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Motor sequence learning in the brain: the long and short of it.

Gonzalez C. C.[1] and Burke M. R.[2]

[1] Department of Psychology, Thomson Rivers University, 900 McGill Road, Kamloops, BC, Canada. V2C 0C8; cgonzalez@tru.ca; phone (250) 828 5316

[2] Department of Psychology, Faculty of Medicine and Health, University of Leeds, U.K. LS2 9JT, UK. m.r.burke@leeds.ac.uk

Corresponding Author: Dr Melanie Rose Burke, Department of Psychology, Faulty of Medicine and Health, University of Leeds, LS2 9JT, U.K.

Email: m.r.burke@leeds.ac.uk

Tel: (+44) 0113 3435738

Abstract

Motor sequence learning involves predictive processing that results in the anticipation of each component of a sequence of actions. In smooth pursuit, this predictive processing is required to decrease tracking errors between the eye and the stimulus. Current models for motor sequence learning suggest parallel mechanisms in the brain for acquiring sequences of differing complexity. We examined this model by comparing shorter versus longer sequences of pursuit eye movements during fMRI. In this way we were able to identify overlapping and distinct brain areas involved in simple versus more complex oculomotor learning. Participants revealed predictive pursuit eye movements from the second presentation of the stimulus in both short and long sequences. Brain imaging results indicated activation of parallel brain areas for the different sequence lengths that consisted of the Inferior Occipital Gyrus and the Cingulate as areas in common. In addition, distinct activation was found in more working memory related brain regions for the shorter sequences (e.g. the middle frontal cortex and dorsolateral prefrontal cortex), and higher activation in the frontal eye fields, supplementary motor cortex and motor cortex for the longer sequences, independent on the number of repetitions. These findings provide new evidence that there are parallel brain areas that involve working memory circuitry for short sequences, and more motoric areas when the sequence is longer and more cognitively demanding. Additionally, our findings are the first to show that the parallel brain regions involved in sequence learning in pursuit are independent of the number of repetitions, but contingent on sequence complexity.

Keywords: fMRI; Human; Learning; Memory; Pursuit

Introduction

Many of our daily activities involve learning new sequences of movements and then executing this learnt behaviour (Lee & Quessy, 2003). Motor sequence learning involves the transition from reactive to predictive processing that is associated with skilled behaviour, resulting in faster, more accurate movements. A good example of this transition is observed during pursuit eye movements of repeated single velocity ramps, double-step ramps and sinusoids (Barnes et al., 2000; Barnes & Donelan, 1999; Barnes & Schmid, 2002; Collins & Barnes, 2005; Wells & Barnes, 1999; Kao and Morrow 1994). The outcome of this learning is revealed by the early initiation (latency) of the movement, with eye velocity increasing towards a moving target prior to the brain receiving the information to drive the movement response (Kowler & McKee, 1987). Furthermore, prediction in pursuit results from the learning of not only timing, but also direction and velocity of the up-coming stimulus presentation (Barnes & Donelan, 1999; Wells & Barnes, 1999). This type of learning is often known as prediction and is inherent to all motor systems, providing compensation for the delays in internal neural processing in the brain. The pursuit system provides an excellent model for investigating early motor learning (such as those used for sequence learning) with the benefits of clear measurable behavioural markers of the acquired sequence.

A number of previous studies have shown that the learning of new motor sequences involves activation in prefrontal, premotor, anterior cingulate, and parietal brain areas (Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Jueptner et al., 1997). In support of these findings Toni, Krams, Turner, and Passingham (1998) were the first to study the time-course of motor learning using fMRI and found that the dorsolateral prefrontal cortex (DLPFC), anterior cingulate and dorsal premotor cortex are involved in early learning of an 8 finger sequence. However, activity in these areas decreased as learning progressed, with a shift in activity to the supplementary motor area (SMA). It is logical to suggest that the

differences in activation across brains region during learning are a result of increased efficiency in the brain (Koch et al., 2006; Schmid, Rees, Frith, & Barnes, 2001), facilitating the acquisition of more complex motor skills. This activation of different brain regions seems to reflect distinct learning stages (early versus late), but also, different aspects of learning such as speed or accuracy (Hikosaka, Rand, Miyachi, & Miyashita, 1995; Rand et al., 2000).

One way in which motor learning can be classified is via a serial process when the flow of information shifts from spatial memory to the development of a motor skill via serial sensorimotor transformations. Another possibility of learning in the brain is via parallel processing, whereby spatial and motor information are acquired independently and processed in parallel (Alexander, DeLong, & Strick, 1986; Hikosaka et al., 1999). Hikosaka et al (1999) proposed that serial processing may apply to simple movements, such as reaching, but that this serial process would be repetitive and inefficient for more complex sequences of actions. To optimise these demanding brain computations, Hikosaka et al. (1999; 2002; Sakai et al., 1998) proposed a motor learning model that supports the notion that prior to learning a sequence, performance relies on the sensorimotor information flow (serial sensorimotor or visual-to-motor processing) and is predominantly a form of 'spatial' memory. However, with practice, performance becomes non-reliant on this serial sensorimotor transformation and is taken over by two parallel acquisition mechanisms: 1) an explicit short-term *spatial* coordinate system; and 2) an implicit longer-term *motoric* storage system. The acquisition by the spatial sequence process occurs early (in the order of milliseconds) and is highly flexible, relying on attention and working memory mechanisms. Acquisition achieved by the motoric system occurs more slowly (i.e. minutes/hours), and in the later stages of learning (i.e. days/months) performance retains speed without awareness (Hikosaka et al., 1999, 2002). There is a gradual shift between these parallel sequential processes from the initial fast acquisition (observed in anticipatory movements associated with pre-SMA and DLPFC

activation in monkeys) towards motor cortices in later stages (automatic movements). Consistent with Hikosaka et al.'s (1999, 2002) findings in humans and non-human primates, Sakai et al.'s (1998) fMRI study also describes a transition between an early "declarative stage" of learning in frontal brain areas (DLPFC and pre-SMA), to a later more procedural learning stage in parietal brain regions (precuneus and intraparietal sulcus).

One important feature of the model described here, is that the learning of a sequence occurs via spatial and motor mechanisms independently and each system has its own input-output (spatial or motor coordinates). However, accurate and quick performance of a sequence means that the two parallel sequence process must communicate (possibly in pre-SMA or PMA), ideally with the spatial sequence guiding the motor sequence (Hikosaka et al., 1999). However, Hikosaka et al (1999) noted that the parallel arrangement means that a sequence can be acquired by either of these mechanisms (initiated by spatial or motor mechanisms), thus, a sequence might be acquired rather implicitly by the motor sequence mechanism, as with implicit SRT learning (Pascual-Leone, Grafman, & Hallett, 1994). Additionally, when disrupting the motor sequence process the spatial process may continue to acquire the sequence, but with errors (e.g., SMA, Hikosaka, Miyashita, Miyachi, Sakai, & Lu, 1998). Nakahara, Doya, and Hikosaka (2001) examined neural network models of sequence learning and also found that the parallel (independent) systems required communication or a 'monitor' (pre-SMA) but that subsequent manipulations of the model suggested that the model is robust even if one mechanism fails (or it is destroyed), as the other can take over and still learn the sequence although not perfectly (i.e., implicit or motor vs. explicit or spatially accurate).

In smooth pursuit tracking, the reactive to predictive transition into a steady state occurs quickly (after a single presentation) when implementing repeated short sequences of ramps (see Collins & Barnes, 2005). This probably reflects the low attention and working

memory requirement needed for such a simplistic task. However, the question still remains as to how the system copes with more complex sequences of actions that require enhanced attention and go beyond the short-term memory (prediction) buffer capacity (see Collins and Barnes 2005). Collins and Barnes (2005) found that the predictive drive in smooth pursuit is optimal during short sequences, but is affected by increased cognitive load (added ramps to a sequence), resulting in learning at a slower rate. They suggested this slower learning may reflect the additional working memory requirements, but that learning was indeed possible in the more complex sequences. This finding provides evidence of the robustness of the sequence learning processing. However, it is still unclear whether the learning of complex sequences results in a slower, spatial to motor serial transition, or alternatively the system immediately implements a more implicit (slower) motor mechanism to acquire the sequence. Furthermore, previous studies have shown areas involved in very short–term predictive mechanisms, including frontal eye fields (FEF), supplementary eye fields (SEF), dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) (Burke & Barnes, 2008; Ding, Powell, & Jiang, 2009; Lencer et al., 2004; Schmid et al., 2001); but have failed to examine longer more complex sequence lengths. Thus, knowledge of how prediction in the early stages of learning is used for more complex motor skills acquisition is not yet understood.

