saccadic and pursuit eye movements. In pursuit tasks, the visual stimulus consisted of a white squared target (15 x 15 pixels) that moved continuously in both horizontal and vertical directions over a black background. The target motion sequences started at the centre of the screen (i.e., zero) and were comprised of 4 or 8 constant speed (30 °/s) ramps, each moving in one of four directions (up, down, left or right). The same target (white square) was used in the saccade tasks, which consisted of 4-step sequences along the four possible directions, also starting from the centre of the screen. The steps and ramps that built the sequences for the saccade and pursuit tasks are referred to as components of a sequence, and each component started at the end position of the previous one to generate a continuous motion target. The direction of each component was randomized to form unique sequences within and between each experimental block.

Saccade and pursuit experimental tasks were conducted in two main conditions to investigate sequence learning in the oculomotor system: predictive (PRD) sequence condition and a random (RND) sequence condition. There were 6 experimental blocks of equal duration consisting of: 1) 4 component PRD saccade sequence (4PRDs); 2) 4 component RND saccade sequence (4RNDs); 3) 4 component PRD pursuit sequence (4PRDp); 4) 4 component RND pursuit sequence (4RNDp); 5) 8 component PRD pursuit sequence (8PRDp); and 6) 8 component RND pursuit sequence (8RNDp). In the PRD blocks each sequence was presented 4 consecutive times and in RND blocks the sequences consisted of single presentations of unique sequences. The order of the experimental blocks was randomized between participants, and all participants performed the same

sequences within each block. Participants' instructions were to follow the target and keep up with it as best they could using their eyes only and they were explicitly aware that in PRD blocks each sequence was repeated 4 times while in the RND blocks all of the sequences were different from each other and from the PRD sequences. Table 2.1 provides details of the experimental blocks used in the experiment.

2.2.4 Procedure

Participants performed saccades to a 4 component stimulus target and pursued a 4 and an 8 component moving target with a constant speed of 30 °/s. Sequence component directional changes occurred every 750 ms during the PRD condition. Previous studies have found that target directional changes within these timing ranges (500 to 1000 ms) and the equivalent target pacing frequency promote learning and prediction (Jarrett & Barnes, 2005; Joiner & Shelhamer, 2006). Stimulus frequency and durations were randomized between 500, 750 and 1000 ms in the RND condition (also see Table 2.1). Each 4 component sequence was 3000 ms in duration, whilst each 8 component sequence was 6000 ms in duration.

Participants were asked to maintain fixation on a central cue for 3000 ms or 6000 ms for the 4 and 8 component trials respectively. The fixation cue then flashed to indicate the start of a sequence (1000 ms or 2000 ms after for 4 and 8 component sequences respectively). This was followed by the presentation of 4 identical sequences in the case of PRD or 4 unique sequences in RND conditions to form a series. The moving target disappeared briefly at the start each component during a sequence. After performing a sequence, participants moved their eyes to

the centre of the screen to start the next new or repeated sequence. Fixations were inserted at the start of a series and participants performed 10 series during the 8 component PRD and RND conditions and performed 20 series during the 4 component PRD and RND conditions. This meant that the overall block lengths were equal in duration. Figure 2.1 displays an example of the saccade and the pursuit PRD and RND sequence task

Table 2.1 Experimental blocks description.

Block	Number of components	Component duration	Number of sequences
4PRDs	4	Duration: 750ms Sequence duration: 3000ms Amplitude: 22.5°	20 sequences x 4 reps 80 trials total *
4RNDs	4	Durations: 500, 750 or 1000ms Sequence duration: 3000ms Amplitudes: 15°, 22.5° and 30°	80 different sequences *
4PRDp	4	Duration: 750ms Sequence duration: 3000ms Speed: 30°/s	20 sequences x 4 reps 80 trials total*
4RNDp	4	Durations: 500, 750 or 1000ms Sequence duration: 3000ms Speed: 30°/s	80 different sequences *
8PRDp	8	Duration: 750ms Sequence duration: 3000ms Speed: 30°/s	10 sequences x 4 reps 40 trials total*
8RNDp	8	Durations: 1000, 1750 or 2000ms Sequence duration: 6000ms Speed: 30°/s	40 different sequences *

Each condition consisted of a 4 component saccade and pursuit sequence task, in addition to an 8 component pursuit task. (*) Does not include fixation cues inserted at the beginning of every 4 sequences in RND and PRD blocks.

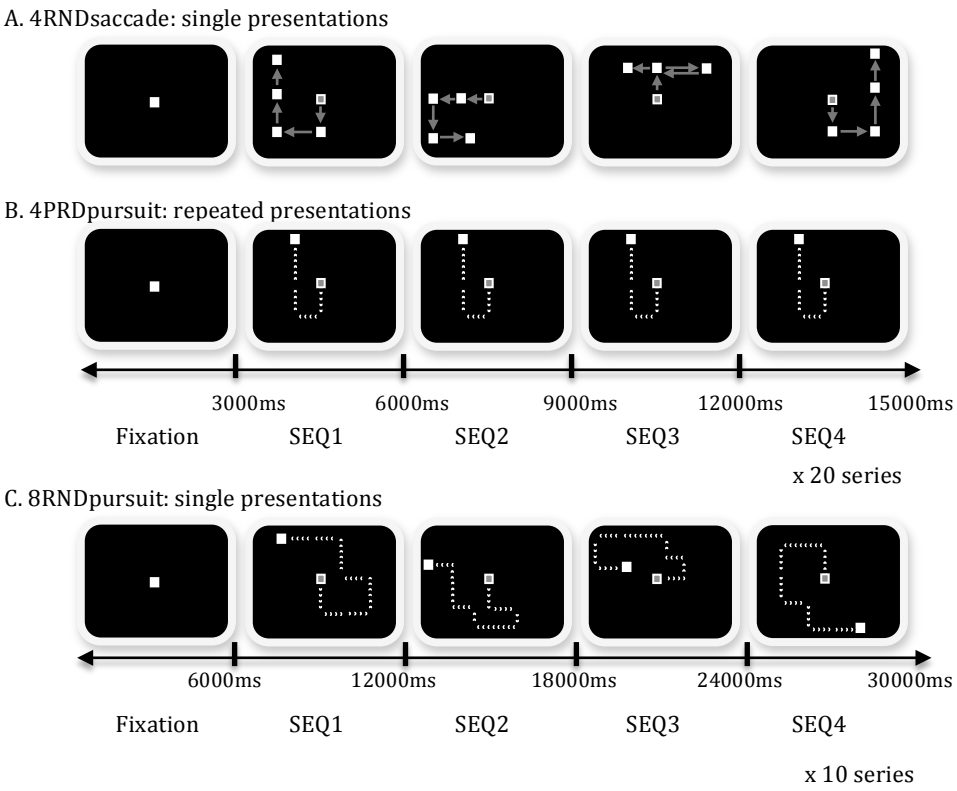

Figure 2.1. A 4RND saccade series (A) and 4PRD pursuit series (B) and an 8RND pursuit series (C). The target moved along the horizontal and vertical axis during 4 and 8 component sequences. The target briefly disappeared between components. A and C show different sequences across the series, whilst B shows identical presentations in the PRD condition. The fixation and sequence durations were equal (3000 ms and 6000ms for a 4 and an 8 component sequence respectively). A total of 10 and 20 series were presented for the 8 and 4 component sequences respectively.

2.2.5 Analysis

Eye movement data sampled at 1000 Hz were obtained from the Data Viewer software (SR research Ltd, Canada). Blinks were automatically eliminated from the raw data prior to analysis. Data Viewer bridged gaps within the missing data using linear interpolation. Eye displacements and velocities were analysed using a custom made programme in MATLAB (version 7.8, Mathworks Inc., USA) designed for each stimulus type and each eye movement type. Eye data was corrected for drifts at the start of every sequence (at position zero) to avoid

contamination from the previous eye movements. Analysis of eye movement data was performed for each component within all sequences in all condition blocks.

Saccades

Saccades were computed from the velocity traces and identified as samples with velocity exceeding 100°/s. Saccade onsets were obtained by identifying the peak velocity (PV) of a saccade and calculating the 2nd derivative to obtain abrupt changes in slope (i.e., peak Jerk). Saccade latencies (t_{SAC}) were then computed from each target onset to the corresponding saccade onset of the 4 components within a sequence. Saccade PVs were plotted and visually inspected for accuracy (Figure 2.2). In addition, time to peak velocity (TTPV), absolute eye end position error to target location and variable error were also measured for each component of all sequences in both conditions (PRD and RND). Predictive responses were identified as latencies smaller than 80 ms, which are responses made prior to any significant visual feedback (Smit & Van Gisbergen, 1989). Conversely, trials with saccade latencies shorter than 80 ms during the RND conditions and during the 1st presentation of a new sequence in the PRD conditions were considered as anticipatory guesses of target location. These guesses only accounted for an average of 3.3 ± 1.7% of reactive trials and therefore were eliminated from the analyses. A repeated measures ANOVA was used to identify significant differences within PRD saccadic sequences and between the PRD and RND conditions. Interactions between variables were evaluated using Bonferroni corrected post-hoc test. A significance level of $p < 0.05$ was established for all statistical analyses. Results are expressed as means ± standard deviations (sd).

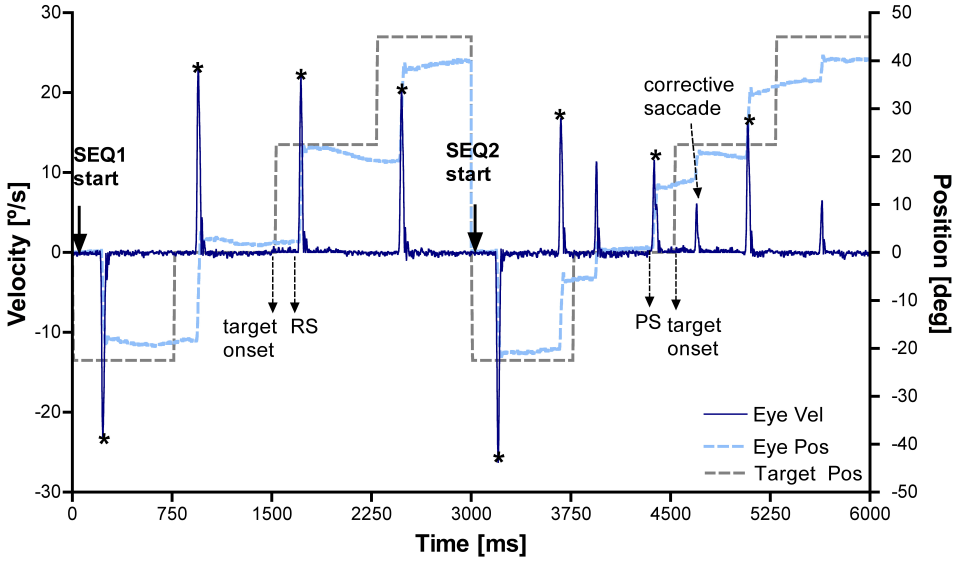

Figure 2.2. Example of a single participant's saccadic eye movements to the first and the second presentation (SEQ1 and SEQ2) of a 4 component PRDs trial. The graph illustrates the eye position and velocity traces and the target position. The start of the sequence is determined by the first component of the sequence. Four saccades and peak velocities are plotted (shown by a asterisk*) for each presentation. The end position of the eye was compared to actual target position to obtain accuracy measures for each component. The graph also shows reactive saccades (RS) during SEQ1 and predictive saccades (PS) during SEQ2. Saccade onsets were calculated using the 1st saccade made to the target's location. Corrective saccades were also observed but were not included in the analysis.

Smooth pursuit

Intrusive and catch-up saccades were eliminated from the smooth pursuit eye movement data using a previously described technique (Bennett & Barnes, 2003). Linear interpolation techniques were used to link the resulting gaps from the removal of the saccades. The velocity traces were then filtered using a 10 Hz low-pass filter. Peak velocity was identified for each component in the sequence and plotted for visual inspection to ensure that the first peak was detected (Figure 2.3). After peak identification, time to peak velocity (TTPV) was calculated from target onset in each component.

Pursuit onsets of a continuous eye movement to individual ramp components can be problematic to identify, as eye velocity at the start of each ramp is influenced by the decaying response to the prior ramp (see Barnes & Schmid, 2002). However, derivation of eye acceleration and peak Jerk, using differentiation methods to determine the rate of change in the PRD and RND eye velocity trajectories, allowed a reliable estimate to be obtained for the initiation of pursuit towards each new target direction within a sequence (Figure 2.3, upper right). Pursuit latencies (t_{PUR}) were then determined as the time from target onset to peak Jerk for each component of the sequences. This latency estimate gives a rather conservative indication of predictive behaviour. A more global temporal assessment was also implemented to calculate the overall differences in timing between eye movements and the target stimulus for predictive sequence trials by performing a cross-correlation analysis across the entire sequences (see Barnes et al., 2000; Barnes & Schmid, 2002). This method is commonly used to compare similarities between 2 waveforms across time. The time at which the maximum correlation was reached was calculated, and used to describe the time delay between the eye velocities and the corresponding moving stimulus. Only correlations above 0.6 were used to obtain latency values. Velocity gain was calculated as the ratio of eye velocity and target velocity. Position error was calculated as the averaged absolute error of each component with respect to the target location. The variability of the response was also calculated for individual trials for each condition and each subject (Figure 2.4).

A repeated measures ANOVA was used to identify significant differences between the PRDs and RNDs conditions and between the identical presentations (SEQ1, SEQ2, SEQ3 and SEQ4) in the PRD tasks. Interactions between variables

were evaluated using Bonferroni corrected post-hoc test. Correlations and deltas (Δ) were used to compare saccadic and pursuit performance between PRD and RND sequences. A significance level of $p < 0.05$ was established for all statistical analyses. Results and graphs are expressed as means ± standard deviations (sd). Statistical analyses were performed using the SPSS package. One participant's eye data was excluded from the 8 component sequence analysis due to issues with the eye tracker signal.

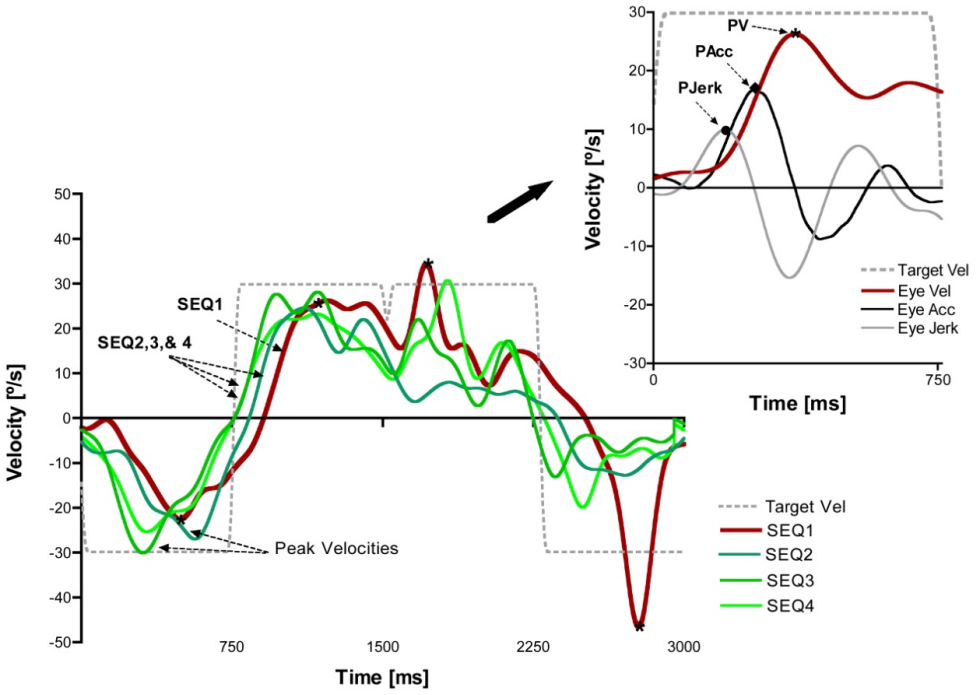

Figure 2.3. An example taken from an individual participant's eye data to a 4PRD pursuit trial showing target (dotted line) and eye velocity traces. The figure ilustrates eye movement responses to the 4 identical sequence presentations. The first peaks of each response were also obtained from all the sequences to each of the 4 target components. Note that the repeated eye velocity traces (SEQ2, SEQ3 and SEQ4) appear to reach PV faster than in SEQ1. Cross correlations were obtained from these eye velocity traces and the target. The upper right graph shows the eye velocity trace of one component of the 4PRD sequence and PV, acceleration trace and peak acceleration (PAcc) and jerk trace and peak jerk (PJerk) as well as target velocity.

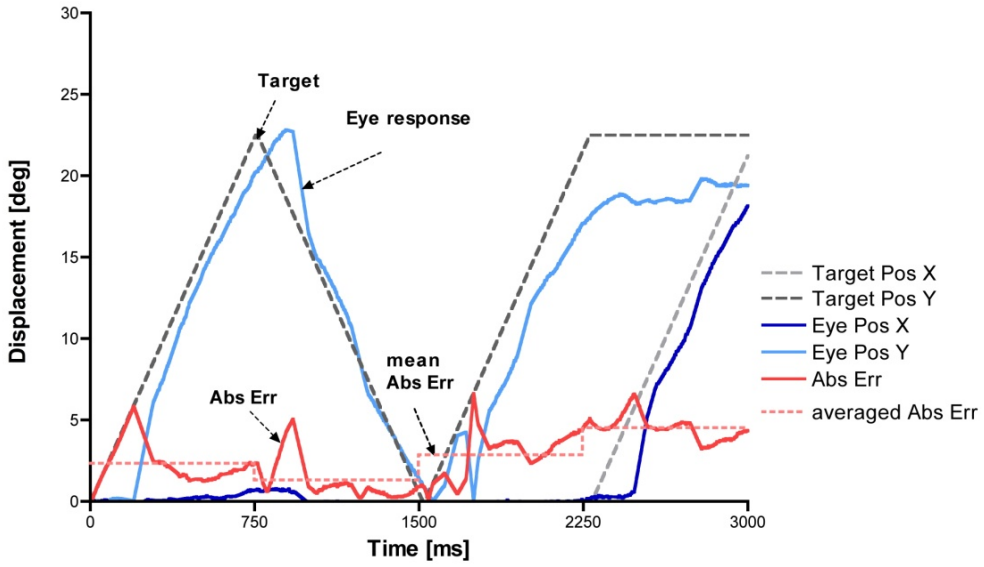

Figure 2.4. An example of eye displacements and target position of a 4PRD sequence trial. Absolute error (Abs Err) was calculated and averaged to obtain 4 averaged accuracy values for each component of the sequence (as indicated for component 3).

2.3 Results

This study analysed pursuit and saccadic eye sequence learning when performing a series of complex sequences during predictive and comparing to random conditions. We also further investigated the effects of additional successive components in a sequence during learning. Two main analyses were carried out: i) to examine the existence of predictive behaviour during the PRD condition compared to RND sequences in both types of eye movements to determine sequence learning differences; and ii) to assess short-term storage capacity and learning effects when adding components to a sequence. Examples of participants' eye data are shown in Appendix B (Figure 1) for the 4PRD and 8PRD blocks.

2.3.1 Effects of repetition: Saccades and pursuit

A within PRD analysis was performed to investigate saccade and pursuit adaptation in the form of reduced latency values while tracking a stimulus with a predictable trajectory. In both saccade and pursuit tasks participants were able to learn the complex sequences and significant temporal shifts were evident within the second presentation. Pursuit latencies (t_{PUR}) from SEQ1 (258 ± 22.76 ms) were significantly longer compared to SEQ2, SEQ3 and SEQ4 (180.75 ± 20.23, 180.02 ± 20.23 and 176.02 ± 20.72 ms respectively) across all components ($F_{(9,108)}$=3.38; p=0.001) (Figure 2.5A). Furthermore, no differences in performance were observed between the second presentation and subsequent presentations of the sequence (i.e., SEQ2, SEQ3 and SEQ4) in pursuit trials (p>0.05). In contrast, saccadic eye movement latencies (t_{SAC}) did show differences between identical presentations ($F_{(9,108)}$=21.843; p<0.001). This meant that participants were able to significantly predict the target after the first presentation, with further improvements in the SEQ3 and SEQ4 presentations (t_{SAC} of 230.75 ± 17.97, 67.89 ± 64.27, 36.16 ± 77.19 and 36.45 ± 89.93 ms for SEQ1, SEQ2, SEQ3 and SEQ4 respectively) of the same sequence (Figure 2.5A). The saccadic predictive responses, however, were more variable between and within participants.

To quantify learning adaptations shown in the PRD sequences in saccadic and pursuit responses, the temporal shifts from the identical presentations were compared by calculating the latency differences (deltas) from SEQ1 and obtain deltas for saccade and pursuit (Δ_{SAC} and Δ_{PUR}). A repeated measures ANOVA revealed a significant interaction ($F_{(2,24)}$=8.41; p= 0.002), where Δ_{SAC} was significantly greater than Δ_{PUR} across repetitions (p<0.001). Figure 2.5(A)

displays the differences between Δ_{SAC} and Δ_{PUR} and (B) also shows that in saccade responses, Δ_{SAC} increased from SEQ2 to SEQ3 and SEQ4 ($p<0.001$, $p=0.015$), whilst pursuit response latency differences did not significantly change across repetitions ($p>0.05$). Note that SEQ1 responses for saccades and pursuit did not show significant differences ($p>0.05$) establishing the same baseline for both eye movement types when computing latency Δ values.

2.3.2 Cross correlations in smooth pursuit

Given the conservative pursuit onset measures, cross correlations of the whole sequences were computed between the identical sequences and the target to better assess timing shifts within identical presentations. The time of maximum cross-correlation (t_{COR}) between the repeated responses and the stimulus and between SEQ1 responses and the stimulus revealed a sequence main effect ($F_{(3,33)}=33.98$; $p<0.001$). The repeated sequences showed reduced t_{COR} values compared to SEQ1 responses ($p<0.001$), but no differences were observed between these repetitions ($p>0.05$) (Figure 2.6). Participants' repeated responses showed a reduced temporal shift after performing SEQ1 with an averaged PRD t_{COR} value that was less than 80 ms which suggests that participants predicted the target's directional changes (Barnes et al., 2000). In addition pursuit adaptations as seen in these t_{COR} results are in accordance with latency results.

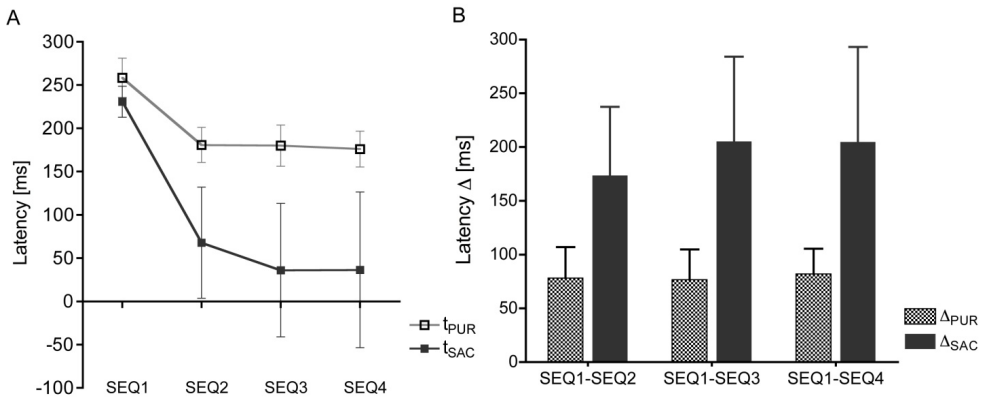

Figure 2.5. (A) Means and standard deviations of saccade and pursuit latencies across the 4 identical presentations and (B) means and standard deviations of saccade and pursuit latency differences between the first presentation and the repetitions of the sequence. Significant temporal shifts were observed following the first presentation of a sequence in both types of eye movements following SEQ1 (A), with greater latency differences in saccadic predictive behaviour compared to pursuit (B).

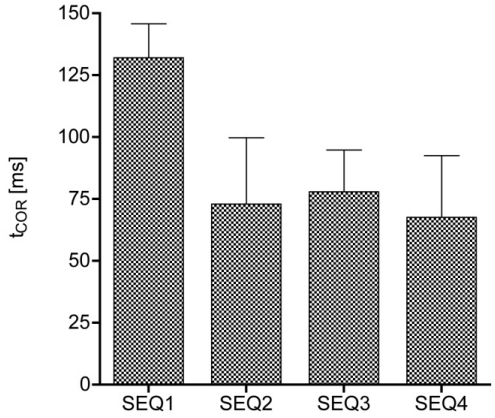

Figure 2.6. Cross-correlation mean timing shifts and standard deviations between the target and the identical presentations. The graph illustrates significantly larger timing shifts for SEQ1 compared to SEQ2, SEQ3 and SEQ4 eye velocity trajectories.

2.3.3 PRD versus RND sequences: Saccades and pursuit

The saccade and pursuit responses from the repeated sequences that displayed significantly reduced latencies were averaged as predictive responses (PRD). PRD responses were compared to the mean visually triggered eye

movement responses (RND conditions) across the 4 component sequences. Preliminary analyses determined that SEQ1 and RND responses were not statistically different in any measure ($p>0.05$), validating SEQ1 pursuit and saccade responses as reactive and supporting their exclusion from this analysis.

Separate analyses were performed to assess predictive behaviour in both eye movement types. Results were then compared between saccades and pursuit as differences between PRD and RND responses (Δ_{SAC} and Δ_{PUR}). To observe whether eye movements kept the same level of prediction across the entire sequences, the individual components were also compared. Overall, visually triggered responses from the RND saccades conditions resulted in t_{SAC} values of 244.5 ± 31.23 ms. Saccade latency analysis revealed a sequence type (RND and PRD) by 4 component interaction ($F_{(3,36)}=64.12$; $p<0.001$). A post-hoc test showed that t_{SAC} values were shorter when performing the PRD sequences compared to the RND sequences across all of the 4 components ($p<0.001$). In addition, post hoc tests revealed latency differences between components. RND latencies increased across components (1 vs. 3 and 4 $p<0.001$; 2 vs. 3 and 4 $p<0.003$ and 3 vs. 4 $p<0.002$) and in contrast, PRD latencies decreased from component 1 to 2 ($p<0.001$) and from components 1 and 2 to 3 and 4 ($p<0.03$) (Figure 2.7A). Saccadic $TTPV_{SAC}$ values also revealed an interaction ($F_{(3,36)}=64.23$; $p<0.001$) where peak eye velocity was reached faster during PRD sequences compared to the RND condition ($p<0.001$). As found with latency, participants also exhibited increases in $TTPV_{SAC}$ between components within RND sequences and decreased $TTPV_{SAC}$ across PRD components ($p<0.05$).

A contrast between t_{PUR} PRD and RND conditions also revealed a significant sequence type by component interaction ($F_{(3,36)}=28.39$; $p<0.001$). Post

hoc analyses indicated shorter latencies in the PRD sequences compared to RND across components ($p<0.001$). Similar to saccades, t_{PUR} values across components increased in RND sequences (1 and 2 vs. 3 and 4; $p<0.03$) whereas in the PRD pursuit sequences, responses displayed larger t_{PUR} values during the 1st component, but were only different to the second component ($p=0.008$) (Figure 2.7B). $TTPV_{PUR}$ measures revealed a similar pattern to latency in pursuit and likewise, an interaction ($F_{(3,36)}=12.63$; $p<0.001$) that showed that participants achieved PV earlier in PRD compared to RND conditions across all components (Post hoc test, $p<0.001$). Differences were only noted between components 1 and 2 in both RND and PRD conditions ($p=0.012$ and $p=0.045$ respectively). Unexpectedly, both eye movement types exhibited an increasing lag across components when performing RND sequences.

To directly assess these timing differences between conditions, the latency differences between RND and PRD responses across components were calculated as Δ_{SAC} and Δ_{PUR} values and entered into a repeated measures ANOVA. A significant interaction between eye movement type delta and component ($F_{(3,36)}=29.592$; $p<0.001$) revealed significant differences between saccadic and pursuit eye movements ($p<0.001$) except at component 1 ($p=0.068$). In addition, Δ_{SAC} values increased across components ($p<0.001$), whilst Δ_{PUR} values increased from component 1 and stabilized after this during components 2, 3 and 4 ($p<0.001$). Figure 2.7(C) indicates Δ_{SAC} and Δ_{PUR} differences across the 4 components of the sequences and presents the differences in predictive timing between saccade and pursuit when performing a 4 component sequence.

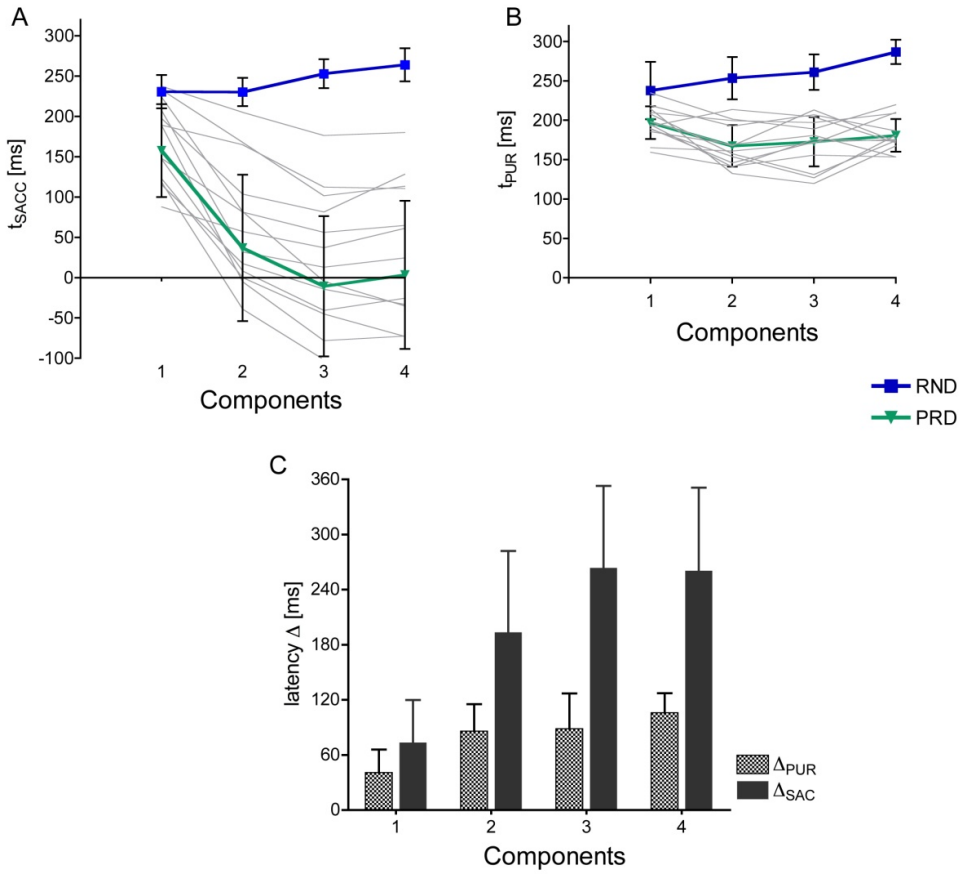

Figure 2.7. Means and standard deviations of t_{SAC} (A) and t_{PUR} (B) of the RND and the PRD conditions and individual participants' PRD responses (light grey) across the 4 components. Saccade responses show a wider latency range between participants compared to pursuit (A and B grey lines). The figure also shows saccadic and pursuit Δ (C) obtained from the latency differences between RND and PRD conditions across the 4 components of the eye movement types. Saccadic movements were temporally closer to target onset than pursuit eye movements and therefore showed greater prediction.

2.3.4 Predictive changes in eye movement trajectories: Saccades and pursuit

To assess magnitude changes in eye movements we investigated eye movement parameters such as accuracy and PV, which have been shown to differ under predictive conditions. Saccade parameters during PRD sequences were in accordance to previously reported predictive saccade characteristics with decreased PV and accuracy. Overall, the PV_{SAC} was significantly lower during the

predictive sequences compared to the RND conditions ($F_{(3,36)}$=7.1; p<0.001) across all components except the 1st component as revealed by an interaction (p<0.02 for components 2, 3 and 4 respectively). In addition, post hoc analysis showed that saccades were similarly slower in components 2, 3 and 4 (p<0.001). We found that end point accuracy of the eye was reduced during predictive saccades (first saccade to target), but was later corrected once the target became visible. An analysis of the absolute error of the first saccade revealed a significant sequence type by component interaction ($F_{(3,36)}$=7.44; p=0.001) and decreased accuracy was observed during the PRD sequences compared to the RND sequence type across all components (p<0.004). Furthermore, accuracy during the PRD and the RND saccade conditions deteriorated across the 4 sequence components (p<0.05). The PRD sequences showed higher error variability compared to RND responses ($F_{(3,36)}$=39.61; p<0.001) . Post hoc analysis indicated that the saccade error variability also increased across components in the PRD condition (p<0.03) but not when performing RND sequences (p>0.05) (Figure 2.8A).

Pursuit absolute errors were calculated as distance from target across the 4 components. In contrast with saccades, no significant differences were found between PRD and RND eye displacement errors (p=0.07). However, a components effect ($F_{(63,36)}$=120.76; p<0.001) indicated that the absolute error of the eye remained significantly increased across components (post hoc test p< 0.004) (Figure 2.8B). Variability of the eye displacement during pursuit also showed a component main effect ($F_{(3,36)}$=9.94; p<0.001) where the first component showed increased variability compared to components 2, 3 and 4 in both PRD and RND conditions (p=0.001, p=0.001 and p=0.004 respectively). PV_{PUR} analysis did not show any significant differences between PRD and RND sequence types (p>0.05).

Furthermore, maximum acceleration for pursuit did not show any significant differences between PRD and RND conditions ($p>0.05$). However, in the PRD conditions, a significant decrease in acceleration was observed during the last component of the sequence ($F_{(3,36)}=4.03$; $p<0.001$). Overall, early TTPV values in PRD conditions were caused by early pursuit onsets, not by increased acceleration.

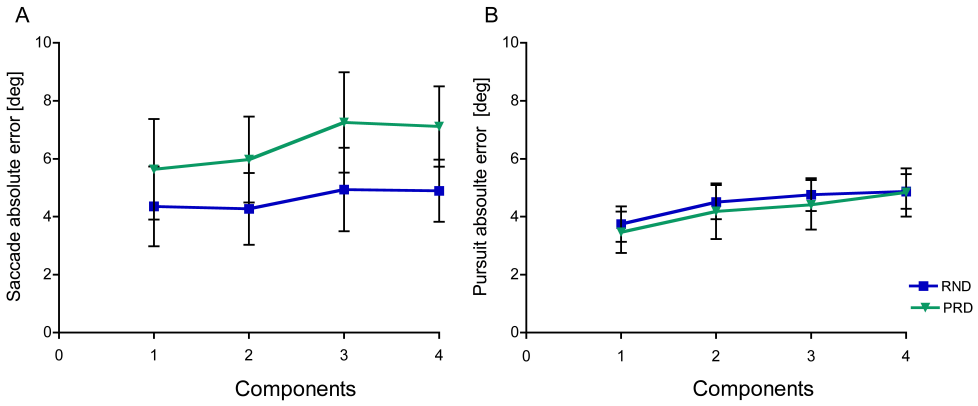

Figure 2.8. Mean ± sd RND and PRD absolute errors for saccadic (A) and pursuit (B) eye movements. The graph on the left illustrates how error is an important factor in saccadic eye movements while in pursuit the error remains almost constant as the target is in motion. In both types of eye movements the error increased across RND sequences.

2.3.5 Effects of repetition in longer sequences

A t_{PUR} main effect ($F_{(3,30)}=46.15$; $p<0.001$) showed that participants' 8PRD latencies significantly decreased quickly after performing SEQ1 ($p<0.001$) and as in the shorter 4 component sequences, performance did not significantly differ between SEQ2, SEQ3 and SEQ4. In addition, an effect for component ($F_{(7,70)}=10.02$; $p<0.001$) indicated an increase in latency across the 8 components regardless of whether participants were performing a sequence for the first, second, third or fourth time. It was observed that the repeated sequence responses were similar

to SEQ1, but performed with shorter latencies across components. Latencies from SEQ1 (268.03 ± 62.34 ms) and RND (281.58 ± 54.43 ms) responses in the 8 component sequences were not significantly different ($p>0.05$). In addition, t_{PUR} from 8 component sequences did not show differences across repetitions, thus the averaged 8PRD response was used to compare with the shorter 4PRD responses (see Figure 2.9A PRD).

2.3.6 Sequence learning in short versus long sequences

The Appendix B (Figure 1) shows examples of participants' 4PRD and 8PRD trials and illustrate eye velocity traces of SEQ1 and the repeated sequences. These examples also demonstrate a timing shift across repetitions in both the 4 and 8 component sequences. A contrast with the 4 component sequences was performed and to see how performance differed, the RND 8 component sequences were divided into two parts: 1) 8PRD1, which consisted of components 1 to 4 and 2) 8PRD2, which consisted of components 5 to 8. It was first established whether RND responses differed between sequence lengths. The analysis of the t_{PUR} values between the RND 4 component and the 8 component sequences revealed a sequence effect ($F_{(2,24)}=25.89$; $p<0.001$) and a component effect ($F_{(3,36)}=14.12$; $p<0.001$). As expected, latencies between 4RND and 8RND1 (i.e., first 4 components of the longer sequence) were not significantly different ($p>0.05$) however; eye movements from the last 5 to 8 components of the 8RND sequences were slower to initiate compared to 8RND1 ($p<0.001$) and 4RND ($p<0.001$) (Figure 2.9A). Overall, pursuit latencies were longer in the last component compared to 1 and 2 ($p<0.001$). These results indicated an increase in latency across the components regardless of whether participants were performing a

short or a long sequence. Analysis also revealed that t_{PUR} values between 4PRD and 8PRD1 ($F_{(2,22)}$=15.32; $p<0.001$) were not significantly different ($p>0.05$) but that latencies from 8PRD2 were longer than both the latencies from 4PRD and the 8PRD1 ($p<0.001$).

The differences between RND and PRD latencies were calculated for the 4 and the two part 8 component sequences. These (RND-PRD) deltas were compared to assess reactive to predictive adaptations in shorter and longer sequences. Analysis did not reveal any significant differences between sequence length deltas ($p>0.05$) (Figure 2.9B). Temporal deltas along the 8 component sequences were similar across the entire sequences and not significantly different from the 4 component sequence responses. Results from these RND to PRD differences showed that even though the 5 to 8 components of the 8PRD sequence responses indicated longer latencies, prediction did not significantly differ between the shorter and the longer sequences.

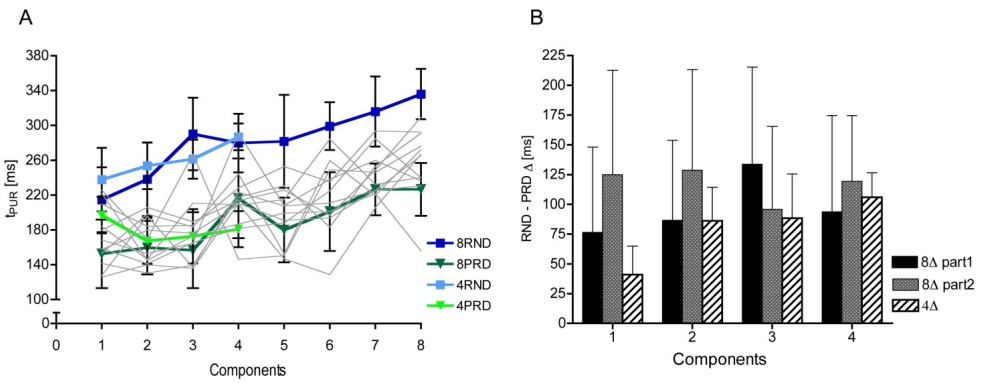

Figure 2.9. Mean ± sd of the RND and PRD latencies for the 4 component sequences and the 8 component sequences and individual 8PRD participant data (grey lines) (A). The graph (B) shows RND and PRD deltas for the 4 and the two parts of the 8 component sequences. The graph on the left shows the 4 component sequences over the first 4 components of the longer sequence and similar latencies. It also shows a continuous increase in pursuit latency as participants track the RND and the PRD sequences. The graph on the right shows the timing differences between a RND and a PRD sequence in the different length sequences. These results show that the temporal shifts were consistent across the 8 component sequences and not significantly different from the 4 component RND to PRD time shifts.

Analysis of TTPV values did not reveal similar findings as the latencies and deltas. TTPV results showed that 8PRD1 and the 8PRD2 reached PV at similar time points and that the 4PRD sequences had overall shorter TTPV values across all components ($F_{(6,66)}$=4.1; p=0.001) (Figure 2.10A). Furthermore, acceleration values showed significant differences between the 4 component and the 8 component predictive sequences ($F_{(6,66)}$=4.14; p=0.001) (Figure 2.10C). However, these effects were also observed in the RND conditions (see Figure 2.10B and 2.10D). RND TTPV and acceleration values between the 4 and the 8 component sequences also revealed that the 4RND sequence responses reached PV faster (sequence length effect $F_{(2,24)}$=74.484; p<0.001) and had larger peak accelerations (sequence length effect $F_{(2,24)}$=88.939; p<0.001) compared to the 8RND responses.

PVs were slightly higher for the 4PRD sequences compared to the 8PRD1 sequence but not different to the 8PRD2 sequences (mean PV of 17.94 ± 3.13, 15.98 ± 3.32 and 16.8 ± 3.18 º/s respectively) ($F_{(2,22)}$=5.23; p=0.014) (Figure 2.11A). Absolute errors from the short 4PRD sequences were significantly different form the 8PRD2 segment ($F_{(2,22)}$=59.96; p<0.001) but not from the 8PRD1 segment (p>0.05) (Figure 2.11B). Similarly, the 4PRD pursuit responses exhibited less variable error ($F_{(2,22)}$=59.96; p<0.001) compared to 8PRD2 segments but were similar to the 8PRD1 sequences (4.19 ± 0.82, 5.39 ± 0.77 and 4.28 ± 0.76 deg respectively).

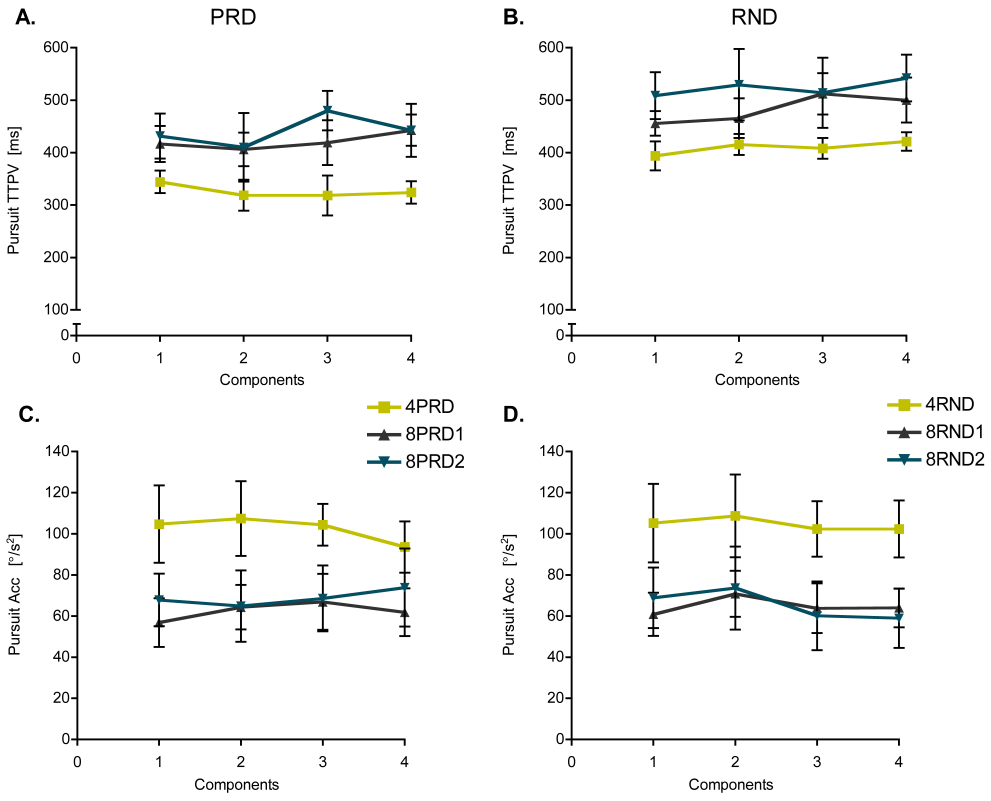

Figure 2.10. Mean ± sd of 8PRD1, 8PRD2 and 4PRD TTPV (A) and mean ± sd acceleration (C); and mean ± sd of 8RND1, 8RND2 and 4RND TTPV (B) and mean ± sd acceleration (D). Pursuit responses from the shorter sequences reached PV faster and had significantly higher acceleration compared to the longer 8 component sequences. Similar effects were observed for both RND (right) and PRD (left) conditions.

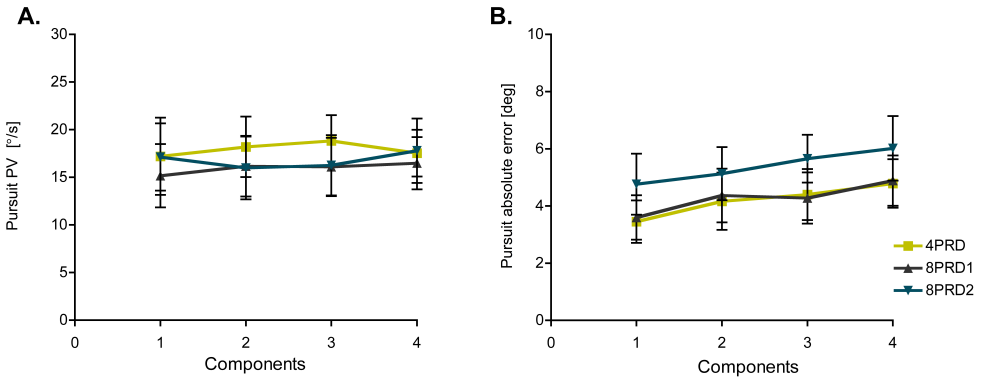

Figure 2.11. Mean ± sd of 8PRD1, 8PRD2 and 4PRD PV in °/ms (A); and the mean ± sd absolute error in degrees (C) over 4 components.

2.3.7 Cross-correlations: 8 and 4 PRD pursuit

A global comparison was performed between the timing shifts of the entire sequences with respect to target velocity. Correlation t_{COR} values from the 4PRD sequence presentations had similar temporal shifts compared to those of the first half of the 8PRD sequence, but different to the second half ($F_{(2,22)}$=6.53; p=0.007) (see Figure 2.12). No differences between components 1 to 4 and components 5 to 8 in the 8PRD sequences were observed (p>0.05).

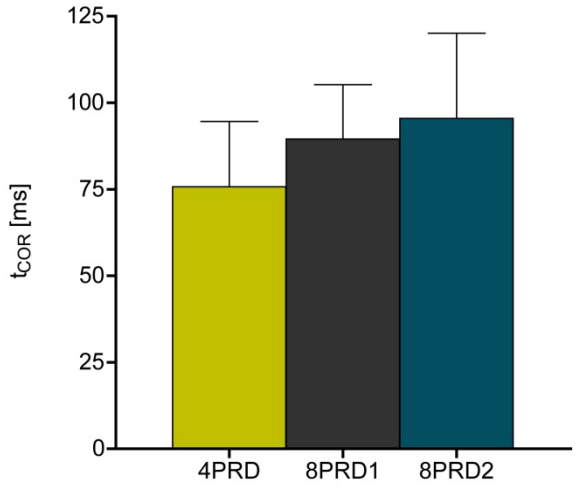

Figure 2.12. Mean and sd pursuit timing effects of the 4PRD sequences and the 8PRD sequences (divided into 2 segments). The graph shows the temporal delays obtained from the cross-correlation analysis between the PRD sequences and the target stimulus. 4PRD was significantly different from 8PRD2 but not to 8PRD1.

2.4 Discussion

A sequence learning task was implemented in which a continuous moving target was presented in predictable (repeated 4 times) conditions and compared to random presentations to investigate short-term oculomotor adaptations modified by prior experience in saccadic and pursuit eye movements. We expected participants to (a) predict directional shifts in order to land on the target as it was illuminated during the saccade sequence learning and (b) also predict and self-initiate smooth pursuit eye movements prior during the repeated PRD sequences. We also hypothesized that these adaptations would be more apparent in shorter vs. longer sequences, with participants exhibiting slower learning rates in the longer compared to the shorter sequences. Overall, participants showed evidence of learning the complex sequences and exhibited predictive eye movements over consecutive, identical sequence trials (PRD). Reduced latencies were observed during PRD conditions in both pursuit and

saccade tasks only after the first presentation of a sequence. However, temporal modifications between reactive and predictive responses differed between the eye movement types supporting earlier findings (Burke & Barnes, 2006). Saccades from PRD conditions were predominantly self-initiated predictive responses with earlier onsets compared to pursuit eye movements also in support of previous findings using single and double ramps (Burke & Barnes, 2006). In addition, once predictive tracking was achieved, saccades continued to decrease in latency between presentations and across components, whilst pursuit eye movements did not show significant changes across the repeated sequences. This is a significant contribution to the understanding of sequence learning in saccades and pursuit, and suggests that more learning occurs in saccadic trials. Furthermore, our data suggests that temporal feedback continues to optimise the response in saccades from trial to trial, but this is not a feature of sequencing learning during pursuit. Results from this study suggest that during the learning of a repeated sequences different timing signals between saccades and pursuit exist to achieve their independent aims. These include matching eye and target location during saccades and matching eye and target velocity during pursuit. We also show important implications in the understanding of the neural mechanisms for saccade and pursuit adaptations. Differences in timing and learning adaptations between saccades and pursuit and between short and long sequences are discussed. In addition, we show that increasing the number of components in a sequence not only increases latency to the later targets (components 5 to 8), but also alters the eye kinematics (lower acceleration and longer TTPV) to target components in the longer sequence when compared to shorter sequence tasks. It is important to note that this effect is observed in both PRD and RND sequences,

and therefore, this effect was not considered learning-related but possibly suggests an overall attentional effect with longer sequence presentations.

2.4.1 Effects of repetition: PRD saccade and pursuit

Saccade latencies obtained during RND conditions and from SEQ1 responses fell within previously reported visually triggered latency ranges (200-250 ms) (Burke & Barnes, 2006; Erkelens, 2006; Saslow, 1967). Saccades with significantly shorter latencies (< 80 ms) occurred after the first presentation of the sequence (SEQ1) and these predictive saccades exhibited reduced PVs and significantly poorer end point accuracy compared to saccades in the RNDs conditions, which are changes previously associated with saccadic predictive behaviour (Collins, Jahanshahi, & Barnes, 1998). Participants also revealed significant pursuit timing shifts during the identical presentations after the first presentation of a sequence in PRD conditions. However, both RND and PRD pursuit latencies were longer than previously reported (t_{PUR} of ~240 ms and ~170 ms for RND and PRD conditions respectively) (Collins & Barnes, 2005). In the present study, a continuous motion stimulus was used compared to the sequences of discrete single ramps used in previous work and differences in these tasks could account for increases in latency values. However, we presented a similar stimulus to elicit saccades and the saccade latencies fell within previously reported ranges suggesting the complexity of the sequences may not account for these delays. These latency differences could be attributed to the more conservative pursuit onset measure, which identified the abrupt change in slope towards PV. Burke and Barnes (2006) also reported longer pursuit latencies in their 2-step ramp task compared to previous reports (~100 ms longer), but found

slowing of pursuit prior to the onset of a second target when the target's directional changes became predictable. This slowing of pursuit velocity has been suggested to occur as anticipation of a subsequent sequence component (Jarrett & Barnes, 2005) and could be considered part of the predictive pursuit response. Finding the accurate turnaround onset in pursuit of a continuous moving target is challenging. To address this, we examined a more global comparison between sequence timings by obtaining cross correlations, which also revealed significant time shifts in the repeated sequences compared to SEQ1. The cross-correlation latency values obtained from this analysis showed similar timing shifts of the PRD responses compared to previous findings (cross correlation timing shift differences of > 30 ms between SEQ1 and the repetitions), which found predictive pursuit eye movements during a 2-ramp sequence learning task with a stimulus velocity of 15 and 30 °/s (Barnes & Schmid, 2002). Our findings of the timing shifts across identical presentations suggest that PRD pursuit responses were predictive in nature, as previously hypothesized. Thus, experimental results demonstrate that repeated presentations of a multiple component predictable stimuli lead to oculomotor temporal shifts that are evident after only a single presentation. However, one main interest in this study was to assess whether analogous saccade and pursuit sequence learning paradigms would reveal similar adaptations.

In pursuit (PRDp) conditions, predictive behaviour occurred and was maintained with no significant improvements after the first presentation. Similarly rapid pursuit adaptations have been observed in previous learning tasks that included repetitions of constant velocity 2 and 4 ramp sequences which led to the build up of a steady-state pursuit response, with no significant

improvements seen only after a few repetitions (Barnes et al., 2000; Burke & Barnes, 2007; Collins & Barnes, 2005). Predictive pursuit during the repeated sequences suggests that the target's velocity was stored after the first presentation and then released as an estimate for the subsequent responses (Barnes et al., 2000; Collins & Barnes, 2005). Differences in saccade latencies across repetitions also suggest predictive behaviour after only one presentation of the new sequence. However, unlike pursuit eye movements, saccade latency adaptations continued after the second presentation and reached a plateau between the 3rd and 4th. Saccadic and pursuit latency differences across identical sequence presentations not only revealed significant differences in the level of prediction and therefore learning.

Shelhamer and Joiner (2003) examined saccadic tracking over predictive and reactive frequencies. They found evidence to suggest that current saccades were made based on past perceived performance, where if the current saccade was made late with respect to target onset, then the future saccades would have decreased latencies. Previous findings from double step (T1 and T2) paradigms have suggested that when saccades are aimed at multiple individual targets, they don't simply rely on the initial motor plan containing information about T1 and T2, but that the saccade to T2 also depends on the motor update following T1 (Quaia, Joiner, Fitzgibbon, Optican, & Smith, 2010). Thus, the saccade system relies on the storage and update of multiple motor commands to be able to produce movements to multiple targets (Quaia et al., 2010; Ray, Bhutani, Kapoor, & Murthy, 2011). This may suggest that the saccadic system needs to compensate for this processing to elicit more automatic responses and make sure the eyes arrive at the right location as the target illuminates. A study by Joiner and

Shelhamer (2006) investigating predictive saccadic tracking timing mechanisms found that the reactive-to-predictive pacing transition exhibited an initial lag, but was quickly corrected to match the predictive pacing frequency and showed a greater-than-chance probability of making repetitive predictive movements once prediction was established. Even though our pacing frequencies did not change like in Joiner and Shelhamer's (2006) study, results clearly showed that responses to the first presentation of the sequence were reactive in nature and may have had to make the transition from RND to PRD. Thus, saccade latency differences between presentations may have been affected by a reactive to predictive lag and once corrected this then facilitated prediction, reflecting an increase in the ability to acquire the timing and position of the target as participants performed the repetitions of a sequence.

It has been well established that pursuit eye movements made prior to attaining stimulus feedback are influenced by the previous acquisition of the target's position, timing and velocity (Barnes & Asselman, 1991). Barnes and Schmid's (2002) pursuit sequence learning experiments revealed that prediction of future responses was highly correlated to the response from the prior sequence. They also found that when unexpected changes to the stimuli occurred, pursuit responses took little time to adapt and revert to a steady state of the new conditions. This behavior is in line with the adaptations found by Joiner and Shelhamer (2006) for saccades. Additionally, Joiner and Shelhamer's (2006) experiments looking at pursuit and saccadic random to predictive transitions and showed that both types of eye movements go through similar transitions and concluded that saccades and pursuit have similar time constraints and similar preparation times. However, even though Burke and Barnes (2006) found

predictive responses during repeated ramps compared to RND stimuli, they observed an overlap between PRD and RND latencies in pursuit, but not in saccades and suggested that saccadic and pursuit eye movements do not share similar timings. In support of this, we did not find similar saccade and pursuit adaptations from reactive responses to the predictive presentations. The Δ_{PUR} values (Figure 2.5) showed similar temporal modifications with repetition, whilst Δ_{SAC} values showed greater temporal shifts from SEQ1 that could be further modified with repetition and across components. It therefore seems that either shared or independent timing constraints for pursuit and saccades are task-dependant and warrant the investigation of the activation of the neural networks involved. Previous studies have found that pursuit and saccades indeed share a common predictive process (Nyffeler et al., 2008), but it may be that the decision signal for both movement types has different response thresholds (Krauzlis, 2003). Different levels of activation have been observed in brain regions common to saccadic and pursuit initiation (Burke & Barnes, 2008; Krauzlis, 2003). Burke and Barnes (2006) also suggested that there are different timing threshold criteria for the movement types, with a more stringent criterion for saccadic compared to pursuit eye movements.

Indeed, findings from saccadic eye movements showed shorter latencies for predictive saccades and greater timings shifts from RND tasks compared to pursuit eye movements. In accordance with Burke and Barnes (2006), these results also suggest differing motor delays and timing threshold levels between the two oculomotor subsystems. PRD saccadic latencies also exhibited greater intra and inter subject variability (see Figure 2.5 and Figure 2.7). Burke and Barnes (2006) also found a wide range of latency responses to PRD targets in

both saccadic and pursuit eye movements compared to reactive responses, with saccades showing a wider range of responses (~ -400 to ~80 ms) compared to pursuit. Self-initiated responses had increased variability and predictive saccade results showed more difficulty in maintaining the same level of prediction. Predictive pursuit movements are facilitated when a gap is presented during the start of an expected ramp stimulus (Barnes & Schmid, 2002). In our pursuit task, the visual stimulus was always present and this has been shown to inhibit predictive behavior and thus allowed pursuit to be influenced by the continuous updating of target position (Burke & Barnes, 2006). This reflected the adaptability of the pursuit system to include visual feedback to guide the eye close to the target (Burke & Barnes, 2006). It is also suggested that these adaptations had equal effects throughout the sequence and may explain why pursuit performance was similar across repetitions and components. In saccadic eye movement responses, the predictive drive occurred across components suggesting minimal visual feedback, whilst pursuit responses were influenced by both prediction and continuous target motion feedback. This was also reflected in the accuracy of the two types of eye movement. Predictive saccades had decreased accuracy compared to reactive RND visually guided responses and kept decreasing as participant showed more prediction across sequence components. In contrast, displacement absolute errors and gain were unchanged between RND and PRD pursuit suggesting some use of feedback during the predictive responses.

Using analogous sequence tasks, we were able to compare RND and PRD behaviour in saccadic and pursuit eye movements; but also, we were able to investigate oculomotor sequence learning using predictable stimuli. To our

knowledge, only one other study has used timing and spatially equivalent stimuli to make comparisons between the two eye movement types (Burke & Barnes, 2006). In addition, unlike previous studies that implement uni and bi-directional 2 step/ramp stimuli (Burke & Barnes, 2006) or repeated series of discrete 4 to 6 ramps stimuli (Collins & Barnes, 2005), we implemented a multiple ramp/step stimuli in which a component started where the previous one had ended using one of four possible directions. Oculomotor responses exhibited evidence of learning these sequences possibly through the use of stored target information in predictive responses and within 2-3 presentations. We also found different levels of adaptation between the eye moment types that suggest different timing signals and/or levels of activation in the neural circuitry between the eye movement types to achieve their independent aims (e.g., reach target location in saccades or achieve target velocity in pursuit) during sequence learning. There was also evidence of past history effects in both types of eye movements, possibly stored in short-term memory. We suggest that overall PRD responses were generated by an internal drive that presumably uses a short-term buffer that can be replayed for subsequent responses (Barnes & Schmid, 2002).

2.4.2 Effects of sequence length: 4 vs 8 component pursuit

One aim of this study was to test this buffer (mentioned above) by adding components to the sequence and assess whether the predictive drive endures throughout longer sequences. Our results from the repeated 4 component sequences are in accordance with previous findings from sequences of interrupted discrete 4-ramp studies, in which stimulus information related to at least 4 components of a sequence can be temporarily stored and released as a

predictive response throughout the entire discrete or continuous ramp sequences (Barnes & Schmid, 2002). In the present experiment, an 8 component sequence task was implemented and it was hypothesized that participants would exhibit difficulties in learning these longer sequences and show decreases in predictive measures. However, as in the 4 component sequences, shorter latencies were exhibited within the second presentation and maintained throughout the repetitions (also see Appendix B: Figure 1). In addition, RND to PRD timing shifts were also similar throughout the 8 component sequences compared to the 4 component sequences. Similar timing shifts compared to the shorter sequences, suggest similar degree of predicting the sequences. Collins and Barnes (2005) found that a steady state response from a 6 ramp stimulus was achieved after more repetitions (3-4) of the sequence compared to a 4 ramp stimulus (2-3). They suggested that these differences reflected the additional cognitive load on short-term memory.

There are several contrasting methods between Collins and Barnes's (2005) study and the present study. Collins and Barnes's (2005) experiment consisted discrete ramps that were uni or bi-directional along the horizontal plane and each ramp started at midpoint following a fixation period. In addition, they used several velocities for each repeated series. In the present experiments, participants had to learn a series of interconnected movements in 4 directions and only one target velocity had to be memorized throughout the experimental sequence learning block. It is suggested that the additional fixation periods between discrete ramps and the use of different velocities forced participants to code, store and retrieve target velocity and may include additional cognitive demands compared to our directional changes and use of a single velocity and

account for the need of more repetitions and different findings between our results and Barnes and Collins (2005). In addition, fixation periods may have introduced more variability in pursuit prediction with participants being able to increase prediction with repetition. In our continuous task, it may be that prediction was more restricted as participants had to predict the next component quickly regardless of the number of components. Moreover, the 8 components repeated responses seemed stereotyped and similar to SEQ1 but temporally shifted. It is also possible that participants learnt the pursuit sequences as a general pattern of continuous movement in contrast to learning each component individually (Collins & Barnes, 2005).

Collins and Barnes (2005) found low velocity predictive responses to the first presentation, which they suggested were "guesses" due to the high degree of expectancy of target movement. Barnes and Asselman (1991) presented periodic target motion stimuli with sudden perturbations to the stimulus parameters (e.g., frequency or amplitude). They observed a quick velocity build-up as part of the predictive drive and when the amplitude and direction of the target was unexpectedly changed, eye velocity exhibited inappropriate velocity and direction, which was corrected once the change was registered and not part of the predictive mechanism (Barnes and Asselman, 1991). We did not observe a consistent guesses or contamination from the previous series during the first presentation of each novel sequence, suggesting that prediction was not only function of high expectancy. It is possible that a transient decay effect was not observed due to the fixation periods inserted between repetitions and that once the subjects became aware of stimulus factors such as velocity, they then used this to predict the occurrence of the target turnarounds by SEQ2. Previous

research described in the present study clearly show that 4 component stimuli are can be easily stored and are within limits of short-term memory. Given that the shorter and longer sequences showed similar adaptation within repetitions and similar temporal shifts from RND to PRD responses and no big gain differences were observed, then it is possible that participants learnt the 4 and 8 component sequences in a similar way. We therefore also suggest that the predictive drive could also persist throughout the longer sequences, indicating participant's ability to store larger amounts of information.

Our results showed behavioral similarities when performing sequences of short and longer lengths. However, there were some differences between sequence lengths (i.e., peak acceleration and TTPV) that were present regardless of whether the sequence was reactive or predictive. Short sequences seemed to exhibit an overall temporal advantage compared to the long sequences and present higher peak accelerations. We also observed an increasing pursuit lag with respect to the target across the longer sequence components and particularly, latencies were greater in the last 4 components of the longer sequences. Again, this lag was present regardless of sequence type (RND or PRD). We then attribute these differences to a systematic effect of the task itself possibly related to decreased attention during longer sequences and not a learning-related effect. Still, RND conditions exhibited longer latencies compared to PRD in both sequence lengths. Timing delays in RND pursuit are suggested to be a result of not being able to match eye velocity to target velocity, while eye displacement is maintained closely to target displacement with the aid of the saccadic system (Barnes & Asselman, 1991; Bennett & Barnes, 2003). Reduced latencies during

PRD conditions suggested that some form of learning or preprogramming was occurring. Further investigations on how these continuous sequences can be pre-programmed and performed with shorter latencies could provide insight into learning in the oculomotor system and the longevity of the storage process.

Typically, sequence learning has been studied through repeated finger tapping tasks and learning is usually measured by a reduction in reaction time and the number of errors performed. Adaptations that occur with repetition are part of a fast learning stage and with extended practice; consolidation of the skill is acquired (Ungerleider et al., 2002). Studies have long suggested the existence of dissociation between short-term storage (over several second) and longer storage delays (over several minutes); but conflicting studies have also suggested links between short and long-term memory formation (Ranganath & Blumenfeld, 2005; Ranganath, Cohen, & Brozinsky, 2005). Our experiments involved learning of longer continuous sequences and thus, learning over longer periods. Investigating the neural circuits involved and comparing them to long-term learning and also to anatomical circuits found in short 2 ramp studies could provide insight into the links between the predictive short-term buffer and learning.

Chapter 3

3 Sequence learning: Eye only fMRI experiment

3.1 Introduction

Prior knowledge of where and when an object will appear or change direction enables preparation for an up-coming event and consequently a faster response. This type of learning has been shown to be true in many motor systems, including the oculomotor system. Repeated presentations of a motion stimulus such as constant-velocity ramps and single frequency interval steps, can elicit anticipatory eye movement responses after only a few repetitions (Barnes & Schmid, 2002; Shelhamer & Joiner, 2003). Eye movements made in the absence of a moving stimulus suggest an interaction between cognitive processes (i.e., prediction) and the preparation and generation of the motor response (Drew & van Donkelaar, 2007). Predictive estimations of the forth-coming target are internally generated and suggest the storage of the target's parameters (e.g., velocity or position) and subsequent release of the appropriate motor command (Barnes & Asselman, 1991; Poliakoff et al., 2005). Recent studies have shown that oculomotor predictive behaviour is modulated in the frontal eye fields (FEF) and supplementary eye fields (SEF) (Drew & van Donkelaar, 2007; Nyffeler et al., 2008). The SEF and FEF have been shown to influence both pursuit and saccadic eye movements (Burke & Barnes, 2006) suggesting a common predictive process (Nyffeler et al., 2008). Transcranial magnetic stimulation (TMS) applied to the SEF has been shown to facilitate prediction in saccadic and pursuit eye movements (Nyffeler et al., 2008). However, electrophysiological lesion and functional imaging studies suggest that within these brain areas, segregated neural

populations exist for saccades and pursuit (Burke & Barnes, 2006; Petit et al., 1997).

Predictive saccades elicited during sequence learning tasks use volitionally driven circuitry implicated in the maintenance of short-term memory, including the dorsolateral prefrontal cortex (DLPFC) located in the central sulcus (Burke & Barnes, 2008; Müri & Nyffeler, 2008). DLPFC activation has been found during the inhibition of reflexive saccades and pursuit, the preparation of a predictive saccade and in directional decision making of subsequent saccadic movements (Burke & Barnes, 2011; Müri & Nyffeler, 2008; Pierrot-Deseilligny et al., 2005). The DLPFC plays an important role in cognitive control (Müri & Nyffeler, 2008) and has direct connections to frontal lobe areas, specifically the FEF in lateral part of the precentral gyrus and SEF in the supplementary motor area region. In addition the DLPFC interacts with the anterior cingulate cortex (ACC), which is involved in on-line control of voluntary saccades (Heide et al., 2001), the posterior parietal cortex (PPC) and the parietal eye fields (PEF) in the intraparietal sulcus (Müri, Iba-Zizen, Derosier, Cabanis, & Pierrot-Deseilligny, 1996).

There are many areas in the brain used to track a moving target including the visual information processing area V5 also known as the medial temporal area (MT) and with the medial superior temporal area (MST) is commonly called the V5 complex (V5+), as well as intraparietal regions and the frontal lobe (Ding et al., 2009). Similar to saccades, the FEF are important structures for the control of smooth pursuit eye movements (Ding et al., 2009; Fukushima et al., 2002; Petit et al., 1997). The FEF and the prefrontal cortex (PFC) have been associated with prediction in pursuit, in visuospatial attention and short-term memory (Ding et al., 2009; Lencer et al., 2004). However, within the FEF, pursuit and saccade related

areas have been distinguished with weaker pursuit activation located more lateral in the precentral sulcus compared to saccadic activity related FEF structures (Ding et al., 2009; Petit et al., 1997). Anterior to the FEF areas, the DLPFC has been previously observed to be involved during predictive pursuit (Burke & Barnes, 2008; Schmid et al., 2001). However, Burke and Barnes (2008) found time-dependant decreased DLPFC activation as pursuit learning occurred during predictive conditions. In addition, Burke and Barnes (2008) found differences between saccadic and pursuit DLPFC activation with higher activity during pursuit compared with saccades. In addition, more saccade activation was found in the frontopolar regions during an analogous sequence learning task. This is a significant finding as the use of short-term memory for the storage of velocity is a lesser-known mechanism compared to the storage of positional information in saccadic eye movements (Burke & Barnes, 2008).

The PEF, equivalent to the lateral intraparietal (LIP) area in non-human primates, in the posterior parietal cortex, has been shown to modulate both saccadic and pursuit eye movements with a few neurons continuing to fire in the absence of a previously viewed moving stimulus, as shown by brain stimulation techniques (Krauzlis, 2003). Studies have suggested that the LIP areas contribute to saccadic spatial remapping when recalling targets from memory (Heide et al., 2001; McDowell et al., 2008). Other examples of parallel but distinct brain areas involved in saccades and pursuit are the basal ganglia and the cingulate cortex. The anterior cingulate (ACC) has not only been associated with the control of intentional saccades in sequence learning (Heide et al., 2001; Pierrot-Deseilligny et al., 2002), but has also been reported to be involved in the planning of pursuit and in the storage of the timing and velocity of previously viewed moving stimuli (Ding et al., 2009; Schmid et al., 2001). It has been shown that both the saccadic

region of FEF and the pursuit FEF region project onto the caudate with similar strengths, but to mostly non-overlapping networks (Krauzlis, 2003). The basal ganglia and caudate nucleus are involved in the control of volitional saccades through inhibitory inputs via the SC in the brain stem (Hikosaka et al., 2000). Clinical studies have indicated that damage to the basal ganglia resulted in impaired pursuit, however, little is known to what extent pursuit is controlled by this structure (Hikosaka et al., 2000; Krauzlis, 2004).

There is a wide structural overlap between saccadic and pursuit eye movements with parallel but distinct neural populations for the control of the two subsystems. The cortical control of the oculomotor system requires further investigation. Investigating areas in the brain during analogous tasks between these subsystems may provide valuable information on how the two differ and/or interact. Burke and Barnes (2006 and 2008) investigated neural and behavioural characteristics of saccade and pursuit during equivalent step and step-ramp paradigms respectively. This task allowed for the direct evaluation of the two types of eye movements during random and predictable conditions. They found that the two subsystems indeed overlap in cortical regions but that these are utilized differently depending on the demands of the task, for example, velocity dependent regions in the right DLPFC and position dependent areas in the ventral PFC and left DLPFC, all located within the frontal lobe (Burke & Barnes, 2008). Burke and Barnes (2008) also compared memory driven responses to more reflexive visually guided behaviour and found learning-related activation during predictable stimuli in areas such as visual area V5, DLPFC and cerebellum, which may be important during motor learning mechanisms.

Extending from this, we designed an analogous saccadic and pursuit sequence task with comparable visual and temporal characteristics. In contrasts

with previous studies that have observed brain activation when using a series of discrete ramps or simple two-step/ramp stimuli to observe predictive behaviour as evidence for learning, we presented a continuous movement of interlinked components that better represent real-world tracking, and was similar to one of Collins and Barnes (2005) experiments for pursuit. Collins and Barnes (2005) sequence learning task consisted of repeated constant velocity ramps in which they measured the build up of predictive responses. We also presented predictable (i.e., repeated) sequences and random sequences to investigate memory-driven and visually guided saccadic and pursuit behaviour and brain pathways. In addition, we tested short-term memory capacity by using 4 and 8 component (i.e., ramps/steps) sequences. The main goals of this study where: i) to contrast memory-driven (PRD) and visually guided (RND) cortical pathways in the saccadic and pursuit system; ii) to determine sequence learning activation in the oculomotor system and to investigate the role of short-term memory in sequence learning in the pursuit system.

From previous findings, we hypothesized that in PRD sequences, memory-related brain activation would be observed compared to the more reactive visually guided areas in RND trials. We suggested that in our paradigm, prediction involved the use of short-term memory based on prior experience to learn the complex sequences (Burke & Barnes, 2008; Schmid et al., 2001). This was in accordance with behavioural results, which showed anticipatory responses after having performed a sequence once and across all sequence components (Poliakoff et al., 2005). We also suggested that the saccadic and pursuit system would show parallel but distinct activation levels in these memory related areas.

A study by Tomasi and colleagues (2007) tested increases in working memory and visual attention load and found that an increase in cognitive load

showed larger BOLD responses in these working memory and visual attention areas. It was then hypothesized that an additional load placed in working memory would show differential brain activation during the short compared to the long sequences.

3.2 Methods

3.2.1 Participants

Thirteen participants 22 to 34 years of age (26.1 ± 3.83 yrs, 9 females) with normal or corrected eyesight and no known neurological conditions took part in the study. All participants gave informed consent prior to experimental sessions. This study was approved by the local and regional NHS ethical committee and by The University of Manchester and Leeds ethical committees and conducted in accordance with the standards laid out in the 1964 Declaration of Helsinki.

3.2.2 Experimental paradigm

Participants performed the same experimental task in a dark laboratory setting 1 week prior to the scanning session. Experimental procedure and eye data analysis are described in detail in chapter 2. In this session, the 6 blocks: 1) 4 component PRD saccade sequence (4PRDs); 2) 4 component RND saccade sequence (4RNDs); 3) 4 component PRD pursuit sequence (4PRDp); 4) 4 component RND pursuit sequence (4RNDp); 5) 8 component PRD pursuit sequence (8PRDp); and 6) 8 component RND pursuit sequence (8RND) were presented in randomized order within and between participants, and all

participants performed the same sequences within each block. New sequences were to those performed in the laboratory session were presented. Participants' instructions were to follow the target with their eyes and keep up with it as best they could and they were explicitly aware that in PRD blocks each sequence was repeated while in the RND blocks all of the sequences were different from each other and from the PRD sequences.

3.2.3 fMRI experimental setup and acquisition

Eye movements were recorded inside the fMRI scanner using an ASL optical video eye tracker (Applied Science Laboratory, Bedford, MA) that sampled at a rate of 60 Hz. Participants were supine on the scanner bed. The head coil provided support for the participants' head and with the addition of cushions helped to minimize head movements during scanning. An image of the eye was reflected via a mirror positioned on the head-coil to the ASL video camera positioned outside the scanner near the head of the participant. A second mirror was used to reflect the image of the experimental paradigm projected on a 180 x 110 mm screen located at the subject's feet in front of the scanner (Figure 3.1). Prior to experimental trials, adjustments to the mirrors where made to obtain a good eye signal and to make sure that the screen was fully visible by the participant. Eye movement data were collected and stored for offline analysis. The same paradigm as in the laboratory experiments was used and again participants completed the 6 blocks in random order. Novel sequences were designed for these scanner sessions and all participants performed the same sequences in the same order within each block. Calibrations for the eye took place in the scanner prior to each experimental block. The room was kept as dark as

possible and the lights were turned on in between blocks to maintain alertness. The scanner experimental sessions lasted about 45 minutes.

The fMRI scanner consisted of a 3T (Phillips 3.0 T Achieva) with an eight-channel sense head-coil (Achieva 3.0 T Neuro Coil) designed to reduce the signal-to-noise ratio (Burke & Barnes, 2011). The BOLD changes in brain activity were measured while participants performed the PRD and RND saccadic and smooth pursuit tasks. Scans were collected using T2*-weighted spin echo pulse (TR of 2000 ms, TE of 35 ms; 90° flip angle, FOV of 250 mm, 1.8 x 1.8 x 4 mm^3 voxel size and a total of 30 slices). Data were pre-processed using SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/) in which spatial realignment, co-registration to each participant's mean EPI fMRI scan, normalization (MNI model) to a local T2* (EPI) template and smoothing using FWHM Gaussian filter (8mm) was applied to all participants' fMRI scans. In addition, fMRI data were high pass filtered at a 128 Hz cut off frequency.

Event analysis was performed over the PRD conditions across the 4 identical presentations of a sequence (i.e., SEQ1, SEQ2, SEQ3 and SEQ4) and over the RND conditions in pursuit and saccadic tasks, which resulted in a 6 condition matrix (Figure 3.2). An event analysis was then performed, which included fixation periods and predictive or random presentations of a sequence (i.e., a series) from each block (Figure 3.3). This analysis allowed us to assess changes in the BOLD signal related to repeated versus random sequence presentations and to include a control (fixation) and separate activity related to the motor response type and not the stimulus itself. The individual participants' contrasts were then entered in a group level analysis where a one-sample t-test was performed for each global contrast ($T > 3$). The resulting MNI coordinates were verified using the SPM8 anatomical toolbox (Eickhoff et al., 2005) and then converted into

Talairach space for anatomical labelling (Talairach Daemon software http://www.nitrc.org/projects/tal-daemon/) (Lancaster et al., 2000). Contrast analysis corresponded to i) the analysis of saccade and pursuit in PRD and RND sequences and ii) the differences in long and short pursuit PRD sequences. One participant's fMRI data had to be excluded due to faulty images.

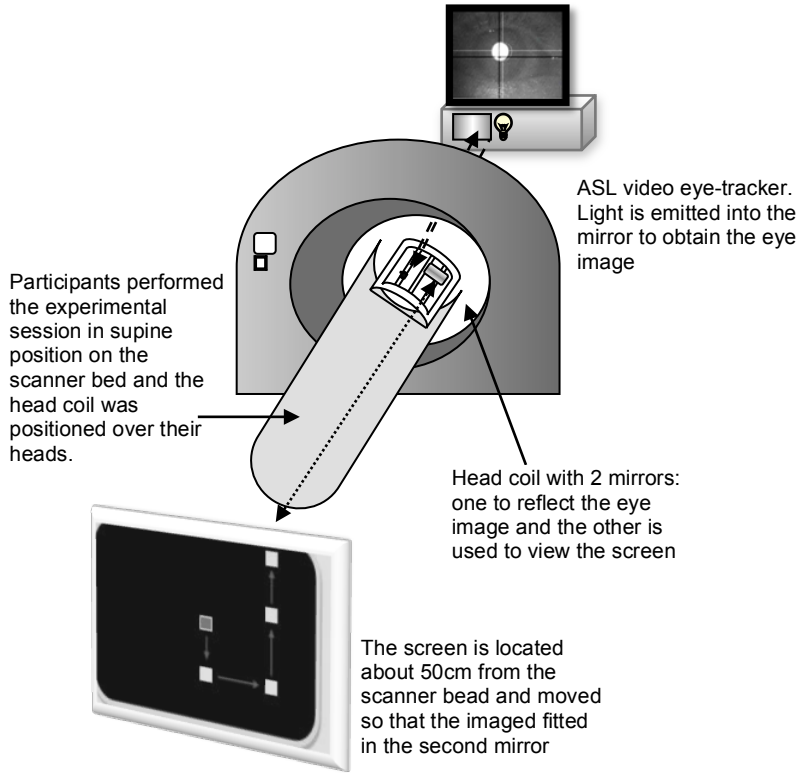

Figure 3.1. Representation of the experimental set up in the fMRI scanner. Eye movements were monitored by capturing the pupil of the video image. The display screen was located at the feet of the participant and viewed by the subject via an adjustable mirror on the head coil. Padding was provided inside the coil to prevent head movements during the scan.

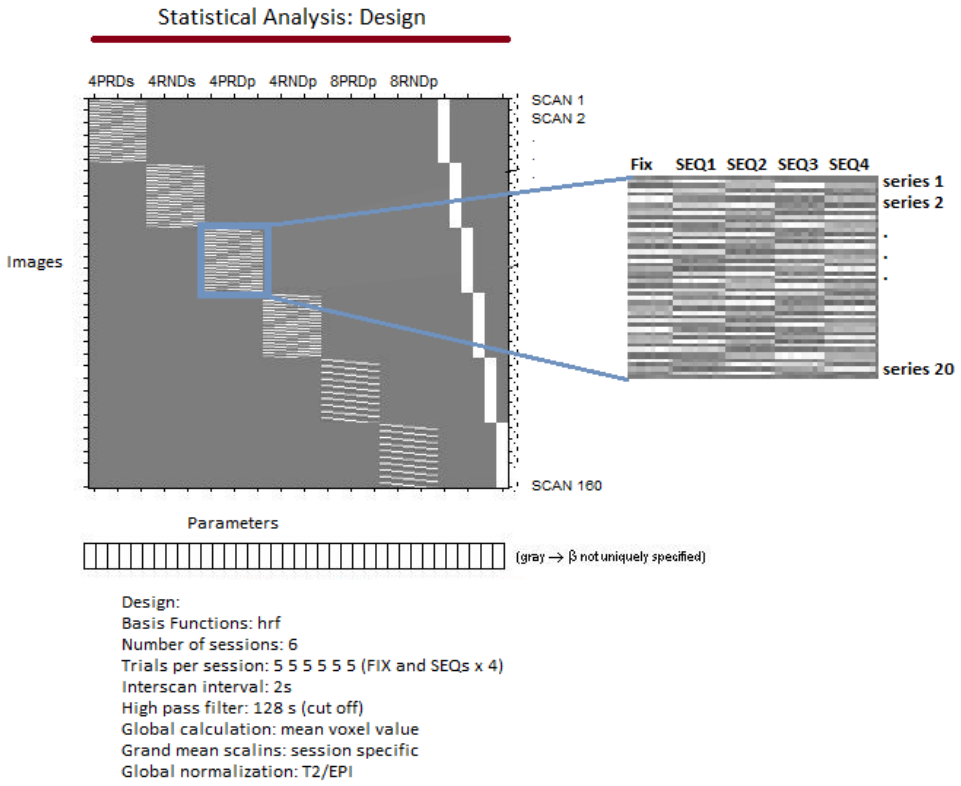

Figure 3.2. Design matrix for the 6 experimental conditions. Each session/condition consisted of a fixation and 4 repetitions of a sequence (SEQ1, SEQ2, SEQ3 and SEQ4) during the PRD conditions or 4 novel RND sequences each time. A total of 20 series and 10 series were presented for a 4 component and an 8 component sequence respectively.

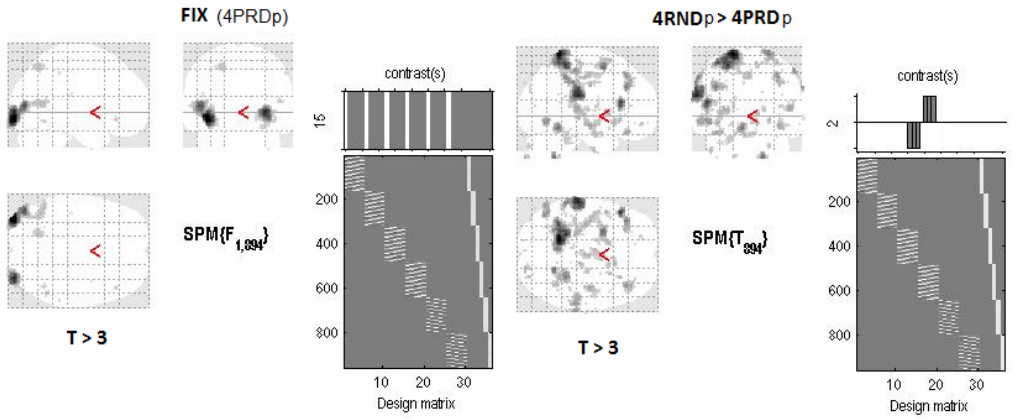

Figure 3.3. Example of a single participant's fixation F contrast (left) and 4 component pursuit T contrast (right). The figures show brain activation corresponding to each contrast and the design matrix, with the events contrasted. Fixation activity was removed from the other contrasts. In addition, SEQ1 was also excluded as this corresponded to a reactive response in the PRD condition. The design matrix on the right shows the allocation of positive activation for RND conditions and negative activation for PRD conditions.

3.3 Results

An analogous saccade (SAC) and pursuit (PUR) sequence learning task using a predictable sequence versus a random sequence were implemented. The temporal and spatial characteristics of the stimulus were equivalent for the PUR and SAC experimental blocks to allow for comparisons. Participants performed this experiment in a dark laboratory environment prior to the scanning session. We monitored their eye movements inside the scanner and after further inspection concluded that they were able perform the task effectively inside the scanner. Eye data results from the laboratory session are reported in full in chapter 2. In summary, oculomotor responses exhibited shorter latencies during repeated sequence (PRD) conditions compared to RND conditions. In addition, timing shifts were evident within the second presentation of the sequence in both eye movement types; but did not change with more repetitions in pursuit, whilst in saccades latencies decreased even more to the third and fourth presentations. Results also revealed timing differences between saccade and pursuit conditions during these tasks. Specifically, saccadic eye movements revealed greater RND to PRD timing shifts compared to pursuit eye movements. We also found that prediction in pursuit was similar across the components of the PRD sequences, whilst saccades showed differences in the level of prediction across components and higher variability. Based on behavioural data, we performed a series of contrasts to identify areas involved in predictive and reactive visually guided saccadic and pursuit eye movements. For the analysis of fMRI images, fixations were used as baseline measures to enable differentiation of the activation of eye movement areas and the stimulus effect on cortical regions. To assess activation of areas associated with sequence learning, BOLD activity from the repeated sequences that showed stable and significant latency decreases in the behavioural

data (i.e., SEQ2, SEQ3 and SEQ4) were contrasted. Activation during PRD and RND sequences was identified as predominantly memory-generated and visually guided respectively and isolated for both types of eye movements.

3.3.1 RND versus PRD contrasts: Saccades and pursuit

4RNDs > 4PRDs (saccade alone)

The 4RNDs > 4PRDs contrast active regions included the early visual areas (V1/V2, BA18/BA17) in the left occipital cortex, right middle temporal gyrus (MTG, BA21), right inferior parietal lobe (IPL, BA40), right superior parietal lobe (SPL, BA7) and bilateral FEF (BA6) in the superior regions of the prefrontal cortex (PFC). Activity that was higher for predictive tasks was found in right V2 (BA18), basal ganglia (caudate nucleus) and left cerebellum (CBM), right anterior cingulate cortex (ACC, BA24) and left SEF (BA6) in the PFC were also observed (Figure 3.4A and Table 3.1).