The present study aimed to identify the brain areas involved in sequence learning in oculomotor tracking, by addressing a previously unexplored effect of varying sequence length on the BOLD activation in these areas of interest. Our study differs from previous studies in that we compared learning in short (4 ramp) and long (8 ramp) sequences of eye movements using directly comparable stimuli, where only the sequence length (i.e., number of ramps) varied within the fMRI scanner. Furthermore, based on previous studies (mentioned above) we predict the outcome will show distinct parallel circuitry for the activation of shorter (more spatial) versus longer (more motoric) sequence lengths.

The present study used a novel paradigm to examine early (explicit) motor learning in the brain by inducing visuomotor learning of a 4 or 8 continuous target sequence in smooth pursuit. In this way we avoided effector-related learning that may have occurred in previous studies in more motor specific regions (such as the hand in a finger tapping task). In addition, our sequences were presented in a more ecologically valid way that better represent linked sequences of actions compared to single ramps. Our approach was different to previous studies that have investigated brain activation to a single repeated motion of a smoothly moving target (e.g. Ding et al., 2009; Schmid et al., 2001), which are linked to anticipation (rather than learning). It is clear these previous studies have focussed on interrogating the early "serial processing" model as introduced by Hikosaka and colleagues in 1999. In contrast, the focus of the current study was to examine how the "parallel model" is altered by implementing more complex sequences of eye movements. To avoid confounds we matched precisely the temporal and spatial (visuomotor) properties of the stimulus between the sequence lengths, and contrasted a novel (1st presentation of the sequence) to an early acquisition (4th repetition) of that same sequence within the design.

Methods

Participants

Twelve participants took part in the study and were included in the final analysis. Participants were aged between 22 to 34 years old (9 females, mean age 26.1 years, SD 3.83) with normal or corrected-to-normal vision and no known neurological conditions. All participants were university students with the same educational status, and successfully completed the medical history questionnaire resulting in no exclusions. All participants were all right-handed with no history of head trauma. Testing involved two experimental sessions:

1) a behavioural testing session in the visuomotor laboratory (Leeds, UK), and 2) an fMRI scanning session (Salford Hospitals, Manchester, UK) approximately 1 week apart. This study was approved by the Salford Royal 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. All participants gave informed written consent and completed a medical history questionnaire prior to the experimental sessions. Please note that all participants also performed an additional block of saccadic eye movements within the fMRI and laboratory sessions. This saccadic data alongside the 4 PRD pursuit data have been reported elsewhere (Gonzalez, Billington, & Burke, 2016) to address the question of the different eye movement types on brain activation during sequence learning.

Laboratory experimental set-up

The first experimental session took place in a dark room and participants were seated, with their heads stabilized by a forehead-and-chin rest (EyeLink, SR Research Ltd, Canada). Eye movement data was sampled at 1000 Hz and the visual stimuli were created using custom-made programs (Psychtoolbox version 3.0.9 for Matlab®, Mathworks, USA) (Brainard, 1997) and presented on a computer screen (19" CRT colour monitor, 1024 by 768 pixel resolution, 75Hz, mean luminance of 50 cd/m^2), positioned 57 cm in front of the participant. A nine-point (3 x 3 target array) eye movement calibration took place prior to each of the 4 experimental blocks presented in this study (plus 1 saccade block published previously: Gonzalez, Billington and Burke, 2016) to ensure accurate eye measurements throughout. 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 sessions lasted for approximately 45 minutes.

MRI Experimental Set-up

In the second experimental session the same paradigm was also employed within an fMRI scanning system. A custom-made program was used to design new sequences and display the stimuli for the scanner session (Psychtoolbox, Brainard, 1997, and COGENT, www.vislab.ucl.uk, Matlab®, Mathworks, USA), similar to the one used for the laboratory session. Data was collected using a 3T (Phillips 3.0 T Achieva) MRI scanner with an eight-channel SENSE head-coil (Achieva 3.0 T Neuro Coil) designed to reduce the signal-to-noise ratio. A T1-weighted anatomical image (256 x 256 x 176, voxel size = $1mm^3$) was obtained prior to the 4 functional imaging blocks (plus 1 saccade block published previously, Gonzalez, Billington and Burke, 2016). Scans were collected using T2*-weighted spin echo pulse sequence (TR of 2000ms, TE of 30ms; 90° flip angle, FOV of 250 mm, 1.8 x 1.8 x 4 mm^3 voxel size and a total of 30 slices) covering the whole brain. An optical video eye tracker (Applied Science Laboratory, Bedford, MA) with a sampling rate of 60 Hz was used to inspect eye data during scanning to ensure correct task performance. Two mirrors were positioned on the head coil, one to reflect an image of the eye to the ASL video camera, and the second to reflect an image of the stimulus from the projector screen (size: 180cm x 110cm, temporal resolution: 75Hz) located at the participant's feet to the participant. The 4 experimental blocks were counterbalanced between participants and new sequences were implemented to avoid learning between sessions. As in the first session, nine-point eye calibrations took place in the scanner prior to each experimental block and the room was kept as dark as possible during testing with the experimental session lasting approximately 60 minutes. Each experimental block contained 212 volumes with the first two volumes excluded as dummy runs. None of the blocks were found to exceed 2mm movement in any direction and no erroneous image artefacts were observed and hence all data was included in the further analysis.

Stimulus

The target (white square, 15 x 15 pixels) started at the centre of the screen and then moved smoothly in one of four directions (left, right, up or down) to build sequences of 4 or 8 constant speed (15°/s) ramps (see Figure 1). Each sequence consisted of 4 or 8 smoothly moving components (ramps), linked to generate a continuous motion target for subjects to follow with their eyes. The short (4 components) and long (8 components) sequences were presented in two conditions: random (RND) and predictive (PRD). The PRD sequence learning task consisted of a sequence (4 or 8 components) that was repeated 4 times in a row. In the RND condition, all sequence combinations (4 or 8) were unique. Experimental blocks (2 sequence lengths x 2 conditions) were counterbalanced between participants and consisted of: (i) 4 component RND sequences (4RND), (ii) 4 component PRD sequences (4PRD), (iii) 8 component RND sequences (8RND), and (iv) 8 component PRD sequences (8PRD). The duration of each component (ramp) was set at 750 ms in the PRD condition and thus, the 4 and 8 component PRD sequences were 3000 ms and 6000 ms in duration respectively. The duration of each component was randomised between ~500, ~750 and ~1000ms in the RND sequences while still maintaining a 3000ms and 6000ms duration for the 4 and 8 component RND sequences respectively (Figure 2). All participants performed the same sequences within each block and instructions were to follow the moving target as accurately as possible with their eyes only. A 1000ms inter-trial interval was implemented between each sequence, and a 3000ms or 6000ms fixation was also introduced between each set of 4 sequences for the 4 and 8 component sequences respectively. A repetition in this context refers to the repeated presentations of the same sequence in the PRD condition only, of which there were 4 repetitions of a single sequence for both the short and long component sequences. The 4 blocks were presented pseudo-randomly to each participant with 80 unique sequences within the 4RND block, 20 unique sequences with each sequence presented 4

times in a row in 4PRD, 40 unique sequences in 8RND and 10 unique sequences presented 4 times in a row in the 8PRD block. Each block had a duration of ~6 minutes. Although the same experiment from the laboratory session was repeated for the fMRI session, new PRD and RND sequences were designed for this fMRI session and thus (short-term) sequence learning of the short and long PRD sequences was examined in both sessions to new repeated sequences.