4RNDp > 4PRDp (pursuit alone)

Activity was found for random pursuit in the left cerebellum (CBM), left middle temporal gyrus (MTG, BA21), right inferior temporal gyrus (BA20), left IPL (BA40), extrastriate visual area 5 in the left occipital cortex (V5, BA19). Also, areas of the PFC consisted of the right DLPFC (BA9) and bilateral FEF (BA8) in the middle frontal gyrus. Brain areas more active for predictive pursuit included the right insular cortex (IC, BA13), left anterior cingulate cortex (ACC, BA24/BA32) and right IPL (BA40). PFC areas consisted of the right DLPFC (BA9), left vetrolateral prefrontal cortex (VLPFC, BA45) in the inferior frontal gyrus, left SEF (BA6) and right FP (BA10) in the middle frontal gyrus (Figure 3.4B and Table 3.1).

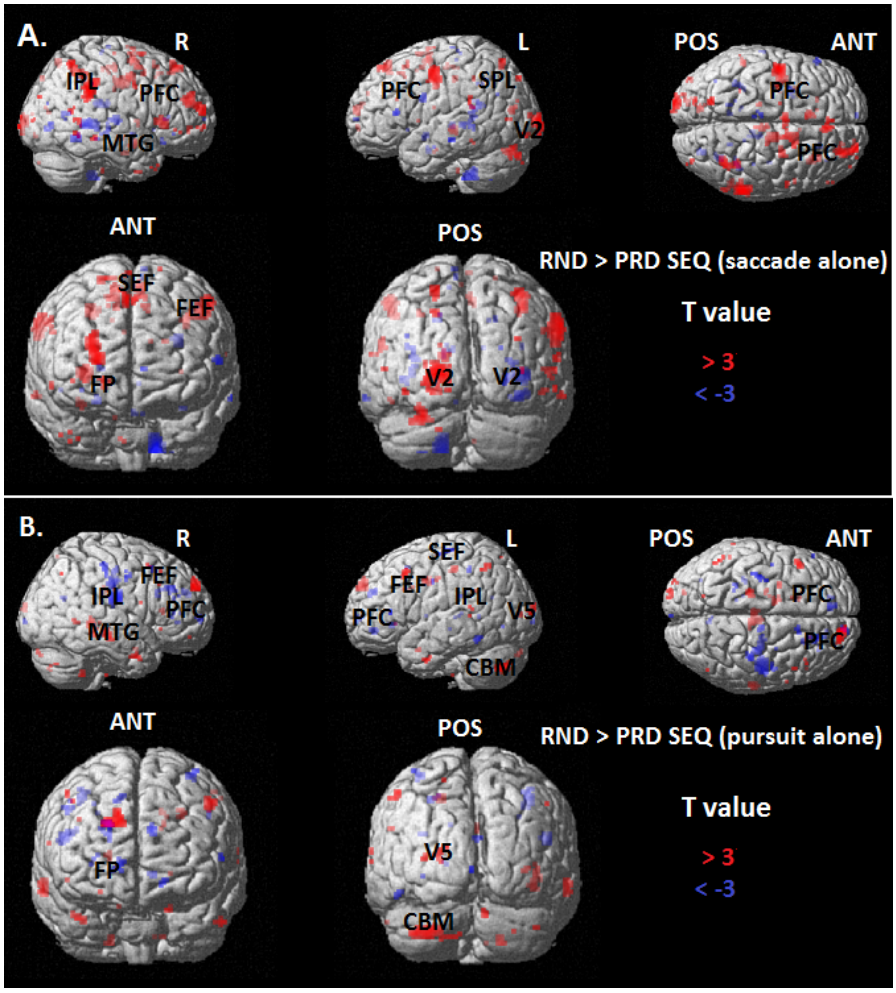

Figure 3.4. Group contrasts for 4PRD vs. 4RND sequences for saccades (A) and pursuit (B) with baseline activity removed. Red areas correspond to positive activation and blue areas show negative activation. Images are labelled according to left (L), right (R), posterior (POS) and anterior (ANT) views.

Table 3.1. Saccade and pursuit PRD > RND group contrasts.

Contrast		cluster size	T	Z	MNIx mm	MNIy mm	MNIz mm	R/L side	Anatomical area	Brodmann area	*location
4RNDs >4PRDs	4RND	2711	7.18	4.17	-20	106	2	L	V1/V2	BA18/BA17	MOG
		3049	6.36	3.94	60	-42	28	R	IPL/SMG	BA 40	
		742	5.77	3.75	24	60	12	R	DLPFC	BA 9	SFG
		1692	5.32	3.58	-46	-12	42	L	FEF	BA6	SFG
		73	4.94	3.44	64	-38	-12	R	MTG	BA21	
		415	4.22	3.13	34	-2	-20	R	PHC		
		469	3.97	3	20	-79	54	R	SPL	BA7	
		632	3.92	2.98	56	-2	38	R	FEF	BA6	
	4PRD	144	6.2	3.89	38	-44	0	R	BG		Caudate
		138	3.52	2.77	-12	-43	-37	L	CBM		
		19	3.8	2.92	26	-76	-2	R	V2	BA 18	
		43	3.39	2.7	12	10	37	R	ACC	BA24	
		32	3.34	2.67	-30	2	26	L	SEF	BA 6	PCG
		101	3.0	2.36	-36	-50	20	L	IC	BA 13	
4RNDp > 4PRDp	4RND	938	6.87	4.09	-28	-70	-32	L	CBM		
		193	5.5	3.65	40	4	-32	R	MTG	BA 21	
		157	5.44	3.63	20	52	32	R	DLPFC	BA 9	SFG
		137	4.14	3.09	-46	18	44	L	FEF	BA 8	MFG
		183	3.53	2.78	-50	-56	50	L	IPL	BA 40	
		101	3.7	2.87	-53	-3	-28	L	ITG	BA 20	
		351	3.44	2.73	-22	-96	18	L	V5	BA 19	
		167	3.37	2.69	48	14	47	R	FEF	BA 6	MFG
		169	3.06	2.51	50	-62	44	R	IPL	BA 40	
	4PRD	973	5.89	3.79	48	-16	22	R	IC	BA 13	
		150	3.98	3.01	20	34	32	R	DLPFC	BA 9	MFG
		13	3.68	2.86	-56	30	8	L	VMPFC	BA 45	IFG
		36	3.4	2.71	28	-39	44	R	IPL	BA40	SMG
		35	3.32	2.66	14	60	2	R	FP	BA 10	
		52	3.13	2.55	-16	48	-8	L	ACC	BA 32	
		65	3.1	2.54	-6	44	28	L	DLPFC	BA 9	MFG
		100	3.01	2.48	-34	-20	64	L	SEF	BA 6	PCG
		21	3.0	2.47	-10	31	-3	L	ACC	BA 24	

Table includes contrast, cluster size, significance level, MNI coordinates and brain areas
** Parahypocampal Gyrus (PHC), precentral gyrus (PCG), superior marginal gyrus (SMG), Middle frontal gyrus (MFG), superior frontal gyrus (SFG), middle occipital cortex (MOC).*

3.3.2 Saccade versus pursuit contrasts

4PRDs > 4PRDp

The 4PRDs > 4PRDp revealed activation of the left cerebellum, parahippocampal gyrus, the right MTG (BA21), right superior temporal gyrus (STG, BA39), left ACC (BA24) and left FEF (BA8), right SEF (BA6) and the right DLPFC (BA9) in the superior and middle frontal gyruses. Activity corresponding to 4PRDp was found in the parahippocampal gyrus, the IC (BA13), precuneus (BA7), right ACC (BA24), and the SEF (BA6/BA4), and the DLPFC (BA46) and VLPFC (BA47) in the inferior PFC (Figure 3.5A and Table 3.2).

Saccade > Pursuit (4PRD and 4RND combined)

Saccade > pursuit contrasts showed activity for saccade tasks in right MTG (BA21) and left superior temporal gyrus (STG, BA22), left ACC (BA24), left CBM, parahippocampal gyrus, bilateral FEF areas (BA6) in the medial frontal gyrus and right SEF (BA6) in the precentral gyrus, as well as the right IFG (BA47). In addition, activation for pursuit was observed in the parahippocampal gyrus, left basal ganglia, the right ACC (BA24), left thalamus, left IC (BA13) and visual areas in the occipital cortex (V2/V5, BA18/BA19) (Figure 3.5B and Table 3.2).

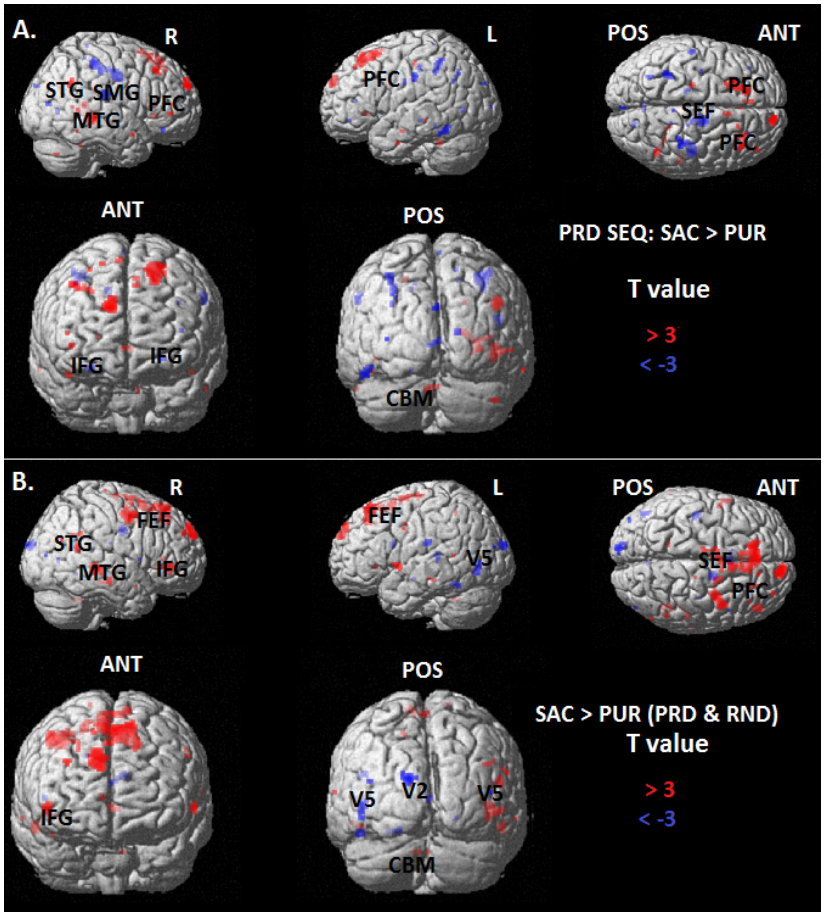

Figure 3.5. Group contrasts for 4PRDs > 4PRDp (A) and overall saccade > pursuit (B). Red areas correspond to positive activation and blue areas show negative activation. Images are labelled according to left (L), right (R), posterior (POS) and anterior (ANT) views.

Table 3.2. 4PRDs > 4PRDp and overall SAC > PUR group contrasts.

Contrast		cluster size	T	Z	MNIx mm	MNIy mm	MNIz mm	R/L side	Anatomical area	Brodmann area	*location
4PRDs > 4PRDp	SAC	411	4.69	3.33	46	-38	-6	R	MTG	BA21	
		1190	4.35	3.19	-16	32	52	L	FEF	BA 8	SFG
		223	4.21	3.12	14	58	28	R	DLPFC	BA9	SFG
		404	4.15	3.09	32	-50	-2	R	PHC		
		490	3.84	2.94	46	-56	32	R	STG	BA 22	
		667	3.82	2.93	38	26	44	R	DLPFC/FEF	BA 8/BA9	MFG
		60	3.75	2.89	0	25	-1	L	ACC	BA 24	
		364	3.66	2.85	-4	-30	-23	L	CBM		Culmen
		102	3.26	2.63	6	10	62	R	SEF	BA 6	SFG
	PUR	918	5.88	3.78	14	-12	38	R	ACC	BA 24	
		350	5.04	3.48	42	-24	22	R	IC	BA 13	
		44	3.99	3.02	26	32	-13	R	VLPFC	BA47	IFG
		612	3.64	2.84	-30	-46	48	L	SPL	BA 7	
		202	3.53	2.78	12	-37	66	R	SMG		
		161	3.31	2.66	-34	-12	30	L	SEF	BA 6	PCG
		47	3.21	2.6	4	-36	68	R	SEF	BA 4	PCG
		170	3.11	2.54	-22	-27	-8	L	PHC		
		52	3.03	2.49	-38	35	12	L	DLPFC	BA 46	IFG
SAC > PUR	SAC	2001	7.19	4.18	50	-34	-4	R	MTG	BA21	
		5209	5.6	3.69	38	-2	44	R	FEF	BA 6	MFG
		209	4.73	3.35	46	34	-2	R	IFG	BA 47	
		210	4.49	3.25	-58	8	0	L	STG	BA 22	
		232	4.38	3.2	0	26	-2	L	ACC	BA 24	
		148	4.31	3.17	26	-28	-16	R	PHC	BA 35	
		115	3.87	2.96	16	-24	72	R	SEF	BA 6/BA 4	PCG
		96	3.3	2.65	-52	0	36	L	FEF	BA 6	
		602	3.21	2.6	42	4	-20	R	STG	BA 22	
		32	3.17	2.58	-12	-32	-18	L	CBM		Culmen
	PUR	110	5.49	3.65	16	-8	36	R	ACC	BA 24	
		662	4.2	3.12	-24	-32	8	L	Thalamus		Pulvinar
		945	4.16	3.1	-12	-96	20	L	V2/V5	BA 19/BA 18	
		232	4.15	3.09	-42	-26	24	L	IC	BA 13	
		230	3.62	2.83	-10	12	22	L	BG		Caudate
		71	3.05	2.51	-18	-8	-14	L	PHC		
		115	3.01	2.48	46	-82	10	R	V5	BA 19	
		17	2.91	2.42	-20	40	0	L	ACC	BA 32	

Table includes contrast, cluster size, significance level, MNI coordinates and brain areas
** Parahypocampal Gyrus (PHC), precentral gyrus (PCG), superior marginal gyrus (SMG), Middle frontal gyrus (MFG), superior frontal gyrus (SFG), middle occipital cortex (MOC).*

3.3.3 Sequence length contrasts

Behavioural data indicated prediction during the 4 component pursuit sequences. We implemented sequences containing 8 constant velocity components and thus, these sequences had twice the items to remember and each sequence lasted twice as long. Pursuit eye movement results from short versus long sequences are explained in full in chapter 2. In summary, participants showed significantly shorter latencies during the 2nd, 3rd and 4th presentations of the sequence during the 8PRD sequences compared to the first presentation, with no significant latency differences between repetitions. In addition, prediction in

the longer pursuit sequences did not differ compared to the 4 component sequences (when comparing latencies). In contrast, TTPV and acceleration values showed differences between sequence lengths with participants exhibiting significantly increased TTPV values and lower accelerations in the 8PRD sequences compared to the 4PRD sequences in pursuit. TTPV results may suggest a decreased ability to store stimulus parameters, such as velocity. To investigate whether longer sequence tasks had effects on the brain areas responsible for learning and prediction we performed contrasts between the 8PRD and 8RND and between the 8 and 4 component PRD sequences. First, we assessed whether the 8 component sequence task rendered similar active areas compared to the 4 component pursuit task.

8RNDp > 8PRDp

8RND > 8PRD pursuit contrasts revealed activity for random conditions in the right cerebellum and areas in the PFC including the bilateral FEF (BA6), right DLPFC (BA9), left VLPFC (BA47) and inferior frontal gyrus (BA44/BA45); as well as the visual areas V5 (BA19) in the left occipital region and V1/V2 (BA18). Activation corresponding to predictive sequences was apparent in the basal ganglia, ACC (BA32) and the left DLPFC (BA9); as well as activation in the right temporal cortex (BA22) (Figure 3.6 and Table 3.3).

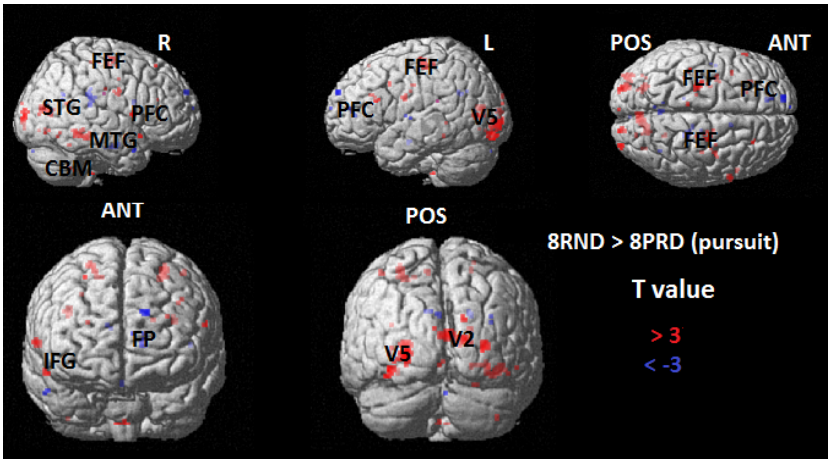

Figure 3.6. Group contrasts for the 8 component RND > PRD sequences. Red areas correspond to positive activation and blue areas show negative activation. Images are labelled according to left (L), right (R), posterior (POS) and anterior (ANT) views.

Table 3.3. The 8 component sequence group contrasts.

Contrast		cluster size	T	Z	MNIx mm	MNIy mm	MNIz mm	R/L side	Anatomical area	Brodmann area	*location
8RND >8PRD	RND	3095	5.02	3.47	-28	-20	56	L	FEF	BA 6	
		630	3.83	2.94	20	-16	56	R	FEF	BA 6	MFG
		89	3.64	2.84	26	-52	-22	R	CBM		
		66	3.37	2.69	26	32	22	R	DLPFC	BA 9	
		133	3.36	2.68	10	-76	19	R	V2/V1	BA18	
		175	3.3	2.65	-27	-93	6	L	V4/V5	BA19	
		243	3.11	2.54	-28	38	-8	L	VLPFC	BA 47	MFG
		355	3.05	2.5	12	-28	2	R	Thalamus		
		97	3	2.42	52	20	16	R	IFG	BA 44/45	
	PRD	36	3.8	2.92	-14	58	30	L	DLPFC	BA 9	
		45	3.24	2.62	12	8	20	R	BG	Caudate	
		8	3.04	2.5	4	-88	-28	R	CBM		
		82	3.03	2.5	-12	44	6	L	ACC	BA 32	
		50	3.03	2.49	48	-10	4	R	STG	BA 22	
		269	3	2.4	0	-60	28	L	ACC	BA 32	

Table includes contrast, cluster size, significance level, MNI coordinates and brain areas
** Parahypocampal Gyrus (PHC), precentral gyrus (PCG), superior marginal gyrus (SMG), Middle frontal gyrus (MFG), superior frontal gyrus (SFG), middle occipital cortex (MOC).*

8PRDp > 4PRDp

Contrasts for the predictive sequences induced activity in similar areas of the brain with activity corresponding to the longer sequences found bilaterally in SEF (BA6), the right DLPFC (BA46), the left IPL (BA40), CBM and thalamus. Activity corresponding to shorter sequences was also found bilateral PFC in SEF (BA6/BA4) and DLPFC (BA46/BA9), and the right ACC (BA32), visual areas V5

(BA19), right inferior temporal gyrus (ITG, BA20), left SPL (BA7) and basal ganglia (Figure 3.7A and Table 3.4).

8 > 4 component (PRD and RND pursuit combined)

Long > short sequence contrasts revealed areas in common between the different sequence lengths. Activity corresponding to the 8 component sequences was found in bilateral PFC in the medial frontal gyrus (BA10), bilateral STG (BA22), right ACC (BA32), right middle temporal (MT), parahippocampal gyrus and the left CBM. More active regions for the 4 component sequences revealed activation in PFC in right FP (BA10); right DLPFC (BA46), right ACC (BA24/BA32), the left IC (BA13), right superior temporal (ST/BA39) and the parahippocampal gyrus (Figure 3.7B and Table 3.4).

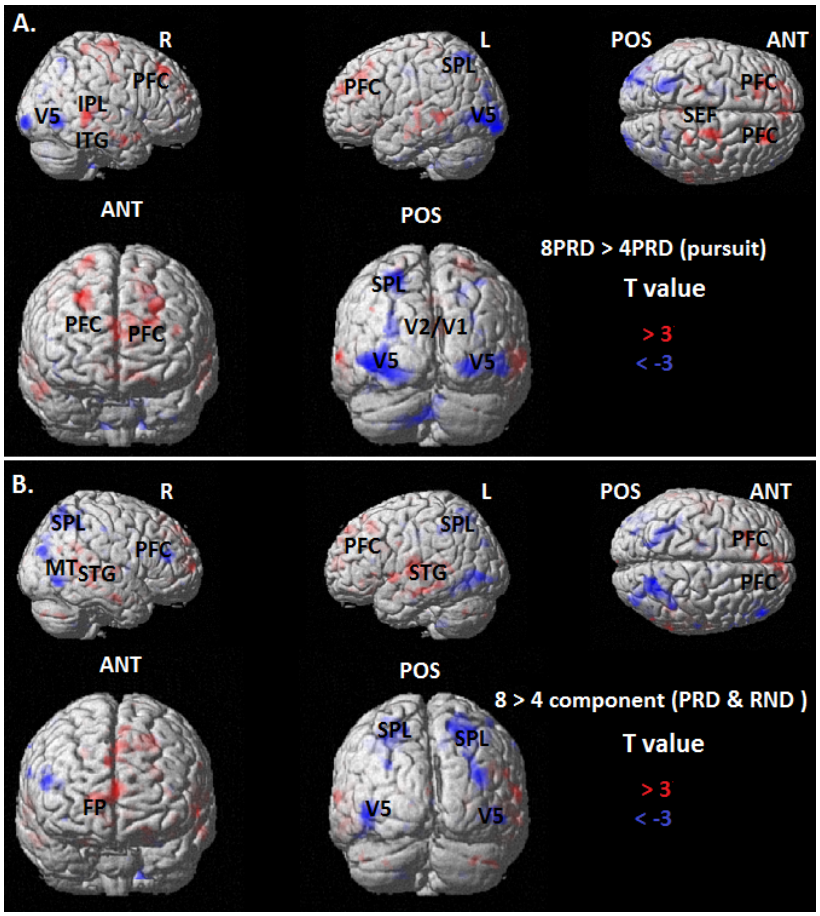

Figure 3.7. Group contrasts for random 8PRDp > 4PRDp (A), and overall 8 > 4 component pursuit PRD and RND sequences (B). Red areas correspond to positive activation and blue areas show negative activation. Images are labelled according to left (L), right (R), posterior (POS) and anterior (ANT) views.

Table 3.4. The 4 and 8 component sequence group contrasts

Contrast		cluster size	T	Z	MNIx mm	MNIy mm	MNIz mm	R/L side	Anatomical area	Brodmann area	*location
8PRD > 4PRD	8PRD	25715	12.77	5.24	22	-22	70	R	SEF	BA 6	PCG
		757	4.94	3.44	-20	-34	70	L	SEF	BA6/4	PCG
		158	4.31	3.17	6	-100	18	R	Cuneus	BA 18	
		450	3.98	3.01	-40	-78	36	L	Precuneus	BA 19	
		26	3.6	2.82	32	-84	-36	R	CBM	Uvula	
		17	3.52	2.78	-34	-32	32	L	IPL	BA 40	
		50	3.31	2.66	-2	-10	8	L	Thalamus		
		71	3.01	2.48	58	30	12	R	DLPFC	BA 46	
		68	3	2.33	16	-94	8	R	BA 18	V2/V1	
	4PRD	19026	17.31	5.75	-40	-78	2	L	V5	BA 19	
		2114	9.61	4.73	-24	-60	52	L	SPL	BA 7	
		1180	6.8	4.07	-22	-12	42	L	SEF	BA 6	PCG
		649	5.1	3.5	43	-71	2	R	V5	BA19	
		182	4.9	3.42	4	2	14	R	BG		
		90	4.13	3.08	34	-4	-46	R	ITG	BA 20	
		191	4.08	3.06	48	42	14	R	DLPFC	BA 46	
		59	3.76	2.9	12	-26	42	R	ACC	BA 32	
		22	3.49	2.76	48	-2	56	R	SEF	BA 6	
		28	3.23	2.61	-58	14	32	L	DLPFC	BA 9	
8 > 4	8	562	6.18	3.88	14	64	6	R	FP	BA 10	
		1435	5.75	3.74	-52	-20	2	L	STG	BA 22	
		422	5	3.46	66	-48	10	R	STG	BA 22	
		374	4.96	3.44	-8	28	-6	L	ACC	BA 32	
		345	4.94	3.44	56	-34	-2	R	MT		
		32	4.34	3.18	-48	-64	-34	L	CBM		
		1026	4.02	3.03	-26	26	26	L	MFG		
		124	3.7	2.87	-20	2	-20	L	PHC		
		137	3.59	2.81	10	44	-4	R	ACC	BA 32	
	4	19170	7.71	4.31	10	-10	36	R	ACC	BA 24	
		2209	5.57	3.68	38	34	18	R	DLPFC	BA 46	
		83	4.62	3.3	-38	26	14	L	IC	BA 13	
		494	4.05	3.04	20	6	42	R	ACC	BA 32	
		71	3.66	2.85	38	56	-8	R	FP	BA 10	
		69	3.5	2.76	32	-16	-24	R	PHC		
		13	3	2.45	34	12	-38	R	ST	BA 39	

Table includes contrast, cluster size, significance level, MNI coordinates and brain areas
** Parahypocampal Gyrus (PHC), precentral gyrus (PCG), superior marginal gyrus (SMG), Middle frontal gyrus (MFG).*

3.4 Discussion

We designed analogous saccadic and pursuit sequence tasks to observe brain activity related to sequence learning by using PRD stimuli conditions and contrasting these to RND sequences. It is important to note that we measured eye movement latencies at each component of a sequence and that predictive behaviour was taken as evidence for learning the sequences. Indeed, behavioural data revealed shorter latencies in both eye movement types when performing

predictable (i.e., repeated) sequences compared to RND sequences and these timing shifts were evident within the second presentation of a sequence. However, these findings also showed some differences in adaptation levels of sequence learning between saccades and pursuit and showed differing timing threshold levels. From this, we hypothesized that the areas in the brain responsible for sequence learning would show parallel but distinct sub-regions and/or levels of activation corresponding to pursuit and saccadic eye movements. To contrast PRD and RND conditions we eliminated SEQ1 activity to avoid contamination of activation corresponding to reactive behaviour during predictable conditions. Contrasts showed many similar active brain regions in pursuit and saccadic PRD and RND tasks, with PRD activity showing memory-related regions and RND activity involving reactive pursuit and saccadic regions that have previously been reported in other lesion and fMRI studies investigating reactive visually-guided behaviour. In addition, RND conditions involved more overall brain activity compared to PRD conditions. Burke and Barnes (2008) demonstrated a time-dependant decrease in BOLD activation in areas such as the DLPFC during predictive conditions in a step/step-ramp paradigm for saccades and pursuit respectively. They suggested that this decrease in activation was correlated to learning the sequence and that a steady-state in predictive conditions represented a more automatic response with less decision-making and use of working memory. With our behavioural results indicating participants were able to learn the sequences quickly, we suggest that RND activation was predominant compared to the more automatic learnt responses in PRD conditions. We also investigated brain areas related to the longer and shorter sequences. We found activation of more memory and attention related areas involved during the longer movements sequences compared to the shorter sequences, possibly due to

additional task-related cognitive load. Overall, our results showed higher PFC activation during the longer sequences suggesting more cognitive processing during these sequences.

3.4.1 4RND versus 4PRD: Saccades

The 4RNDs > 4PRDs contrast higher activation in areas corresponding to the RND conditions. Specifically, higher BOLD activation was observed in the PFC for the randomized task. Early visual areas V1 and V2 were also more active during RND trials compared to the PRD conditions and areas of the parietal cortex (SPL and IPL). During our task visual stimulus information is registered in the primary visual cortex (V1) and sent to the extrastriate cortical regions (V2), in the middle occipital gyrus. These visual areas are involved in the mapping of the stimulus in visual space (McDowell et al., 2008). Our results present similar findings to Burke and Barnes (2008) who found increased activation in early visual areas (V1 and V2) during random stimuli presentations compared to predictable stimuli. Higher RND activation in these areas possibly reflects higher attentional demands to perform the unknown sequences (Büchel et al., 1998; Burke & Barnes, 2008).

From the occipital cortex and visual areas, stimulus information such as position travels to areas in the parietal cortex including the superior parietal lobe in Brodmann's area 7 and the parietal eye fields, and has direct connections with the superior colliculus (SC) and the frontal motor regions SEF and FEF (McDowell et al., 2008). The parietal cortex has been associated with saccade related sensori-motor transformations and visuospatial updating (Heide et al., 2001) and thus, most fMRI studies have shown greater parietal activation during volitional

compared to reflexive saccades (McDowell et al., 2008). Nonetheless, disruptions to the parietal cortex using TMS have shown to increase the latency of visually-guided saccades (Kapoula, Isotalo, Müri, Bucci, & Rivaud-Péchoux, 2001) and similarly, lesion studies have shown that damage to the parietal cortex increases pro-saccade latencies (Gaymard, Lynch, Ploner, Condy, & Rivaud-Péchoux, 2003; McDowell et al., 2008). Furthermore, Heide and colleagues (2001) identified multiple saccade areas within the posterior parietal cortex, including the SPL, which were active during visually guided saccadic tasks and not during memory or triple-step saccadic tasks. Surprisingly, in the present 4RNDs > 4PRDs contrast, inferior parietal lobe activity was also associated with RND saccades and was not found to be significantly active in the PRD saccade paradigm. Activation of IPL has been previously observed during more volitional driven circuitry involved in saccadic sequence learning tasks (McDowell et al., 2008) compared to stimuli driven visually guided tasks (Heide et al., 2001; Müri et al., 1996; Pierrot-Deseilligny, Milea, & Müri, 2004). The IPL is suggested to be involved in the remapping of spatial location to compensate for eye displacements during saccadic sequences (Heide et al., 2001). Contrasting fMRI studies have also shown that the parietal eye fields (PEF) in IPL (and equivalent to non-human primate IPL , see Müri et al., 1996; Pierrot-Deseilligny, Milea, & Müri, 2004) is mainly involved in reflexive behaviour, triggering saccades by disengaging fixation (Müri & Nyffeler, 2008), but this region may not be as important during intentional saccades, which are suggested to be mainly controlled by FEF (McDowell et al., 2008; Pierrot-Deseilligny et al., 2004). The FEF and parietal regions are heavily interconnected and play an important role in the interphase between sensory input and motor output (O'Shea, Muggleton, Cowey, & Walsh, 2006). Indeed, the visual hierarchy and roles of the FEF and parietal areas are still disputed. FEF has

been suggested to have a perceptual role in vision that is independent of eye movement programming (O'Shea et al., 2006). The role of FEF may have to do with patterns of interconnections with the extriastriate visual areas and with FEF neurons showing earliest activation; which places the FEF temporarily on par with the visual areas. Our results showed more activation in FEF and visual areas during the RND conditions. Higher activation of FEF during RND conditions is in accordance with previous studies. In addition, we found SEF activity and not FEF to be more significant during PRD conditions. Burke and Barnes (2008) also found the FEF more active during RND compared to PRD conditions and SEF more active during PRD conditions. Petit et al. (1996) also found SEF activation associated with sequence learning activities compared to self-paced saccades. Studies comparing saccadic SEF and FEF found that the SEF was more involved in the planning and updating of sequential saccades and FEF was more involved in determining the direction of the upcoming saccade (Isoda & Tanji, 2003). Our results showed activation of the early visual areas and the DLPFC and FEF as well as the parietal cortex during visually guided sequence saccades, which may reflect the acquisition of the multiple targets and visuospatial attentional shifts required during the RND trials. Finally, activity of the parietal cortex may not be exclusive to memory-guided behaviour as our results show significant activity during RND sequences (Heide et al., 2001).

Activity corresponding to the PRD sequences was found in the caudate, cerebellum, insular cortex and ACC. Lesion studies have demonstrated the involvement of the ACC in the control of saccades (Gaymard et al., 1998). More precisely, the posterior part of the ACC has been found to be active during volitional but not reflexive saccades (Pierrot-Deseilligny et al., 2004), with ACC lesion studies showing increased latencies and decreased gain during memory

guided tasks, and increased errors in the anti-saccade and saccadic sequence tasks (Gaymard et al., 1998). The existence of reciprocal and bilateral connections between the "cingulate eye field" (CEF) in the ACC and the frontal oculomotor areas (e.g., FEF, SEF and DLPFC) suggests that the CEF prepares these motor areas for the upcoming motor response (Pierrot-Deseilligny et al., 2004). ACC involvement in this sequence learning paradigm was likely to be important for facilitating frontal motor areas early and prior to the actual motor response.

3.4.2 4RND versus 4PRD: Pursuit

As in saccades, the 4RNDp > 4PRDp contrast showed generally higher BOLD activity for the random trials. PFC activation was observed during both predictive and random conditions, with bilateral DLPFC and left VLPFC activation during prediction and right DLPFC activation during random sequences. DLPFC has been involved in decision-making and the use of working memory (Burke & Barnes, 2008). Higher DLPFC activation during random sequence conditions compared to predictive conditions has been previously reported (Burke & Barnes, 2008; Koch et al., 2006; Schmid et al., 2001). An imaging study by Schmid and colleagues (2001) investigated anticipation and learning of smooth pursuit eye movements during predictive and non-predictive conditions. They showed a time-dependent decrease in DLPFC activation during the predictive trials. More recently, Burke and Barnes (2008) also determined a time-dependent decrease in DLPFC activation during predictable pursuit and similar to the present study findings, they found higher DLPFC activation during random compared to predictable stimuli conditions. These activation differences are suggested to mirror practice-related behavioural changes and are attributed to an enhanced

efficiency of signal processing as a result of short-term learning (Koch et al., 2006; Schmid et al., 2001). The observed signal differences in task-relevant regions such as the DLPFC represented an automatic transition to the quickly learnt PRD pursuit sequences (Burke & Barnes, 2008). The VLPFC in the inferior frontal gyrus (IFG) has been previously involved in working memory and attention processing (Wolf et al., 2009) and has also been demonstrated to be activated during pursuit (Burke & Barnes, 2011). Lesions to the frontal and the parietal cortex have resulted in working memory and attentional impairments (Nee & Jonides, 2009). An fMRI study by Nee and Jonides (2009) examined the neural networks involved in (i) a perceptual word selection task and (ii) a memorial word selection task. In each task, participants were cued as to what word they needed to attend to or remember. They found overlapping neural networks in perceptual and memorial selection, but also showed distinct functionalities within these regions. Results from their imaging study revealed the DLPFC as a structure is involved in both attention and short-term memory, the parietal lobe and the FEF were mostly involved in task-related attentional demands and the left VLPFC was mostly associated with behaviour measures of memory selection (Nee & Jonides, 2009). Our results also showed activation of left VLPFC only during the PRD pursuit suggesting a mainly memory-related role, while the DLPFC was associated with both RNDp and PRDp conditions consistent with the greater attentional role.

We found PFC, FEF and SEF activity to both the RNDp and the PRDp conditions. SEF role in pursuit has been previously associated with pursuit initiation and during predictable target directional changes (Gagnon et al., 2006). More specifically, microstimulation of SEF during fixation has been shown to elicit smooth pursuit eye movements (Tian & Lynch, 1995) and stimulation during on-

going pursuit has been shown to increase eye velocity (Missal & Heinen, 2004). Gagnon et al. (2006) delivered TMS to SEF during the pursuit of a sinusoidal target and found that stimulation of SEF has strongest effects during directional reversals but not during the mid-cycle of the movement to maintain pursuit. Drew and van Donkelaar (2007) also delivered TMS over SEF and FEF during unpredictable and predictable pursuit. They found SEF to have contributed mostly to predictable directional changes of on-going pursuit whilst FEF contributed to the preparation of pursuit during unpredictable target motion. In accordance with these and Burke and Barnes (2008) findings, we found the SEF to be more involved in predictable pursuit, whilst FEF mostly contributed to visually guided RND responses.

Activity was found in the MTG during RND pursuit tasks. Activity in MTG was also observed in Burke and Barnes (2008) findings from a PRD versus RND oculomotor task. In Burke and Barnes (2008) MTG was observed in both saccadic and pursuit eye movements, but how MTG modulates oculomotor control was not evident. Burke and Barnes (2008) suggested that the MTG appeared to be involved in the processing of velocity information in pursuit eye movements when compared to MTG activity during an analogous saccadic eye movement task. Activation during RND conditions, suggests that MTG was not involved in learning-related behaviours but was active when trying to track and match target velocity during random trials. Further understanding of the role of MTG in pursuit is currently lacking and warrants further study.

Surprisingly, activation of the cerebellum was specific to the RND pursuit trials. Previously thought to modulate and control saccadic eye movements, the cerebellum has also been found to be involved in the control of pursuit with Purkinje cells discharging according to gaze velocity during smooth tracking

(Krauzlis, 2004). Contrary to the findings presented here, previous functional imaging studies have shown changes in cerebellar activation during motor sequence learning and motor adaptations to changing sensorimotor inputs (Doyon et al., 2002). Still, the relative contributions of the cerebellum to motor skill learning remains unknown (Doyon et al., 2002; Seidler et al., 2002). Seidler and colleagues (2002) investigated cerebellar activity during an implicit motor learning task and found no cerebellar activity associated with the learning of the motor skill, when learning was evident and performance was held constant. This suggests that sequence learning may involve other areas than the cerebellum (Miall & Jenkinson, 2005). It is also possible that the neural representations of sequence learning become less dependent on the cerebellar-cortical circuit as learning occurs (Doyon et al., 2002), and this also explains why the cerebellum was not significantly more active during the learnt 4PRDp sequence trials.

There was a clear distinction of active areas in PRD and RND sequences in saccadic and pursuit tasks, such as activation within the PFC. We found learning pursuit sequences activated more anterior PFC than learning saccadic sequences. IFG and frontopolar activation was observed during predictive pursuit conditions, possibly contributing to a pursuit memory-driven circuitry (Braver & Bongiolatti, 2002) of the learnt sequences. Activity in ACC, DLPFC and SEF in saccades also showed evidence of the transition from sensory to memory-driven circuitry when the sequences were predictable. Furthermore, circuitry previously associated with the control of saccades and pursuit eye movements was also found, such as V5 and MTG, associated with the processing of visual motion information, visual areas in the PFC, and the cerebellum (Krauzlis, 2003). However, activation of some areas associated with memory-driven circuitry was either attenuated or not

significant during PRDp and PRDs conditions (e.g., DLPFC was not significantly active during PRDs). Previous imaging studies have suggested that practice effects are associated with shifts in brain circuitry to areas responsible for more automatic processing (Petersen, van Mier, Fiez, & Raichle, 1998). Recent findings, however, suggest a decrease in the spatial extent of the task-specific activation patterns, reflecting increased neural efficiency and enhanced connectivity between these regions (Koch et al., 2006). It is possible that a decrease in activation in task-relevant areas occurred since participants were able to acquire a steady state after the first presentation in pursuit and after the second presentation in saccade, reflecting a more automatic processing during the learnt responses.

3.4.3 Sequence Learning: Saccade versus Pursuit

This contrast allowed for the observation of areas that were exclusive to the predictive condition but differed between the eye movement types. The 4PRDs > 4PRDp contrast showed many areas in common between the two types of eye movements associated with sequence learning. Specifically and as expected, the ACC, SEF and DLPFC areas within the PFC were active during pursuit and saccadic predictable sequence conditions. However, within these areas there were distinct activation sites for pursuit and saccades, such as activation of PFC areas associated with working memory and attention in the inferior PFC (BA46/BA47) compared to more superior and middle frontal regions (BA8/BA9) for pursuit and saccades respectively. As previously reported, we also found that the overall level of activation differed between saccades and pursuit (Burke & Barnes, 2008). Indeed, the level of PFC activation was higher in saccadic compared to pursuit

sequence learning tasks. Higher activation in DLPFC in saccadic tasks maybe due to this area's involvement in spatial working memory and saccade inhibition (Pierrot-Deseilligny et al., 2002). TMS studies have determined that the DLPFC is associated with the control of short-term spatial memory, with the parahippocampal cortex involved in visuospatial memory (Müri et al., 2000; Pierrot-Deseilligny et al., 2002). Furthermore, lesions of the DLPFC have resulted in a reduction of the percentage of predictive saccades. The observed impairments in short-term spatial memory in lesion studies show that the DLPFC is thus involved in the control of predictive saccades (Müri & Nyffeler, 2008; Pierrot-Deseilligny et al., 2003). Our results also show the DLPFC and the parahippocampal gyrus to be involved not only in saccade but also in pursuit sequence learning. Other studies have shown that the DLPFC and the ACC were active when predicting smooth pursuit eye movements of occluded moving targets and also show that the more predictable the stimulus became, the less activation was observed in DLPFC and ACC (Ding et al., 2009). This finding may explain why activation was decreased during PRD conditions compared to RND conditions.

Activation of SEF was also associated with saccade and pursuit tasks, as previously described, and also corresponds to a key area during predictive oculomotor responses (Nyffeler et al., 2008). SEF is suggested to be a higher order structure of prediction that is independent of oculomotor output (Müri & Nyffeler, 2008; Nyffeler et al., 2008). In accordance with previous findings, our results showed that the DLPFC, ACC, parahippocampal gyrus and the SEF are important structures for the timing and preparation of predictive oculomotor responses. We also support the notion that distinct subregions for pursuit and saccades can be identified within these. Distinct subregions of FEF have been previously identified

during the execution of pursuit and saccadic eye movements. We found smaller pursuit-related activation than saccade-related activation in our task supporting finding that the pursuit FEF area may be smaller than the saccadic FEF area (Petit et al., 1997). This could also be true for other areas of the PFC, which could reflect distinct decision criteria for the two movements and different processing steps to construct the motor commands.

In addition to finding common regions between the two eye movement types, we also found non-overlapping areas possibly used for either position-dependent (saccades) or velocity-dependent (pursuit) processing and storage of the learnt sequences. Burke and Barnes (2008) found the superior marginal gyrus (SMG, BA40) active in pursuit and the temporal lobe and CBM active in saccadic tasks. Previous studies have also shown that lesions over SMG have resulted in increased in saccadic and pursuit latency (Heide, Kurzidim, & Kömpf, 1996). Lencer and colleagues (2004) found SMG to be more active during the blanking of pursuit compared to continuous target presentation. This and our results suggest that the SMG is an important area for the control of pursuit when internally generating a predictive response within a sequence without using stimulus feedback. The superior and middle temporal areas seemed to be more important during PRD saccadic sequence tasks.

Activation of MTG was also observed during pursuit and saccade RND conditions compared to PRD conditions. The role of the temporal lobe in oculomotor control is not yet fully understood (Burke & Barnes, 2008). Burke and Barnes (2008) found increasing BOLD activity in STG during saccadic eye movements and sustained activation in MTG during pursuit eye movements suggesting a dissociation between saccadic and pursuit regions in temporal lobe; however, these results were obtained when combining RND and PRD conditions

together. Typically, lesions to middle temporal areas impair the tracking of a moving target, shown by a reduced gain (Leigh, 1989). Indeed, activation of temporal regions in pursuit may have been more automatic during PRD sequence learning resulting in lower activity when compared to saccadic activation. Results suggest that MTG and STG may play a role in the generation of both reflexive visually guided and predictive sequences of saccades. However, the specific function of these temporal regions in saccadic control remains unclear and warrants further investigation.

In pursuit, cerebellar activation was attributed to RND conditions and was suggested to have decreased during the pursuit steady state responses to a more automatic network as a result of implicit learning. In contrast, cerebellar activation was attributed to predictive saccades compared to RNDs and to pursuit. It is possible that additional cerebellar activity was required during saccadic tasks. The CBM has been involved in the processing of saccadic errors and more specifically, involved in saccadic amplitude and timing (Thier, Dicke, Haas, Thielert, & Catz, 2002). Imaging studies have observed links between cerebellar volume activation and saccade accuracy, which may account for the higher activation for saccades observed here (Ettinger et al., 2005).

Results showed higher PFC activation associated with saccades compared to pursuit tasks. In addition, these findings show overlapping cortical regions associated with saccade and pursuit sequence learning. However, within these areas, distinct subregions for saccade and pursuit were identified. These results are in accordance with previous findings that suggest a partial overlap and sharing of important group of neurons between the two types of eye movements (Erkelens, 2006). These regions include the DLPFC, SEF, ACC and other frontal

regions, implicated in the maintenance of short-term memory. The distinct saccade and pursuit subregions and non-overlapping significant active brain regions, may reflect the different behavioural outcomes and threshold requirements of the two types of eye movements, such as the mentioned cerebellum predominant activation in saccade and not pursuit.

Our experimental design allowed us to make direct contrasts between RND and PRD conditions in the different eye movement types. To our knowledge, only one other experiment performed direct saccade and pursuit comparisons. Burke and Barnes (2008) used repeated series of discrete return step/ramps stimuli in contrast to our repeated continuous 4 component (i.e., step/ramp) sequences. For this experiment, we isolated and compared pursuit and saccade PRD areas, in contrast to combining PRD and RND like in Burke and Barnes (2008). This allowed us to identify saccade and pursuit common brain areas associated with performing predictive sequences and the learning that occurs within these; areas for random sequences between the two eye movements and areas that were eye movement type specific. We found important areas for performing random continuous sequences such as IPL, FEF, DLPFC, MTG, which showed activation in pursuit and saccade RND > PRD contrasts and predictive areas such as DLPFC, SEF, IC, parahippocampal gyrus and the ACC; which were common areas active from PRDs > PRDp contrasts.

We found similar anatomical areas to Burke and Barnes (2008), such as activation of memory-related areas DLPFC, SEF and SMG (pursuit). We suggest that predictive activity induced in behavioural experiments, required short-term memory as behavioural results demonstrated the influence of prior experience with no additional directional or timing cues given. To test the longevity of the

predictive buffer and investigate longer term learning in the oculomotor system, we then implemented a longer sequence and compared the anatomical areas of activation to the shorter sequences.

3.4.4 8 versus 4 component pursuit sequences

The 8 component pursuit sequence conditions rendered activation of similar brain regions compared to the shorter sequences. More specifically, both RND conditions (8 and 4 components) showed activation in FEF, DLPFC, V5 and the cerebellum. However, some differences exist with additional BOLD activation for the 8RND task, in the inferior and medial frontal gyruses, early visual areas and the thalamus. It is suggested that these reflect the additional cognitive and perceptual (i.e. visual) demands of the 8 component sequences. In addition, as shown for the 4 sequence tasks, a general decrease in activation was also observed in the 8PRD condition compared to the 8RND condition and again are suggested to reflect practice-related behavioural changes since participants also exhibited shorter latencies during 8PRD compared to 8RND conditions.

Common activation areas during PRD conditions included the SEF and the DLPFC, whilst the CBM and thalamus and early visual areas were active only in the longer sequences. In pursuit, the brain must keep track of on going velocity to reconstruct target velocity from retinal motion signals as a negative feedback control system that requires neural circuits to match target velocity for the maintenance of pursuit (Tanaka, 2005). It has been suggested that the networks in charge of the velocity memory for pursuit include the cerebellum, brain stem and thalamo-cortico-pathways that link subcortical and cortical eye movement areas (Tanaka, 2005). Anatomical studies have found the existence of pursuit

signals in the central thalamus and many projections into the frontal eye field areas (Tanaka, 2005). We therefore support the finding the thalamus is involved in the control of 8PRD and 8RND pursuit. It is possible that the longer sequences required additional neural activation related to the attentional (PRD and RND) and the use of short-term memory (PRD) demands of the longer length pursuit tasks. Tomasi and colleagues (2007) compared activation patterns between working memory and visual attention tasks. Their visual attention task consisted of covert attentional pursuit with no working memory load requirements, while the working memory task required holding a number of recently presented letters. Tomasi and colleagues (2007) found that increased working memory load caused larger activation in fronto-parietal networks consisting of IFG, MFG and IPL, while increased attentional processing was observed in the PCC and superior occipital region (BA19). Common networks for visual attention and working memory also included the IPL, inferior occipital gyrus, thalamus and the cerebellum. Our results are consistent with these findings and additional cognitive load also increased activation in frontal lobe areas such as IFG and MFG, which have also been associated with memory processing and were active during the 8PRD conditions (Manoach et al., 1997; Tomasi, Chang, Caparelli, & Ernst, 2007). We also found a similar network to Tomasi et al. (2007), with the involvement of fronto-parietal (IPL) and thalamic network, which may reflect the greater attentional and memory demands of the longer sequences.

Behavioural results did not show significant differences in temporal shifts between the shorter and longer sequences when performing predictive sequences compared to random sequence responses. However, BOLD contrasts did show activation dependent of sequence length. Since behavioural results did not point to prediction differences between the sequence lengths, then it cannot be argued

that the shorter sequences were better learnt compared to the longer sequences, but that more brain activation was needed to achieve a similar performance. We observed similar areas of activation associated with prediction and internally generated pursuit responses such as SEF, DLPFC, SMG and ACC (Burke & Barnes, 2008; Krauzlis, 2004). As in serial reaction time tasks (SRT), we used sequences in which the future location is predicted based on the current and prior locations. SRT sequence tasks measure reaction time decreases with increased learning of the motor sequence and have been shown to involve activation of right PFC, MTG, caudate and thalamus (Fletcher et al., 2005). Our results also show activation of these areas as evidence for sequence learning in pursuit (Figure 3.6 and 3.7A). In addition, recent studies suggest that the contribution of brain regions engaged in sequence learning (i.e., cerebellum, striatum and motor cortical regions) are not likely to be confined to a particular learning stage (i.e., short term vs. longer term) (Penhune & Steele, 2012).

These findings provided insight into the pursuit brain areas associated with attention and short-term memory, important for sequence learning. We also observed how the pursuit system adapts to additional cognitive demands to achieve a steady state. It has been previously suggested that the networks involved in attention and short-term memory have limited capacity. However, our results showed similar learning and overlapping brain activation between short and long sequences and compared to previous studies investigating short-term predictive behaviour (step/ramp stimuli) and longer-term sequence learning (SRT). Behavioural SRT results have indicated a temporal restructuring of a motor sequence during learning through the basic motor adaptation of individual units with practice, but also through the development of chunking or enabling grouping

individual items into larger units (Orban et al., 2010). Although controversial, the basal ganglia (BG) has been proposed to play an important role in implicit learning (Penhune & Steele, 2012) and also has been proposed to be associated with chunking multiple movements into groups defined by quicker onsets within a subgroup. Experiments that modify task difficulty load and cognitive task demands could enhance knowledge on how the system deals with the overload of information and how we achieve learning.

Chapter 4

4 Sequence learning: Eye and hand behavioural experiment

4.1 Introduction

The skilled coordination of sequences of eye and hand movements is central to many activities such as driving, writing and when performing sports. Even though the eye and the hand can respond separately and at times show decoupling during visually guided responses (Henriques, Medendorp, Khan, & Crawford, 2002), evidence suggests that performance improves when they move together during coordinated movements (Maioli et al., 2007). For example, studies have demonstrated that the amplitude of a saccade influences manual pointing movements (van Donkelaar, Lee, & Drew, 2000). Similarly, smooth pursuit eye movements are more accurate and exhibit fewer catch-up saccades when accompanied by manual tracking of a sinusoidal moving target (Koken & Erkelens, 1992). In addition, hand movements have been found to be more accurate when eye movements follow the same spatial trajectory, suggesting that the oculomotor system assists in manual tracking (Miall & Jenkinson, 2005).

There is extensive research that has investigated the interactions between eye and hand movements, particularly during visually guided pointing and reaching (Binsted et al., 2001; Lewis et al., 1998; Wilmut et al., 2006). Goal directed reaching studies usually show saccadic eye movements preceded by a hand movement with improved end-point accuracy, particularly when a sequence of movements to known targets are performed (Vidoni, McCarley, Edwards, & Boyd, 2009). It has been suggested that, in these cases, learning seems to occur in parallel, with the eye leading the hand (Binsted & Elliott, 1999). In eye and hand

motor learning information related to current sensory input must be obtained and compared to an internal plan based on prior experience to enable the generation of a faster and more accurate response (Philip et al., 2008). Motor learning of coordinated sequences of movements have primarily been studied using stereotyped finger-press or finger-tapping movements during serial reaction time tasks (SRT) (Philip et al., 2008; Robertson, 2007). During a SRT task, a repeated sequence becomes predictable and evidence of learning the sequence is determined as a decrease in motor (finger) reaction time. SRT studies typically show hand performance (e.g., hand latency and accuracy) and little attention is given to eye and hand interactions.

Barnes and Marsden (2002) investigated eye and hand predictive responses during the oculo-manual tracking of a repeated constant velocity target. Barnes and Marsden (2002) observed similar pre-programmed predictive responses of the eye and the hand with a similar build up of storage of the target's velocity. Engel and colleagues (2000) also showed similarities in the kinematics of the eye and hand during abrupt directional changes when tracking a moving target. This effect was observed despite the differences in the mechanics between the two response types. In both studies, an anticipatory slowing of the eye and hand was observed at the end of the stimulus movement (Barnes & Marsden, 2002) and when a moving target changed direction (Engel, Anderson, & Soechting, 2000), suggesting common functional features in the neural mechanisms of manual and smooth pursuit tracking (Engel et al., 2000). Pursuit eye movements are not typically performed without stimulus feedback, however studies have shown pursuit eye movements to exhibit predictive behaviour during repeated presentations of a continuous motion stimulus (Barnes & Asselman, 1991). In predictive pursuit, target velocity is internally stored and subsequently released

to generate anticipatory movements without the use of stimulus feedback (Barnes & Donelan, 1999).

The present study aimed to gain more understanding of eye and hand coordination and sequence learning during oculomanual tracking. Extending the work of Graham and Marsden (2002) we also aimed to assess manual and oculomotor predictive behaviour as evidence for sequence learning and to investigate eye and hand coupling during learnt sequences. The sequence tasks consisted of a multiple of connected constant speed ramps. These stimuli represented many ecologically relevant situations in which a connected sequence of multiple movements needs to be learned to avoid the inherent neural time delays (e.g., sequences of movements while driving or movements made to hit a tennis ball). Similar to SRT and pursuit tasks, we investigated eye and hand coordination effects of presenting predictable (i.e., repeated) sequences and compared them to random sequence presentations. Studies have shown that the pursuit system can exhibit rapid learning and is capable of storing multiple components of a sequence in short-term memory and predict each component only after a few repetitions (see Burke & Barnes, 2007). There is also evidence to suggest that this "buffer" memory system is limited, however, studies examining the limitations of rapid sequence learning and how these also affect eye and hand coordination are lacking. Our primary goal was to compare eye and hand coordination during sequence learning compared to random responses during a multiple component sequence. In addition, our secondary goal was to investigate eye and hand sequence learning in situations of high versus low cognitive load by adding components to the sequences.

Based on previous research we hypothesised that eye and hand responses would show similar predictive responses and similar temporal adaptations to

repeated sequences. We also hypothesised that hand performance would improve over the repetitions alongside pursuit responses when compared to the more reactive, visually guided responses. Given that temporal coupling of the eye and the hand has been shown to be task-dependent, aimed at optimizing feedback when guiding the hand (Crawford, Medendorp, & Marotta, 2004), we suggested that this coupling would show differences between conditions. Specifically, we expected that during random sequences, hand movements would show a larger lag due to visual feedback delays compared to predictive responses in which both eye and hand movements would be pre-programmed to a known location. Finally, we expected that a decrease in predictive eye movements would also disrupt hand movement performance during the longer sequences.

4.2 Methods

4.2.1 Participants

A total of twelve right handed participants 22 to 34 years of age (26.3 ± 3.9 yrs, 9 females) with normal or corrected eyesight and no known neurological conditions took part in this study. Informed consent was obtained prior to the experimental sessions and was approved by The University of Leeds local ethical committee and conducted in accordance with the standards laid out in the 1964 Declaration of Helsinki.

4.2.2 Experimental set up

Testing took place in a dark room and participants were seated with their foreheads and chins supported to avoid head movements during testing. Eye

movements were measured using an eye-tracker (Eyelink 1000, SR research, Canada), which sampled at 1000 Hz. The experiment was presented on a monitor (17 in CRT colour monitor, 1024 by 768 pixel resolution, 75Hz) located 57 cm in front of the participants. A fibre optic joystick (angular range of 30 and zero impedance) (Cambridge Research Systems Ltd, Kent, UK) was secured in front and lateral to the participants' dominant arm, which was held at 90° with their forearm positioned over a padded surface, allowing them to grip the joystick's vertical bar (grip =11.5 x 3 cm). Participants performed wrist movements to rotate the joystick with almost no resistance and were given a series of practice trials to become familiarized with the joystick prior to the experimental blocks. Experimental trials were designed using custom-made programmes (COGENT, Psychtoolbox, and MatLab, Mathworks, USA). Eye movement calibrations took place prior to each experimental blocks and a joystick calibration was performed once for each subject at the beginning of the experimental session. Rest breaks were given between each block, in which the lights were turned on in order to avoid dark adaptation and fatigue. The experimental session lasted approximately 60 minutes.

4.2.3 Experimental task and procedure

Participants were asked to track a continuously moving stimulus along the vertical and horizontal axis using a joystick. A green circle (15 x 15 pixels) represented the joystick's position and the pursuit target consisted of a white square (20 x 20 pixels). Experimental trials were made up of 4 or 8 constant speed (15°/s) ramp sequences, with each ramp in the sequence occurring in one of 4 possible directions (up, down, left or right). In contrast to previous

experiments described in chapters 2 and 3, the target speed in the present experiment was decreased to ensure that participants could track it effectively using the joystick. The individual ramps that built the sequences were referred to as components of a sequence, and each component started at the end position of the previous one to generate a continuous sequence of moving ramps. The direction of each component was randomized to form unique sequences within and between each experimental block. Both target and joystick cursors were positioned at the centre of the screen prior to any movement of the target. Once the movement sequence started, participants had to attempt to align and maintain the green cursor in the middle of the white pursuit target (Figure 4.1). Tasks were performed under two main conditions: predictive (PRD) sequences in which each sequence was repeated 4 consecutive times, and a random (RND) sequence condition, which consisted of novel sequence presentations. There were a total of 4 experimental blocks: 1) 4 component PRD sequence (4PRD), 2) 4 component RND sequence (4RND), 3) 8 component PRD sequence (8PRD), and 4) 8 component RND sequence (8RND) (Table 4.1). Experimental blocks were randomized between participants, and all participants performed the same sequences in the same order within each block. Participants were explicitly aware that in PRD blocks each sequence was repeated 4 times while in the RND blocks all of the sequences were different from each other and from the PRD sequences.

PRD sequence component directional changes occurred every 1.5 s during the PRD condition while in RND conditions, directional changes were randomized with durations of 1, 1.5 or 2 s. The 4 component sequence was 6 s in duration, whilst the 8 component sequence was 12 s in duration. Central fixations were inserted prior to a PRD series of 4 identical sequences and before each series of 4 unique RND sequences. The fixation cue then flashed to indicate the start of the

PRD or the RND series. The target briefly disappeared at the start of each components throughout the presented sequence. After performing each sequence, and when the target disappeared, participants repositioned the joystick at centre of the screen at the start position. The white pursuit target and green joystick cursor then appeared at the centre of the screen to start the next new or repeated sequence.

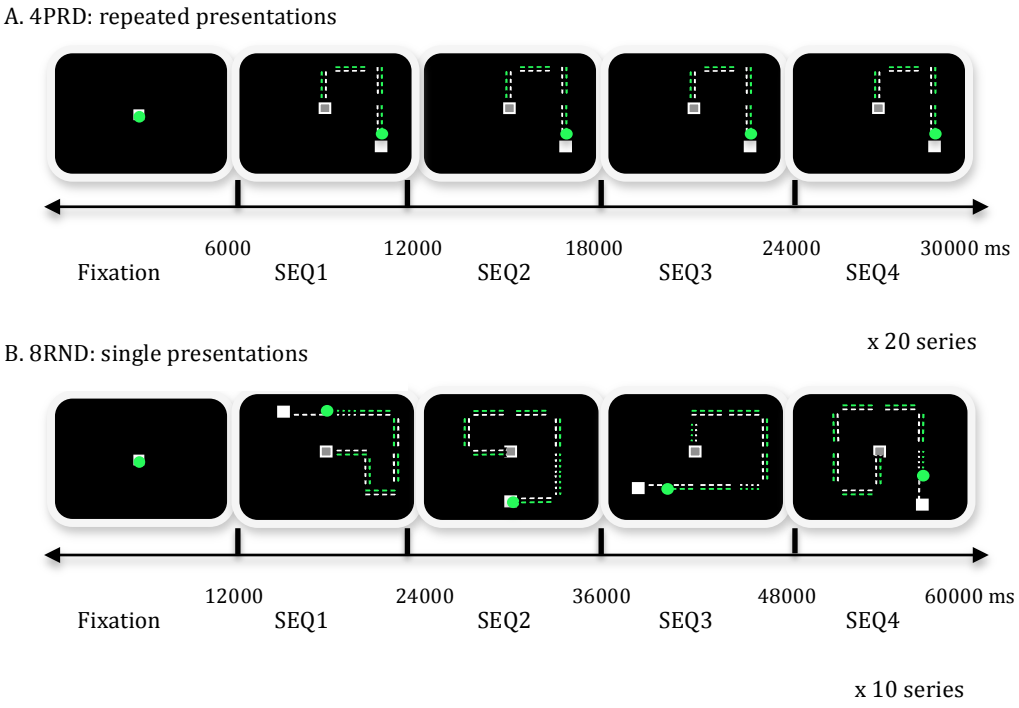

Figure 4.1. Examples of experimental paradigms for the 4 component PRD sequence task (A), the 8 component RND sequence task (B). The figure illustrates a series of 4 sequences that starts with a fixation cue and is followed by several target presentations that were either repeated for predictable trials, or novel for randomized trials. The white square is the target and the green circle represents hand movements of the joystick and both target and hand started at the centre of the screen. Each component (ramp) of a PRD sequence had the same duration (1.5 s), whilst in the RND condition durations were randomized (1, 1.5, and 2 s). The target briefly disappeared at the start of a new component. All experimental blocks had the same overall duration.

Table 4.1. Experimental blocks.

Block	Number of components	Component (ramp) duration	Number of sequences
4PRD	4	Duration: 1.5 s Sequence duration: 6 s Speed: 15°/s	20 sequences x 4 reps 80 trials total*
4RND	4	Durations: 1, 1.5 or 2 s Sequence duration: 6 s Speed: 15°/s	80 different sequences *
8PRD	8	Duration: 1.5 s Sequence duration: 12 s Speed: 15°/s	10 sequences x 4 reps 40 trials total*
8RND	8	Durations: 1, 1.5 or 2 s Sequence duration: 12 s Speed: 15°/s	40 different sequences *

(*) Does not include fixation cues inserted at the beginning of every 4 sequences in RND and PRD blocks.

4.2.4 Analysis

Eye movement data were obtained using the Data Viewer software (SR research Ltd, Canada). In DataViewer, blinks were automatically eliminated from the raw data prior to analysis and also bridged the gaps of missing data by linear interpolation. Joystick (hand) data were sampled at 60 Hz and then interpolated to create equivalent length vectors for both eye and hand. Eye and hand displacements and velocities were analysed using a custom made programme in MATLAB (version 7.8, Mathworks Inc., USA). The data were corrected for drifts at the start of every sequence to avoid contamination from the previous trial. The eye and hand velocity traces were filtered using a 10 Hz low-pass filter. Peak velocity was identified for each component in the sequence and plotted for visual inspection to ensure that the first peak was detected. After identification of the peak for each component, time to peak velocity (TTPV) was calculated from target onset to this peak for each component in a sequence. Eye and hand onsets were then calculated by using differentiation methods to measure the rate of change in

velocity (i.e., peak jerk) (Figure 4.2). The latencies for the eye (t_{Eye}) and hand (t_{Hand}) were then determined as the time from each component onset to eye peak jerk during the RND and PRD sequences. Gain was calculated as the ratio of eye or hand velocity to target velocity. Eye and hand displacement errors were calculated as the averaged absolute error of each component with respect to the target location (see Figure 4.3). A coefficient of variation (CV) was used to determine the variability of the hand velocity of each target component and calculated as the ratio of the standard deviation over the mean velocity of each ramp. This measure assessed the smoothness of the hand movements in each component of the sequence.

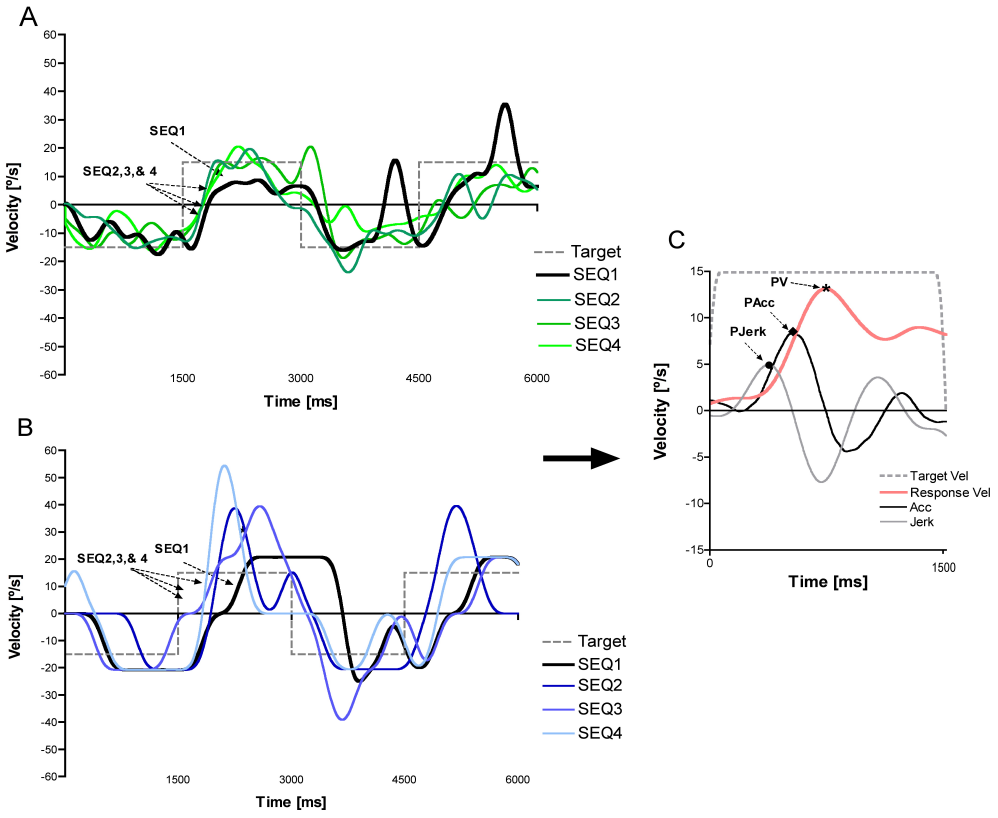

Figure 4.2. Example of a participant's 4PRD series showing eye (A) and hand (B) velocities of the identical presentations and eye and hand analysis of PV and latency (i.e., time to peak jerk) (C) across component timings. Graph C displays the 15°/s moving target, the participant's response across one component (1500 ms) and the corresponding acceleration (PAcc) and jerk (PJerk) used to calculate t_{Eye} and t_{Hand} of each component of the 4 identical presentations during 4PRD.

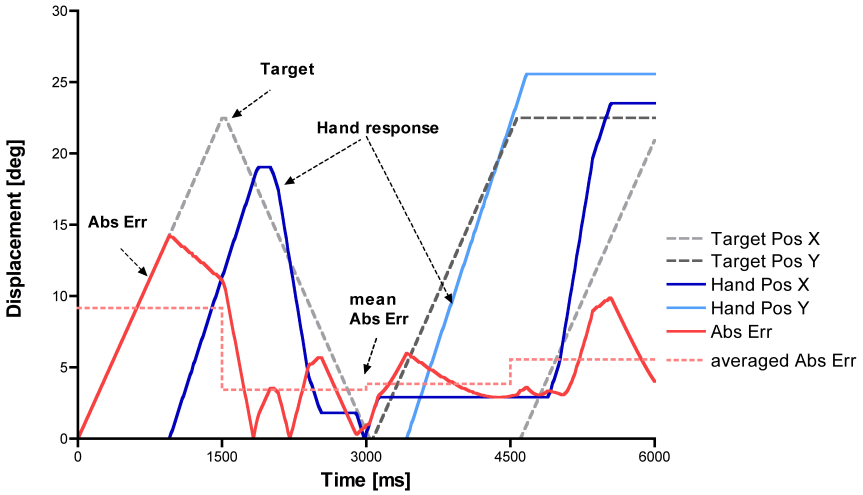

Figure 4.3. Example of a 4PRD SEQ1 trial showing hand displacement in X and Y and the absolute error from the target.

A repeated measures ANOVA was used to identify significant differences between the PRDs and RNDs conditions and between the identical presentations (SEQ1, SEQ2, SEQ3 and SEQ4) in the PRD tasks. Interactions between variables were evaluated using Bonferroni corrected post-hoc test. Deltas (Δ) were used to compare eye and hand performance between PRD and RND sequences. A significance level of $p < 0.05$ was established for all statistical analyses. Results, including graphs, are expressed as means ± standard deviations (sd). Statistical analyses were performed using the SPSS software package (IBM SPSS Ltd.). Two participants eye movement data were removed from the analyses due to issues with image capture of the eye. Real time feedback of the participants' performance was available throughout all experimental sessions to make sure they were performing the tasks correctly.

4.3 Results

In the present experiment, we investigated hand and eye responses when tracking a moving stimulus during predictive and random conditions. We assessed whether predictive responses would be revealed by repeated presentations of identical sequences and if these predictive responses would exhibit similar features between the eye and the hand. We also investigated the effects of oculomotor prediction on the hand responses by adding cognitive demands to the coordination of eye and hand during longer sequences.

Analysis of identical presentations revealed similar behaviour between the eye and the hand. As hypothesized, learning was evident during the 4 component sequences with participants showing a decrease in eye and hand latencies as well as TTPVs from the first presentation of the sequence that was maintained throughout the repetitions. In addition, the differences between eye and hand timings were also shorter when performing predictive sequences compared to the random sequences. Furthermore, in contrast to the eye, hand accuracy improved with repetition.

Latency significantly decreased between the RND and PRD condition during the longer 8 component sequences, and coupling between the eye and hand timings was not significantly different from the shorter 4 component sequences. Hand accuracy also improved in the 8 component PRD sequences; however, there were differences between the last 5 to 8 components of the 8PRD compared to the 4PRD sequences. Even though eye and hand latencies do seem to increase with each component when performing longer sequences, the overall timing shift between PRD and RND conditions (i.e., prediction) was maintained. In

addition, latency increased across components regardless of whether the sequence was PRD or RND.

4.3.1 Effects of repetition in 4PRD sequences: Eye

A comparison between identical presentations of a sequence revealed that pursuit eye movement latencies (t_{Eye}) were significantly shorter in the repetitions compared to the first presentation (SEQ1) ($F_{(3,33)}$=24.104; $p<0.001$) but t_{Eye} did not continue to decline with repetition (SEQ2, SEQ3 and SEQ4) ($p>0.05$) (Figure 4.4A). A similar effect was observed in $TTPV_{Eye}$ ($F_{(3,33)}$=16.311; $p<0.001$), with a post hoc analysis suggesting that PV was reached earlier during subsequent repetitions compared to SEQ1 ($p<0.001$, $p=0.009$ and $p=0.002$ for SEQ2, SEQ3 and SEQ4 respectively). As with latencies, no significant $TTPV_{Eye}$ differences between the repetitions were obtained from post the hoc test ($p>0.05$) (Figure 4.4B). In addition to timing shifts, eye displacement analysis showed that pursuit eye movements were less accurate ($F_{(3,33)}$=39.059; $p<0.001$) in the repeated sequences ($p<0.001$ for SEQ1 vs. SEQ2, SEQ3 and SEQ4) but not between the repeated sequences ($p>0.05$) (Figure 4.5).

4.3.2 Effects of repetition in 4PRD sequences: Hand

Participants' hand timing responses showed similar effects to pursuit eye movements during PRD sequence conditions. As with pursuit latencies, participants showed a significant decrease in hand latencies (t_{Hand}) quickly after performing the first presentation ($F_{(3,33)}$=38.527; $p<0.001$). A post hoc test showed that t_{Hand} from the repeated sequences significantly decreased from SEQ1 (all $p<0.001$), but performance did not improve further with repetition ($p>0.05$)

(see Figure 4.4A). TTPV showed a similar trend ($F_{(3,33)}$=30.374; p<0.001) (Figure 4.4B).

In contrast to pursuit eye movements, hand accuracy increased during the repeated sequences compared to SEQ1 ($F_{(3,33)}$=29,753; p<0.001) and did not significantly change with repetition (p>0.05) (also see Figure 4.5). Further investigation into hand performance across the repetitions showed that the coefficient of variance (CV_{Hand}) of the hand velocity profile decreased during the repeated PRD sequences ($F_{(3,33)}$=14.708; p<0.001) compared to the first presentation. The post hoc test revealed that when repeating the sequences hand velocity traces became less variable (CV = 1.045 ± 0.8, 0.89 ± 0.11, 0.89 ± 0.14 and 0.91 ± 0.13 for SEQ1, SEQ2, SEQ3 and SEQ4 respectively) (p<0.009).

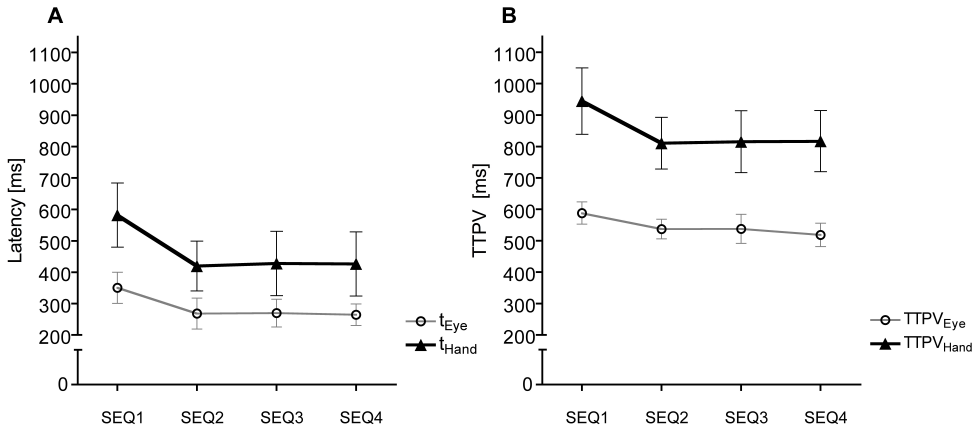

Figure 4.4. Timings from Eye and hand responses across identical 4PRD sequences. Mean ± sd of the eye and hand latency (A) and TTPV (B) values are displayed together, however, a separate analysis was conducted to assess differences between identical SEQ1, SEQ2, SEQ3 and SEQ4 presentations for each response type.

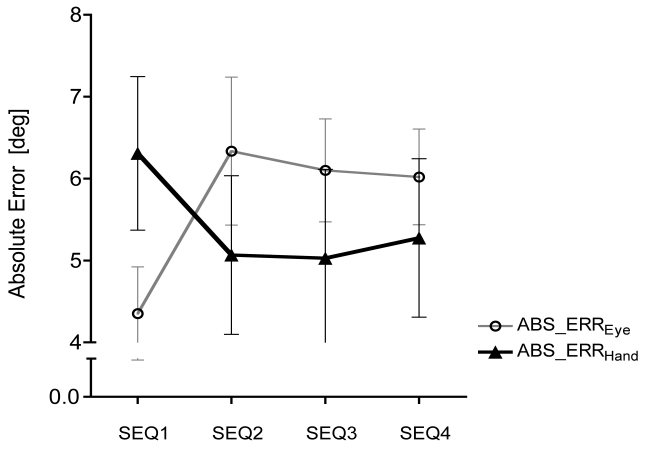

Figure 4.5. Mean eye and hand absolute errors ± sd across identical sequence presentations. Separate analyses revealed that eye displacements became less accurate across repetitions, while hand accuracy increased in SEQ2, SEQ3 and SEQ4 compared to SEQ1.

4.3.3 4PRD versus 4RND: Eye and hand coordination

Our results have established that eye and hand responses exhibited shorter latencies across all sequence components during PRD compared to RND conditions. We then assessed whether eye and hand coordination is affected by learning during PRD versus RND conditions. To determine this, latency deltas between the eye and the hand were calculated for both PRD (Δ_{EH_PRD}) and RND (Δ_{EH_RND}) conditions and compared. PRD responses were averages obtained from the responses to sequence presentations SEQ2, SEQ3 and SEQ4. No significant differences were observed between SEQ1 and the averaged RND sequences.

Analysis of the eye and hand latency deltas revealed a significant difference between the deltas in the PRD and RND conditions ($F_{(1,12)}$=7.755; p=0.017) with Δ_{EH_PRD} latencies being smaller compared to the Δ_{EH_RND} latencies across all components. This significant finding shows that the temporal difference between eye and hand was significantly smaller during the PRD condition (Figure

4.6). A similar trend was found with eye and hand TTPV deltas in PRD versus RND conditions ($F_{(1,12)}$=13.428; p=0.003).

Achieving target velocity was also an important factor for tracking the moving target. As well as investigating whether participants timed the directional changes and initiation of the movement with the target, we also assessed velocity gain with respect to the target (15°/s) and whether gain was affected by a predictable stimulus compared to random conditions. Overall, pursuit velocity was stable across conditions with average PV_{Eye} values of 13.34 ± 1.89 °/s and 12.90 ± 1.69 °/s for PRD and RND sequences respectively; while PV_{Hand} values were 23.8 ± 2.37 °/s and 24.258 ± 1.833 °/s for PRD and RND respectively. To avoid including joystick reaction delay effects on acceleration and peak velocity (which possibly caused the higher PV_{Hand} values) we then compared eye and hand velocity gain separately during PRD and RND conditions. No significant differences were obtained in hand velocity gain (p>0.05). A separate analysis did reveal significant differences between PRD and RND eye velocity gains (condition effect of $F_{(1,12)}$=7.317; p=0.019) with a positive gain difference of 0.04 and PRD values larger and closer to target velocity (15°/s).

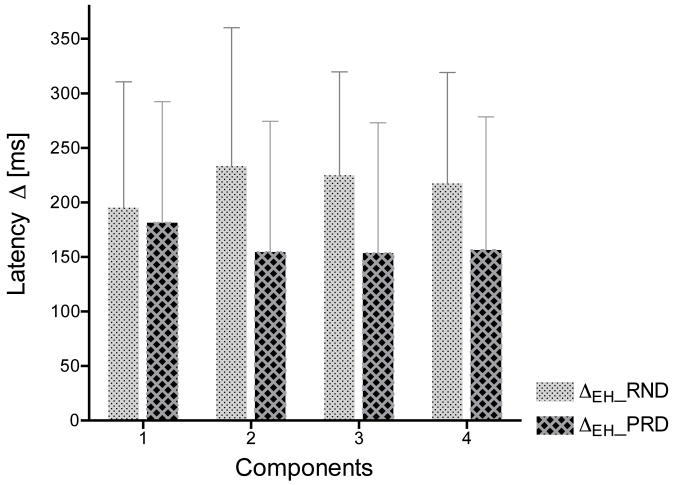

Figure 4.6. Mean + sd of the differences between eye and hand latencies compared between PRD and RND conditions across the 4 components of the sequence. Results revealed a smaller eye and hand latency difference in PRD compared to RND conditions.

4.3.4 Effects of repetition of 8 component sequences: Eye and hand

Prior to making comparisons between longer and shorter sequences, it was assessed whether timing shifts had occurred with repetition during the longer sequences. Analysis of 8PRD t_{Eye} revealed significant timing shifts ($F_{(3,30)}$=12.345; p<0.001) during the repeated sequences compared to SEQ1 (p=0.024, p<0.001 and p=0.022), but no further latency differences between the repeated sequences (p>0.05) (Figure 4.7A). $TTPV_{Eye}$ results also revealed an effect for sequence presentation ($F_{(3,30)}$=3.842; p=0.019), however, a post-hoc revealed these differences were only significant between SEQ1 and SEQ2 (p=0.034) (Figure 4.7B). Pursuit eye movement also revealed a significant increase in displacement error (absolute error) during the repeated sequences compared to SEQ1 ($F_{(3,30)}$=39.586; p<0.001) (Figure 4.8). During the 8 component PRD sequences, pursuit latency was significantly decreased after only one presentation of the

sequence. However, these effects were attenuated in TTPV values (mentioned above).

Analysis of the 8PRD t_{Hand} and $TTPV_{Hand}$ also revealed significant timing shifts during repeated sequences compared to SEQ1 ($F_{(3,30)}$=23.167; p<0.001 and $F_{(3,30)}$=20.193; p<0.001), with no further differences between SEQ2, SEQ3 and SEQ4 (p>0.05) (Figure 4.7). Hand absolute error decreased from SEQ1 during the repeated sequences ($F_{(3,30)}$=24.428; p<0.001) (Figure 4.8) as shown for the hand data in the shorter 4 component sequences, but no significant differences were observed in hand velocity trace variance (CV) between repetitions (p>0.05).

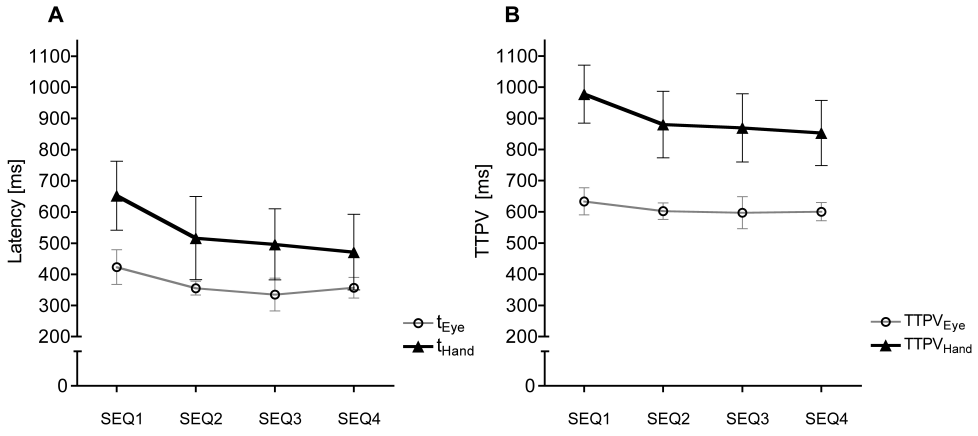

Figure 4.7. Mean ± sd of the eye and hand latency (A) and TTPV (B) across the identical 8PRD sequence presentations. Separate analyses were performed for the eye and the hand. Overall, the latency graph (left) shows significant timing decreases during the repeated sequences compared to SEQ1. The TTPV graph (right) shows timing shifts during the repeated sequences compared to SEQ1 in the hand but not the eye.

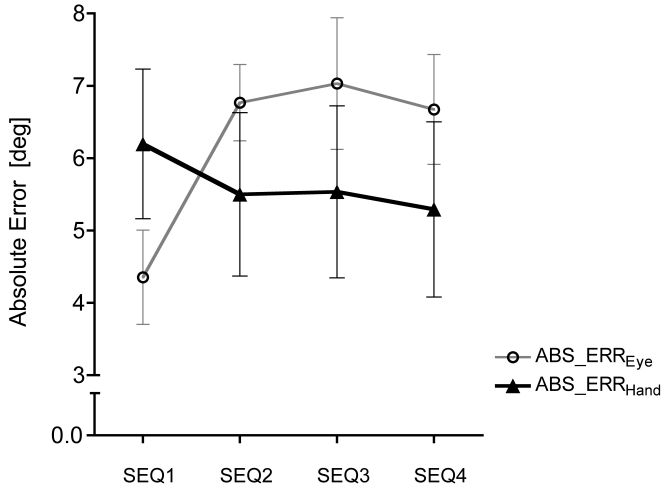

Figure 4.8. Mean ± sd 8PRD eye and hand absolute error across identical sequence presentations. Separate analyses indicated an increase in hand accuracy and a decrease in eye accuracy from SEQ1 and both were maintained across repetitions.

4.3.5 Effects of sequence length in PRD sequences: Eye and hand

The eye and hand responses from the 8 component repeated sequences (i.e., SEQ2, SEQ3 and SEQ4) were also averaged to obtain a mean 8PRD of the hand and a mean 8PRD of the eye for each participant. To investigate whether sequence length had an effect on the coordination of eye and hand, comparisons of the hand and eye deltas (Δ_{EH}) were performed between the shorter and longer PRD sequences. In addition, the longer sequences were divided into two parts and comparisons were made across (i) the 4 components of the 4PRD sequences and (ii) components 1 to 4 of the 8PRD sequence and (iii) 5 to 8 of the 8PRD sequences (i.e., 8PRD1 and 8PRD2 respectively).

Analysis showed that eye and hand latency timings were not altered by sequence length. Hand-Eye latency deltas did not reveal any significant differences between the 4PRD and the 8PRD1 or with 8PRD2 (mean latency Δ_{EH} of 140.53 ± 98.76 ms, 151.21 ± 89.7 ms and 172.6 ± 100.3 ms respectively; p=0.26)

nor were there any differences in TTPV deltas (mean TTPV Δ_{EH} of 253.63 ± 104.8 ms, 256.57 ± 88.72 ms and 227 ± 121.8 ms respectively; p=0.099) (Figure 4.9).