Analysis

Eye Data

Eye movement data was sampled at 1000 Hz and initially pre-processed using the Data Viewer software (SR research Ltd, Canada). This pre-processing involved removing blinks and bridging the gaps within the missing data using linear interpolation. Eye velocities were analysed using a custom made programme in Matlab® (version 7.8, Mathworks Inc., USA). Intrusive and catch-up saccades were eliminated from the smooth pursuit eye movement data using a previously described linear interpolation technique to link the resulting gaps (Bennett & Barnes, 2003). The velocity traces were then filtered using a 10Hz low-pass, zero phase filter. Eye movement data were also obtained from the scanning environment (60Hz), which revealed an identical pattern of responses from the participants during all conditions (i.e. prediction shows earlier onsets than the random condition), although the signal was significantly noisier. For the purpose of clarity, we will present only the laboratory based data within this section (see Gonzalez, Billington, & Burke, 2016 for examples of eye data inside and outside the scanner in supplementary material).

A cross-correlation analysis was performed on the pre-processed eye and target velocity traces as a global temporal assessment to calculate the overall differences in time between eye movements and the target stimulus for PRD and RND sequence trials (for previous examples of this analysis see Barnes et al., 2000; Barnes & Schmid, 2002). This was

implemented due to the fact that pursuit onsets of a continuous eye movement to multiple components (ramps) can be problematic to identify, as the eye velocity at the start of each ramp is influenced by the decaying response to the prior ramp (Barnes & Schmid, 2002) (see Figure 2). Thus, the cross-correlations between the target and eye movements of each sequence were performed to obtain the overall temporal delays from the target for RND and for each repeated PRD sequence (SEQ1, SEQ2, SEQ3 and SEQ4). The time at which the maximum correlation was reached (t_{COR}) was calculated and used to describe the time delay between the eye velocity during the repeated sequences and the corresponding moving stimulus. Eye velocity X and Y traces were combined to create a single eye velocity trace, which was used to compare with the target velocity (see Figure 2). This resulted in a t_{COR} value independent of direction.

A repeated measures ANOVA (IBM SPSS statistics, version 20, NY, USA) with 2 levels was used to identify significant differences for the 4PRD and 8PRD conditions (level 1) and between the identical sequence presentations (level 2: SEQ1, SEQ2, SEQ3 and SEQ4). Interactions between variables were evaluated using a Bonferroni corrected post-hoc t-test. A significance level of $P < 0.05$ was established for all statistical analyses. Results and graphs are expressed as means and standard deviations (SD).

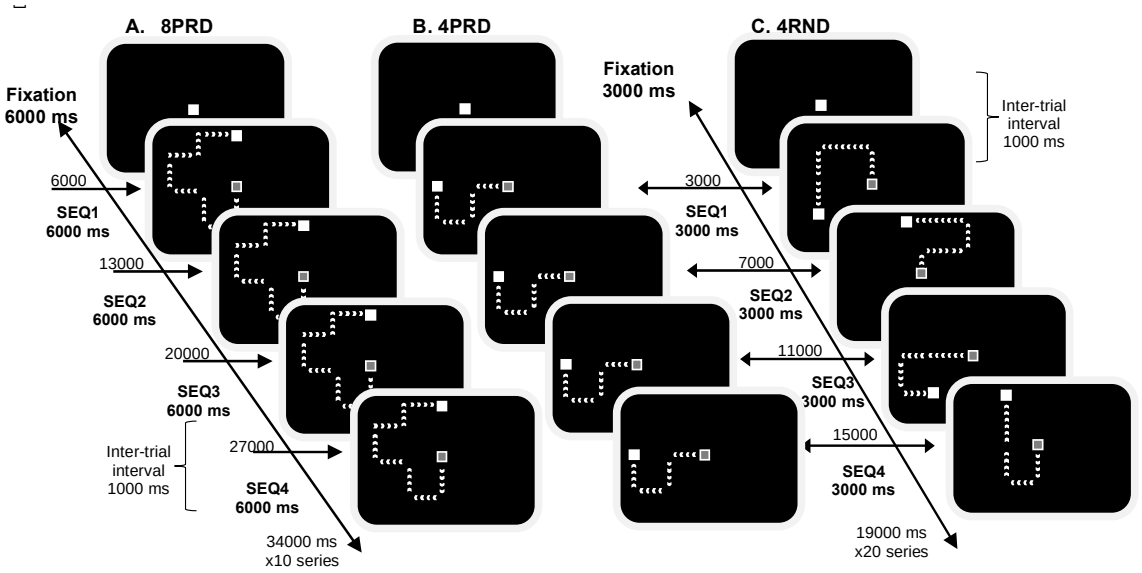

Figure 1. Examples of sequence presentations for the 8PRD (A, left), 4PRD (B, middle) and 4RND (C, right) experimental blocks. In PRD conditions (A and B), each sequence was repeated 4 times following a fixation and all sequences started in the centre of the screen. In RND conditions, each sequence was unique (C). Each series of 4 repeated sequences and 4 unique sequence presentations (i.e., SEQ1, SEQ2, SEQ3 and SEQ4) started with a fixation of equal duration to the sequence trials (6000 ms and 3000 ms for the long and short sequences, respectively). A 1000 ms inter-trial interval was implemented between each sequence and between a fixation and a sequence. Participants performed 10 series of the long sequences (40 sequences + 10 fixations in total) and 20 series in the shorter sequences (80 sequences + 20 fixations in total). Stimuli were designed for schematic purposes and are not to scale.

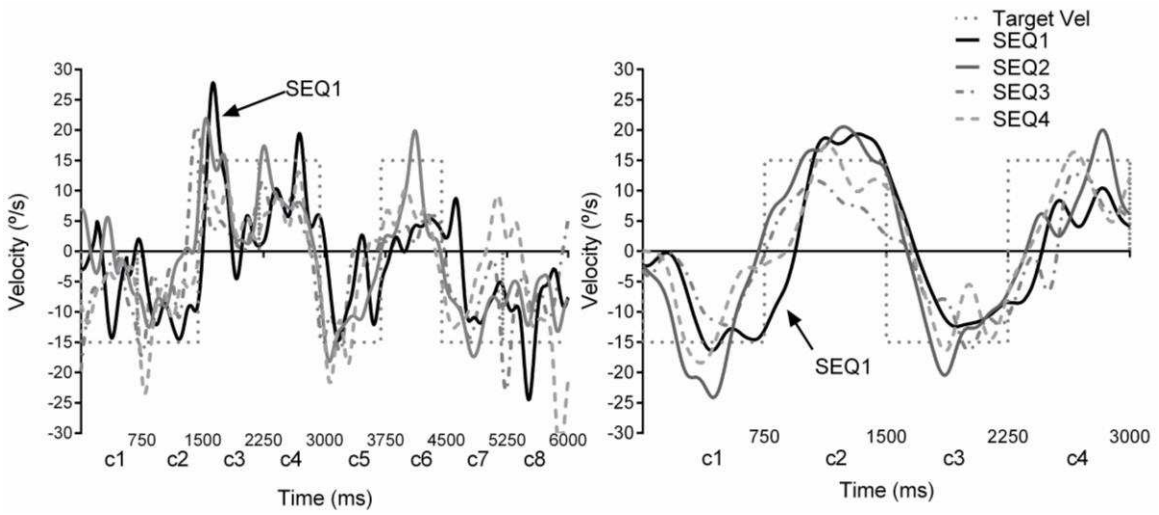

Figure 2. The graphs show examples of eye velocities to the repeated sequences, across a 8 (A, left) and 4 (B, right) PRD sequences. Each component (ramp) lasted for ~750ms and resulted in 6000ms and 3000ms sequences in the 8PRD and 4PRD trials respectively. The eye velocity traces show temporal shifts of the repeated sequences (SEQ2, SEQ3 and SEQ4, grey and dotted traces) towards the target (dotted squared trace), compared to the first sequence (SEQ1, black line). All sequences started in the centre of the screen or zero position, thus the velocity of each component shows upward, rightward (15°/s), downward or leftward (-15°/s) directional changes. NB c1, c2, etc. denote the components within a sequence; c1 to c4 for the 4PRD sequences and c1 to c8 for the 8PRD sequences.

fMRI

Data was pre-processed in the standard way using BrainVoyager QX version 2.8 (Brain Innovations B.V. Maastrict, the Netherlands), and included; slice time correction, motion correction, spatial realignment, co-registration with individuals anatomical images, normalized and transformed into Talairach space (Talairach Daemon software, http://www.nitrc.org) (Lancaster et al., 2000). Finally, data was filtered using a temporal high-pass filter at 128 Hz cut-off frequency to remove scanner drift, and smoothed using a 3d

Gaussian kernel with a FWHM of 8mm. No participant exceeded the cut-off of 2mm in any direction for head motion and so were included in the final analysis.