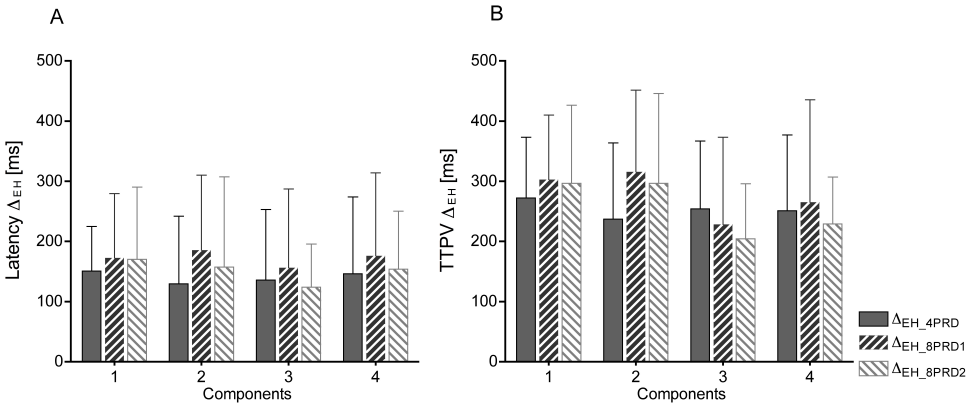

Figure 4.9. Mean ± sd of latency (A) and TTPV (B) Hand – Eye deltas from the 4 and 8 component sequences across 4 components. The longer sequences were divided into two parts with 4 components. Eye and hand latency and TTPV differences did not significantly differ in the longer versus the shorter sequences.

4.3.6 Effects of sequence length in PRD sequences: Eye

Further analyses showed differences in eye movements during longer 8PRD sequences compared to 4PRD sequences. Specifically, analysis showed that pursuit latencies for the shorter sequences were smaller, followed by 8PRD1 and the largest corresponding to 8PRD2 ($F_{(2,20)}$=67.129; p<0.001) (see Figure 4.10A upper graph). TTPV analysis showed differences in 8PRD2 compared to 8PRD1 and 4PRD ($F_{(2,20)}$=26.190; p<0.001). TTPV values from the last 4 components (8PRD2) were the highest compared to the first 4 components (8PRD1) and compared to the shorter sequences (TTPV values of 626.91 ± 23.6, 573.44 ± 44.5 and 538.5 ± 34.16 ms respectively) (p<0.05). In addition, the highest eye peak acceleration for the 4PRD condition and lowest for 8PRD2 with a significant difference between them ($F_{(2,20)}$=8.182; p=0.003) (peak acceleration of 45.0 ± 3.2, 41.84 ± 3.97, 46.99 ± 3.58 °/s^2 for the 8PRD1, 8PRD2 and 4PRD respectively)

($F_{(2,20)}$=8.182; p=0.003). Gain was also higher for the 4PRD compared to the 8PRD1 and 8PRD2 ($F_{(2,20)}$=7.792; p=0.003) (gain of 0.82 ± 0.08, 0.82 ± 0.07 and 0.87 ± 0.07 for the 8PRD1, 8PRD2 and 4PRD respectively).

To assess whether these effects were sequence learning specific, we also made sequence length comparisons in RND conditions. RND pursuit also revealed sequence length differences with the 4 component RND sequence exhibiting shortest latency (condition effect $F_{(2,20)}$=66.68; p<0.001), shortest TTPV ($F_{(2,20)}$=45.629; p<0.001) and highest peak acceleration ($F_{(2,20)}$=5.054; p=0.016) compared to the longer sequences. These results showed overall differences between sequence lengths regardless of condition.

4.3.7 Effects of sequence length in PRD sequences: Hand

Similar to the eye, hand latency analysis revealed that shorter sequence responses had the smallest latencies, followed by 8PRD1 and 8PRD2 showing the highest ($F_{(2,20)}$=23.137; p<0.001) (Figure 4.10B). Analysis also revealed that PV were reached faster during the 4PRD sequences compared to the TTPV values of the longer sequences (8PRD1 and 8PRD2) (TTPV values of 792.14 ± 102.7, 851.37 ± 126.7 and 883.48 ± 66.15 ms respectively) ($F_{(2,20)}$=13.263; p<0.001). Given that an important task goal was to maintain the joystick cursor on target, we assessed hand error in shorter versus longer sequences. Hand absolute error was greater ($F_{(2,20)}$=8.149; p=0.003) in the last 5 to 8 components of 8PRD2 compared to 4PRD (p=0.013), but was not significantly different from 8PRD1 (p=0.082) (absolute error of 5.39 ± 1.3, 5.6 ± 1.1 and 5 ± 1.1 deg for the 8PRD1, 8PRD2 and the 4PRD respectively). In addition accuracy during 4PRD and 8PRD1 was also not significantly different (p>0.05). No effects for sequence lengths were observed in

hand velocity gain, hand peak acceleration or variance in hand velocity CV ($p>0.05$).

Similar to eye analysis, in order to assess whether these effects were sequence learning specific, we also made sequence length comparisons in RND conditions. Hand RND latency and TTPV also revealed timing differences with 4RND exhibiting shorter latencies ($F_{(2,20)}=29.349$; $p<0.001$) and TTPV values ($F_{(2,20)}=46.569$; $p<0.001$) compared to the longer sequences. However, RND accuracy was not significantly different between sequence lengths ($p>0.05$).

4.3.8 Effects of sequence length in RND to PRD timing shifts: Eye and the hand

Figure 4.10 also demonstrates RND and PRD latencies across the 8 and 4 components for the eye and the hand separately as well as the PRD and RND differences across the short and long sequences in the two types of responses. The latency differences between the RND and PRD responses (RND – PRD deltas) were calculated for the 4 component and 8 component sequences. Pursuit eye movement latency deltas revealed a significant difference between the short sequences and the last 5-8 components of the longer 8 component sequences ($F_{(2,18)}=5.74$; $p=0.012$). A component effect also revealed an overall increased temporal difference in the last component compared to the rest ($F_{(3,27)}=9.901$; $p<0.001$). No significant interaction between sequence length and the components was observed ($p>0.05$).

In contrast, hand deltas post hoc analysis did not show significant differences between sequence lengths (interaction $F_{(6,72)}=2.457$; $p=0.032$). However, there was high variability in hand latency deltas between participants (Figure 4.10B). In addition, component differences were only observed in the

shorter 4 component sequences, with component 1 exhibiting the smallest difference ($p<0.03$) and component 4 showing larger differences from 2 and 1 ($p<0.03$). No differences between components were observed in the longer 8 component sequences ($p>0.05$).

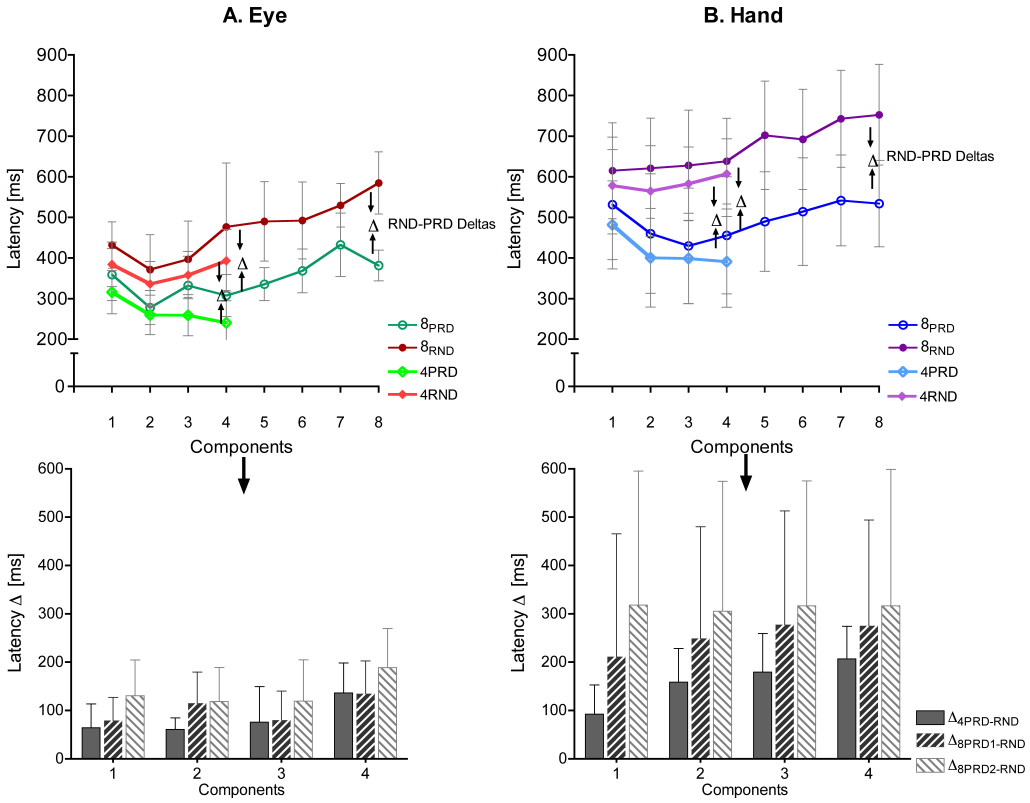

Figure 4.10. Mean ± sd eye (A) and hand (B) 4 component and 8 component PRD and RND latencies across components (upper graphs) as well as RND – PRD deltas from these sequences (lower graphs).

4.4 Discussion

Eye and hand sequence learning was identified by comparing performance during predictable with random sequence presentations. Participants exhibited significant shorter latencies of the eye and hand during repeated sequence presentations in comparison to RND. In addition to temporal improvements in performance, manual tracking accuracy also improved rapidly

with a decrease in displacement error and smoother velocity profiles. Rapid adaptations to predictable stimuli have been previously observed in pursuit eye movements (Burke & Barnes, 2006; Collins & Barnes, 2005). However, manual tracking responses are less documented and also exhibited similar behaviour to the ocular tracking suggesting similarities in the way the two motor systems pre-program motor responses during predictable sequence conditions. In addition, differences between the latencies in the eye and hand were reduced during PRD compared to RND sequences. This suggests a tight coupling between the eye and the hand that may serve to enhance motor sequence performance, and avoid visual feedback delays in the coordination system. We also suggest that both motor systems interact and share common processing features, but that the eye and hand also have separate sequence-specific encoding to enhance learning and add flexibility to the system. Comparisons between sequence lengths showed that RND to PRD temporal shifts were maintained and did not differ between sequence lengths. Results showed similar adaptations (i.e., prediction) in the longer compared to the shorter sequences. Coupling between the eye and the hand was also maintained throughout the PRD longer sequences and again did not differ from the shorter sequences. We found that eye and hand tended to increase in latency with increased sequence components regardless of condition, which may be attributed to an attentional effect and not a learning effect. Overall, our results showed similar sequence learning effects in the eye and the hand during coordination.

4.4.1 Effects of repetition in the eye

Pursuit latencies were longer than previously reported latencies for single discrete ramps (t_{PUR} of ~367 ms and ~269 ms for RND and PRD conditions respectively) (Collins & Barnes, 2005). A recent study by Gonzalez and Burke (2012) (see Appendix A) investigated memory and visually guided eye and hand movements when coordinated together (eye and hand) or when performing movements alone (hand only and eye only). Results from Gonzalez and Burke (2012) showed delayed eye responses when making eye and hand movements compared to eye movements alone (also see Bekkering, Adam, Kingma, Huson, & Whiting, 1994). This suggests that pursuit delays could be caused by the higher degree of coordination needed when responding with the eyes and the hand. A study by Miall and Reckess (2002) found that eye and hand tracking of a target is optimal at a certain lead time of the eye from the hand (75 to 100 ms) and that timings below or above (200 to 300 ms) this eye and hand offset degrade tracking performance. Our results showed eye and hand latency differences in predictable conditions (~ 160 ms) compared to random conditions (~ 200 ms). It could be suggested that the eye movement is delayed for optimal visual guidance of the hand and so that the eye and hand offsets are optimal for coordinated tracking. In addition, latency differences could also be attributed to the more conservative pursuit onset measure, which identified the abrupt change in slope towards PV (i.e. time to turnaround). Burke and Barnes (2006) also reported longer pursuit latencies in their 2-step ramp task compared to previous reports (~100 ms longer than single ramps), but found slowing of pursuit prior to the onset of a second target when the target's directional changes became predictable. This slowing of pursuit velocity has been suggested to occur in anticipation of a subsequent sequence component (Jarrett & Barnes, 2005; Wells & Barnes, 1999) and could be

considered part of the pursuit onset response. Finding the accurate turnaround point in pursuit of a continuous moving target (especially if the target continues in the same direction) is challenging. For this reason our latency measures were obtained through previously used techniques to investigate movement onsets (see Grierson, Gonzalez, & Elliott, 2009; Konczak & Karnath, 1998). In addition, we used the same method of latency calculations for both RND and PRD sequences and therefore our differences between conditions cannot be attributed to the methodology.

Similar to previous findings, pursuit eye movements exhibited significantly decreased latencies that were evident from the second presentation of the sequence and did not change with further repetitions. Studies have shown evidence of predictive behaviour during ocular pursuit of repeated discrete ramps, double-step ramps and sinusoids (Barnes et al., 2000; Barnes & Donelan, 1999; Barnes & Schmid, 2002; Collins & Barnes, 2005; Wells & Barnes, 1999). From these studies, it has been suggested that predictive behaviour presents evidence of the oculomotor system's ability to store velocity information from prior experience and subsequently use this information as an estimate of target velocity to generate a predictive response (Barnes & Asselman, 1991; Collins & Barnes, 2005). Collins and Barnes (2005) investigated predictive pursuit during identical presentations of a series of 4 or 6 discrete constant velocity ramps. In their study, evidence of pre-programming of pursuit responses each sequence component was observed and a steady state (i.e., maintained performance) was achieved quickly after the second presentation of the stimulus for single direction stimuli and third or fourth presentation for bi-directional stimuli. Unlike most previous studies our sequence learning task used multicomponent interconnected sequences in which each new component started where the previous one had

ended. It could be argued that the addition of connected components and multi directional (4 directions) changes would increase cognitive demands and this would produce longer learning (Burke & Barnes, 2007). Instead, our results showed a very quick pursuit adaptation (i.e., changes in performances that are maintained such as decreases in latency) after only one presentation of the sequence. These results suggest that in our task, it was possible to store a number of components in short-term memory and make predictions based on these for the upcoming sequence. Also, in our task, participants were able to quickly learn each sequence since only directional changes needed to be stored, as velocity and timing in the predictive sequences remained constant. It is not clear, however, which features of the task-related differences (i.e. task instruction, continuous motion or multi-directional components) resulted in the quick adaptation observed in our study when compared to previous findings (for discussion also see chapter 2).

4.4.2 Effects of repetition in the hand

Hand latency values were obtained through similar methods to eye movement latencies. In this method, peak velocity was an important feature for finding the latency of a response. We observed very high peak velocities during hand movements (~24 °/s) that exceeded the eye (~13 °/s) and the target's velocity (15 °/s). Barnes and Marsden (2002) also observed that participants' hand peak velocities exceeded that of the eye movement, possibly due to catch-up movements. Since the hand does not have the ability to correct positional errors as rapidly as the eye, we would suggest that one of the strategies participants used was to quickly move the joystick to peak velocity to compensate for the hand

lag during the target's directional changes. Indeed, in support of this theory, hand velocities after the target changed direction was consistent throughout the RND and PRD sequence trials with values that typically reached higher than 20°/s. We suggest that this strategy was performed to catch-up with the target after the directional change. Given this observed strategy, using peak velocity to estimate the latency of the hand proved to be very conservative (as with the eye) and may have resulted in late hand latency measures. In addition, we also suggest that in our task, each component of a sequence was connected and therefore the build-up of anticipatory responses in the brain may have been more restricted compared to discrete ramp presentations. It should also be noted, however that the higher hand velocity and conservative latency measure does not affect the comparison of latency differences between conditions and within repetitions in our task.

Similar to the eye movements, predictive hand responses exhibited significant decreases in latency to all components in the sequence after the first presentation and was maintained throughout the repetitions. Our measure of learning in hand movements is in accordance with previous findings in both tracking and SRT tasks where sequence learning is inferred once changes in performance, typically shorter reaction times, are observed (Barnes & Marsden, 2002; Engel et al., 2000; Robertson, 2007). It has been previously shown that participants are able to make short-term predictions of target motion based on target parameters (e.g., velocity or frequency) to pseudorandom targets (Foulkes & Miall, 2000). It is possible that participants were able to store target velocity input independently and that latency decreases were a result of participants learning the components of the sequence based on prior performance. Presumably participants were able to prepare a motor response and improve their hand tracking using stored information. Indeed hand absolute displacement

error also significantly decreased during repeated presentations, improving manual tracking. Participants also exhibited less variability in the hand velocity traces in the predictable sequence learning trials. Spikes or "jerky" traces during manual tracking have often been observed when positional corrections are performed (Barnes & Marsden, 2002; Xia & Barnes, 1999). These corrections could be similar to catch-up saccades during pursuit tracking and have been shown to decrease after repeated presentations (Collins & Barnes, 2005; Miall, Weir, & Stein, 1993; Vercher, Lazzari & Gauthier, 1997). Our findings suggest that not only were participants more on time during target turnarounds, but also presented smoother and more accurate tracking.

Our results also showed similar adaptations of the eye and the hand during predictable sequence presentations. Unlike SRT tasks, where learning occurs over many repetitions, our task showed quick changes in performance, which were maintained. These results have been previously observed in pursuit sequence learning tasks. Burke and Barnes (2007) investigated pursuit sequence learning during ramps to vertical and horizontal predictable and random targets. Their results showed that optimal performance could be reached and maintained within the first two presentations. This predictive behaviour was suggested to occur in a short-term storage "buffer" prior to the consolidation of the motor action in longer-term learning (Burke & Barnes, 2007).

Barnes and Marsden (2002) found that predictive responses of the hand were similar to those in the eye and exhibited a slowly rising velocity profile that were scaled to target velocity after only a few repetitions. This idea of pre-programming eye and hand movements was further supported by trials in which the target failed to appear, but predictive behaviour was still observed (see

Barnes & Marsden, 2002). Barnes and Marsden (2002) compared tracking behaviour of the eye and the eye and hand together and suggested that similar predictive behaviour could indicate a common predictive drive between the two motor systems (also see Xia & Barnes, 1999). Similarly, Engel et al (2000) showed similar speed modulation between the eye and the hand during abrupt directional changes in eye and hand tracking, also suggesting common mechanisms. Our results also show quick adaptations in hand behaviour to predictable sequence presentations. In addition, participants were able to perform complex motion sequences with improved hand tracking (shorter latencies and more accurate) that approached the performance of the eye.

4.4.3 RND versus PRD sequences in eye and hand coordination

Presumably, the eye and hand coordination system entails the use of visual input to guide movements of the hand accurately (Crawford et al., 2004). In our experiments, direct comparisons between the eyes alone, hand alone and eyes and hand to assess the use of visual information were not performed. Instead, our goal was to determine sequence learning effects during predictable (repeated) sequences compared to random sequences in the coordination of eye and hand. However, contrasts of our results with previous literature may help explain how the eye and hand movements systems affect each other during coordination tasks.

Direct comparisons of oculomotor and manual latencies were not performed since the eye is typically faster than the hand, due to processing delays and mechanical differences in the two modalities. By analysing the motor systems separately, we established that eye and hand responses both exhibited decreases in latency during repeated sequences compared to random sequences. Responses

showing performance changes, attributed to sequence learning (i.e., PRD), were averaged and compared to visually guided responses from the RND sequence presentations. In PRD sequences, pursuit eye movements showed decreased latencies across all sequence components (Figure 4.6) and overall higher gain and PV that matched target velocity more closely compared to RND eye velocities. However, it was also observed that eye movements exhibited poorer accuracy during PRD sequence presentations. Deterioration of accuracy of the pursuit system is generally associated with the inability to match eye velocity with target velocity. For example, when tracking high velocity targets as the system mechanics of the eye have limitations (Meyer et al., 1985). However, it has been observed that eye displacement can usually be corrected to match target displacement using quick saccadic corrections (Barnes & Asselman, 1991; Bennett & Barnes, 2006). Decreased accuracy could be explained by an anticipatory decrease in eye velocity at the end of a sequence component, however RND responses exhibited longer delays at the beginning of each component and therefore displacement absolute errors should have cancelled each other during condition comparisons. A possible explanation could be that the eye was also focusing on the hand cursor or somewhere in the middle between target and cursor during PRD sequence presentations. This suggests that during PRD pursuit, participants were able to enhance tracking performance and better match eye and target velocity without needing to directly fixate on the target.

Hand performance showed decreased temporal shifts (latency and TTPV) of almost 200 ms and improved tracking performance during PRD compared to RND sequences. Interestingly, the difference between the latency of the eye and hand was shorter overall in PRD compared to RND conditions. We suspect that temporal eye and hand differences between the PRD and RND sequence

responses revealed a positive change in eye and hand interaction. Thus, the larger temporal lag resulted in less accurate performance due to poorer temporally relevant visual feedback during the unknown sequences. In contrast, PRD results showed a decrease in this time lag between eye and hand responses, thus optimising the use of visual feedback. In-line with our findings, prediction or pre-programming of a response to a stimulus is known to eliminate or decrease errors in latency, velocity and/or position caused by a delay in visual feedback (Philip et al., 2008). Other studies investigating visually guided movements have principally found that making eye movements provides more useful information to guide a hand movement compared to when eyes are fixated (Wilmut et al., 2006). The explanation for this may involve an efference copy signal from the eye movement, which can then be used as a calibration for the hand movement (feed-forward) and/or for the effective use of oculo-propioceptive signal of the eye to estimate and update the target location (feedback) for use with the hand (Wilmut et al., 2006). A recent study by Gonzalez and Burke (2012) investigating eye and hand coordination during memory-guided GoGo and NoGo saccade/touch tasks found evidence that efference copy of a previously performed response (GoGo) provided a motor advantage during the memory-guided response. Their results showed improvements in hand performance during GoGo (when efference copy information was available) in both the eye and hand and hand only (eyes fixed) conditions, when compared to NoGo task (no efference copy information was provided). Thus, overall better performance was observed in the GoGo eye and hand responses when efference copy information was available in support of the work presented here. Improved eye and hand performance demonstrates that the eye is a strong feature of the hand response, generating an efference copy feed-forward signal or providing ocular proprioceptive feedback signal or both. Feed-

forward mechanisms have been previously suggested during tight coupling of the eye and hand when performing sequential movements (Wilmut et al., 2006). It has been observed that the eyes move ahead (saccade) to the target location and at the same time, the hand initiates or reaches PV (Wilmut et al., 2006). The lack of visual feedback delays supports a feed-forward system for the control and execution of fast and accurate hand movements (Wilmut et al., 2006). In addition, during multiple target sequences, the eyes often move ahead to a second target before the hand has reached the first target (Wilmut et al., 2006). The authors suggested that a temporal short-term buffer allows the eye to look ahead as the hand follows closely behind. A study by Miall and Reckess (2002) investigating eye and hand tracking timings showed that optimal performance was obtained without the use of effective visual feedback (lag <100 ms) strongly supporting a feed-forward system. In addition, they also observed a breakdown in performance when the hand moved closer to the eye (time lag < 75 ms), suggesting that at this time predictive information is not available to be used by the manual tracking system. The later could also support the presence of a short-term buffer in which the oculomotor system feeds into the manual system (also part of a feed-forward model) and improves hand tracking. Miall and Reckess (2002) also suggested a breakdown in eye and hand tracking when the visual feedback was delayed between 200 and 300 ms. Our results showed similar results in which the eye an hand offset was optimal below this range (< 200 ms). Together, these findings suggest that improvements in performance are dependent on the tight coupling of eye and hand movements and that both systems appear to send input signals (efference copy) to provide predictive information of the required response.

We acknowledge that learning a motor sequence is possible without the use of visual information (e.g., using proprioception). However, in our experiment,

vision was available and we suggest that an interaction between the two motor effectors since this interaction would result in enhanced performance. It is possible that pursuit tracking facilitated manual tracking in the pre-programming of a known sequence compared to RND conditions and that indeed the two systems may share information of upcoming movements. The coordination of the eye and hand involves a large network of brain areas including the cerebellum. Miall and Reckess (2002) suggested that the cerebellum features as part of a feed-forward model important for prediction during eye and hand coordinated movements (tracking). Furthermore, Miall and Jenkinson (2005) showed activation of the cerebellum associated with minimizing performance errors and errors related to the temporal relationship between the eye and the hand.

Lazzari, Vercher and Buizza (1997) developed a model for the coordination of arm and eye motor systems, which consisted of three parts: the eye controller (containing smooth pursuit and saccadic system), arm motor controller and a coordination control system. The latter resulted from an exchange of sensory-motor information between the motor systems involved in the same task, such as tracking a visual target. Lazzari et al's (1997) model showed results that closely matched human behaviour during visuomotor tracking and provided evidence that eye-arm coordination control is based on the integration of visual, premotor and proprioceptive signals. Vercher, Lazzari & Gauthier (1997) also suggested the cerebellum as a structure where this integration occurs and similarly to Miall and Jenkinson (2005), that this area controls corrections for arm trajectory. Since comparisons between eye only and eye and hand were not performed it is then difficult to establish to what extent the hand movements aided pursuit tracking in our task. In addition, we implemented hand movements that were constrained to a joystick and may not

provide significant afferent signals for the eye to take full advantage compared to arm movements.

Further analysis of the anatomical areas involved in oculomanual sequence learning compared to random conditions would provide more insight into the neural networks needed for eye and hand interactions and for performing these coordinated actions in a faster and more accurate manner. Our results showed tight coupling between the eye and the hand and also showed similar sequence learning adaptations between the two motor systems as observed previously (Barnes & Marsden, 2002; Engel et al., 2000; Miall & Reckess, 2002). In addition, our results provide further evidence that eye and hand tracking elicits similar pre-programmed behaviour to that observed in the pursuit system (Barnes & Marsden, 2002; Xia & Barnes, 1999). However, there were some performance differences between the motor systems (e.g., in latency thresholds, ocular gain and positional accuracy) and indeed the predictive drive was released at different times. We propose that each motor system may share information about the target, but may also learn sequence-specific elements separately (Vidoni et al., 2009). This allows for increased flexibility in the system and overall sequence acquisition allowing each motor system to contribute and improve motor performance (Vercher et al., 1997; Vidoni et al., 2009).

4.4.4 Long versus short sequences in eye and hand coordination

We first investigated whether sequence repetitions would elicit similar adaptations compared to short sequences. Results showed that, as with short sequences, participants exhibited significant decreases in pursuit latencies during the second presentation of a sequence with similar performance throughout

repetitions. However, these effects were attenuated in TTPV values. Accuracy was also decreased across repetitions as seen in the shorter sequences, suggesting that in our tasks, it was more important to maintain velocity rather than keeping the eye strictly on the target. This could be explained by the instructions given to maintain the cursor on target and without specifying whether the eye had to be maintained on target as well. Also, as mentioned above, this could have been a strategy used by participants to achieve optimal manual tracking. Interestingly, hand performance also exhibited similar adaptations to those of the eye by exhibiting significant decreases in latency and TTPV after only one presentation and maintaining performance throughout repetitions. These findings indicate a similar time course for learning the sequences in both shorter and longer sequence lengths. In addition, accuracy improved quickly and was maintained throughout the repetitions equally in both longer and shorter PRD sequences. It was hypothesized that performance improvements and maintenance would be different between the sequence lengths (4 and 8) and that the longer sequences would show evidence of requiring more repetitions to show improvements in performance, however, this was not the case. We then compared these longer sequence adaptations to the shorter sequence adaptations to investigate if sequence length had an effect on the eye and hand predictive drive and overall interaction between the two systems. We predicted that close interactions between the eye and the hand would be altered by the longer and more complex sequences. Our results revealed that PRD eye and hand offsets (< 200 ms) were not altered by sequence length. The eye and hand also exhibited similar tight coupling during PRD tasks throughout the longer sequences. Our rationale was that participants would exhibit a breakdown in the pre-programming of all the components of the longer sequences, and that this in turn would revert eye and

hand offsets into RND behaviour and thus exhibit longer delays, as the system would rely on visual feedback. Instead, participants showed temporal shifts in the eye and the hand during repeated sequences and were able to maintain similar PRD timing differences between the eye and the hand despite the sequence containing more components and being temporally longer (12 s x 4 reps).

Further analysis into the effects of the longer versus shorter sequences in the hand and the eye revealed some performance differences in both motor systems. In particular, results suggested shorter latencies in eye movements in the shorter sequences. In addition, pursuit presented an increasing lag behind the target across the components of the longer sequences and overall, the last components of the longer sequences showed the largest latency differences. It could be suggested that a breakdown in pursuit occurred due to the inability to maintain a similar predictive drive across all the components of the sequence. However, similar effects were observed in RND conditions indicating that this lag effect was not due to pre-programming differences and in both the eye and hand suggesting it was not modality-specific. As these effect is prominent in both modalities it is unlikely to be sequence learning specific, we therefore suggest that these effects were attention-related. We predict that this increasing lag with increasing sequence component number is most probably due to an attentional-related fatigue, and that pursuit may become less efficient at following temporally and spatially longer movements. The changes in attention throughout the sequence would affect both the eye and hand, as it would adjust processing time.

We did observe some small differences between the eye and hand conditions, which do not fit with this idea. For instance, hand accuracy did not seem to change during the RND sequences, but differences were observed between sequence lengths in the PRD condition. It could be argued that higher

displacement error in the PRD longer sequences were due to the increased lag. However, this was not the case in the RND condition and increases in lag behind the target were also observed. Thus, although accuracy improved in the PRD sequences, shorter PRD sequences maintained this accuracy better than longer sequences with repetition. In addition, variability of the hand velocity trajectory did not improve with repetition during the longer sequences as shown in the hand CV values of the 4PRD sequences. This is in-line with our attentional fatigue suggestion as decreases in accuracy and increase in variability are also features of decreased attention of the added components.

Analysis of the overall temporal shifts between RND and PRD sequences showed significant differences between the short and long sequences. Pursuit latency differences were larger during the added components of the longer sequences. We suggest that the participants maintained a similar predictive drive throughout the longer sequences, but it was a decrease in attention that caused the increased lag and the increased RND to PRD difference during the last components. In the hand, temporal shifts between conditions were highly variable but did not reach significance between sequence lengths. We suggest that the predictive drive in the hand was also maintained and did not differ between sequence lengths.

Results showed that oculomotor and manual systems exhibited temporal improvements across all components of short sequences. However, when the sequences were longer a systematic temporal lag occurred in pursuit that also translated downstream to the hand, without affecting the coupling of these systems. Indeed, despite the increased lag effects, the eye and the hand exhibited consistent RND to PRD temporal shifts attributed to sequence learning. RND to PRD timing shifts seemed larger for the hand compared to the eye. However, in

general it is not efficient for the eye to jump ahead of the target since optimal position of the eye should be on or just after the target for accurate vision (i.e., in the foveal region).

The results presented here demonstrate that participants are able to show rapid improvements in performance during multiple component sequences. Unlike SRT tasks where learning takes place over many (>10) repetitions of a sequence, participants showed rapid adaptations presumably through the use of a short-term storage buffer (Burke & Barnes, 2006, 2007). Previous research has suggested that there are limitations to this buffer and that learning deteriorates when components are added to the sequence. We agree that it is unlikely that the number of repetitions used in this study would invoke long-term response consolidation and it is possible that added repetitions would show further performance improvements. However, our task allowed us to observe rapid adaptations in eye and hand behaviour that were sequence learning specific in very short time intervals.

Collins and Barnes (2005) found that a steady state response from a 6 ramp stimulus was achieved after more repetitions (3-4) of the sequence compared to a 4 ramp stimulus (2-3). They suggested a breakdown in the predictive drive and that these differences reflected the additional cognitive load on memory. Their task consisted of individual discrete ramps while in our task a series of continuous interconnected movements were made. Previous findings of pursuit sequence learning have shown that a 4 component stimuli of discrete ramps can be quickly learnt and are within the limits of short-term memory (Barnes & Schmid, 2002; Collins & Barnes, 2005). It could be argued that in our task a 4 component sequence could also be easily and quickly stored and since no significant differences were observed in prediction between sequence lengths,

and that the system was also able to store the added components with only small performance decrements (e.g., increased eye and hand lag and poorer hand accuracy). We therefore suggest that the predictive drive could also persist throughout the longer sequences, indicating participant's ability to store large amounts of information. It is possible however, that sequence learning occurred using a different strategy in this unique continuous movement paradigm. Participants may have learnt the pursuit sequences as a general pattern of continuous movement in contrast to learning each component individually (see also Collins & Barnes, 2005). Since participants only had to store a single velocity, it could also be argued that decreases in latency in a sequence may have been based on only having to store target direction. Also performance changes may have been influenced by prior component performance of that sequence and not solely based on prior presentation of that sequence (Barnes & Schmid, 2002). Future studies with sudden perturbations to this type of stimulus parameters (e.g., blanking or changes in velocity or amplitude), similar to those employed by Barnes and Asselman (1991), would provide further insight into how continuous sequences are learned.

We have demonstrated that eye and hand coordination performance changes depending on learning-specific parameters by implementing a novel tracking task. Sequence learning was determined for the two motor systems when comparing the repeated and random conditions. We also revealed the similarity in the two system's ability to rapidly learn both short and long sequences. This supporting novelty contributes to the literature by suggesting that the two motor systems share similar predictive brain mechanisms to enhance sequence learning.

Chapter 5

5 Sequence learning: Eye and hand fMRI experiment

5.1 Introduction

Many skilled behaviours of every day life require the coordination of eye and hand movements such as driving, writing and performing sports. Motor learning entails the ability to perform movements in a faster, more accurate and automatic manner. Thus, learning is inferred when improvements in motor performance have been observed through prior experience. Furthermore, changes in performance have also been associated with decreases and shifts in neural activation associated with the acquisition of a motor skill (Penhune & Steele, 2012).

Past studies have primarily used stereotyped finger-press or finger-tapping movements during serial reaction time tasks (SRT) to investigate behavioural and neural changes that occur during implicit (procedural) learning (Philip et al., 2008; Robertson, 2007). During a SRT task, a repeated sequence becomes predictable and learning is measured as a decrease in motor (finger) reaction time and thus sequence learning elicits predictable responses to each element of the sequence. Even though single cell recordings and fMRI studies have not yet reached a consensus on the anatomical areas needed for motor learning, and have not yet determined the temporal dynamics of the different stages of learning (i.e., encoding, consolidation and retention), the majority of studies have identified a common network involved in this process. This network includes the primary motor cortex (M1), the supplementary motor areas (SMA), the basal ganglia (BG) and the inferior parietal lobe (IPL) (Grafton, Fagg, Woods, & Arbib,

1996; Halsband & Lange, 2006; Seidler et al., 2005; van Donkelaar et al., 2000). Discrepancies between studies in the anatomical areas associated with SRT sequence learning are often described as being task-related differences. For example, there is controversy surrounding the involvement of the lateral prefrontal cortex and the cerebellum during these SRT tasks (Halsband & Lange, 2006; Miall & Jenkinson, 2005; Seidler et al., 2005). Some studies suggest that the discrepancy is due to the nature of the learning process being either implicit or explicit, whilst a TMS study by Robertson et al (2001) suggested that lesions to the PFC prevents learning of SRT sequences. However, this was apparent only when learning was based on spatial information (Robertson et al., 2001; Seidler et al., 2005). Similarly, cerebellar activation has been associated with learning, with studies showing learning-related increases in activation (Halsband & Lange, 2006). However, some studies suggest that the cerebellum might not be involved in actual learning per se and that cerebellar activation is possibly related to error correction (Miall & Jenkinson, 2005; Orban et al., 2011; Seidler et al., 2002). A study by Boyd and Weinstein (2004) found that cerebellar patients had intact learning of spatial but not temporal features, suggesting that tasks that require a temporal component may elicit cerebellar activation (Penhune & Doyon, 2005), but not during spatial sequence learning. Even though these SRT studies have provided valuable insights into the neural correlates involved in skill acquisition, these tasks may involve more abstract learning rather than motor learning per se (Philip et al., 2008). In addition, learning-based improvements are usually measured without assessing the effects of visual information or eye and hand interactions (Philip et al., 2008).

Visually guided pointing and reaching has been extensively studied (see Binsted et al., 2001; Lewis et al., 1998; Wilmut et al., 2006) to investigate the

interactions between static eye position and motor responses. Studies investigating the dynamic interactions occurring during eye and hand movements have suggested that a network of premotor areas in both frontal and parietal lobes play important roles in the coordination of eye and hand movements. Specifically, the posterior parietal cortex (PPC) is involved in the integration of signals related to eye amplitude (saccades) and limb movement (van Donkelaar et al., 2000) when planning a motor response (Engel et al., 2000).

It has been found that oculomotor processing modulates motor actions, suggesting a common drive between the eye and the hand (van Donkelaar et al., 2000). For example, single cell recordings and imaging studies have demonstrated that the amplitude of a saccade influences manual pointing movements (van Donkelaar et al., 2000). Similarly, smooth pursuit eye movements are often more accurate and require fewer catch-up saccades when accompanied by a limb movement. In addition, manual tracking becomes more accurate when the eye also follows the same trajectory (Maioli et al., 2007). Engel and colleagues (2000) found that smooth pursuit and manual tracking were modulated in a similar manner, with each modality displaying similar reductions in speed during target directional changes. This suggests common neuronal functional elements between pursuit and manual tracking. Likewise, Barnes and Marsden (2002) found predictive responses in the eye and the hand when performing oculomanual tracking of a constant velocity moving target and suggested similar predictive mechanisms in the eye and the hand. Typically, pursuit eye movements are strongly reliant on visual feedback to maintain the eye on the stimulus. However, behavioural studies have shown that similar to pre-planned fast hand/finger reaction times, predictive pursuit eye movements can be

elicited through repeated presentations of identical stimuli (Barnes & Asselman, 1991) showing evidence of stimulus velocity/position encoding.

The anatomic pathways of smooth pursuit have been described in detail and involve the extrastriate visual areas (V5), middle and superior temporal areas (MST complex), which are responsible for sending visual information to the frontal eye fields (FEF) and supplementary eye fields (SEF) in the frontal cortex. The dorsolateral prefrontal cortex (DLPFC) together with the parietal eye fields (PEF) in the PPC are involved in attention and monitoring, with the cerebellum also playing a role in the control of pursuit eye movements (Drew & van Donkelaar, 2007; Lencer & Trillenberg, 2008). Particularly, predictive pursuit eye movements have been shown to involve activation of the SEF, parietal cortex, cerebellum and the anterior cingulate (ACC) (Burke & Barnes, 2008; Drew & van Donkelaar, 2007; Lindner et al., 2006; Schmid et al., 2001). The DLPFC and the ACC have additionally been shown to be more active during predictive tracking of occluded moving targets (Ding et al., 2009). More recently Burke and Barnes (2008) investigated anatomical areas involved in oculomotor tracking of double-step tasks in random and predictive (repeated) conditions and their experimental design allowed for the differentiation of brain activation linked to visually guided (random) and memory-guided (predicted) responses. For example, they found the SEF to be more active during predictive pursuit compared to higher activation of the FEF during visually guided pursuit, suggesting learning-related cortical activation shifts.

Extending from this, we have designed a multiple component sequence task to investigate neuronal activation during eye and hand coordinated tracking of a constant velocity stimulus. Similar to a SRT, our primary goal was to examine sequence learning by implementing predictable sequences and to compare them

with random sequences. Sequences consisted of multiple connected constant velocity ramps moving in two dimensions. Our task therefore presents a more ecologically relevant comparison of motor sequence learning that maybe applicable to both early development when learning to draw a shape or write, to later stages of learning a skilled motor action such as serving a ball at tennis. All these examples comprise a series of repeated and learned movements of the hand. We hypothesized that improvements in performance would be observed with the repetitions of an identical sequence in the predictive conditions compared to the random sequences. We also expected that eye and hand coupling would show learning-related changes in both behavioural and fMRI BOLD signals. Temporal coupling between the eye and the hand varies in a task-dependent mode, presumably to optimize visual information (Crawford et al., 2004). In Barnes and Marsden (2002), eye and hand tracking exhibited similar trajectories and showed evidence of storage of timing and velocity-coded information based on prior experience and these responses were scaled appropriately for subsequent events. It may be that even though information is released at different times (faster for the eye than for the hand) common networks may be found during oculomanual compared to oculomotor tracking (Barnes & Marsden, 2002). We therefore expected to identify brain activation associated with the learning of eye and hand coordinated responses and also to find areas common for predictive pursuit.

To further investigate learning effects and brain activation changes, we increased cognitive load by comparing short sequences with more complex longer sequences. Therefore, our secondary goal was to identify anatomical brain areas and networks associated with memory and cognitive load that were essential for acquiring motor sequences. Studies have suggested that short-term and working memory processes and timing mechanisms contribute to sequence learning (Bo &

Seidler, 2009). However, short-term memory capacity and how it contributes to oculomanual tracking has not yet been established.

fMRI studies have shown brain activation shifts during visuomotor learning tasks, whereas the frontal cortex is activated during the early stages of learning and a shift to parietal areas is observed during more advanced learning stages (van Mier et al., 1998). Other studies have found learning-related decreases in activation in the supplementary motor area (SMA), premotor area (PMA) and the cerebellum (van Mier et al., 1998). Learning-related increases in PMA have been observed in tracking tasks, pursuit tasks, visually guided reaching and writing tasks, but not during finger tasks (Halsband & Lange, 2006). Thus PMA activation may not only reflect the representations of motor actions but rather the representation of sensory cues and motor commands for the storage of motor skills (Halsband & Lange, 2006). We therefore predict higher activation of PMA during predictive sequence presentations compared to the random conditions.

5.2 Methods

5.2.1 Participants

Eleven right handed participants 22 to 31 years of age (25.5 ± 3.4 yrs, 8 females) with normal or corrected eyesight and no known neurological conditions took part in the study. All participants gave informed consent. This study was approved by the local and regional NHS ethical committee and by The University of Manchester and Leeds ethical committees and conducted in accordance with the standards laid out in the 1964 Declaration of Helsinki.

5.2.2 Experimental paradigm

Participants performed the same experimental task in a dark laboratory setting 1 week prior to the session performed in the fMRI using the same joystick but different eye tracker. Experimental paradigm used in this session is explained in full detail in chapter 4. Experimental blocks: 1) 4 component PRD sequence (4PRD), 2) 4 component RND sequence (4RND), 3) 8 component PRD sequence (8PRD), and 4) 8 component RND sequence (8RND), were randomized between participants, and all participants performed the same sequences in the same order within each block. The sequences presented during the fMRI session were different but equivalent to those presented during the previous laboratory session. Participants were explicitly aware that in PRD blocks each sequence was repeated 4 or 8 times while in the RND blocks all of the sequences were different from each other and from the PRD sequences.

5.2.3 fMRI experimental setup and acquisition

Eye movements were recorded inside the fMRI scanner using an ASL optical video eye tracker (Applied Science Laboratory, Bedford, MA) that sampled at a rate of 60 Hz. Participants were supine on the scanner bed. The head coil provided support for the participants' head and with the addition of cushions that helped to minimize head movements during scanning. A fibre optic joystick (angular range of 30° and zero impedance) (Cambridge Research Systems Ltd, Kent, UK) was secured on the scanner bed lateral to the participants' semi-pronated dominant arm allowing them to grip the joystick's vertical bar (grip =11.5 x 3 cm). Participants performed wrist movements to rotate the joystick with almost no resistance. An image of the eye was reflected via a mirror

positioned on the head-coil to the ASL video camera positioned outside the scanner near the head of the participant. A second mirror was used to reflect the image of the experimental paradigm (and joystick cursor) projected on a 180 x 110 mm screen located at the subject's feet in front of the scanner. Prior to experimental trials, adjustments to the mirrors were made to obtain a good image of the eye and to make sure that the screen was fully visible to the participant. Eye and hand movements were inspected to make sure that participants were performing the task correctly. The same paradigm as in the laboratory experiments was used and participants completed the 4 blocks in random order (see chapter 4). Participants performed the same sequences in the same order within each block. Calibrations for the eye and the hand took place in the scanner prior to each experimental block. The room was kept as dark as possible and the lights were turned on in between blocks to maintain alertness. The fMRI experimental sessions lasted about 50 minutes.

The fMRI scanner consisted of a 3T (Phillips 3.0 T Achieva) with an eight-channel sense head-coil (Achieva 3.0 T Neuro Coil) designed to maximise the signal-to-noise ratio (Burke & Barnes, 2011). The BOLD changes in brain activity were measured while participants performed the pursuit tasks. Scans were collected using T2*-weighted spin echo pulse (TR of 2000 ms, TE of 35 ms; 90° flip angle, FOV of 250 mm, 1.8 x 1.8 x 4 mm^3 voxel size and a total of 30 slices). Data were pre-processed using SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/) in which spatial realignment, co-registration to each participant's mean EPI fMRI scan, normalization (MNI model) to a local T2* (EPI) template and smoothing using FWHM Gaussian filter (8mm) was applied to all participants' fMRI scans. In addition, fMRI data were high pass filtered at a 128 Hz cut off frequency.

5.2.4 fMRI analysis

Event-related analysis was performed over the PRD conditions across the 4 identical presentations of a sequence (i.e., SEQ1, SEQ2, SEQ3 and SEQ4) and over the RND conditions in pursuit and saccadic tasks, which resulted in a 4 condition matrix (Figure 5.1). Within each condition, this event-related analysis included fixation periods and presentations of a sequence (i.e., a series) from each condition block. This analysis allowed us to assess changes in the BOLD signal related to repeated versus random sequence presentations and to include a control (fixation) and separate activity related to the motor response type and not the stimulus itself. The individual participants' contrasts were then entered in a group level analysis where a one-sample t-test was performed for each global contrast ($T > 3$). The resulting MNI coordinates were verified using the SPM8 anatomical toolbox (Eickhoff et al., 2005) and then converted into Talairach space for anatomical labeling (Talairach Daemon software http://www.nitrc.org/projects/tal-daemon/) (Lancaster et al., 2000). Contrast analysis corresponded to 1) the analysis of PRD and RND sequences and 2) the differences in long (8 component) and short (4 component) sequences.

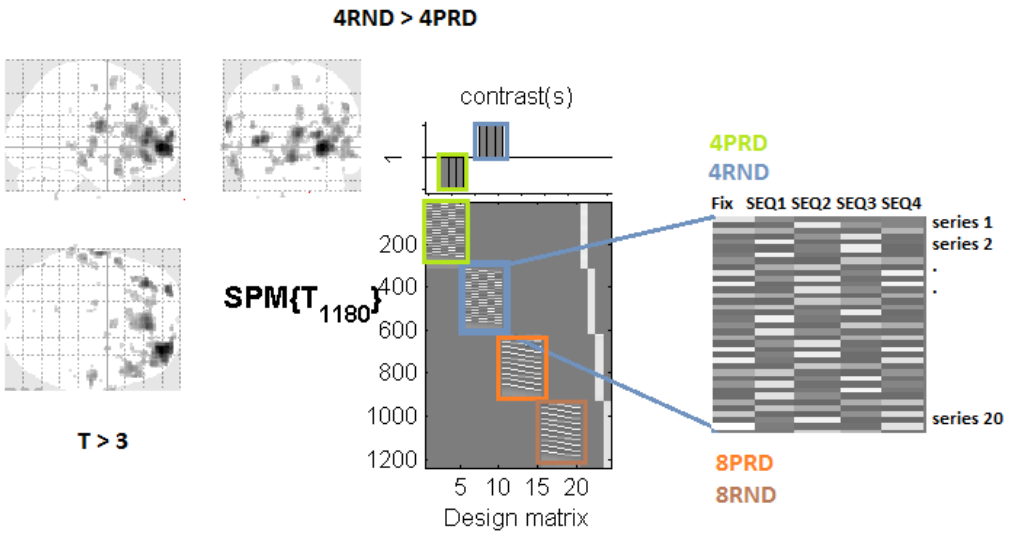

Figure 5.1. Example of a single participant's 4RND > 4PRD contrast. The figure shows brain activation corresponding to each 4 component sequence (left) and the overall 4 condition design matrix (middle), with the events (i.e., fixation, SEQ2, SEQ3 and SEQ4) contrasted (right). Activity corresponding to fixations was used as baseline. Activity from these fixations and from SEQ1 was excluded from further analysis respectively as SEQ1 corresponded to a reactive response as observed in the RND condition. The design matrix on the right shows the allocation of positive activation for RND conditions and negative activation for PRD conditions.

5.3 Results

Participants performed eye and hand coordinated responses to predictable and random sequence stimuli. Predictable conditions consisted of the repetition of a multiple component sequence (i.e., a series), whilst random conditions consisted of tracking single novel multiple component sequences. Contrasts of BOLD responses between RND and PRD sequences were performed. Inspections of the eye and hand data confirmed that participants were performing the task correctly in the scanner. In addition, examples of participants' hand velocity traces in the laboratory and fMRI sessions are graphed in Appendix B (Figures 2.1 and 2.2 for the 4PRD and 8PRD blocks respectively). Behavioural results are reported and discussed in full in chapter 4. We assessed whether participants would exhibit sequence learning during PRD conditions by showing

significant decreases in eye and hand latencies. Overall, participants exhibited learning of up to 8 component sequences. Hand responses were very similar to the effects observed in pursuit eye movements and also showed significant decreases in latencies during the predictive or repeated sequences compared to the random presentations. Evidence of temporal shifts in the eye and in the hand were observed from the second presentation of the repeated sequence, and performance did not improve with additional repetitions. Accuracy of the hand increased and jerkiness decreased with repetition. In contrast, the eye did not seem to improve in accuracy with repetition. An important finding was that timing differences between the eye and hand decreased during PRD sequences compared to RND sequences. Comparisons with the longer sequences showed that eye and hand coupling also changed during the longer PRD sequences. No major differences were observed between eye and hand behaviour of the long sequences, compared to the shorter sequences.

We examined the brain areas activated when coordinating the eye and hand during PRD and RND sequences. For these contrasts, we excluded BOLD fixation activity from all conditions and also excluded SEQ1 activity to make sure that PRD behaviour was not contaminated by activity related to reactive responses. We also compared brain activation when learning short and long sequences.

5.3.1 RND versus PRD contrasts

4RND > 4PRD

When comparing RND and PRD brain activation for the shorter sequences higher activity for the RND task was observed in the right prefrontal cortex (PFC),

particularly in pre-motor area (PMA, BA6), FEF (BA8), DLPFC (BA9) and the FP area (BA10). Furthermore, additional activation for random sequences was observed in the right IPL (BA40), left superior temporal gyrus (BA22), thalamus and the basal ganglia (BG). Greater activation for the predictable sequence learning task was found in left FP (BA10), left pre-motor and supplementary motor areas (PMA and SMA, BA6), right DLPFC (BA46), bilateral SPL (BA7), left IPL (BA40), bilateral extrastriate visual areas V5 (BA19) and subcortical areas included the left thalamus, BG, the right parahippocampal cortex (PHC) and the left ACC (BA32), with additional activation observed in the right cerebellum (Figure 5.2A and Table 5.1).

8RND > 8PRD

Contrasts performed between PRD and RND tasks for the longer sequences revealed higher activation in bilateral FP (BA10), left precuneus (BA7), early visual area V2 (BA18), left PCC (BA30), the right thalamus, right BG and right STG (BA22). Greater activation for the PRD task was found in left DLPFC (BA46), left SPL (BA7) and left IPL (BA40), right MTG (BA21) and bilateral STG (BA22), visual areas V2/V5 (BA18, BA19) and subcortical areas including the left cerebellum, left IC (BA13), right ACC (BA24) and left parahipocampal cortex in the medial temporal lobe (MTL) (Figure 5.2B and Table 5.1).

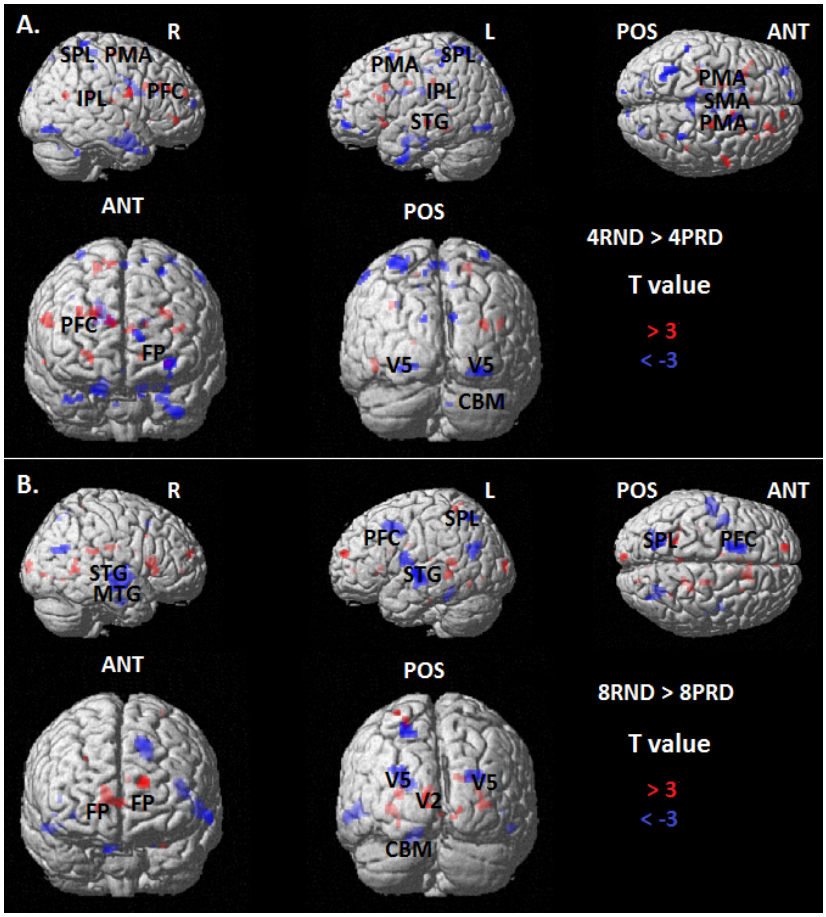

Figure 5.2. Group contrasts for (A) 4RND vs. 4PRD sequences and for (B) 8RND vs. 8PRD with baseline (fixation) activity removed. Red areas correspond to greater activation in the RND task and blue areas show greater activation in the PRD task. Images are labelled according to left (L), right (R), posterior (POS) and anterior (ANT) views and activation threshold T > 3.

Table 5.1. Anatomical areas for 4RND vs. 4PRD and for 8RND vs. 8PRD group contrasts.

Contrast		cluster size	**T**	Z	MNIx (mm)	MNIy (mm)	MNIz (mm)	R/L side	Anatomical area	Brodmann area	*
4RND > 4PRD	4RND	126	5.3	3.5	56	0	22	R	PMA	BA 6	PCG
		55	5.1	3.4	22	36	30	R	DLPFC	BA 9	
		127	5.0	3.4	20	-14	60	R	FEF	BA 8	MFG
		261	4.2	3.1	-44	-26	-6	L	STG	BA 22	
		112	3.8	2.8	52	-38	56	R	IPL	BA 40	
		218	3.5	2.7	26	44	-2	R	FP	BA 10	MFG
		81	3.5	2.7	8	-18	18	R	Thalamus		medial
		72	3.3	2.6	-10	20	-2	L	BG		caudate
	4PRD	284	6.4	3.8	-32	-58	64	L	SPL	BA 7	
		1424	5.5	3.6	16	-16	-22	R	PHC	BA 28	
		756	5.3	3.5	-10	-20	22	L	Thalamus		dorsal
		216	5.0	3.4	-28	-78	-10	L	V5	BA 19	
		66	5.0	3.4	-30	54	-8	L	FP	BA 10	MFG
		299	4.6	3.2	36	-68	-12	R	V5	BA 19	
		45	4.4	3.1	-4	-32	64	L	PMA/ SMA	BA 6	PCG
		46	4.1	3.0	34	-46	70	R	SPL	BA 7	
		48	3.7	2.8	44	-50	-34	R	CBM		
		57	3.6	2.8	-52	-38	56	L	IPL	BA 40	
		122	3.4	2.7	-12	-28	46	L	ACC	BA 32	
		72	3.2	2.6	16	-36	20	R	BG		caudate
		32	3.0	2.4	46	24	30	R	DLPFC	BA 46	
8RND > 8PRD	8RND	230	6.1	3.75	24	-32	22	R	BG		caudate
		82	5.02	3.38	-16	56	18	L	FP	BA 10	
		96	3.89	2.91	-6	-44	14	L	PCC	BA 29	
		94	3.58	2.75	-20	-50	60	L	Precuneus	BA 7	
		186	3.36	2.64	32	-54	12	R	STG	BA22	
		572	3.15	2.52	-6	-98	10	L	V2	BA 18	
		68	3.0	2.43	4	-28	16	R	Thalamus		
		150	3.0	2.31	18	50	22	R	FP	BA 10	
	8PRD	1293	6.1	3.7	-36	-6	-26	L	PHC		MTL
		380	5.1	3.4	18	-22	36	R	ACC	BA 24	
		335	5.0	3.4	-20	-62	58	L	SPL	BA 7	
		550	4.4	3.1	46	-20	-2	R	STG	BA 22	
		74	3.9	2.9	66	-30	2	R	MTG	BA 21	
		239	3.8	2.9	-14	-44	-16	L	CBM		
		60	3.8	2.9	-38	-40	10	L	IPL	BA 40	
		75	3.8	2.9	-34	32	10	L	DLPFC	BA 46	
		73	3.7	2.8	-44	-20	24	L	IC	BA 13	
		94	3.7	2.8	-64	-48	14	L	STG	BA 22	
		572	3.2	2.5	34	-74	22	R	V5	BA19	
		32	3.0	2.4	-44	-70	-14	L	V5	BA19	y

Table includes contrast, cluster size, significance level, MNI coordinates and brain areas. (*) MTL = middle temporal lobe; MFG = middle frontal gyrus, PCG = precentral gyrus.

5.3.2 8 versus 4 component sequence contrasts and 4 and 8 component sequences combined

4PRD > 8PRD

Comparisons of the shorter and longer predictive sequences were performed and revealed differences associated with increased cognitive demands. Higher activation for the 4 PRD task was observed in left FP (BA10), right PMA (BA6), bilateral SPL (BA7), visual areas V2/V5 (BA18, BA19), and the basal ganglia, left thalamus, right ACC (BA32) and right cerebellum. Higher activation for the 8 PRD task was observed in left FP (BA10), bilateral DLPFC (BA9, BA46), left premotor cortex (BA6), right SPL (BA7), bilateral ACC (BA24) and the left parahippocampal cortex (BA30) (Figure 5.3 and Table 5.2).

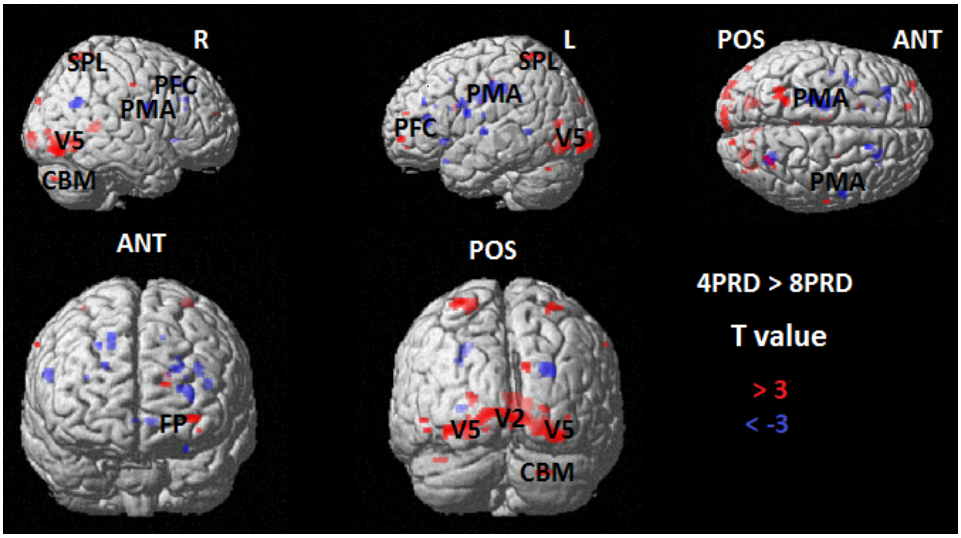

Figure 5.3. Group contrasts for 4PRD vs. 8PRD sequences with baseline fixation activity removed. Red areas correspond to the brain areas more active for the shorter 4 predictable sequences, and blue areas show the areas more active for 8 component predictable sequences. Images are labelled according to left (L), right (R), posterior (POS) and anterior (ANT) views. Activation threshold T > 3.

Table 5.2. Anatomical areas for 4PRD vs. 8PRD sequences group contrasts.

Contrast		cluster size	T	Z	MNIx (mm)	MNIy (mm)	MNIz (mm)	R/L side	Anatomical area	Brodmann area	*
4PRD > 8PRD	4PRD	5669	10.0	4.6	30	-76	-12	R	V2/V5	BA 18/19	
		237	5.1	3.4	-20	-48	64	L	SPL	BA 7	
		88	5.0	3.4	-32	58	-2	L	FP	BA 10	MFG
		115	4.1	3.0	14	-92	30	L	V5	BA 19	
		124	4.1	3.0	26	-66	62	R	SPL	BA 7	
		44	3.9	2.9	62	-14	42	R	PMA	BA 6	PCG
		234	3.9	2.9	24	-6	16	R	BG		Putamen
		138	3.6	2.8	-2	-14	14	L	Thalamus		Pulvinar
		78	3.3	2.6	34	-56	-22	R	CBM		Declive
		170	3.2	2.6	2	16	36	R	ACC	BA 32	
	8PRD	533	7.3	4.1	-22	-24	36	L	ACC	BA 24	
		135	5.6	3.6	-36	6	28	L	PMA	BA 6	MFG
		83	4.1	3.0	-8	22	-6	R	ACC	BA 24	
		361	4.1	3.0	26	-62	26	R	SPL	BA 7	
		196	3.9	2.9	56	-2	24	R	PMA	BA 6	PCG
		207	3.9	2.9	-32	34	10	L	FP	BA 10	MFG
		23	3.9	2.9	-28	20	-22	L	DLPFC	BA 46	
		30	3.9	2.9	-26	-50	4	L	PHC	BA 30	
		232	3.6	2.7	16	18	46	R	DLPFC	BA 9	

Table includes contrast, cluster size, significance level, MNI coordinates and brain areas. (*) MTL = middle temporal lobe; MFG = middle frontal gyrus, PCG = precentral gyrus.

4 > 8 (RND and PRD combined)

Contrasts performed on all trials (both PRD and RND) for the short (4) and long (8) sequences revealed higher activation for the short sequences in bilateral PMA and SMA (BA6), right M1 (BA4), bilateral DLPFC (BA9) and IFG (BA47), as well as right temporal activation in ITG (BA20) and MTG (BA21), right V5 (BA19), right basal ganglia and the right thalamus. Higher activation for the longer 8 component sequence was observed in bilateral parahippocampal gyrus (BA30/BA36), right basal ganglia, left cerebellum, right ACC (BA24) and right PFC areas such as DLPFC (BA9) and FP (BA10) (Figure 5.4A and Table 5.3).

RND > PRD (8 and 4 SEQ combined)

Contrasts between the random and predictive sequences showed greater activation in right DLPFC (BA9), right FP (BA10), left SPL (BA7) and bilateral basal ganglia for the random task. Higher activation for the predictive sequences

was found in the left PMA (BA6), right M1 in the precentral gyrus (BA6), right DLPFC (BA9), right V5 (BA19), right MTG (21) and STG (BA22), left SPL (BA7), left IPL (BA40); bilateral IC (BA13) and bilateral ACC (BA24); bilateral parahippocampal cortex and the right cerebellum (Figure 5.4B and Table 5.3).

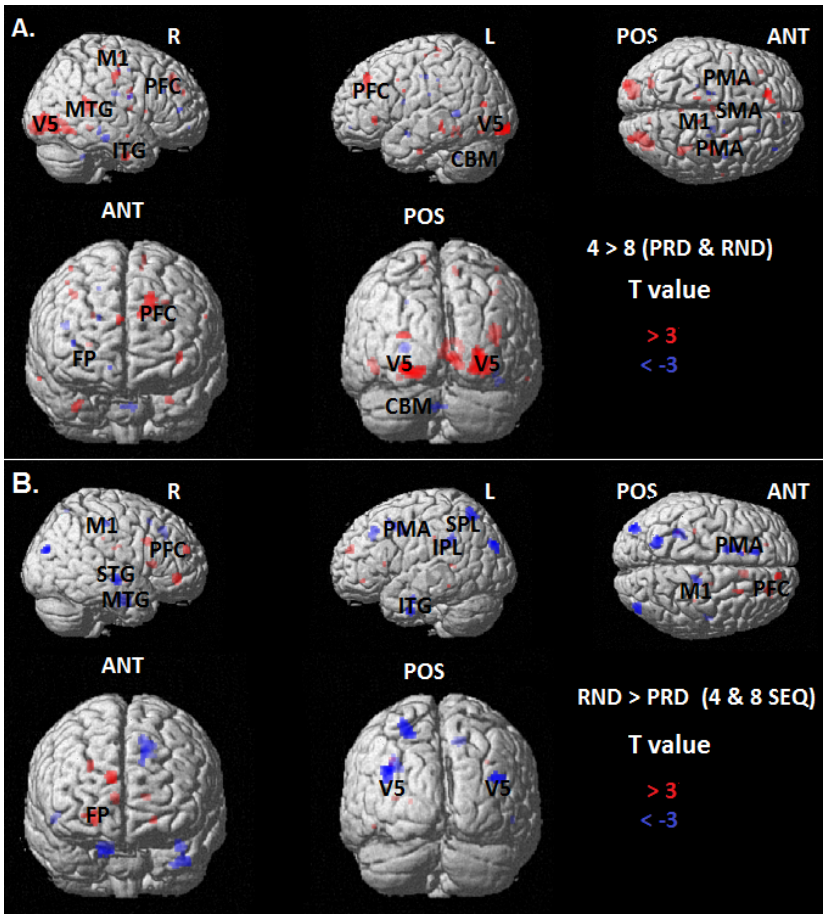

Figure 5.4. Group contrasts for (A) 4 vs. 8 component sequences (RND and PRD conditions combined) and for (B) RND vs. PRD sequences (both long and short combined) with baseline activity removed. Red areas correspond to greater activation for 4 (upper image) and RND sequences (lower image) respectively and blue areas show higher activation for 8 (upper images) and PRD (lower images) respectively. Images are labelled according to left (L), right (R), posterior (POS) and anterior (ANT) views. Activation threshold T > 3.

Table 5.3. Anatomical areas for overall 4 vs. 8 and RND vs. PRD sequences group contrasts.

Contrast		cluster size	T	Z	MNIx (mm)	MNIy (mm)	MNIz (mm)	R/L side	Anatomical area	Brodmann area	*
4 > 8	4	3794	7.3	4.1	30	-78	-8	R	V5	BA 19	
		204	5.6	3.6	-18	38	40	L	DLPFC	BA 9	
		47	4.7	3.3	36	-8	-36	R	ITG	BA20	
		153	3.9	2.9	36	-18	42	R	M1	BA 4	PCG
		160	3.6	2.8	-26	8	38	L	FEF	BA8	
		105	3.6	2.8	-46	-36	-12	L	V5	BA 19	
		102	3.4	2.7	6	48	26	R	DLPFC	BA 9	
		185	3.4	2.7	24	-2	10	R	BG		
		98	3.3	2.6	-38	32	-2	L	IFG	BA 47	
		71	3.2	2.6	62	-6	-16	R	MTG	BA 21	
		110	3.2	2.5	0	-14	16	R	Thalamus		
		100	3.2	2.5	-12	-30	68	L	PMA/SMA	BA 6	MFG
		192	3.2	2.5	10	-28	62	R	PMA/SMA	BA 6	MFG
	8	60	4.2	3.1	-24	-48	6	L	PHC	BA 30	
		104	3.7	2.8	40	-26	-18	R	PHC	BA 36	
		45	3.7	2.8	16	-16	24	R	BG		Putamen
		32	3.7	2.8	-16	-18	42	L	ACC	BA 24	
		62	3.1	2.5	44	-4	22	R	DLPFC	BA 9	
		54	3.0	2.4	-4	-50	-36	L	CBM		
		29	3.0	2.4	38	46	10	R	FP	BA 10	
		85	3.0	2.4	20	-14	48	R	ACC	BA 24	
		36	3.0	2.4	20	30	28	R	DLPFC	BA 9	
RND > PRD	RND	252	5.1	3.4	8	20	10	R	BG		Caudate
		128	4.4	3.1	24	42	-4	R	FP	BA 11	
		141	4.1	3.0	10	52	22	R	DLPFC	BA 9	
		66	3.3	2.6	-20	42	-8	L	ACC	BA 32	
		78	3.2	2.6	-30	-44	8	L	BG		Caudate
		50	3.1	2.5	-32	-28	36	L	SPL	BA 7	
	PRD	246	5.6	3.6	-36	-84	28	L	V5	BA 19	
		991	5.5	3.6	-38	-6	-26	L	ITG	BA 20	
		325	5.3	3.5	-16	4	42	L	ACC	BA 32	
		56	5.2	3.5	-12	12	24	L	BG		Caudate
		170	5.1	3.4	48	-14	-8	R	STG	BA 22	
		282	4.9	3.4	-22	-64	54	L	SPL	BA 7	
		110	4.9	3.3	-14	30	44	L	PMA	BA 8/6	MFG
		84	4.9	3.3	30	8	-36	R	STG	BA 38	
		82	4.7	3.2	30	-10	-30	R	PHC		Hippocampus
		168	4.3	3.1	16	-26	48	R	ACC	BA 32	
		60	4.2	3.1	16	-36	-26	R	CBM		Culmen
		190	4.2	3.0	42	-82	24	R	V5	BA 19	
		111	4.2	3.0	-12	-14	54	L	PMA	BA 6	
		52	3.9	2.9	40	-4	30	R	M1	BA 6	PCG
		100	3.6	2.8	-44	2	14	L	IC	BA 13	
		135	3.5	2.7	-50	-40	48	L	IPL	BA 40	
		66	3.3	2.6	48	-2	16	R	IC	BA 13	

Table includes contrast, cluster size, significance level, MNI coordinates and brain areas. (*) MTL = middle temporal lobe; MFG = middle frontal gyrus, PCG = precentral gyrus.

5.4 Discussion

We investigated eye and hand coordination during predictable and randomized sequence conditions. Behavioural results showed temporal shifts in the eye and the hand during the repeated predictive sequences compared to the

random sequences, showing evidence of learning. In addition, latencies significantly decreased after only one presentation of the sequence and performance was then maintained throughout the repetitions. Rapid adaptation has been previously observed in pursuit eye movements following repeated stimulus presentations (see Barnes et al., 2000; Barnes & Donelan, 1999; Barnes & Schmid, 2002; Collins & Barnes, 2005). Predictive smooth eye movements suggest the storage of velocity information derived from prior experience, which is subsequently released as an estimate of the targets velocity and compensates for feedback processing delays in the system (Collins & Barnes, 2005). In addition, a study by Barnes and Mardsen (2002) investigating oculomanual tracking of a moving target in predictable and random conditions, showed evidence of comparable behaviour in the eye and the hand and suggested similar predictive mechanisms between the two. We also found similar behaviour in the eye and the hand and tight coupling when participants performed pre-programmed coordinated responses during PRD conditions. Overall, behavioural results showed motor performance changes in the eye and hand with sequence learning. Comparisons between hand velocity traces during the laboratory and fMRI sessions showed that participants were performing the task similarly in the two sessions (Appendix B: Figures 2.1 and 2.2). Evidence of motor planning and programming of each component of a sequence was attributed to learning-induced changes in the brain.

The resultant contrasts showed brain activation associated with eye and hand coordinated movements when tracking random and predictable stimuli. The BOLD results revealed activation in areas related to unskilled responses that were dependent on feedback (RND) versus areas related to skilled motor responses where feedback became less important and memory-related processing was used

(PRD) (Halsband & Lange, 2006). In addition, BOLD responses also showed distinct activation in areas linked to higher cognitive loads during the learning of longer sequences compared to shorter sequences.

5.4.1 RND versus PRD in short sequences

The *4RND > 4PRD* contrasts showed higher BOLD activation in the right PFC for RND conditions. Specifically, there was activity in the right DLPFC (BA9), right FEF, right FP (BA10) and right premotor areas located in the precentral gyrus (BA6). In our task, the PFC was important for visual attention, which is essential to guide the eye and hand response close to the target during the sequence. The PFC has been shown to control a wide range of goal-directed behaviours. Areas of the PFC receive inputs from a wide range of brain areas the superior temporal regions. In particular the DLPFC is interconnected with supplementary motor areas, premotor areas (along the lateral precentral gyrus), the anterior cingulate, the cerebellum and the superior colliculus (Miller & Cohen, 2001). Furthermore the DLPFC sends projections onto the FEF (BA8) (Miller & Cohen, 2001). The PFC has been found to be the major output of the basal ganglia (via the thalamus). The PFC/thalamus/BG network highlighted in these previous studies appears to be important for the short sequence learning tasks presented here. Tanaka (2005) found thalamic activation during pursuit eye movements in a non-human primate study. He found that lesions to neurons in the thalamus decreased eye velocity during pursuit initiation and maintenance in visually guided and blanking tasks, suggesting that this area is also important for the control of predictive pursuit. In eye and hand tasks, the cerebellar/ thalamic/ cortical (PMA and SMA) pathway has been implicated in guiding the hand to keep

on the target even when the hand cursor is reversed (mirror) from the actual hand position (Miall & Reckess, 2002). Furthermore, thalamic lesions have been shown to interrupt the route of visual information from PPC (via cerebellum and thalamus) to the frontal lobes suggesting that the thalamus is also involved in sensorimotor integrative functions and an important subcortical area of the dorsal stream (Oreja-Guevara et al., 2004). Even though RND and PRD conditions exhibited activation of similar areas, distinct sub-regions within these areas were identified and were dependent on whether the task was visual (RND) or memory-driven (PRD).

Interestingly, right PFC and PMA were found active during RND sequence responses. Previous studies have shown the importance of DLPFC and the posterior parietal cortex (PPC) during processing of visuospatial information and in the orientation of spatial attention, with the PPC receiving information from visual areas before projecting onto the PFC via bottom-up processing (Katsuki & Constantinidis, 2012). Other imaging studies suggest that information about the saliency of a stimulus is first registered in the PPC, before being later acknowledged in the PFC (FEF and DLPFC) in a serial fashion (Buschman & Miller, 2007; Katsuki & Constantinidis, 2012). A recent electrophysiological study by Katsuki and Constantinidis (2012) however, presented evidence of early involvement of the PFC and suggested a similar timing of activation in both the inferior IPL and FEF areas in a parallel network during visual attention rather than strictly serial (Katsuki & Constantinidis, 2012). Our results also show frontal (DLPFC) and parietal (IPL) activation for the randomized sequences, which is a common network active during coordinated eye and hand motor responses (van Donkelaar et al., 2000). In particular, the right frontal and parietal areas are

thought to be important in visuospatial orientation of attention (Rushworth, Paus, & Sipila, 2001), which are possibly more in demand in the RND task.

Rushworth and colleagues (2003) investigated the role of certain regions within the parietal cortex in movement control. They suggested that a limited region in parietal cortex (IPL) is concerned with visuospatial attention and other regions (such as SPL) are concerned with "motor attention" or the control and preparation of limb movements. For example, when dissociating eye and hand movements and re-directing attention without the intent to make a limb movement, a BOLD signal increase occurs in non-human primate LIP (equivalent to human IPL), whilst re-directing a hand movement in the absence of a change of visuospatial attention results in activation of the more posterior region, the SPL, and the anterior part of the IPL (Rushworth, Johansen-Berg, Göbel, & Devlin, 2003). These findings suggest distinct contributions to the guidance of movement intentions and control within the parietal cortex (Rushworth et al., 2003). Our 4RND > 4PRD contrast showed higher SPL and left IPL activation during PRD conditions, which as previously mentioned, has been associated with attention (Rushworth, Nixon, & Passingham, 1997). We also found IPL to be more active during RND trials in which FEF was also active. Taken together, our results provide further evidence for a specialization between the visuospatial attention network that is more a feature of the RND task, and motor-related attention that becomes more prominent once the sequence is familiar (PRD task). This additional activation in the motor-attention network could account for the better coupling between the eye and hand movements in PRD conditions compared to RND conditions. We also suggest that during RND sequence presentations, PFC involvement was distinct from the motor response itself and becomes important for information integration and re-directing visuospatial attention (bottom-up) to

subsequently produce an eye movement (via FEF) followed by a hand movement (via M1). Overall, the RND tasks involved complex sequences that required the focus of attention to be continually shifted (IPL/FEF network) when compared to the repetitive sequences (Rushworth et al., 1997).

The orbital and medial PFC (BA10 and BA46) are structures also associated with memory processing and include (via the dorsal thalamus) connections with the hippoccampus (Miller & Cohen, 2001). A decrease in right DLPFC activity was observed during PRD conditions compared to RND conditions. The DLPFC has been previously associated with the control of short-term memory and in eye movement studies (Pierrot-Deseilligny et al., 2005; Ranganath, Johnson, & D'Esposito, 2003). A time-dependant decrease in BOLD activation has been previously observed during repeated presentations of a stimulus (see Burke and Barnes, 2008). These previous results and our current findings suggest a transition to more automatic responses as a result of short-term learning that creates a reduction in the need for the DLPFC (Burke & Barnes, 2008; Koch et al., 2006; Schmid et al., 2001).

Human lesion studies have identified the medial temporal lobe (MTL) as a region containing neurons involved in medium-term learning (delays > 20 s) (Pierrot-Deseilligny et al., 2002). This anatomical region includes the hippocampus and the parahippocampal cortex (PHC), with the latter having interconnections with the PPC and the DLPFC (Pierrot-Deseilligny et al., 2002). Furthermore, lesion studies have shown that the right PHC contributes to spatial memory at delays beyond the short-term memory storage duration (> 6 s) of DLPFC (Pierrot-Deseilligny et al., 2002). The decrease in DLPFC BOLD activation and increase in right PHC (BA28) may reflect a shift from early learning stages towards the observed motor adaptation of the learnt sequences. The SPL is

directly connected to the parahippocampal gyrus and has also been implicated in spatial working memory of visually guided actions (Sommer, Rose, Weiller, & Büchel, 2005). We suggest that both the SPL and the PHC are important for the encoding of our visuomotor sequence learning task, however a time-based analysis would provide more detailed information about which stages of the learning process they are mostly involved in.

An important finding of this study was the observation of SMA and pre-motor activation during PRD sequence presentations. In particular, lesion studies have implicated the SMA in voluntary movement initiation of previously learnt movement sequences that require precise timing (Halsband & Lange, 2006; Lee & Quessy, 2003). In addition, there is evidence of practice-related increases in SMA during implicit learning tasks and left hemisphere activation is evident regardless of training hand side (Halsband & Lange, 2006). A similar effect in pre-motor cortex has been observed. Specifically, the PMA has been implicated during tracking and writing tasks. Our findings suggest that the left PFC is mainly involved in the early storage of motor skills during an acquisition phase of learning (Halsband & Lange, 2006).

The results presented here show similar anatomical activation to previous SRT tasks involved in sequence learning such as; the PFC, the PPC, the SMA, the basal ganglia, the thalamus and the cerebellum (Fletcher et al., 2005; Lee & Quessy, 2003). The role of the cerebellum during visually guided sequence learning has been attributed to complex processing of the timing component of motor sequences (Orban et al., 2011). Lesions to the cerebellum result in difficulty maintaining movements on-time, which is an essential part of sustaining rhythmic responses, and possibly chunking of components during sequence learning tasks

(Orban et al., 2011). Miall and Jenkinson (2005) investigated neural activation during an eye and hand-tracking task in human participants, with a special interest in cerebellar activation. The results from this previous study suggest that sequence learning may involve other areas of the brain than the cerebellum, but that cerebellar contributions are important for established or learnt eye and hand coordinated movements. The cerebellum seems to be critical in minimizing performance errors and errors related to the temporal relationship between the eye and the hand (Miall & Jenkinson, 2005). In our task, eye and hand latency differences were decreased during the repeated PRD sequence presentations and this maybe indicative of an internal forward signal that can facilitate learning during coordinated eye and hand tracking.

We also found activation in areas not typically observed in SRT tasks including FP areas, ACC, visual areas V5. ACC activation is frequently observed with PFC activation during pursuit, but its role in motor learning is different to that of the frontal regions (Halsband & Lange, 2006). In these previous pursuit studies, the ACC has been reported to be involved in motor planning and memory of stimulus timing and trajectory proving important for predictive eye movements (Ding et al., 2009; Schmid et al., 2001). In motor learning tasks, the ACC was also active during learning, but not during performance of pre-learned sequences (Jueptner, Flerich, Weiller, Mueller, & Diener, 1996).

We also found hemispheric shift from attention-dependent circuitry (e.g., right DLPFC and FP) into anatomical areas for storage of visuomotor skills (e.g., ACC and SMA). Van Mier and colleagues (1998) investigated changes in brain activity (PET) during a tracing-learning task with practice, no practice, high and low complexity and high and low speeds. Interestingly, their results showed activation of ipsilateral premotor cortex with no contralateral hand movement.

They also observed activity in the right parietal cortex during unskilled performance, whilst skilled performance showed activation in SMA. Finally, they also reported practice-related activation occurring in the same hemisphere regardless of hand used (van Mier et al., 1998). Our results showed right cerebral activation during the more attention-demanding RND sequences and a shift to left PMA and activation of the SMA during responses that showed evidence of learning. When comparing our results to previous oculomotor learning studies, our BOLD results revealed areas previously reported to be linked to the control of pursuit such as ACC, DLPFC, IPL and cerebellum (see Burke & Barnes, 2008) and thalamo-cortical areas (Tanaka, 2005).

5.4.2 RND versus PRD in long sequences

The 8RND and 8PRD sequences revealed predominant subcortical activation during presentations. In particular, higher activation corresponding to RND responses was found in the basal ganglia, thalamus, PCC, precuneus (BA7) and bilaterally in FP areas (see Table 5.1). Activation of the FP areas, the thalamus and the BG was also observed during the shorter sequence presentations (4RND). We found additional cortical activation in DLPFC, IPL, FEF and pre-motor cortex during the shorter random sequences, which did not reach significance during the longer random sequences. The precuneus was an area revealing higher activation in the longer, but not the shorter, random sequences. The precuneus, located in posterior parietal cortex, is suggested to be an association area sub-serving a variety of behaviours (for review see Cavanna & Trimble, 2006). It has reciprocal connections with other parietal areas (SPL and IPL) known to be involved in visuospatial attention processing (Cavanna & Trimble, 2006). The precuneus also

has connections with the frontal lobes (FEF and medial frontal cortex), the SMA and the ACC. Non-human primate studies have shown precuneus involvement in the control of eye movements and visually guided reaching (Cavanna & Trimble, 2006; Thier & Anderson, 1993). An imaging study by Petit (1999) found precuneus activation during pursuit and saccadic eye movements suggesting that this area is important for oculomotor control. Furthermore, Luo et al (2004) found activity in the precuneus during a sequential key-press task and also suggested that the left precuneus was involved in the control of attention during finger movements, but not in movement preparation. This area of the brain is also interconnected with the dorsal thalamus and structures in the basal ganglia (Cavanna & Trimble, 2006), which are areas that were also significantly active in the 8RND task. The connectivity amongst these areas imply some involvement in higher-order cognitive functions, and this gains support from fMRI studies that suggest an important role for this network in locating and shifting attention to a target of interest (Cavanna & Trimble, 2006). The BOLD responses for the 8RND task revealed involvement of this brain network (precuneus, thalamus, basal ganglia and PCC) during the longer sequences only. This demonstrates higher spatiotemporal demands of the longer and more complex sequences, when compared to our shorter or predictable sequences.

BOLD activity corresponding to the PRD 8 component sequences showed both cortical and sub cortical activation. Similar to the short sequence contrast, higher activation in the 8PRD task was observed in the parahippocampus, the ACC, cerebellum as well as the DLPFC, IPL, SPL and extrastriate visual areas (V5). Similar to the shorter sequences, we found that parahippocampal activation (in MTL) was higher in predictable condition than the random condition, and likewise the DLPFC was higher for random trials. Studies have found structures

of the MTL to be involved in memory and learning possibly during encoding or retrieval or both (Eldridge, Engel, Zeineh, Bookheimer, & Knowlton, 2005; Schacter et al., 1999; Suthana, Ekstrom, Moshirvaziri, Knowlton, & Bookheimer, 2011). The activation observed during the longer sequences also supports these previous findings of the role of parahippocampal activation during the early stages of medium term learning, but adds to the literature by revealing the storage role of this area in eye and hand sequence learning tasks (Pierrot-Deseilligny et al., 2002). The observed cerebellar activation for the 8PRD task was also consistent with the shorter sequence contrast and seemed more important during PRD conditions. The nature of the involvement of the cerebellum in learning is still a subject of much debate with some studies suggesting little involvement. These task-dependent differences between studies have shown both increases and decreases in BOLD activation during learning (Doyon et al., 2002; Miall & Jenkinson, 2005; Seidler et al., 2002). For example, Seidler and colleagues (2002) investigated cerebellar activity during an implicit motor learning task and found no cerebellar activity associated with the learning of the motor skill, when learning was evident and performance was held constant. However, Miall and Jenkinson (2005) found that for learnt eye (pursuit) and hand (joystick) coordinated tracking; the cerebellum plays a major role in supporting learning (for more examples see Miall & Reckess, 2002; Miall, Weir, & Stein, 1988; Miall et al., 2000). Our results also showed that the cerebellum was important during improved eye and hand tracking, possibly through cerebellar involvement in experience-dependant coordinated responses (Miall & Jenkinson, 2005).

Prefrontal activation was only observed in left DLPFC and no activation of motor regions was observed during the PRD conditions in the longer sequences. Interestingly, the premotor and SMA areas have been previously reported during

sequence learning tasks and associated with experience-related changes; however, activation of the SMA during learnt sequences has not been consistent across studies (Lee & Quessy, 2003). Seidler et al. (2005) investigated early and late encoding during a key-press SRT task in either an early or a late stage of learning and reported that SMA was not involved in learning the SRT task itself. Other studies have observed increased SMA activity during implicit learning tasks, and in particular tasks that require sequential movements with precise timing (Halsband & Lange, 2006). In contrast with other SRT studies, our 8PRD task did not show significant motor cortex activation. It may be that in these areas a decrease in PMA and SMA activity is associated with increased learning, maybe by a long-term depression (LTD) mechanism in the brain. It is also possible that our 8 sequence task induces some habituation effect of performing automatic movements. However, habituation effects are usually observed over a whole network involved in the task, and not a single area, and therefore it is unlikely to be the cause of the effects observed in these premotor regions. Our results indicate that memory-related areas play a more important role for improving performance during the more complex sequences.