We used a general linear model to gain estimates of voxel activation to each condition (4PRD, 4RND, 8PRD and 8RND). The regressors in the design matrix were boxcar functions designed to model the activity during each sequence (3s or 6s seconds for 4 and 8 sequence lengths respectively) with the fixation also modelled independently (also 3 or 6 seconds). These boxcar functions were then convolved with an individual estimate of the haemodynamic response function.

We extracted the mid-sequences from each trial (2nd and 3rd presentation within the set of 4 sequences) and for each participant (1st level analysis) for both the RND and PRD conditions. We then did a 2nd level contrast of the resultant RND versus PRD data (group level) in order to identify areas important for sequence learning (PRD condition) and remove low level visual processing (RND condition). After applying a threshold of $t >= 3.5$ and cluster size > 20 (based on previous studies: Gonzalez et al., 2016) and a FWE ($p < 0.05$) to correct for multiple comparisons, we identified a number of regions of interest (ROIs) that confirmed our previous a priori findings (Gonzalez et al., 2016). From these regions of interest, we extracted normalized beta weights for each participant from the first (1st sequence) and final (4th) repetition of each sequence within the PRD condition (as reported in figures 4-7). We used this method to try and avoid any circulatory bias in our data when extracting beta weights allowing more accurate comparisons of reactive (SEQ1) and predictive (SEQ4) behaviour. Although we accept that since the ROI are from the 2nd and 3rd sequences a greater bias towards the predictive weighting will be predicted. Finally, regression analyses were performed to compare beta weight activation of each ROI during SEQ1 and SEQ4, with the corresponding SEQ1 and SEQ4 behavioural t_{COR} values for each sequence length. In addition,

Separate regressions were also performed between common ROI beta weights for short and long sequences.

Results

Eye movements

Cross-correlation analysis revealed that the temporal delays of the eye velocities from SEQ1 in PRD conditions compared with the overall RND responses were not statistically different in either the short ($p = 0.3$) or long ($p = 0.24$) sequences. Thus, the first presentation of a sequence (SEQ1) in PRD conditions was determined as a reactive response in both sequence lengths. A repeated measures ANOVA was then used to compare between this SEQ1 and the repeated sequences and within PRD sequence repetitions (SEQ2, SEQ3 and SEQ4) and determine learning effects, both within and between the sequence lengths (4PRD vs. 8PRD).

Cross-correlations: temporal shifts

Analysis revealed a significant interaction between repetition and sequence length ($F_{(1, 11)} = 5.106$; $p = 0.04$). Post hoc tests revealed a decrease in t_{COR} values of the repeated sequences (SEQ2, SEQ3 and SEQ4) compared to SEQ1 in the 4PRD condition (all $p < 0.001$), however, there were no differences between the repeated sequences ($p = 1.0$, $p = 1.0$ and $p = 0.59$ for SEQ2, SEQ3 and SEQ4 respectively) (Figure 3, left).

Similar to the 4PRD, the 8PRD sequences all had shorter t_{COR} values compared to SEQ1 (all $p < 0.001$), but with no differences between the repeated sequences ($p = 1.0$, $p = 0.15$ and $p = 0.06$ for SEQ2, SEQ3 and SEQ4 respectively) (Figure 3, right). Post hoc tests also showed that t_{COR} values of SEQ1 in 4PRD and 8PRD trials were not statistically different ($p = 0.15$)

confirming participants were performing equally regardless of sequence length to the random trials. Differences were evident between the sequence lengths in the repeated (predictable) sequences (p = 0.018, p = 0.04 and p = 0.005 for SEQ2, SEQ3 and SEQ4 respectively), with shorter temporal lags (aka better prediction) in the 4PRD sequences (mean across repetitions = 75.7 ± 18.8 ms) than the 8PRD (mean across repetitions = 91.8 ± 17.2 ms).

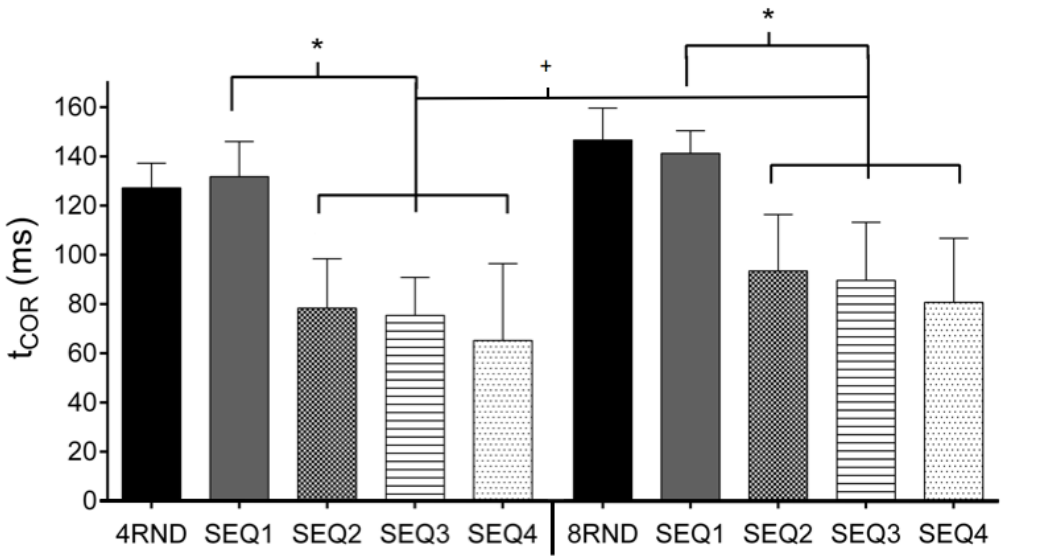

Figure 3. The Mean (and SD) t_{COR} values of the blocked 4 component tasks (left) and 8 component (right) sequences for all participants is shown for both RND (solid colour bars) and repeated PRD (patterned bars) conditions. The first presentation of the predictive sequence (SEQ1; solid grey column) was determined as reactive with no significant differences between these responses and the RND responses (solid black bar) in either sequence length condition. Both graphs show that eye velocities of the repeated sequences (SEQ2, SEQ3 and SEQ4) had shorter temporal delays (closer to the target) compared to SEQ1, with smaller t_{COR} values corresponding to predictive behaviour (*, $p < 0.001$) and a shorter lag (lower t_{COR} value) behind the target. In addition, the repeated sequences (SEQ2-4) in the 4

component sequence condition showed smaller t_{COR} values compared to the repeated 8 component sequences (+, $p < 0.05$).

fMRI results

We performed contrasts between overall PRD and RND conditions, identifying basal ganglia (BG) and frontal regions (BA 11, BA 9) for random sequences and inferior occipital gyrus (IOG), frontal eye fields (FEF), cerebellum (CBM), anterior cingulate cortex (ACC), as well as motor regions and the inferior parietal lobe (IPL, BA40) for predictive sequences (Table 1). In addition, contrasts between the overall short versus long sequences (4SEQ vs. 8SEQ) resulted in activation, at a threshold of T>3.5, of frontal (BA9, BA8), motor (BA4, M1) and inferior occipital gyrus (IOG) for shorter sequences, whilst higher activation in BG and the subcortical areas of the anterior cingulate cortex (ACC) and the parahippocampus (PHC) were observed during the longer sequences, see (Table 1).