V5 activation was higher in PRD tasks than randomized tasks, and it is not an area that is usually associated with sequence learning. This is probably due to the majority of previous sequence learning studies using finger key press tasks and stationary stimuli compared to our motion task, which consisted of tracking a continuously moving target. Our task would therefore invoke visual motion signals that travel to V5 via early visual areas. TMS delivered to V5 causes temporary visual motion "blindness", suggesting a key role for V5 in the perception of human motion (Beckers & Hömberg, 1992; Maunsell & van Essen, 1983). Bilateral V5 activation was also observed during the shorter predictable

sequences also revealing that this area was more active during the learnt sequences. This was unexpected since previous studies have suggested that V5 is mostly active during visual feedback and therefore a decrease in activation during PRD conditions would show less dependence on visual input (Lencer & Trillenberg, 2008). Other studies however, have found V5 active during PRD pursuit and in particular neurons in the medial superior temporal (MST) area are active during predictive eye movements, in the absence of visual feedback (Burke & Barnes, 2008; Lencer & Trillenberg, 2008; Schmid et al., 2001). Despite these previous findings it remains unclear how this area contributes to predictive eye movements or to eye and hand motor learning when tracking a moving sequence stimulus.

Nagel et al (2008) investigated cortical mechanisms of retinal and extraretinal visually guided pursuit compared to pursuit during target blanking. They found V5 was important for coding target velocity and the superior temporal gyrus alongside the cerebellum was also implicated in the control of the velocity of the response. Nagel et al (2008) also found that the inferior parietal lobe was implicated in sensorimotor transformations in the absence of a visual target indicating that this area contains an internal representation of target velocity in extraretinal pursuit. Taken together, previous findings from studies showing activation during the absence of visual feedback indicate V5 (see Lencer & Trillenberg, 2008) and IPL (Nagel, Sprenger, Hohagen, Binkofski, & Lencer, 2008) as sources for efference copy information. Other studies have shown the dorsal stream (including the SPL) also plays an important role in spatial localization, with contributions from the parahippocampus (Sommer et al., 2005). Interpreting our data alongside these previous studies provides evidence that the network V5, IPL, SPL, STG and parahippocampus, all are important for the

internal representation of position/velocity information used for predicting the sensory consequences of the motor response.

During the longer sequences we found higher BOLD activation of sub-cortical memory-related areas suggesting a greater demand on this resource. Differences with other sequence learning studies are task related and possibly because learning occurred quicker compared to other studies. BOLD signals also showed activation of areas for the voluntary control of pursuit that we now have shown are also important in eye and hand coordinated movements (Burke & Barnes, 2008). Results from these longer sequences highlighted a network of brain activity in the parietal cortex related to the integration of visuospatial attention, orientation and short-term memory. In addition, visual area V5 was also important for encoding stimulus information when performing eye and hand coordinated tracking during PRD responses.

5.4.3 4 versus 8 components: Effects of sequence length

We specifically designed our paradigms to allow comparisons between the longer and shorter sequences and also ensured that the visual stimulus parameters for these different lengths were equivalent. We observed a number of similarities in BOLD activation for both the short and long sequence RND > PRD contrasts. A more direct comparison (4PRD > 8PRD) confirmed that similar areas were active during both sequence length tasks. Common areas of activation included the ACC, SPL and prefrontal areas FP and PMA. However, despite these similarities, higher V5 activation was observed to the shorter 4PRD sequences. The basal ganglia, thalamus and cerebellum also showed higher activation in 4PRD compared to 8PRD task. In contrast, the 8PRD showed overall higher

activation of prefrontal areas with bilateral PMA, DLPFC, right FP compared to the 4PRD responses. In addition, significant activation of the PHC was exclusive to the longer PRD sequences.

Oreja-Guevara et al (2004) investigated brain activity using fMRI during visually guided hand responses (joystick) while tracking a continuously moving target and a ballistic stationary stimulus. Participants were asked to maintain fixation throughout the trials therefore removing the visuomotor tracking component and thus allowing only hand tracking to occur. Overall, their results showed activation of V5, PMA and SMA, PPC areas, basal ganglia (putamen), thalamus and cerebellum. They suggested that V5 not only contributes to oculomotor tracking, but also to the control of visually guided hand movements. V5 and parietal connections have shown to be integrative areas of spatial visuomotor information and visuomotor control. Our short PRD sequence results showed similar areas to Oreja-Guevara et al (2004), However, it is difficult to compare weather learning or predictive responses took place in this latter study since reaction times were not reported. Their task consisted of single ramps on the horizontal axis. It is possible that participants were able to create a spatial representation of target direction/velocity and responses could have been pre-programmed. Our RND sequences were temporally and spatially randomized and only elicited reactive eye and hand latencies and when contrasted with the PRD sequences did not show significant V5 activation. Indeed, we and others (Burke & Barnes, 2008) have found that extrastriate visual areas are important during the repeated sequence presentations in PRD conditions compared to RND presentations and more active for the shorter sequences. It is also considered that during predictive motion, the eye could rely more on target visualization leading to higher motion stimulation and therefore more V5 activation was observed that

was not related to memory per se. Activation of the thalamus was also observed in the shorter, but not the longer sequences. In particular, activation of the pulvinar was observed during 4PRD and has been suggested to be involved in sensorimotor integrative functions. Oreja-Guevara et al (2004) and others (Ellermann, Siegal, Strupp, Ebner, & Ugurbil, 1998; van Donkelaar, Stein, Passingham, & Miall, 1999) have shown increased thalamic activation during visually-guided hand movements as an important subcortical area of the dorsal stream. It is possible that as Oreja-Guevara et al (2004) found, our PRD task also required a larger scale sensorimotor network (V5, thalamus, parietal and pre-motor areas) to transform visual input into motor behaviour, supporting the notion of increased visual input use.

Longer sequences showed higher activation of memory-related areas in general (DLPFC, PHC, FP, ACC) compared to the 4PRD sequences possibly demonstrating a more automatic response for the shorter sequences and suggesting that higher order structures were needed during the 8PRD sequences to acquire similar performance levels to the shorter sequences. Fletcher et al (2005) investigated neural activation during a spatial SRT and found PFC, PPC, BG (putamen) and thalamic activation, also consistent with our 4PRD findings. We suspect that the parahippocampal activation (in MTL, BA30) during the 8PRD sequences demonstrates the higher order learning during the more demanding sequences perhaps since more memory capacity is needed.

Higher activation of premotor areas was observed during the longer sequences compared to the shorter sequences. Premotor areas have been known to process temporal information during complex timing tasks and have been linked with the control of movements performed in a sequence (Nakamura, Sakai, & Hikosaka, 1998; Orban et al., 2011). Learning-related changes in PMA have been

reported previously (Hikosaka et al., 1996; Orban et al., 2011; Sakai et al., 1998). Non-human primates activation of pre motor areas has been demonstrated during the pre-movement period in sequence learning tasks (Nakamura et al., 1998). Similar paradigms implemented in humans show that learning of new sequences elicited activation of pre motor areas as well as DLPFC, precuneus (SPL) and PPC (Sakai et al., 1998). The PMA received signals from the DLPFC to aid in the generation of a motor response during our sequence tasks. We suggest that areas of the PFC were important for the encoding (via DLPFC) and retrieval (via PMA) of the sequence to achieve improved accuracy and timing of the eye and hand coordinated responses.

Overall, results from the 4PRD > 8PRD contrast showed increased activation in memory-related areas during the longer sequences compared to the shorter sequences. These differences are suggested to be due to additional cognitive loads in the longer sequences alongside possible learning-related behavioural changes resulting in enhanced efficiency of signal processing and shorter reaction times (Koch et al., 2006; Schmid et al., 2001). The observed higher parahippocampal activation during the longer sequences could also reflect the longer periods of learning (> 6 s) and/or the need to remember more items that go beyond the limits of short-term memory. Also, higher activation of the PFC in the longer sequences could also represent the larger amount of sequence components that had to be pre programmed (DLPFC) in advance when compared to the 4PRD trials. The need for increased memory capacity resulted in a higher level of motor processing occurring in parallel in PMA in order to execute an effective response (Bo & Seidler, 2009).

5.4.4 RND versus PRD: short and long sequences

All trials for the RND versus PRD contrasts in both longer and shorter sequences were compared and showed the overall activation between these conditions. Higher activation corresponding to PRD sequence presentations only revealed activation of V5, IPL, temporal cortex, parahippocampus, pre and motor areas (PMA and M1), cerebellum and the insular cortex. The motor cortex is strongly interconnected with somatosensory and spatial processing regions in the parietal lobe (IPL), premotor and supplementary motor areas (BA6, BA8), the basal ganglia and the cerebellum. We support the findings that sub-cortical activation (ACC, IC, caudate and the PHC) is important for the acquisition of sequential motor actions and that the cerebellum is not exclusively dedicated to learning since it did not feature in the 8PRD responses. However, it may be involved in optimizing the response through error correction and temporal processing for the sequential response (Orban et al., 2010). Our results also support findings of fronto-parietal activation and its importance in attention and short-term memory. The M1, parietal and pre motor region networks are part of the storage of motor representations of a learnt sequence (Penhune & Steele, 2012), possibly with M1 acting as a low-level area (as effector) in the network (Orban et al., 2010). The premotor cortex participates in the planning and selection of actions by integrating signals containing spatial and temporal information possibly generated from V5 (MST) to PPC and from the cerebellum (Lencer & Trillenberg, 2008; Orban et al., 2010).

To summarize, our results showed many areas in common with previous sequence tracking and SRT tasks (that include spatial components), mostly related to learning and skill acquisition. In our study, premotor activation was

involved in the planning and selection of appropriate responses by integrating spatial/velocity and temporal information for producing accurate motor sequence movements (Orban et al., 2010). Even though our task of complex novel sequences was quickly learned by our participants, we observed areas that have been shown to be active during skill acquisition and consolidation, such as pre motor areas and fronto-parietal areas. We also observed areas that were not only important for oculomotor performance, but also for the control of coordinated eye and hand movements. This suggests that even though eye and hand responses are performed at different times, they may share common predictive mechanisms. In support of this, our behavioural results obtained from the eye and the hand showed similar latency and accuracy effects when performing the sequences exhibiting evidence of equivalent timing and velocity-coded storage from prior experience. Further studies are needed to identify oculomotor and hand neural correlates during motor sequence learning and examine how we learn sequences of actions. We also reported distinct activation related to motor sequence complexity. We suggest that differential activation between sequence lengths could be attributed to a shift in neural mechanisms from memory processing of the complex longer sequences to a more automatic network of the learnt shorter sequences.

Chapter 6

6 General discussion

In summary, we performed experiments that investigated i) sequence learning in the oculomotor system and compared saccadic and pursuit eye movements using an analogous task, ii) sequence learning in eye and hand coordination, and iii) the effects of cognitive load in eye only and eye and hand coordination tasks. We used a novel task to investigate the behaviour and the anatomical areas involved in motor sequence learning in the eye, and in eye and hand coordination. Our experiments add to the knowledge of the oculomotor's ability to store and use prior information to internally generate an appropriate response; whist avoiding the inherent neural processing delays in the system.

The behavioural findings are in accordance to previous finger-press and finger-tap SRT experiments (Nissen and Bullemer, 1987), and also align with findings on predictive saccades (Ross & Ross, 1987; Shelhamer & Joiner, 2003) and predictive pursuit (Barnes & Asselman, 1991; Barnes & Donelan, 1999). However, our novel task allowed direct comparisons of saccadic and pursuit eye movements by using equivalent stimuli for both eye movement types. Furthermore, we used continuous sequences of movement that could be considered ecologically more valid. This approach however proved technically difficult, as establishing an onset for on-going pursuit is challenging. Some studies suggest that a predictive slowing down of eye velocity occurs in anticipation of a predictable target directional change (Burke & Barnes, 2006), however when no directional change occurs this can prove difficult to identify. Other studies have calculated the onset of pursuit using regression techniques to determine when

eye velocity is zero, however, this method cannot be applied to our continuous pursuit sequence task as the eye velocity is rarely zero. Finally, studies have also looked at eye velocity at a moment in time, after onset and prior to the presumed time when the eye receives visual input (< 100 ms). Thus, if the velocity gain is similar or close to target velocity this is then defined as an anticipatory response (Barnes & Donelan, 1999; Barnes & Schmid, 2002; Collins & Barnes, 2005). This technique is also difficult to apply during continuous pursuit as it assumes that initial eye velocity equalled zero, which is not the case in the experiments outlined here. Another consideration in our study was it's comparability with saccadic metrics, which again are difficult as saccades can reach high velocities quickly and in saccades, the final goal is not matching target velocity but arriving at target location (velocity ~ 0 °/s). We did not intend to directly compare saccade and pursuit latency and to overcome these problems, we compared performance changes (e.g., differences in latencies) between RND and PRD conditions using a similar analysis approach to investigate oculomotor adaptations, and thus identify oculomotor sequence learning behaviour in both saccadic and pursuit systems.

6.1 Sequence learning in the oculomotor system: Behaviour

In experiment one, we presented a series of identical sequences (predictable) for participants to track by making a saccadic or pursuit eye movement to match target position. In both types of eye movements there was evidence of participants quickly learning a 4 component sequence by showing significant decreases in pursuit and saccade latency after only one presentation of the sequence. However, distinct levels of adaptations (i.e., when changes in

performance are maintained) were observed between saccades and pursuit. Indeed latency differences were noticeable during the second presentation compared to SEQ1 in both eye movement types. In pursuit performance did not change across repetitions, suggesting that a pursuit steady-state was reached very early in the presentations. In contrast, saccades made improvements with subsequent presentations (SEQ3 and SEQ4) and showed continued decreases in latency. This is in line with previous findings that suggest that once saccadic prediction occurs it continues and may also improve over time (Shelhamer & Joiner, 2003). Not only were the temporal differences from reactive to predictive eye movements higher in saccades compared to pursuit (as found previously by Burke and Barnes in 2006), but we additionally found learning adaptations were also different between the eye movement types. These differences could be accounted for by the fact that saccadic eye movements can be voluntary initiated without visual information, and given that saccades are ballistic, they do not rely on feedback during the movement. It is therefore possible that saccades can pre-program a response well in advance and then correct any errors once the first saccade is made. In contrast, pursuit eye movements are more reliant on visual feedback and may have limited predictive behaviour due to need for the visual stimuli to drive the response. Indeed gap tasks (i.e., when fixation disappears prior to target onset) have been shown to facilitate pursuit initiation and prediction when the target is not present, but expected. Introducing a gap during turnarounds could have elicited more prediction in our pursuit trials. Nevertheless, we suggest that high target expectancy and responding to a known or learnt sequence resulted in decreases in pursuit latency in the experiments described. However, due to the continuous, visible target presentations, prediction was limited in our experiments when compared to the discrete ramp

presentations used in previous research (Burke & Barnes, 2006; Collins & Barnes, 2005).

We cannot determine whether pursuit response would have improved following more repetitions, however, from past research it is evident that the pursuit system can indeed reach a steady-state quickly with repeated sequences of 2 to 4 components (one or two presentations) (Collins & Barnes, 2005). We also do not suggest that saccades and pursuit would reach similar prediction level, as there is much evidence to suggest that these two types of eye movements have very different latency thresholds due to differing functional demands. For example, during PRD pursuit, responses need to store the velocity and direction of the moving target and are therefore reliant on visual feedback. The system needs to combine the storage of velocity/direction information of the target with the constant updating of the eye. In contrast, saccadic responses only needed to recall the target's location and not timing. This processing is much less demanding, and hence may be reflected as shorter latency responses. This also suggests that the pursuit system requires more adaptability and flexibility to accommodate visual feedback compared to saccades (also see Burke & Barnes, 2006). These findings provide further evidence that these two systems could activate different subregions and/or different levels within the shared anatomical pathways, already established in functional imaging investigations (e.g., FEF, SEF and SC). Krauzlis (2003) found distinct activation levels within the superior colliculus for triggering saccades and pursuit, with lower activation needed to trigger the latter. Missal and Keller (2002) also suggested distinct activation within the OPN in the brain stem. In addition, different saccade and pursuit related subregions have been found in shared areas such as those found in the FEF (Petit et al., 1997).

6.2 Sequence learning in the oculomotor system: fMRI

We further compared the brain areas associated with the two oculomotor systems during our sequence learning tasks. Results from fMRI experiments reflected the observed behavioural changes, and showed distinct areas of activation reflecting sensory and memory-driven circuitry particular and common to both saccadic and pursuit responses. Individual saccade and pursuit contrasts showed that RND conditions required greater sustained activation of attention-related areas such as the DLPFC, FEF and IPL suggesting a preference for these areas in our visually guided responses when learning was not apparent. In addition, individual contrasts also confirmed that the SEF plays a key role in saccadic and pursuit prediction during sequence learning, although activation of this area seemed more important during pursuit than in saccades. Another area in common was the ACC, which has connections with SEF and FEF and possibly facilitated these areas for the preparation of a short latency response during predictable trials. Activation of the DLPFC, an area also connected with FEF and SEF and associated with the use of working memory, was either not significant (in PRDs) or attenuated (in PRDp) during repeated presentations. This at first seems at odds with previous literature, however we suggest that these changes reflected an enhanced processing efficiency as a result of short-term learning. Previous studies have found activation in DLPFC when storing single items for a short period of time (Baddeley, 2000; Halsband & Lange, 2006). However we propose that as the sequences are repeated the need for memory mechanisms are reduced which is reflected in the reduction in DLPFC activity. In pursuit, activation of additional frontal regions was observed in predictable tasks when compared to saccades, with activation of frontopolar, dorsal and ventro-lateral prefrontal cortex. This could indicate additional attention and memory requirements during

pursuit to recall target velocity and update the motor response (visual feedback) during PRD conditions, compared to the more simplistic spatial storage required in saccades. In contrast, temporal regions were more active during saccadic PRD tasks possibly reflecting a learning-related updating of spatial information during the trials (also see Burke & Barnes, 2006). Figure 6.1 summarizes the fMRI data during eye only experiments in a model that shows brain areas specific for saccades and pursuit during sequence learning. We provide new evidence of frontal/temporal distinctions between pursuit and saccades during the learning of sequences that also reveals a shift from prefrontal to more sub-cortical regions of the anterior cingulate during learning.

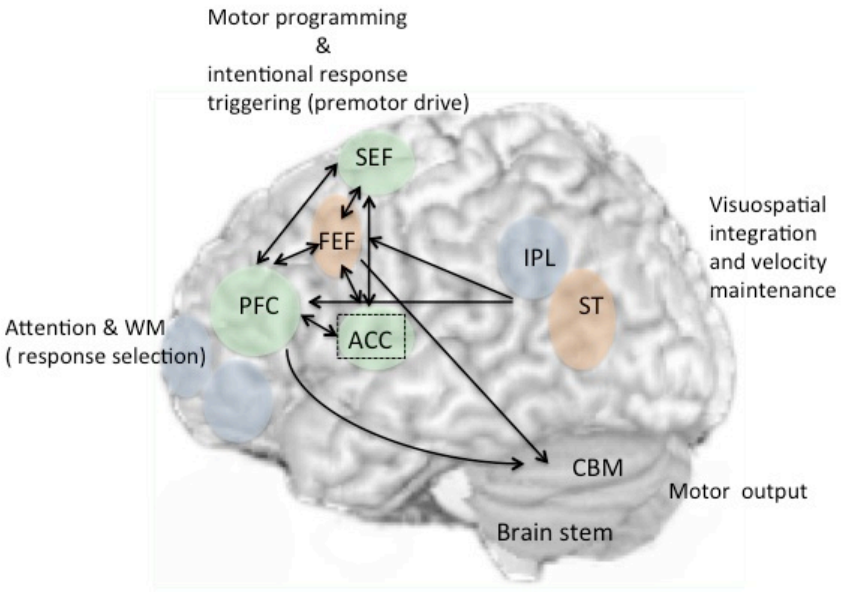

Figure 6.1. Cortical areas found in this study to be involved in predictive saccades (orange) and pursuit (blue) and common pursuit and saccade areas (green). In prefrontal cortex (PFC) areas including the DLPFC, FP and VLPFC are associated with attention and the short-term storage (WM) of stimuli information (velocity and position). These areas with frontal eye fields (FEF) and supplementary eye fields (SEF) are involved in the pre-programming of predictive saccades and pursuit. Inferior parietal lobe (IPL) and the superior temporal gyrus (STG) are areas that receive input from visual cortex and are associated with monitoring and updating of visuospatial information. The anterior cingulate cortex (ACC) is enclosed in dotted lines demonstrating a deeper structure. These areas are taken primarily from the 4 component saccade and pursuit sequences.

6.3 Sequence learning in eye and hand coordination: Behaviour

In our eye and hand coordination experiments, we used the pursuit sequence learning task to examine eye and manual tracking of a continuous motion stimulus. Similar to eye only experiments, behavioural results showed significant decreases in pursuit and hand latency that were evident from the second presentation of a sequence with no further improvements in performance throughout the repetitions. In addition, manual tracking improved in the predictive responses not only temporally with respect to the target onset and directional changes, but also showed decreased displacement errors and smoother tracking. These maintained improvements in performance showed evidence of the ability to store target velocity and make predictions for the upcoming sequence based on prior experience in both the eye and hand. RND to PRD analysis revealed that the lag of the hand behind the eye was decreased during the repeated sequences suggesting tight eye and hand coupling as a result of learning indices changes. Hand movements differ from pursuit eye movements in that they do not require visual target information to generate a movement, and thus produce a response at will, as in saccades. We also established in the eye only experiment that pursuit adaptation occurred quickly and performance was maintained throughout repeated sequences. The fact that the hand was consistently and similarly delayed (to eye and target) across repetitions, suggests that hand tracking was influenced very strongly by eye movements. We also suggest that this was the preferred strategy used by participants to keep up with the target. It has been previously observed that keeping a certain hand lag with respect to the eye improves tracking perhaps due to a short-term buffer that feeds

from the oculomotor system into the manual system as part of a feed-forward system (Miall & Reckess, 2002). Our results support the notion that input from eye movements may be used to make predictions of subsequent hand movements. Furthermore, our findings provide new evidence that improvements in oculomanual tracking during prediction were the result of tight coupling between eye and hand movements. Indeed both systems appear to send related input signals (efference copy) in the same coordinate frame to provide predictive information of the required response.

6.4 Sequence learning in eye and hand coordination: fMRI

Results from our imaging experiment revealed activation in brain areas that are important for eye and hand sequence learning but also, revealed areas in common between the RND and PRD conditions. Thus, these areas may play a key role in the coordination of eye and hand responses regardless of whether the target is presented in a predictable or random manner. We found common brain activation between conditions in a network that included DLPFC->PMA->PPC->thalamus and BG. It has been suggested that this network of brain areas is involved in both sensory guided and internally guided responses (van Donkelaar & Staub, 2000). Our study provides new evidence that this network of brain areas additionally contributes to the integration of sensory and motor signals (PPC and thalamus) and response selection (PMA, PFC and BG). Despite these similarities in activity between PRD and RND eye movements, we did find distinct activation within these areas between conditions, suggesting some specialization does exist. Indeed, from the eye only experiments it was observed that random presentations also required sustained attention and, similar to oculomotor contrasts, DLPFC

activation was often active during both PRD and RND eye and hand conditions. However, similar to eye only findings, DLPFC activation was decreased during eye and hand PRD conditions. Distinct PMA lateralized activation between conditions (right vs. left PMA in RND and PRD respectively) and activation of the SMA showed specialization and learning related changes in brain activation during PRD conditions in the eye and hand experiment. Activation of SMA appeared to be more important during PRD conditions and has been involved with executing previously learned movement sequences specifically, SMA neurons become active during a particular known sequence of movements (Nakamura et al., 1998).

Learning-related changes during PRD conditions also included activation in the FP, SPL/IPL, ACC, V5, cerebellum, and parahippicampal gyrus (BA28), which are all also associated with prediction in pursuit. We suggest that these areas play a key role in the encoding, storage and pre-programming of predictive responses, but may not be important for enhanced coupling between the eye and the hand. Figure 6.2 describes the brain areas involved in eye and hand coordination during sequence learning. PFC, V5, IPL and the ACC are areas that have also been observed during predictive pursuit (Ding et al., 2009; Lencer et al., 2004; Lencer & Trillenberg, 2008) and may support the notion that input signals from the oculomotor system have important contributions to control hand performance. It's also possible that information about eye or hand movements are shared between both systems for optimal eye-hand control (Miall et al., 2000; van Donkelaar et al., 1999). We propose that the same predictive network is used in both eye and hand sequence learning and include the PFC and ACC. However, SMA and PMA are preferentially recruited for the hand, whereas FEF and SEF are significantly more important during eye movements.

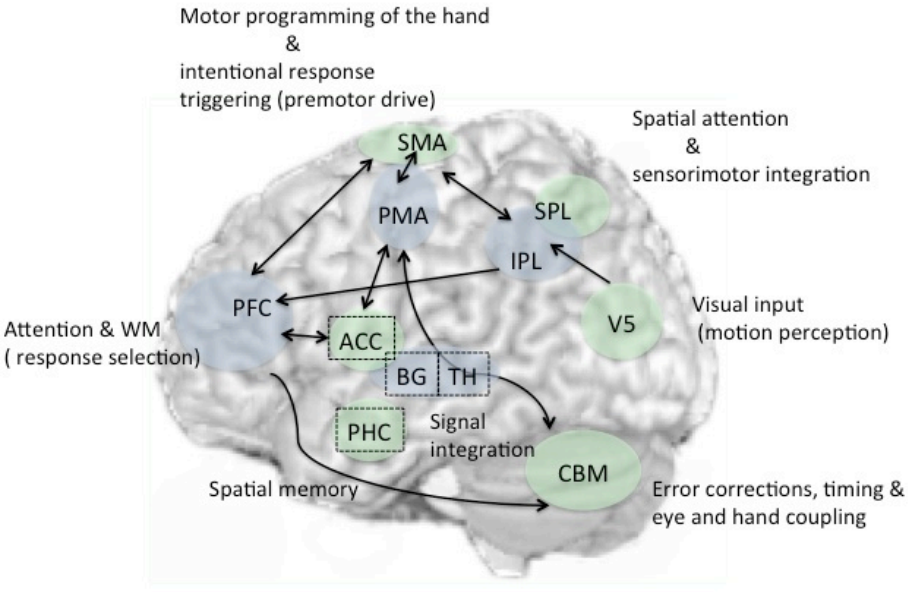

Figure 6.2. Cortical areas found in this study to be involved in eye and hand sequence learning (green) and common RND and PRD areas (blue). In prefrontal cortex (PFC) areas including the DLPFC and FP associated with attention and the short-term storage (WM) of stimuli information. These areas connect with premotor areas and supplementary motor areas (PMA and SMA), involved in the pre-programming of eye and hand movements. Inferior parietal lobe (IPL) and the superior parietal (SPL) are areas that receive input from visual cortex. The anterior cingulate cortex (ACC), basal ganglia (BG), parahippocampus (PHC) and thalamus (TH) are enclosed in dotted lines demonstrating deeper structures. The ACC and PHC are also involved in memory and learning of new motor skills. The cerebellum is connected to motor areas via the thalamus and is associated with timing of rhythmic movements, error corrections and in coordinated eye and hand tracking. These areas are taken primarily from the 4 component sequence comparisons.

We investigated the brain areas associated with PRD and RND in eye and hand coordinated tracking. As previously reported, eye and hand behaviour exhibited behavioural similarities as well as tight coupling which may provide further evidence of a common predictive drive between the two motor systems (Barnes & Marsden, 2002). However, due to our distinct experimental design features (event timings, trial length and number of experimental blocks), it was not possible to perform analyses in SPM between the eye only and the eye and hand results. However, qualitative observations and comparisons between behavioural and the related brain activity results revealed interesting findings. In

particular we observed similar behaviour and similar activation of brain areas during sequence learning for both the eye only and eye and hand analysis. In addition, similar behaviour was found when comparing short and longer sequences in the eye only and in the eye and hand experiments. The latter provides new insight into the capacity of sequence learning and the effects of increasing the cognitive demands on a system. It also provided new insights into how participants' behaviour adapted during the learning of longer sequences and their overall strategy for learning short and long sequences.

6.5 Eye only versus eye and hand coordination: Behaviour

It is difficult to compare between experimental modalities (EO and EH) since in each task the target parameters were altered. For instance, target size was increased (15 x 15 pixels to 20 x 20 pixels for EO and EH respectively) so that participants could position the joystick cursor (15 x 15 pixels) on target and still see the target and target velocity was decreased (30°/s to 15°/s) for the EH so that participants could manually track the trajectory target. This meant that the eyes were slower and exhibited greater variability (oscillation) when compared to tracking the faster target. In addition, target parameters could have had an effect on pursuit parameters. For instance, overall increased latency in EH compared to EO (~ 367 ms and ~ 267 ms for EH 4RND and 4PRD and ~240 ms and ~179 ms for EO 4RND and 4PRD) may not be solely attributed to the hand slowing the eye, but may also be an effect of the slower velocity. However, we did observe similar performance between the EO and EH experiments. In particular, pursuit eye movements displayed equivalent decreases in latency (~100 ms) and TTPV values that reached significance between PRD and RND tasks in both EO

and EH experiments. Both experiments also showed that these temporal improvements did not change across repeated sequence presentations. Pursuit displacement errors across repetitions did differ with the inclusion of the hand, and pursuit absolute errors revealed increases across repetitions for EH experiments but not in EO. We have attributed this to a strategic mechanism of guiding the hand towards the target and shows that the eye does not necessarily have to be on the target to maintain predictive performance when guiding the hand. We also suggest that the addition of the hand does not affect the ability to perform predictive eye movements but that coordinated responses may result in delays eye movement latencies to achieve optimal coupling with the hand and may result in decreased eye accuracy towards the target, possibly to monitor hand location when available.

6.6 Effects of sequence length: Behaviour

We found similar behavioural results during both types of tasks when comparing sequence lengths. Overall, participants showed evidence of learning the longer sequences with significantly decreased eye (pursuit) and hand latencies and TTPVs during PRD conditions, compared to the visually guided RND sequence presentations. Hand responses in the eye and hand experiment not only exhibited temporal shifts, but also showed improved accuracy in the 8PRD conditions compared to RND conditions. Improvements in eye and hand performance, in both EO and EH experiments, were also maintained throughout repetitions. These results are similar to behaviour in the shorter sequences. In addition, overall pursuit temporal shifts and hand temporal shifts seemed to be consistent throughout the longer sequences suggesting that participants were

able to maintain a similar predictive drive throughout the 8 components in the sequence. These results were contrary to our hypothesis since we expected that 8 components would be difficult to store in short term memory and that participants would show evidence that more repetitions would be required to achieve a steady-state. We particularly expected this to be the case during the eye and hand tasks in which a longer sequence of presentations lasted around 48 seconds. Also, when making comparisons with the shorter sequences, pursuit eye movements as well as hand movements revealed that RND to PRD timing shifts did not differ between sequence lengths.

Further analysis of shorter versus longer sequences revealed that some differences do exist and that eye and hand TTPV and eye peak acceleration were indeed affected by sequence length. Closer inspection however, revealed that some of these differences were found regardless of whether the task was PRD or RND. Results showed that during longer sequences, eye and hand movements exhibited increasing lag relative to the target. We suggest that the lag of the eye was possibly translated downstream into the hand, which may explain why performance during the longer sequences seemed to be poorer compared to shorter sequences. During the long sequences it may also be the case that participants exhibited spatial attention decreases that were apparent in both visually-guided and predictive responses and thus, these decreases in performance were not learning-related, but attentional. In addition, timings between eye and hand latencies decreased during the 8PRD conditions compared to the 8RND responses indicating that the tighter coupling between the eye and the hand observed in sequence learning was not affected by sequence length.

Some differences in performance that could be attributed to the level of learning between the shorter and longer sequences could be the observed

increased in hand accuracy during the shorter versus the longer sequences. This difference in accuracy was only observed in the PRD conditions and not during the RND task and so cannot be attributed to the increasing lag seen across the longer sequences.

Previous research has shown that in pursuit sequence learning participants are able to show prediction to 4 discrete ramps after one or two repetitions (Burke & Barnes, 2007, 2011; Collins & Barnes, 2005), thus participants in our study would not have difficulties in storing the 4 component sequences presented. Similar behaviour between the 4 and the 8 components sequences suggests that participants were also able to learn each component of the longer sequences by the second presentation of the sequence. However, our continuous stimuli might elicit a different type of learning compared to the discrete ramps used previously, and our results suggest that participants used the same learning strategy for both sequence lengths by possibly learning the sequences as a whole and storing mainly timing of directional changes.

Our task involved learning of longer continuous sequences than previously reported and thus, learning over longer periods. We observed rapid adaptations in eye and hand behaviour that were sequence learning specific in very short time intervals. Further investigation is needed into how continuous sequences or movements can be pre-programmed and learnt to provide insight into learning in the motor systems and the longevity of the storage process. Indeed, fMRI results indicated the short and long sequences rendered activation of similar areas that also reflected learning-related circuitry during the 8PRD sequence presentations. However, contrasts between sequence length types also revealed different areas associated with memory and learning.

6.7 Eye only versus eye and hand coordination: fMRI

BOLD signals in the human brain revealed activation of similar areas associated with sequence learning. EO and EH 4RND > 4PRD contrasts revealed higher activation corresponding to PRD in the DLPFC, FP, ACC and the IPL. Activation of PFC has been associated with attention and memory during the early learning stages (Sakai et al., 1998), and indeed attenuation of the activation in DLPFC was observed when making 4RND > 4PRD contrasts. These PFC areas as well as the ACC may constitute to a more central memory processing independent of motor task. EH contrasts also showed 4PRD activation in the PHC, thalamus, CBM, BG, V5, SPL and SMA/PMA. When eliminating the effects of RND activation and comparing only PRD conditions, EO 4PRD tasks also exhibited activation of the SPL, V5, BG, and SEF. However, we suggest that these areas (PHC, thalamus, CBM, BG, V5, SPL and SMA/PMA) were more prominent in the EH experiment and therefore possibly more involved in eye and hand coordination during sequence performance compared to oculomotor learning per se. It was clear to see that SMA/PMA were important areas for prediction during eye and hand coordination, whilst SEF was more important for prediction in the oculomotor system. Surprisingly, even though V5 was active during 4PRD pursuit in EO, V5 seemed to be more prominent during the PRD eye and hand experiment, suggesting a role in the exchange of signals between the oculomotor system and the manual system during coordinated learning tasks. Similarly, activation of the CBM was more noticeable during the EH experiment, supporting the notion of its involvement in eye and hand coordination (Miall & Jenkinson, 2005) and more so when coordinating a specific sequence of movements. PHC activation seemed also more prominent in EH PRD tasks, but mainly during the longer EH sequences, supporting the notion that the PHC plays a key role in longer term memory

needed to perform the 8 component sequences (Pierrot-Deseilligny et al., 2002). The IPL was active in both EO and EH tasks, however, the SPL seemed to play a larger role in learning during EH tasks. The SPL may play an important role in visuospatial localization and attention by guiding hand movements and possibly enhancing the coupling between eyes and hand during PRD conditions (see Rushworth et al., 2003). These suggestions, are supported by both previous findings on the importance of PFC->PPC networks during eye and hand coordination in sequence learning (Battaglia-Mayer, Archambault, & Caminiti, 2006; Katsuki & Constantinidis, 2012; Pammi et al., 2012; Sakai et al., 1998; van Donkelaar et al., 2000). Figure 6.3 provide a summary of our findings for the brain areas involved in EO and EH sequence learning.

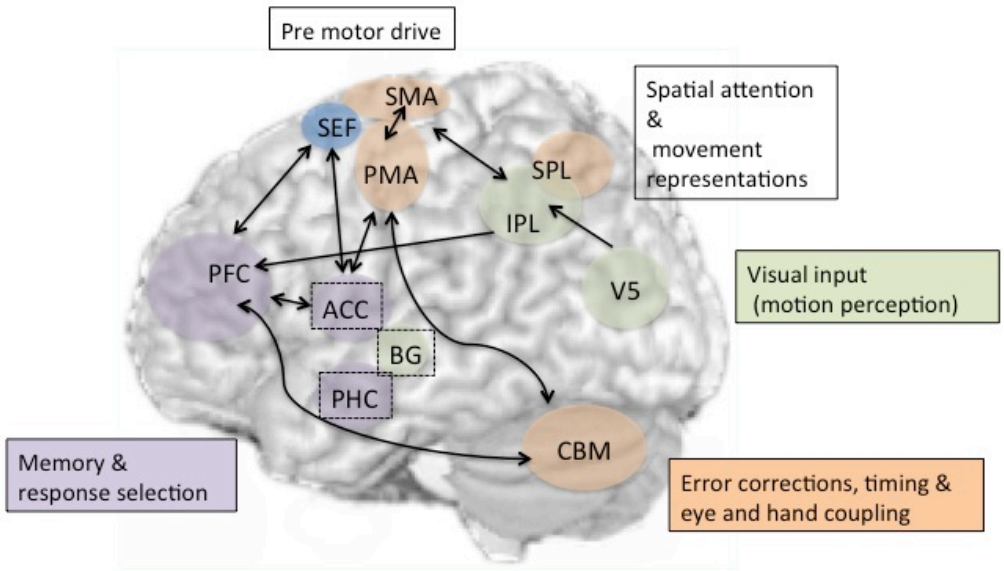

Figure 6.3. Cortical areas found in this study to be involved in eye only and eye and hand sequence learning. Blue areas represent areas that were only active during the EO experiment, green areas are shared between EO and EH responses and orange areas were found more active during EH tasks. The figure also shows areas important for short-term and possibly longer term learning in PHC (as seen in EH PRD results). V5 was active during 4PRD pursuit in EO tasks as well as throughout EH 4PRD and 8PRD tasks and more prominent during the latter. Specialization of parietal areas may also show EO vs. EH related differences in activation (more SPL in eye and hand and more IPL in eye only).

6.8 Effects of sequence length: fMRI

It has been previously suggested that the networks involved in attention and short-term memory are of limited capacity (Baddeley, 2000). However, our behavioural results showed similar learning between short and long sequences as well as activation of some overlapping brain areas between short and long sequences (such as SEF in EO and the ACC and PMA in EH experiments). In the oculomotor system, the limitations of short-term memory have mainly been tested though memory guided saccade tasks by increasing the timing in which a response has to be made following the presentation of a target/s (Ploner, Gaymard, Rivaud, Agid, & Pierrot-Deseilligny, 1998). Pierrot-Deseilligny et al (2002) observed improvements (accuracy) in memory guided saccades at delays longer than 20s, previously thought to be beyond the WM limitations, and suggested the existence of a medium-term spatial memory relevant for these longer delays independently of the short-term memory processing. They also suggested that the medium temporal lobe containing the parahippocampal cortex (PHC) might be involved in these medium-term learning processes, with the parahippicampus previously found to carry visual memory functions and possibly spatial memory functions. The MTL and the PHC have been also associated with encoding of object locations, suggesting a role in associative memory (Sommer et al., 2005). Indeed the PHC and other memory-related areas such as ACC, DLPFC and PMA were more bilaterally active during the longer EH PRD sequence presentations compared to the shorter sequences. Similarly, during the 8PRD EO tasks, SEF and DLPFC activation was increased compared to the shorter sequences. It is suggested that differences in brain activation between sequence lengths reflected the additional use of attention and use of memory needed for the

longer sequences and possibly the temporal restructuring of brain areas associated with short-term and longer-term learning.

Our findings provide evidence of the ability to store multiple components to achieve oculomotor and manual adaptations during sequence learning. Indeed, learning-related improvements in ocular and manual tracking persisted throughout the added sequence components, however, some decreases in the ability to perform overall longer sequences of movements was evident in both ocular and manual systems. Participants seemed to exhibit improved performance, but also seemed to be working harder to achieve these improvements, which may also explain the changes in brain activation such as higher activation in SEF and PMA areas in the EO and EH longer sequence tasks respectively. Our results may also provide evidence that these short-term adaptations can be extended for more time and for more sequence elements than previously thought.

7 Conclusions

Our findings show the complex interactions between behaviour and brain activation associated with motor sequence learning. We used a novel continuous sequence learning task by presenting predictable stimuli and identified learning-related behaviours and changes in brain activation by making comparisons with random sequence presentations. These comparisons (random versus predictive) in functional imaging studies are critically important for identifying brain areas that support higher order cognitive operations such as prediction, short-term memory and learning.

Using an analogous task, we made direct comparisons between saccadic and pursuit adaptations during sequence learning, and have provided important evidence of how these oculomotor systems interact. As expected, we found that both pursuit and saccade oculomotor systems attained short-term learning of the sequences, by demonstrating predictive responses when performing PRD sequences after only one presentation. This suggests a common predictive drive between the systems. However, saccades and pursuit also showed some behavioural differences in adaptation and timing thresholds, with saccades exhibiting earlier predictive mechanisms and more learning across repetitions. The fMRI results reflected these behavioural effects by showing areas that are common for prediction across eye movements such as the SEF, DLPFC, ACC and IPL, but also distinct activation associated with positional-related (saccades) and velocity-related storage. Together, these results provide further evidence of the existence of distinct subregions and distinct activation levels within common brain areas in saccades and pursuit.

Similarly, eye and hand motoric findings revealed learning-dependent changes in eye and hand coordination. In particular, participants exhibited shorter latencies in both motor systems, and improved hand tracking. Tight coupling between the eye and the hand was also observed in PRD sequence presentations. During these PRD presentations, the eye still led the hand, however the lag of the hand from the eye was decreased and maintained across the repetitions. Longer temporal lags between the eye and the hand reflect visual feedback delays during the RND conditions. The shorter eye and hand lag obtained in PRD conditions (tight coupling) may allow for optimized eye movement input to be used by the hand for preparatory motor commands and thus, result in enhanced tracking. Indeed, we found significant overlap between the brain areas involved in prediction during both EO and EH experiments (e.g., ACC, DLPFC, IPL), but we also found areas that may specialise in the integration of signals between the two motor systems (coordination) and enhance coupling during motor sequence learning (e.g., SPL/IPL and CBM). We provide further evidence of common neural networks between the two systems, and demonstrate that the oculomotor system is an important feature of eye and hand sequence learning. Future studies are needed to look at the time-course of the signals within areas of the brain during sequence learning to determine the function of these common networks and identify areas that are involved in general learning (e.g., ACC and DLPFC) versus areas corresponding to motor learning per se, such as PMA and SMA in the hand and SEF and FEF in the eye, as shown in our results.

Finally, findings from our longer sequences suggested that participants were able to show equivalent prediction during both longer and shorter sequences, and that this prediction was prompted by the first presentation of a sequence. However, there were some indications of poorer performance during

the longer sequences, such as an increase in the eye and hand lag from the target with the additional sequence components and decreased hand accuracy when compared to the shorter sequences. We observed additional neural activation during longer sequences, which could be related to the greater demands on attention and memory (PFC, PMA, ACC and PHC) resources. This study shows evidence of a longer-term learning than has been previously reported during predictive behaviour indicating no limit to this short-term store. These findings may be indicative of one memory process that occurs in series towards longer-term memory and motor skill consolidation; however, additional studies may provide insights into the possible limitations in this system.

8 Future directions

As mentioned above, further investigations into the limits and links between short and longer-term memory will provide insights into how individuals are able to learn and perform a wide range of movements. In addition, direct comparisons between eye only and eye and hand performance could indicate whether learning in the motor system involves common neural features and explain how each motor system may share information during learning to enhance performance and this may also be important for coordinated movements. We also suggest that our sequence learning task and findings could provide further insight into certain changes that occur during aging and in developmental disorders, as well as in certain learning disabilities. For example, children with developmental coordination disorder have difficulties learning new motor skills and are overall, less accurate and more variable in their movements compared to typically developed children (Zwicker, Missiuna, Harris & Boyd, 2010).