Table 1: The table reports the overall significant effects from the group level (RFX analysis) to the RND versus PRD and 4 versus 8 sequence (SEQ) conditions, showing the number of voxels; T value; x, y and z coordinates of peak activation; Hemisphere (Hemi); Brain region and Brodmann Area. The cut-off used was T > 3.5.

CONTRAST		Voxel size	T value	Z score	X	Y	Z	Hemi	Brain Region	Brain Area
RND vs. PRD	**RND**	252	5.08	3.41	8	20	8	R	BG	
		128	4.41	3.14	24	41	-5	R	FP	BA 11
		141	4.1	3	10	51	18	R	DLPFC	BA 9
		25	3.73	2.83	6	-22	18	R	Thalamus	
	PRD	246	5.58	3.58	-36	-80	30	L	IOG/V5	BA 19
		325	5.26	3.47	-16	6	38	L	ACC	BA 32
		56	5.22	3.45	-12	13	21	L	BG	
		110	4.89	3.33	-14	31	39	L	FEF	BA 6
		168	4.31	3.1	16	-23	45	R	ACC	BA 32
		60	4.2	3.05	16	-36	-20	R	CBM	

		190	4.18	3.04	42	-78	26	R	V5	BA 19
		111	4.18	3.04	-12	-11	50	L	SMA	BA 6
		52	3.87	2.9	40	-2	28	R	SMA	BA 6
		100	3.61	2.77	-44	3	13	L	IC	BA 13
		135	3.53	2.73	-50	-37	46	L	IPL	BA 40
4SEQ vs. 8SEQ	**4SEQ**	3794	7.27	4.07	30	-76	-3	R	IOG/V5	BA 19
		204	5.61	3.59	-18	39	35	L	DLPFC	BA 9
		153	3.92	2.92	36	-16	39	R	PCG	BA 4
		160	3.62	2.77	-26	9	35	L	FEF	BA8
	8SEQ	60	4.22	3.06	-24	-46	8	L	PHC	BA 30
		104	3.7	2.81	40	-26	-14	R	PHC	BA 36
		45	3.67	2.8	16	-14	23	R	BG	
		32	3.66	2.79	-16	-16	39	L	ACC	BA 24

X, Y and Z correspond to Talairach coordinates in mm. BG= basal ganglia; FP= fronto-polar; DLPFC= dorsolateral prefrontal cortex; IOG= inferior occipital gyrus; ITG= inferior temporal gyrus; SMA= supplementary motor area; FEF= frontal eye fields; IC= insular cortex; IPL= inferior parietal lobe; PCG= precentral gyrus; PHC= parahippocampus, ACC= Anterior Cingulate, CBM= cerebellum.

ROI results

We contrasted sequence 2 and 3 of each set of PRD tasks, with RND sequences for the 4 and 8 component sequence tasks individually. This was done to identify which areas of the brain are principally involved in sequence learning during our predictive tasks and for each sequence length and to remove activation related to low level visual processes (as observed for the RND condition). Please note, since these ROIs were generated using specific sequences within the 4PRD and 8PRD tasks only, these regions are not the same as the global activations observed in Table 1, but do show considerable overlap. We found some regional similarities for the different sequence lengths, but also identified a number of distinct activations. Regional commonality for both the 4 PRD and 8 PRD sequence tasks included: the inferior occipital gyrus (IOG/BA19) and parts of the cingulate cortex. Areas that revealed more activation for the shorter 4PRD were more rostral in location (PCC/BA31), whereas the longer 8PRD task showed more caudal activity (ACC/BA24). We found activation in the

frontal lobe for both sequence lengths, however, these corresponded to distinct sub-regions of the prefrontal lobe for 4PRD (DLPFC/BA9 and middle frontal gyrus/BA10) and 8PRD (FEF/BA6) respectively. In addition, we found brain areas that were more active in the learning of the 8 component sequence learning task when compared to the shorter sequences along the precentral gyrus (BA4 and 6) related to motor and premotor areas.

Areas for both Short and Long Sequences:

(i) Inferior Occipital Gyrus (IOG)

We found the IOG to be involved in both the shorter and longer sequence learning tasks, when compared to the 1st presentation of the predictive trials (behaviourally equivalent to the random condition). Both sequence lengths revealed right hemisphere activation above threshold during learning in BA19. A regression analysis revealed a significant relationship between IOG (beta weights) and behavioural data (t_{COR}) during the 4th sequence, in both short ($R^2 = 0.41$, $p = 0.03$) and long ($R^2 = 0.58$, $p = 0.01$) sequences. Regression analysis between the beta weights of the short and long sequence during the 4th repetition did not reach significance for IOG ($R^2 = 0.23$, $p = 0.13$).

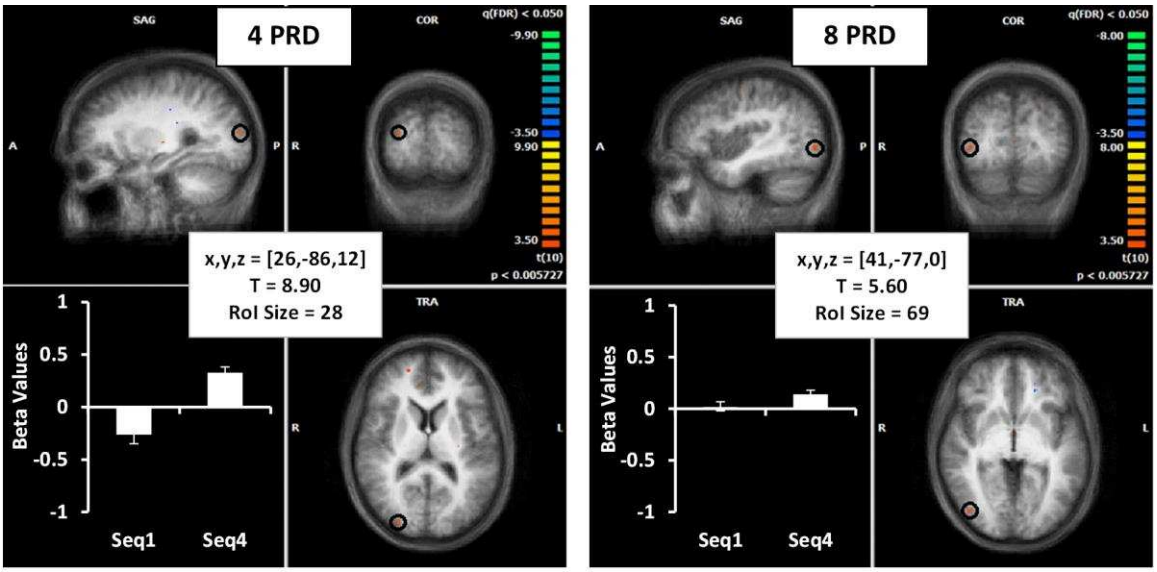

Figure 4. Brain images show location of activation (from seq 2-3) in red (highlighted with a black circle) for the **4PRD** (left) and **8PRD** (right) task on an averaged brain of all participants. In addition, corrected average beta weighting across all participants (for the 1st and 4th sequence) are presented in the lower left corner of each image for the first (SEQ1) and final sequence (SEQ4). Details of Talairach coordinates, T value and cluster size are given in the central white boxes.

(ii) Cingulate

The cingulate was also identified as an important structure for the learning of sequences, however shorter sequences revealed a more posterior location (PCC, BA31) (Figure 5, left) and longer sequences were more anterior (ACC, BA24) (Figure 5, right). Regression analysis between ACC beta weights and t_{COR} values showed a higher R^2 for ACC in the longer sequences during the 4th repetition; however, this did not reach statistical significance ($R^2 = 0.24$, $p = 0.08$). Additionally, regression analysis between short and long sequence beta weights in this region was also not found to be significant during the 4th repetition of the sequence ($R^2 = 0.27$, $p = 0.10$).