A first fMRI study by Zwicker et al (2010) found that children with coordination disorder have "under-activation" in areas associated with visuospatial learning compared to typically developed children during a tracing task. They also suggest that due to the variability that this population presents, more studies and larger samples correlating neural networks with impaired performance are needed. We suggest that our task may provide further insight into whether impairments occur in areas associated with attention and memory such as DLPFC and/or the ACC or in areas associated with the integration of visuomotor information, such as the cerebellum. In addition, our tasks are easy to perform and include the effects of visual input and eye and hand interactions, which are lacking in the literature.

It has also been observed that eye and hand coordination, as well as sequence learning is impaired in certain aging populations. In particular, individuals with Parkinson's disease have been shown to exhibit slow visually-guided aiming movements (Boisseau, Scherzer & Cohen, 2002) and delayed learning associated with attentional and memory demands (Ghilardi, Eidelberg, Silvestri & Ghez, 2003). Investigation of the neural networks responsible for motor sequence learning would shed light on the changes that occur in aging populations and in disease.

We suggest that a fMRI time-course analysis during this sequence learning task would show changes in BOLD signal levels that occur during the learning process. This analysis will show either an increase or decrease in neural activation that may reflect improvements in performance associated with sequence learning. Additional electrophysiological analyses such as implementation of TMS and EEG are also useful techniques that would complement fMRI findings. TMS is a tool that has been extensively used to prove

causality of certain areas of interest and follow-up experiments will provide further insight into the role of anatomical areas found active during the task, such as the involvement of SMG in the encoding of a sequence or the role of SPL in the integration of visuospatial information during sequence learning. Furthermore, due to the temporal limitations in fMRI, EEG techniques would also provide valuable input into the temporal resolution of brain activity during sequence learning tasks and describe the stages of processing in motor learning.

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Appendix A

The Brain uses Efference Copy Information to Optimize Spatial Memory

Gonzalez, C. and Burke, M.R.

Abstract

Does a motor response to a target improve the subsequent recall of the target position or can we simply use peripheral position information to guide an accurate response? We suggest that a motor plan of the hand can be enhanced with actual motor and efference copy feedback (GoGo trials), which is absent in the passive observation of a stimulus (NoGo trials). To investigate this effect during eye and hand coordination movements, we presented stimuli in two formats (memory guided or visually guided) under 3 modality conditions (eyes only, hands only (with eyes fixated), or eyes and hand together). We found that during coordinated movements, both the eye and hand response times were facilitated when efference feedback of the movement was provided. Furthermore, both eye and hand movements to remembered locations were significantly more accurate in the GoGo than the NoGo trial types. These results reveal that an efference copy of a motor plan enhances memory for a location that is not only observed in eye movements, but also translated downstream into a hand movement. These results have significant implications on how we plan, code and guide behavioural responses, and how we can optimise accuracy and timing to a given target.

Keywords: Eye-hand coordination, Short-term memory, Go/NoGo, Eye movements, Hand movements, Vision.

Introduction

Carrying out every aspect of daily living is mediated by the ability to accurately move through the world. Accurate spatial memory of objects in the world relative to oneself is essential when needing to build a map of the surrounding environment for navigation. This process is done often subconsciously through the use of memory and the oculomotor and motor systems.

In order to see, and subsequently reach for objects in the environment, we employ a series of saccadic eye movements which typically move the fovea of the eye onto objects of interest. The distance to be moved is calculated by combining information about the retinal distance of the object on the fovea with information about the position of the eye or limb. This informs the brain of the direction and amplitude of the movement to be made. Eye movements can be either reflexive, in response to an external salient stimulus (such as visually guided saccades), or voluntary (internally generate) with the latter making up the majority of eye movements made in everyday life. A tested method of exploring voluntary movements is "the memory guided" trial type, which involves participants moving their eyes or hand to a previously indicated location (Becker and Fuchs, 1969). Classically during these experiments, a target is flashed in the periphery while the subject maintains fixation in the centre. This provides a retinal image of the object location to later move to once instructed. If a saccade is made to the target when it appears, presumably afferent feedback of a motor movement generates an efference copy that can be stored in short term memory and then later recalled when needed to assist in the generation of a movement to the remembered location. It has been previously found that this efference copy (which is an internal representation of the motor command) can be used by the brain to improve accuracy to the remembered target location during eye movements

(Burke et al., 2010). This is providing the saccade to the target, and then to the remembered location, is not temporally too close (i.e. between 100-300ms) and thus avoids "inhibition of return" (Klein, 2000). The following study aims to understand if this same strategy can be used to improve accuracy or shorten reaction time of the hand to a remembered target.

During eye-hand coordination tasks it is usual for the eye to foveate the object of interest, and then provide the spatial information needed to guide the hand movement (Johansson et al., 2001). Indeed, actually looking at the target will provide the visual feedback necessary to successfully guide the hand (Berkinblit et al, 1995). Herman and Maulucci (1981) showed a clear a temporal correlation between eye and hand movements with the eye preceding the hand by around (60-100ms). It was later suggested that this temporal correlation may enhance visual guidance of hand movements (Neggers and Bekkering, 2002). Following this, Bock (1986) and later others have found that restricting eye movements to a target reduces accuracy of the hand (Prablanc et al, 1979). Despite these findings, spatial information can be provided to the hand without an eye movement, and alternatively the eye can move freely without the need to guide the hand.

To investigate how the eye movement affects the accuracy and response time of the hand, and vica versa, when afferent information is provided to a remembered location, we employed 3 conditions: (i) eyes only, (ii) hand only, and (iii) eye and hand together. Alongside these conditions we used three trial types that were randomized and cognitively cued using a colour: a green cue instructed participants to make a saccade (and/or touch response) to the target on appearance (GoGo) as well as making a response to the remembered location on cue expiration; a red cue indicated participants should maintain fixation on target appearance and only make a response to the remembered location on cue

expiration; the white cue indicated a visually guided trial type in which participants simply made a response to the visual target. This current study extends previous work on encoding across modalities because it investigates the use of afferent feedback (of the muscle fibres) on the accuracy and timing of a memory-guided eye and/or hand movement, to temporally and spatially equivalent locations on a touch-screen.

This study and design were based on two main experimental hypotheses: (i) that participants would be more accurate in a memory guided trials when afferent information of target location was available (GoGo trials) versus non-available (NoGo trials), (ii) and that coordinated movements of eye and hand in memory trials would further improve accuracy. We suspected that the experimental manipulation would have no significant effect on reaction times of the responses of the eye or hand.

Method

Participants

Twenty-five participants (11 females and 14 males) with a mean age of 20.96 years and an age range of 20-22 took part in the experiment. All but one of the participants were right-handed, (all used their preferred hand in the experiment), and all had normal or corrected to normal vision and no neurological deficits. The experiment was ethically approved and all participants were briefed about what the trial type involved before giving informed consent. Participants were given information sheets describing the paradigms and instructed to perform the experiment as accurately and quickly as possible.

Apparatus and Set-up

All stimulus material was presented on a 21" CRT monitor in a dark room absent of external light sources and thereby removing external allocentric cues (so subjects could not see the edges of the display screen). Participants were seated 38cm from the monitor with their heads rested on a forehead and chin rest, which was part of the EyeLink 1000Hz eye-tracker tower mount set-up (SR Research Ltd, Osgoode, Canada). A 9 point calibration was performed followed by a validation of which eye fixation needed to fall within a 0.5° window of the calibration target. A drift correction was performed prior to each block of trials ensuring the recording of accurate eye position throughout the experiment. A touch-screen (Magic Touch Touchscreen, KEYTEC Inc., Texas, USA) was positioned on top of the CRT monitor and was used to collect touch data via a USB input, and recorded by the Experimental builder software. The touch resolution was 4096 x 4096 pixels, with a 10ms maximum response time and a 3mm maximum error and was calibrated prior to the experiment.

Design

A 3 x 3 repeated measures design was used with three modality conditions (Eye only, Hand Only, and Eye and Hand), and three trial types (GoGo, NoGo and VGS) this resulted in 72 trials for each condition that comprised an equal number of each condition i.e. 216 trials in total lasting approximately 40 minutes testing time.

Paradigms

In the Eye Only (EO) condition, participants looked at targets or remembered target locations, with their eyes while resting their hands on the table in-front of

them. In the Hand Only (HO) condition, they were asked to continually fixate with their eye's a target in the centre of the screen for all trials, and touch (with their preferred hand) the target locations. In the Eye and Hand (EH) condition, participants looked and touched the visible or remembered target locations. Participants performed 3 blocks of trials (1 for each condition: EO, HO or EH) with the order counterbalanced between participants. Participants performed 3 trial types (VGS, GoGo and NoGo as outlined below) with 72 trials in each block, resulting in 24 repetitions of each trial type for each condition. All targets were circular and 0.5° in diameter.

- Visually guided saccades (VGS) trials were visually guided movements and required participants to fixate a centrally positioned white target (fixation point) either with the eye (and the hand for HO and EH trials) for 500ms. A peripheral target then appeared (with simultaneous expiration of the central target) and participants were required to either look at (EO) and/or touch (HO and EH trials) this target location, positioned at 10° or 20° from fixation (equivalent to 5 and 10cm on the screen) in a leftward, rightward, upward or downward direction (see figure 1). The distances and directions were used to minimise prediction to the target onset.
- GoGo trials were indicated by a green fixation cue and participants were instructed to fixate (and touch in HO and EH trials) this central point. After 500ms a target appeared in 1 of 8 locations (up, down left or right at either 10° or 20° from the central point). Participants were instructed to move to the peripheral target with either eyes, hand, or both, depending on the block (EO, HO or EH). This peripheral target expired and participants moved back to the centre with their eye and/or hand for a random period of time. Once the fixation point expired participants were

asked to look at (and/or touch) the remembered location of the peripheral target (see figure 1).

- NoGo trials were indicated by a red fixation cue, and participants looked and/or touched this central target. A peripheral target appeared after 500ms in 1 of 8 locations (as above). Participants were instructed to maintain fixation (and touch) on the central fixation point during the target presentation. After a random period of time the fixation point expired, and participants were instructed to look at and/or touch the remembered location of the target (please refer to figure 1).

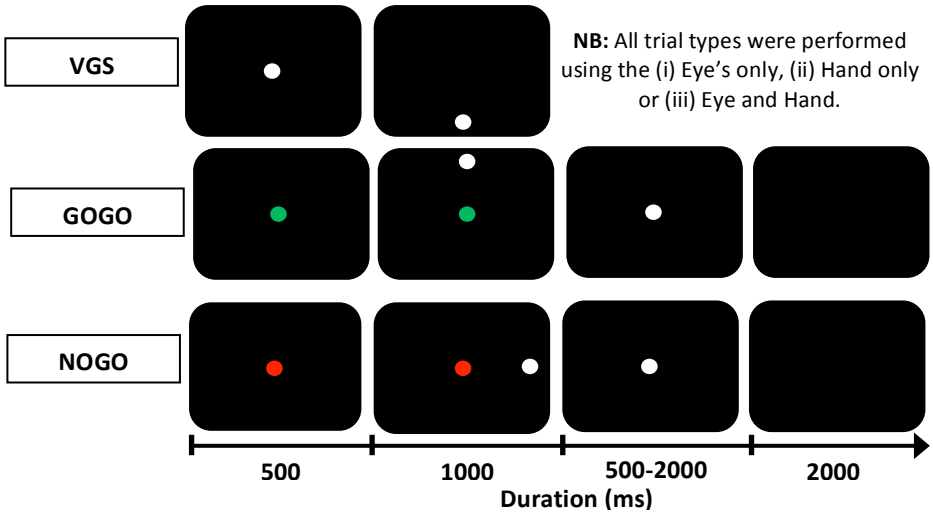

Figure 1: The diagram shows screen diagrams of the 3 trial types (GoGo, NoGo and VGS). The GoGo trial type was identified to the participant by using a green colour fixation, the NoGo by the red coloured cue and the VGS condition by the white coloured fixation cue.

Data Analysis

We collapsed data across target distance, as distance effects have been reported extensively previously and were not of interest in the current study. Reaction time for saccades was derived automatically using DataViewer (SR research Ltd, Osgoode, Canada) and calculated from expiration of the central fixation point to saccade onset. Saccade onset was taken as eye velocity and acceleration exceeding

30°s^{-1} and 8000° s^{-2} respectively. We then selected saccades in which participants made movements >5˚ in the correct direction of the target. Mistakes were rare in the visually guided trial types; however occasionally during the Hand only trial type a subject may have made an eye movement to the target resulting in this trial being excluded. Furthermore, if participants looked at the peripheral target in the NoGo trial type or touched this target, the trial was excluded. Finally, if participants neglected to touch or look at the target in the GoGo trial type the trial was also excluded. Data with RT less than 100ms (VGS only) or greater than 700ms (EO) or 1000ms (EH) were also excluded due to them being anticipatory or delayed respectively. Corrective secondary saccades were not included as we were interested in motor plans of saccades to remembered locations and not in corrective (feedback) mechanisms once the first saccade has been made. In hand trials, all participants touched the centre of the screen just below fixation prior to target onset. Touch response time was calculated as the time delay between fixation offset (also target onset in visually guided responses) until the participant touched the screen, this was therefore a combination of reaction time and movement time. Accuracy was determined by subtracting amplitude of the response from the amplitude of the actual target for both eye and hand (***constant error***) in degrees (°). We placed the mean error responses for each participant to each condition for the eye (RT and error) or hand (response time and error) into 4 individual repeated–measures ANOVAs with two levels: Condition (EO, EH and HO) and Trial type (VGS, GoGo and NoGo). Post Hoc analyses were also performed to identify which of the 3 trial types significantly differed from each other. The same procedure was performed on the variance of the data (standard deviation from each subject) in order to evaluate statistical significance in ***variable error***. Significance was set to $p < 0.05$.

Results

Eye Responses

Reaction Time

The results of the eye movements during the eye only (EO) and eye & hand (EH) conditions for each of the trial types (VGS, GoGo and NoGo) are presented in Figure 2 (left). We found significant differences between the EO and EH conditions ($F_{(1,27)} = 45.05$, $p < 0.001$), a significant difference was found between the trial types ($F_{(2,26)} = 10.866$, $p<0.001$). There was also a significant condition x trial type interaction ($F_{(2,26)} = 7.91$, p 0.002) that showed shorter RTs in the EO condition compared to the EH condition across all trial types ($p < 0.001$ and $p = 0.024$ for VGS and NoGo trial types respectively). Post hoc analysis that when performing with eyes and hand (EH), RTs from the GoGo trial type were significantly shorter than RTs from the VGS trial type ($p < 0.001$), and the difference between the VGS and NoGo trials type approached significance ($p = 0.057$). Post-hoc tests also revealed no significant difference for the different conditions for the EO task.

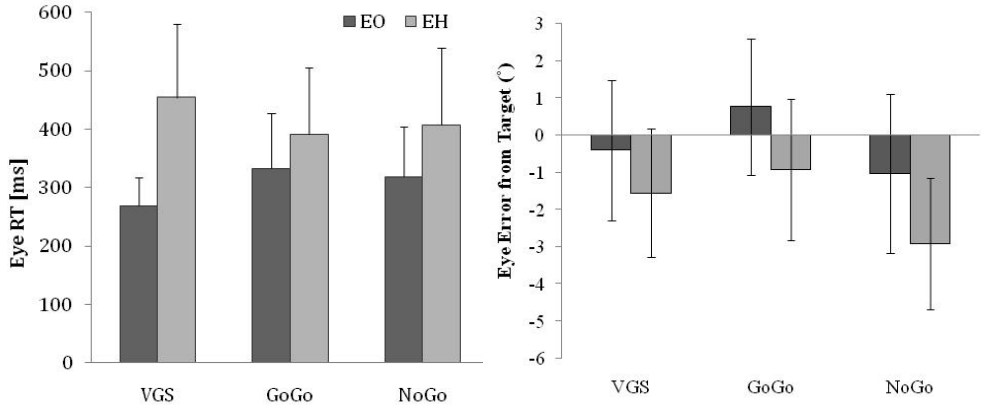

Figure 2: The left graph displays the mean saccadic reaction times from all participants for each trial type (VGS, GoGo and NoGo) for the eye only condition (EO in dark grey) and eye and hand condition (EH in light grey). RTs were significantly longer when including hand movements in the VGS and NoGo trial types but not during the GoGo trial types. The right graph displays the mean saccade amplitude error for all participants for the visually guided (VGS) and memory-guided saccade (GoGo and NoGo) trials in degrees, for each condition: EO (dark grey) and EH (light grey). A negative value represents an undershoot of the eye and a positive value an overshoot. Errors in NoGo trial types were large compared to GoGo and VGS. Also, the addition of the hand significantly decreased eye accuracy in the NoGo trials. Standard deviations of the response are shown as error bars.

Position Error

Constant Error: We found a significant effect of condition ($F_{(1,26)}$ = 20.109, $p < 0.001$) and a significant difference between the trial types ($F_{(1,25)}$ = 21.772, $p < 0.001$)(see figure 3). The EH condition produced a larger error (undershoot) than the EO condition. A post-hoc analysis of the trial types revealed significant differences between all trial types, but with the greatest difference between the memory conditions (VGS and GoGo; p = 0.007, VGS and NoGo; p = 0.003, and GoGo and NoGo; $p < 0.001$). We found no significant interactions (Figure 2: right).

Variable Error: We found no significant difference in the variance between the eye movements in the EO and EH conditions. However a marginal significance was observed between trial types ($F_{(2,26)}$ = 3.344, p = 0.051). A post-hoc test revealed

that the VGS and the GoGo were significantly less variable than the NoGo trial type ($p = 0.026$, and $p = 0.041$ respectively).

Trial Error

Table of Omitted Trials in Eye Data.

Eye data	**EH: GoGo**	**EH: NoGo**	**EO: GoGo**	**EO: NoGo**
Error trials (%)	8.5	13.5	10	10.1

Table 1: The data shows the average number of trials omitted from the data due to errors as a % of the overall number of trials for all participants. EH is the eye and hand task and EO is the eye only task. We found a difference between GoGo and NoGo task in the EH condition, but not the EO condition.

Touch Reponses

Reaction Time

The results revealed no significant differences between the conditions (EH and HO), but significant trial type effects were observed ($F_{(2,25)} = 9.921$, $p = 0.001$) (see Figure 3: left). A post-hoc analysis revealed a significant difference between the response time of the GoGo and NoGo trial type ($p = 0.001$) with the NoGo trial type displaying a significantly longer response times (Figure 3: left). No significant interactions were observed.

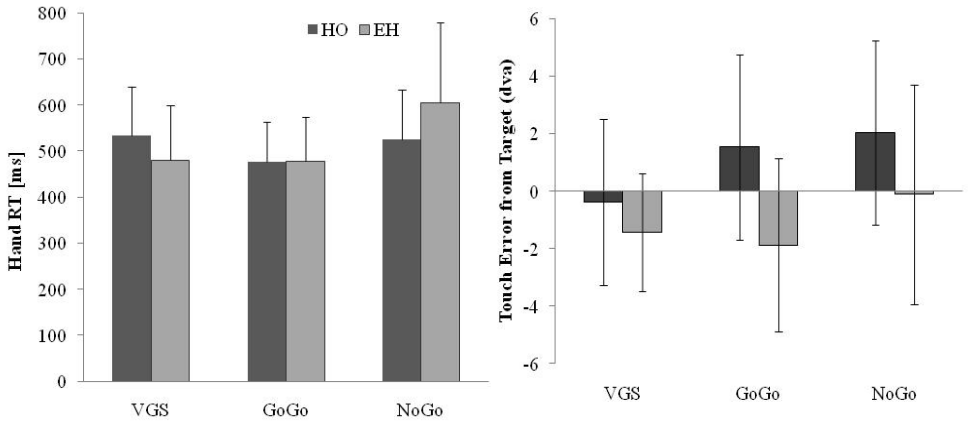

Figure 3: The left graph displays the mean reaction time data from all participants (± std) for the conditions HO (dark grey) and EH (light grey) for each of the trial types (VGS, GoGo and NoGo). Combining reaction time of the eye and hand during the EH condition revealed overall longer reaction times during the NoGo trial type compared to the other trial types with a significantly longer timing for the touch response. This effect however was not reflected in the eye movement for the NoGo trials. The right graph shows the mean touch for all participants (± std) from the actual target location with the trial types presented on the horizontal axis and error (in dva) on the vertical axis. A negative value represents an overall undershoot of the target and a positive value represents an overshoot of the target location.

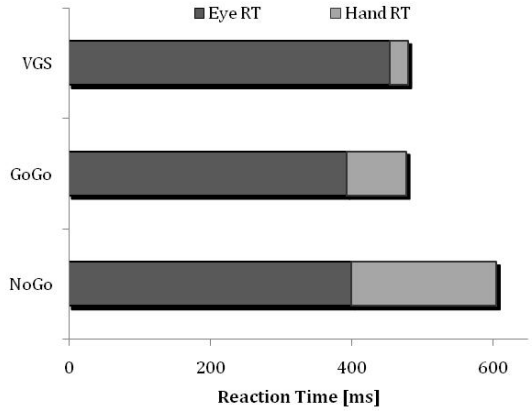

Figure 4: The graph displays the EH mean reaction time data for all participants from the eye and hand movements when performing coordinated responses across the trial types. The light gray bars show the overall hand RT and the dark bars show when the eye starts to move. The graph shows that on average during VGS trials the hand arrives shortly after the eye starts moving. In contrast, during the NoGo trials, the hand arrives much later.

Position Error

Constant Error: We found significant effects for condition ($F_{(1, 24)}$ = 20.082, p < 0.001) and for trial type ($F_{(2,23)}$ = 4.592, p < 0.05) in touch accuracies (see Figure 3: right). As expected, participants were significantly more accurate in the VGS and GoGo trial types than in the NoGo trial types (VGS and NoGo; p = 0.009, GoGo and NoGo; p = 0.038). In addition, participants exhibited more accurate touch responses when performing with both eyes and hand (EH) compared to when maintaining central fixation (HO). No interaction between trial type and condition was observed.

Variable Error: We found no significant effect of condition but a highly significant difference between the trial types was observed ($F_{(2,22)}$=10.643, p = 0.001). A post-hoc analysis revealed that all trial types were significantly different in variance from each other (VGS and GoGo, p < 0.001; VGS and NoGo, p = 0.001; GoGo and NoGo, p = 0.015). A condition x trial type interaction was also observed ($F_{(2, 22)}$ = 4.836, p < 0.05).

Trial Error

Table Omitted Trials in Touch Data.

Touch data	**EH: GoGo**	**EH: NoGo**	**HO: GoGo**	**H0: NoGo**
Error trials (%)	6.3	11.5	7.9	11.1

Table 2 shows the average percentage of omitted or error trials in the touch data for all participants. The GoGo task revealed a lower number of errors than the NoGo task, but no difference was found between conditions.

Discussion

We used directly comparable eye and hand trial types in which the position change of the eye and hand were matched in time and space. Furthermore we included a cognitive cue to signal the response required by the subject in order to

ensure maintained attention n and avoid past history effects interfering with the data. We sought to evaluate two main experimental hypotheses:

1) Participants would recall target locations more accurately in GoGo than in NoGo trials in the eye and hand.
2) Participants would be more accurate when both eye and hand performed the trial type compared to a single modality (condition).

The next sections will discuss each hypothesis in turn.

GoGo versus NoGo

The eye movement analysis revealed that there was no significant difference in the reaction time between GoGo and NoGo trial types. However, the GoGo was significantly more accurate to the remembered target locations and less variable, which further supports findings from a previous paper from this lab (Burke et al., 2010). This paper adds to the literature on this subject by finding a clear improvement in accuracy and latency of the hand during this trial type, indicating that an efference copy of the movement provides a motor advantage in both eye and hand modalities. This finding has clear implications for how best to optimize motor performance and motor skills learning. In support of the findings here, another study on saccadic eye movements found that a better estimation of the target leads to better accuracy (Varizi et al., 2006). Interestingly, we found that the movement time differences between the GoGo and NoGo trial type specifically in the coordinated condition, was mainly due to the lag of the hand behind the eye in the NoGo trial type (see figure 4). This lag varied from 40ms in the VGS condition to around 200ms in the NoGo condition. This nicely falls into line with the Varizi et al (2006) findings, in that the NoGo trial type provides greater uncertainty compared to the GoGo and VGS trial types respectively.

We also found that EH coordination trial types also improved accuracy in memory guided touch responses, suggesting the benefits of using the eye during a touch response is also a feature of spatial memory for the hand. Interestingly, this improvement in the touch response with the addition of an eye movement was not observed during the HO memory guided trial types. This suggests that the eye is providing the spatial memory for hand guidance, and not the extra-retinal information provided by the movement of the hand. However, further investigation is needed to isolate what component of the eye movement contributes to the hand accuracy during memory guided tasks. We also found that an efference copy of the motor plan (in the GoGo trial type) also seems to reduce RT (temporal benefit) to the target, but does not improve accuracy (no spatial benefit) compared to the NoGo trial type during memory guided trial types. This is in agreement with Wilmut and colleagues (2006), who found that an existing motor plan held in short term memory and generated by the efference copy of the first eye movement, could be incorporated prior to the response of the second target, thus improving performance. It is possible that in the present study, the GoGo trial type provided participants initially with an efference copy of the pre-motor plan that could then be stored and later used to recall the location of the target (i.e., the second movement), and hence revealed a tighter coupling between the eye and the hand in coordinated movements when compared to the NoGo trial type in which no efference copy is provided (Goodale et al., 1986). The data presented here is also supported by a previous study from this lab, which also revealed improvement in RT and accuracy in a GoGo saccade trial type compared to the NoGo trial type in both young and middle-aged adults (Burke et al., 2010).

Single (EO and HO) versus combined (EH) modalities

The touch latencies are consistent with previous literature (Makovski and Jiang, 2011), but in general appear longer than standard keypress responses. This is principally because the experiment presented here incorporates both reaction time and movement time to a very precise location (i.e. response time), whereas reaction times of a keypress need not be spatially precise. In addition, we used a highly demanding cognitive trial type where participants needed to process a visual cue before deciding on the required response. The trial type design used here avoids common confounds associated with previous experiments including past-history effects, and ensures the amplitude of the touch movement was directly equivalent to that of the eye.

We found the addition of an eye movement with a hand movement (EH) improved accuracy when compared to hand only (HO) conditions in visually guided trial types, but only a trend was observed in the response time ($p = 0.05$). This benefit from the eye movement in guiding the hand has been found previously (Abrams et al., 1990; Vercher et al., 1996) and is thought to be due to high acuity foveal representations of the target in the brain leading to more accurate spatial information optimising guidance of the hand. Evidence suggests that the additional extra-retinal information provided by the eye movement to the target in the EH can be used to more accurately direct the hand (Soechting et al., 2001). It should be acknowledged that goal-directed movements are strongly coupled to spatial attention. However, spatial attention was controlled in this trial type, as location of the target was provided to the subject in both the GoGo and NoGo conditions. We must therefore assume that spatial attention is not the principal source of the shorter response time.

An experiment by Liesker et al. (2009) looked at EO, HO and combined EH conditions during visual search, both with and without distracters. They found that EO search was more optimal than HO in the absence of distracters, but HO was faster with distracters. In support of Liesker et al. (2009) we found EH combinations of this trial type revealed an improved performance than compared with using only one modality (i.e. shorter response times and a reduction in errors), suggesting that the coordination of modalities can be used to optimize performance.

The use of a cue is an unusual element to this trial type of which its principal aims were to randomize the trials, maintain attention and prevent what is known as a "past history effect" between trials within the data (Kowler, 1989). The use of cognitive cues to randomize the trial typess within a block has been successfully used previously in both saccades (Burke et al., 2010) and smooth pursuit (Burke and Barnes, 2008). Furthermore we have found the colored cognitive cues used in the present study have consistent effects in all conditions, and hence we are confident this manipulation does not account for the differences observed between the EO, HO and EH conditions.

Flanagan et al. (2008) also looked at reach behaviour in a lit room to remembered targets during conditions in which fixation was maintained during encoding, or inspection of the target was allowed (i.e. similar to our memory guided NoGo and Go trial types respectively). Flanagan et al (2008) found that looking towards the visible target during encoding in the memory guided condition did not influence recall behaviour, and thus concluded that gaze was largely decoupled from movement goals during memory guided actions. This study however, did not explicitly report latency or accuracy of the NoGo versus the GoGo responses and therefore comparisons are significantly limited.

Van Donkelaar and Staub (2000) performed a similar experiment in which they blocked conditions of (i) visually guided and (ii) memory guided, eye and pointing hand movements to visual targets. They found that in visually guided conditions, the hand started later than the memory guided conditions, but did not assess modality effects in EO and HO conditions. In this study by van Donkelaar and Staub (2000), participants performed hand only and coordinated eye and hand movements to visually guided targets and remembered target locations (similar to the VGS and NoGo trial type used in this experiment). They overall found the cumulative eye and hand movement RT to the target was longer in visually guided trial types than memory guided trial types. However, we did not find this to be the case. Participants were overall faster in the visually guided condition with the eyes and the hand than in the NoGo condition where larger hand temporal delays with respect to the eye movement onsets were observed. In our GoGo memory guided the overall response duration was faster than in the visually guided trial type (in support of above), but the NoGo trial type (which is more comparable with van Donkelaar and Staub (2000) trial type took longer. One major difference between the experiments by van Donkelaar and Staub (2000) and the experiment presented here is that we randomised trial types and used a cognitive cue at the beginning of each trial to indicate the response requirement of the participants. The van Donkelaar and Staub (2000) experiment blocked trials according to condition, which may have resulted in some past-history effect as there is a need during NoGo trial type to inhibit a reflexive response to the target, presenting the trials in blocks may have change attentional demands and caused habituation to the trial type and overall reducing the effects if inhibition on the system.

A number of studies suggest that individuals are fairly accurate in instances where vision is limited and they rely on other forms of information to guide the

hand (Binsted and Elliot, 1999; Sorrento and Henriques, 2008). In our study, participants performed better when full vision of the target was available to guide the hand, however, touch error differences between these conditions were very small (<0.2 degrees) suggesting that the participants were very accurate at guiding the hand, even when their eyes were fixed. The eye latencies are consistent with previous literature on VGS (Saslow, 1967; Kimming, et al., 2002) and this type of memory guided saccade task (Burke et al., 2010). The results presented here show that making a hand movement with an eye movement (EH condition), when compared to the EO condition, delays the eye response. Contrary to the touch data we found the addition of the hand significantly increased the latency of the eye movement to the target. This effect has been found previously (Bekkering et al., 1994) and our data supports these previous findings. This finding is somewhat logical as a higher degree of coordination is needed during a EH response when retinal information of the target location and extra-retinal information, such as proprioceptive feedback of eye and limb position, must be integrated for the preparation of a motor command (Binsted and Elliot, 1999, Ren et al., 2006). In contrast, during EO conditions ocular coordinates prior to initiation of a saccade and retinal input are easily interpreted and can be used quickly without the need to be translated into arm coordinates (Binsted and Elliot, 1999). However, we found this increase in RT during EH conditions to be more apparent during the visually guided trial type, as this increase in RT in the EH condition compared to EO was somewhat diminished in memory guided conditions. We suspect that allowing time for a motor plan to be developed in the brain, prior to movement execution, may help compensate for this initial delay in processing.

Saccades to remembered target locations are generated based on stored input of the previously flashed target in retinal coordinates, that need to be converted into the hand movement command should a hand movement be required (Crawford et al., 2004). We found, EH conditions revealed faster eye RT in GoGo trial types compared to the VGS, which were also translated into the response time of the hand. It is sensible to suggest that the faster RT in the eye and hand during the GoGo trial type may be due to the efference command generated during the first movement, and was subsequently released prior to the second movement i.e. causing anticipation.

Flanagan and colleagues (2008) conducted a study of the accuracy of memory guided hand movements with free gaze to multiple targets. They found that compared to reaching to visible targets, participants did not especially look to target locations when reaching to remembered locations. Additionally, they found that when participants did gaze close to remembered target locations, this did not improve the accuracy of their hand movements suggesting a decoupling of the eye and hand during memory guided saccades. Our results suggest that including a hand movement in the instruction to the subject in which both eye and hand must reach the target decreased the accuracy of the eye to the target. It should be noted that Flanagan et al. (2008) did not find an interaction between the hand and arm's motor system and the saccadic motor system in this previous study, but also did not require participants to make a saccade to the remembered location at the same time as touching it. Flanagan et al. (2008) reported that most participants did not look at remembered locations when marking locations manually and hence it could be the case that the hand and eye motor systems only interact when working in concert. In addition, Flanagan's study was conducted under ambient light conditions (meaning the edges of the screen were visible) and a

visible origin was present during recall. This meant target location information could have been encoded relative to other visual cues (an allocentric frame of reference) and so gaze did not need to rest on the remembered location to coordinate the hand position. Finally, in Flanagan et al's (2008) study, participants were not required to mark the target visually at the same time as manually; rather they could look where they wanted during manual recall, or were required to centrally fixate. It is possible that in the absence of visual references in recall, coordinated hand and eye movements to the remembered locations interact with the accuracy of either touch positions, or eye positions. This was something the present study aimed to investigate.

In summary, this study has identified that short-term memory for a spatial location can be enhanced by making a single movement of the eye and/or hand to the location that needs to be remembered. This enhancement can be observed in both accuracy and latency of the motor response. In addition, coordinating the eyes and hands during memory guided trial types increases the reaction of the eye, but has little effect on the touch responses in memory guided trials. Results also showed clear motor advantages for the storage of spatial information from motor representations of the target in the memory guided trials (GoGo) providing evidence active following in order to optimize motor skill learning. These results have important implications in the way individuals may optimize the planning and execution of eye and hand movements and how improvements in performance can be achieved in individuals with motor control deficits.

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Appendix B

1.0. Examples of PRD eye behavioural data: Eye Only

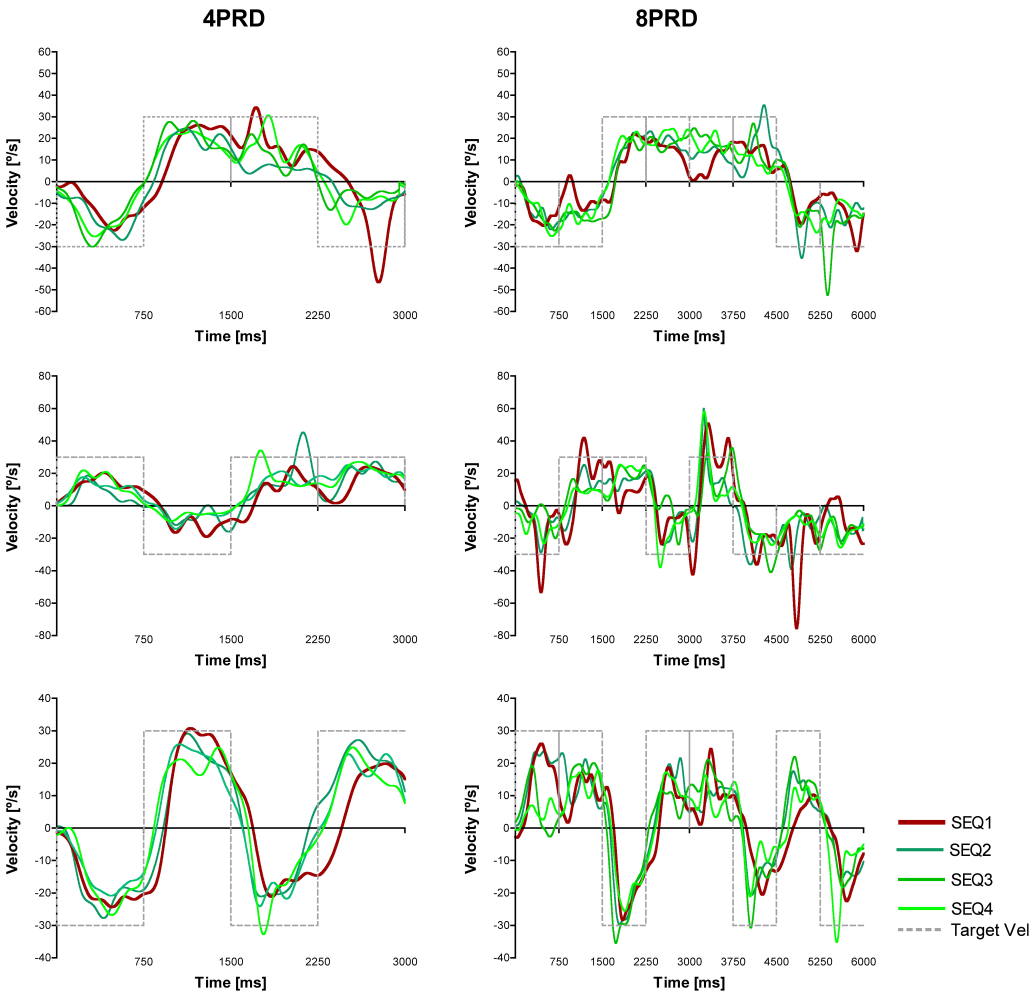

Figure 1. The graphs show examples of eye velocity traces from 4PRD (left) and 8PRD (right) series from 3 participants (upper graph, middle and lower graphs). The graphs also illustrate pursuit velocities from SEQ1 and repetitions as well as target velocity across the 4 and 8 components. Participants' examples show a phase shift in the velocity traces corresponding to the repeated sequences (green traces) compared to SEQ1 (red trace) evident in the 4 and 8 component PRD conditions. These examples were taken from selected random trials corresponding to 3 participants whose data was reported as less noisy during data collection.

2.0. Examples of PRD hand behavioural data: Eye and Hand

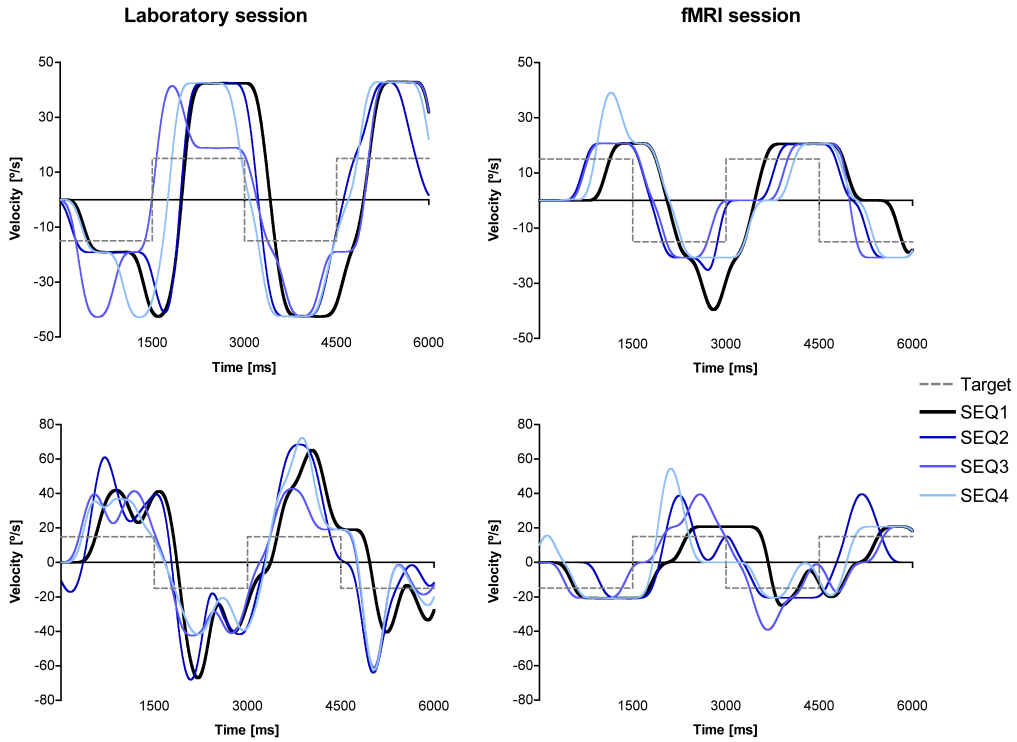

Figure 2.1 The graphs show examples of hand velocity traces from 4PRD trials in the laboratory session (left) and the fMRI session (right) chosen randomly from 2 participants (upper graph and lower graphs). The graphs also illustrate hand velocities from SEQ1 and repetitions as well as target velocity across the 4 components. Participants' examples show a phase shift in the velocity traces corresponding to the repeated sequences (blue traces) compared to SEQ1 (black trace) evident in the two sessions one week apart. New sequences were designed for each session.

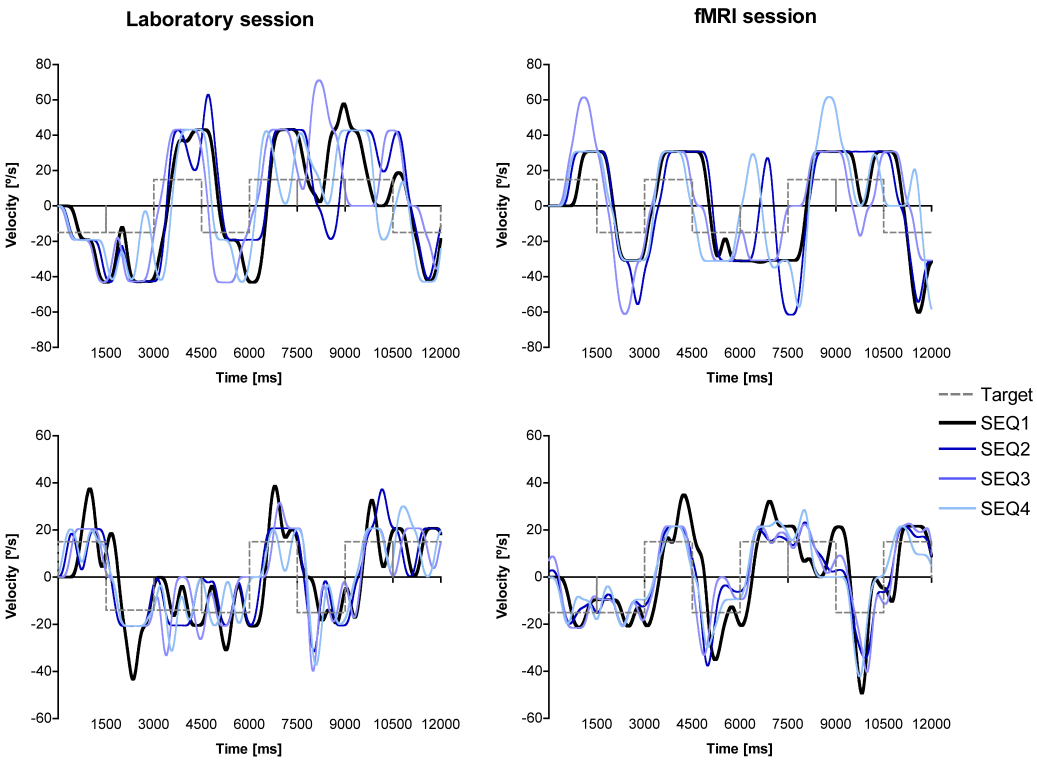

Figure 2.2 The graphs show examples of hand velocity traces from 8PRD trials in the laboratory session (left) and the fMRI session (right) chosen randomly from 2 participants (upper graph and lower graphs. Participants' velocity traces show a phase shift in the velocity traces corresponding to the repeated sequences (blue traces) compared to SEQ1 (black trace) in the two sessions.