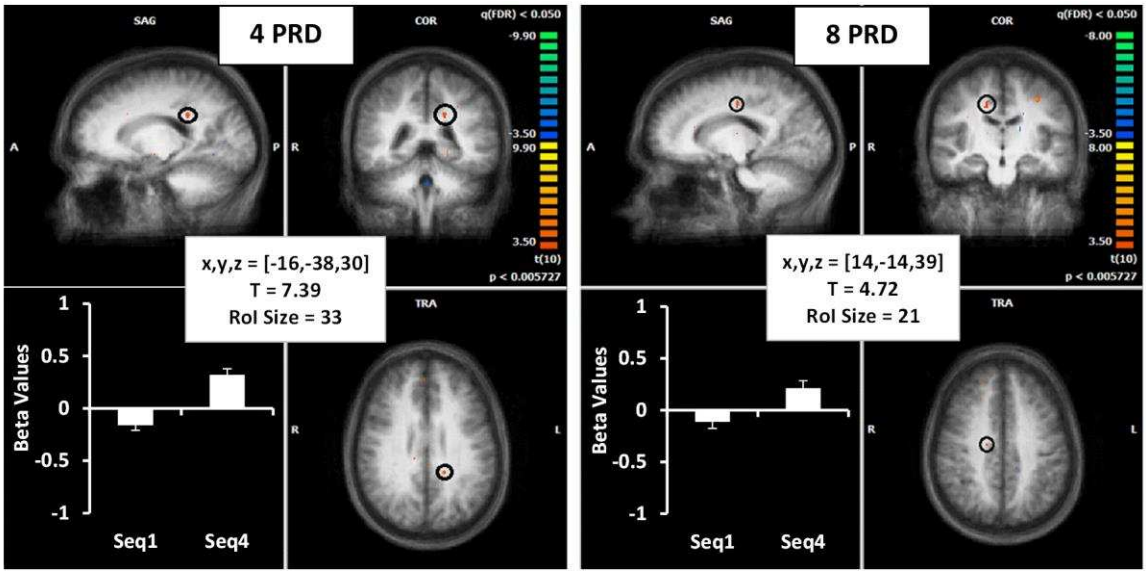

Figure 5. Convention for image is the same as figure 3: activation for the **4PRD** (left) and **8PRD** (right) from sequence 2 and 3 is shown. The activation in the posterior cingulate (BA31) is shown for the shorter sequence in red (with a black outline) on the left image and the ventral anterior cingulate (BA24) found in longer sequences is highlighted on the right image. Averaged beta weights (for the 1st and 4th sequence) are displayed in the graphs on the lower left quadrant of each image and details of regions of interest (ROI) are presented in the box in the centre. Details of Talairach coordinates, T value and cluster size are given in the central white boxes.

Activation for Longer Sequences

(i) Frontal Cortex

The frontal cortex was found to be involved in motor learning for both the shorter and longer sequence lengths. However, longer sequences (Figure 6) revealed greater activation compared to the 1st presentation of the PRD (reactive) condition in frontal eye fields (FEF).

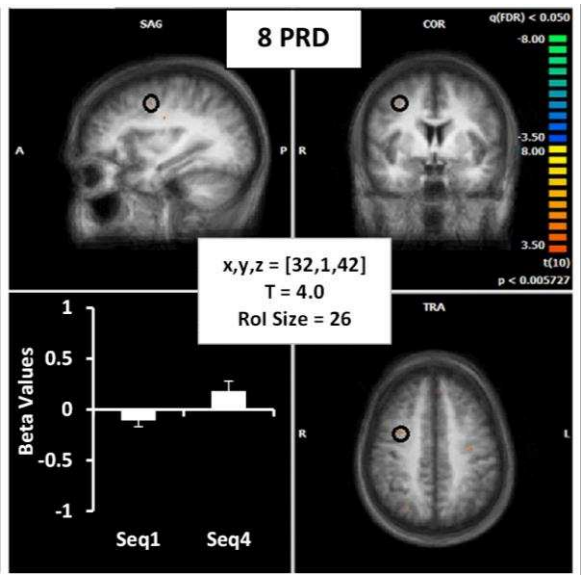

Figure 6. As above, activation (from Sequence 2 & 3) in frontal cortex for all participants to longer sequences (**8PRD**) only corresponding to FEF. Averaged beta weights (for the 1st and final sequence) are displayed in the graphs on the lower left quadrant of each image and details of regions of interest (ROI) are presented in the box in the centre of each image. Details of Talairach coordinates, T value and cluster size are given in the central white box.

(ii) Precentral Sulcus

In addition to areas in common for shorter and longer sequence lengths, we also found bilateral activation in the precentral gyrus that was specific for longer (8PRD) sequences. In the right hemisphere of the motor cortex (BA4), whereas in the left hemisphere the activation was more towards the midline (between middle frontal and precentral gyrus) and identified as part of the supplementary motor area (SMA, BA6). A regression analysis revealed a significant relationship between SMA (beta weight) and behavioural data (t_{COR}) during the 4th sequence, for the long sequences only ($R^2 = 0.49$, $p = 0.02$).

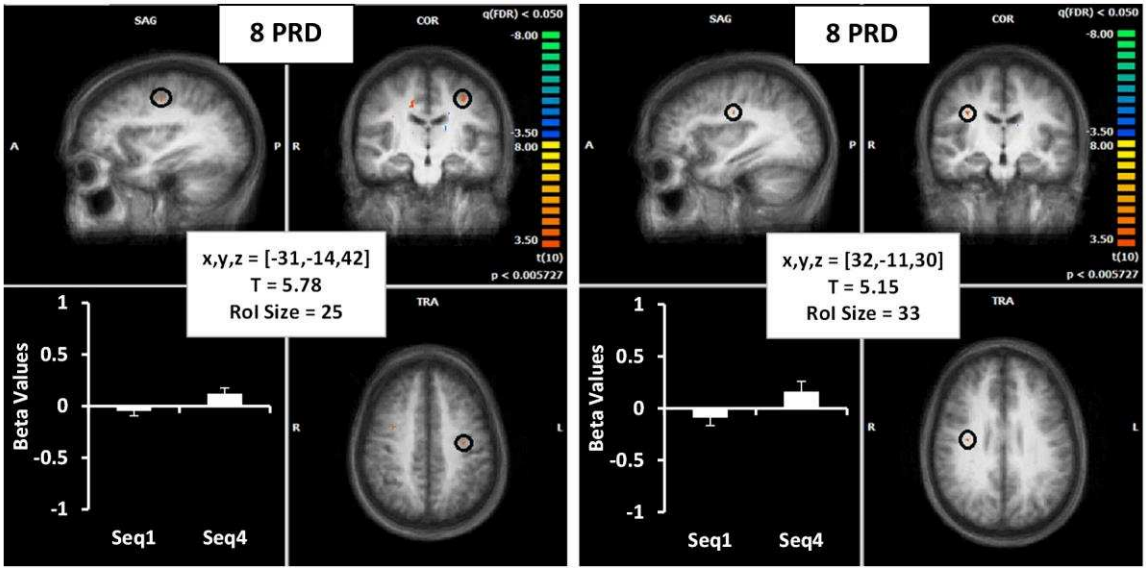

Figure 7. The brain images show the mean activation for the **8PRD** task from all participants in the supplementary motor area (SMA, BA6) (left image) and the motor cortex (BA4) (right image). Beta weights for these ROIs are displayed in the lower left quadrant of each image. Details of Talairach coordinates, T value and cluster size are given in the central white boxes.

Activation for Shorter Sequences

(i) Frontal cortex: Middle Frontal Gyrus and Dorsolateral Prefrontal Cortex

Short sequences revealed more prefrontal activity in the dorsolateral prefrontal cortex (DLPFC) (Figure 8, left). Additionally, shorter sequence lengths revealed one area (that did not reach above threshold for the 8 sequence task) that was identified as an anterior and medial part of the prefrontal cortex, known as the frontopolar cortex (BA10) (Figure 8, right). Despite this, a regression analysis failed to find a significant relationship between the beta weights (fMRI data) and behavioural (t_{COR}) data in either the 1st or final presentation of the stimulus in these shorter sequences ($R^2 = 0.22$, $p = 0.66$; $R^2 = 0.004$, $p = 0.85$ respectively).

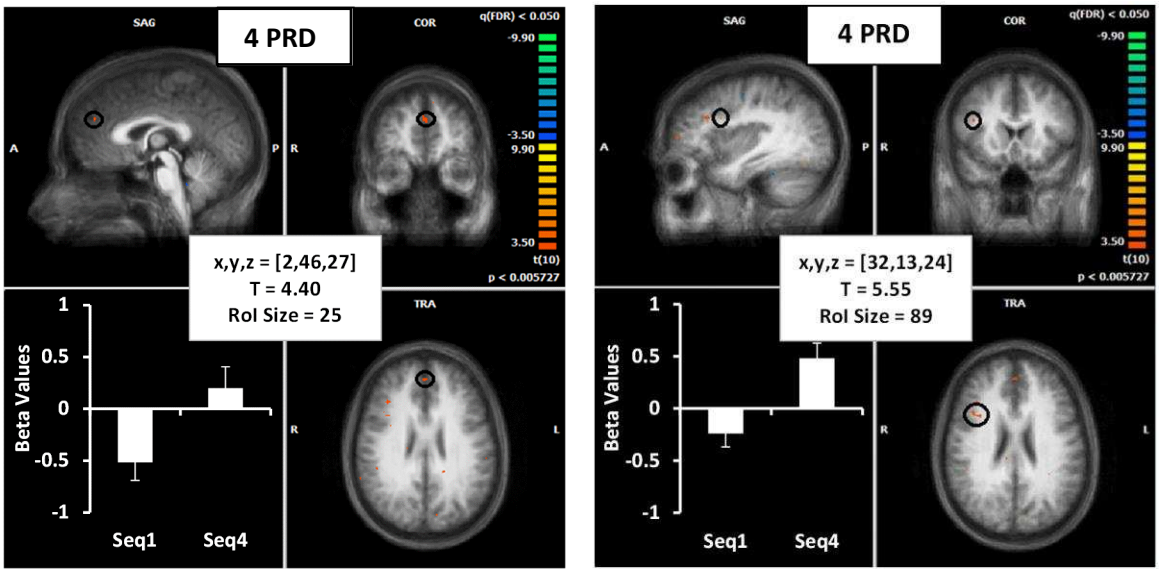

Figure 8. The brain image shows the average activation location for the **4PRD** task (in red with a black circle) overlaid on an averaged brain from all participants. Activation of DLPFC is shown on the left and frontopolar cortex (BA10) on the right, corresponding to the shorter sequences. The averaged (normalized) beta weights are shown in the lower left quadrant and details of the ROI displayed in the centre of the image. Details of Talairach coordinates, T value and cluster size are given in the central white boxes.

Discussion

Prediction in pursuit serves as an excellent example for early learning in the brain, since it has also been shown to contribute to the learning of more complex trajectories (Barnes & Schmid, 2002; Burke & Barnes, 2008; Collins & Barnes, 2005; Gonzalez et al., 2016). In the current study we aimed to identify the brain areas that aid the understanding of how sequence length affects brain activity by implementing a shorter sequence of eye movements, to more complex sequences that can be used for more naturalistic motor learning skills such

as playing the piano or writing. To achieve this, we designed a task that involved participants following a sequence of smoothly moving targets of either 4 or 8 continuous ramps in predictive and random conditions. In addition, we identified the brain areas of interest based on previous studies (Burke & Barnes, 2008; Gonzalez et al., 2016), and the contrast between randomly generated sequences and the predictive sequences independently for the shorter and longer sequences. Furthermore, we used different sequences to the ones within the block design to identify levels of activity within these regions of interest to the 1st and last (4th) presentation of the sequence for each sequence length. This novel approach allowed us to interrogate if parallel (i.e. both spatial and motor circuits are active simultaneously during acquisition) in both shorter and longer sequence length acquisition, or the shorter versus longer sequences activate the same (overlapping) brain areas.

Our data revealed clear learning of our continuous velocity sequences, since their tracking abilities improved rapidly (by the second presentation of the sequence or SEQ2) regardless of sequence length/complexity. More specifically, the sequences of repeated targets (PRD) elicited predictive responses by our participants as shown by smaller temporal lags (t_{COR}) of the eye velocity from the target's velocity in the repeated sequences, when compared to the first (reactive) sequence presentation. This indicated that our task was inducing motor learning and a motor plan of these continuous target motions in the brain, as found previously in tasks where a series of single ramps (i.e. smooth movement from left to right) were presented (Burke & Barnes, 2008; Collins & Barnes, 2005). Furthermore, in support of a previous study by Collins and Barnes (2005), we found a significant difference between the repeated shorter (4 component) and longer (8 component) sequences with a shorter temporal lag in the shorter sequence condition. This result indicates that there was less anticipation occurring in the longer sequences compared to the shorter sequences and this state was maintained throughout the 4 repetitions. Our fMRI data revealed some

overlapping areas which correspond to pursuit activation (V5 and cingulate cortex), as well as a distinct brain activations for these different sequence lengths. In particular, we observed activation in more memory related areas (DLPFC and BA10) during the shorter sequences, whereas in the longer sequences the activated areas that corresponded to pre-motor and motor cortex. These results may reflect the behavioural differences observed in the level of prediction or timing between sequence lengths.

Brain areas for longer versus shorter sequences

M1and SMA: Activation in pre-motor, SMA and motor cortex reflects movement planning and advance preparation of a movement that are vital when learning a new motor skill (Georgopoulos, 1994; Pascual-Leone et al., 1994). Hikosaka et al., (1999, 2002) described the two parallel information processing loops for sequence learning that comprise of the prefrontal cortex (corresponding to the spatial process), and a motor sequence process that utilizes the premotor and motor cortices.

The fact that prediction did not reach equal levels and that distinct brain areas were found active between the two sequence lengths could indicate that different mechanisms are being used during the early stages of learning that both resulted in predictive behaviour; one guided by spatial memory and another via implicit motor networks (for review, see Penhune & Steele, 2012), and thus is ultimately dependent on the task demands. According to the motor sequence model presented in the introduction (Hikosaka et al., 1999; 2002), early learning takes place in memory and attention networks involved in the accurate acquisition of the sequence, whilst motor sequence acquisition takes place in a parallel, but slower network of brain areas and contributes to longer-term learning and retention. This hypothesis is supported by findings showing that spatial/sequential components develop early and that motor control and optimization keeps developing over longer-term practice

(Penhune & Steele, 2012). Our task explored early learning of both sequence lengths and revealed more complex longer sequences involved predominantly motor/premotor activation, whereas shorter sequences utilized frontal activation. However, in contrary to earlier findings (e.g., Toni et al., 1998) this effect was not contingent on the number of repetitions (or practice) since both sequence lengths were repeated 4 times. Interestingly we did not find higher activation in the parietal cortex for the longer sequence length compared to the shorter, however we did find higher activation in this region (specifically BA40) for predictive versus random conditions indicating a similar role for this area in both our learning tasks.

Activity of the motor cortex has not yet been clearly delimited to a specific stage of learning, with some studies revealing activation in the early stages that decreases with practice, and others showing activation increasing with practice (Karni et al., 1995; Penhune & Doyon, 2002). It is possible that the motor cortex could be active in both early and late stages, and activity is more dependent on the complexity of the task (i.e. if the sequence being learnt is beyond the scope for WM mechanism in PFC) (Seidler et al., 2005; Wu, Kansaku, & Hallett, 2004). The parallel sequence learning model, specifies that activity of the motor cortex and other regions (e.g., cerebellum) could emerge at distinct learning stages depending on task demands, and that sequence parameters (i.e., velocity, timing and accuracy) may be optimised over distinct time frames (see Penhune & Steele, 2012) supporting Hikosaka et al.'s model.

The fact that there was less prediction observed in the behavioural measures during the longer sequences may be due to the higher requirements for storage of information (more components). This is in agreement with the notion of a limited capacity memory buffer in storing eye velocities as suggested by Collins and Barnes (2005) and the general notions of a working memory capacity (Baddeley, 2000). Given the shorter sequence was 3 seconds in

duration this clearly involved a short-term memory system for generating predictive responses and was supported by the data revealing higher levels of activity within PFC. However, the longer sequence was 6 seconds in duration and the reliance on this short-term working memory system was weak (reduced activity in PFC), suggesting an alternative mechanism for more complex learning. Furthermore, brain areas for more complex sequences also included the precentral gyrus or more specifically the premotor (BA6) and motor cortices (BA4), providing new evidence that segregates brain area involvement in the learning of a task based on the complexity and duration of a stimulus.

Motor areas are not typically reported as core brain areas for pursuit and only a few researchers have found activation of these areas during predictive conditions (e.g., PMA in Lencer et al., 2004; pre-SMA in Schmid et al., 2001). The fact that these areas are not typically found in previous studies using short, repeated ramps (for review see Burke & Barnes, 2008; Lencer & Trillenberg, 2008) and were not significantly active during our shorter sequences, may indicate that these motor regions were only needed for more complex sequences of longer duration. Our findings provide novel insight into how the pursuit predictive (short-term buffer) system copes with additional sequence components. We further describe how these brain areas differ below.

Frontal cortex: The prefrontal cortex is commonly reported as an active area during the initial stages of learning, when decision making, selection of movements, working memory and attention are required (Halsband & Lange, 2006; Miller, 2000; Miller & Cohen, 2001; Pierrot-Deseilligny et al., 2002). In particular, the DLPFC, active in our shorter sequences, has been shown to be involved in both the encoding and retrieval of stimuli information, however right DLPFC is more important for retrieval in-line with the findings presented here (Halsband & Lange, 2006; Sakai et al., 1998). This area is more typically reported in memory saccade tasks (e.g., Müri & Nyffeler, 2008; Nyffeler et al., 2002), but has

also been associated with learning and prediction in pursuit eye movements (Burke & Barnes, 2008; Ding, Powell, & Jiang, 2009; Lekwuwa & Barnes, 1996; Schmid et al., 2001). In addition to the DLPFC, we found the frontopolar region (BA10) in the rostrolateral prefrontal cortex was significantly active for the shorter repeated sequences in contrast to the 1st presentation of the predictive task, which was behaviourally equivalent to the randomized condition. However, this area did not reach significant thresholds in the longer sequence. The orbital and medial prefrontal cortex are also structures for memory processing (Miller & Cohen, 2001). BA10 has been identified as an area important for executive function and for anticipatory eye movements (Burke and Barnes, 2008). In Hikosaka et al's (1999, 2002) model, the prefrontal cortex (DLPFC) is suggested to be involved in the early stages of learning in which learning occurs via a spatial mechanism, reliant on attention and working memory. After longer practice, the procedure will be acquired by the motor sequence mechanism involving pre-motor and motor cortex, which we observed in the longer sequences. We expect that continued practice in the shorter sequences, in which these frontal areas were more prevalent, would show a decrease in DLPFC and FP activity as previously shown (Burke & Barnes, 2008; Ding et al., 2009; Hikosaka et al., 1999; Koch et al., 2006), and studies identifying learning-related transitions (e.g., Koch et al., 2006).

We did not observe any pre-frontal activation reaching our thresholds in the longer sequences, however we did observed activity in the FEF, located in frontal cortex. Classically FEF is more associated with reactively driven eye movements. For example, Burke and Barnes (2007, 2008) found that FEF was more active for random pursuit, whilst activation of supplementary eye fields (SEF) corresponded to predictive pursuit, in accordance with previous studies (Heide, Kurzidim, & Kömpf, 1996; Schmid et al., 2001). However, a brain stimulation study revealed that predictive pursuit (gain) is controlled by FEF, whilst stimulation to SEF yielded effects on tracking directional changes (Gagnon, Paus, Grosbras,

Pike, & O'Driscoll, 2006). Similarly, Drew and Van Donkelaar (2007) found that SEF was involved in predictable changes of on-going pursuit and FEF controlled the initiation and maintenance of pursuit in both predictable and non-predictable pursuit, which may explain why FEF was not a feature of the shorter predictable sequences (after contrasting 4RND vs 4PRD). Notably, lesions to pursuit FEF have been shown to decrease acceleration, steady state velocity and seem to hinder predictive eye movements during periodic stimuli (MacAvoy, Gottlieb, & Bruce, 1991). This suggests a more prevalent role of FEF in the maintenance of pursuit (also see Fukushima, Fukushima, Warabi, & Barnes, 2013) that are needed during longer sequence presentations. Other studies have found that the FEF is largely unaffected by cognitive factors or increased cognitive load (Culham, Cavanagh, & Kanwisher, 2001; Paus, 1996). However, our study suggests that this area is utilized during more complex sequence acquisitions, and that these types of sequences may form a network with ACC and V5 for the learning of longer sequences in pursuit.

Regional similarities for both sequence lengths

The pursuit system is efficient at integrating both feedback and prediction, a process which occurs during visually-guided tracking as well as in predictive pursuit. The brain areas found here for predictable and non-predictable pursuit is in accordance with previous studies, but with the addition of prefrontal and cingulate areas during prediction (Burke & Barnes, 2008; Schmid et al., 2001). We isolated activation for the learning of a sequence by removing visual and motor related effects by performing a contrast with the 1st (reactive) presentation of the sequence. In addition, we examined these areas more closely for comparisons between the shorter and longer sequence lengths. The data revealed similarities to previously reported data on prediction with the brain areas involved in sequence learning including: (i) a region between the temporal and occipital lobe (BA19), (ii) the cingulate, and (iii) the

frontal cortex. However, we also identified distinct sub-regional differences within these regions for simpler versus more complex sequences. Interestingly, higher cerebellum activation was observed in the overall for PRD conditions when contrasted with the randomized trials (Table 1), indicating a role for this area in sequence learning during pursuit (for review see Lencer & Trillinberg, 2008). However, we did not see differential activation for the shorter versus longer sequences, indicating complexity does not impact this level of activity.

V5: The medial temporal lobe is a key area not only for motion perception, but also for the maintenance of smooth pursuit (for review, see Lencer & Trillenberg, 2008). This area connects with frontal cortex and in particular, FEF where the motor commands for pursuit initiation, maintenance and prediction are generated (Fukushima, Yamanobe, Shinmei, & Fukushima, 2002; Gagnon et al., 2006; Heide et al., 1996). According to non-human primate studies, V5 receives corollary eye movement information as well as a pursuit command that corresponds to an efference copy (or motor plan) that is vital for prediction (Barton et al., 1996). It is however, still not clear how this area aids prediction and/or learning. Dukelow et al (2001) and Schmid et al (2001) both found that V5 in humans was active during anticipatory eye movements in the absence of visual targets. Also, Nagel, Sprenger, Hohagen, Binkofski, and Lencer (2008) examined the cortical mechanisms of visually-guided pursuit and pursuit during target blanking and found that V5 was important for coding target velocity important prediction. Similarly, Burke and Barnes (2008) fMRI findings revealed learning-related activation during predictive eye movements in V5, among other areas related to prediction in pursuit compared to random target presentations. In the current study the activation of this area (regardless of sequence length) could be due to the nature of our continuous sequence presentations. One explanation is that foveal tracking observed in PRD pursuit could be better than during reactive trials and that this is equivalent in both sequence

lengths. Another option is that this area is involved in very early stimulus acquisition (of the efference copy) and therefore has a role for both the short and longer sequence. Given that other researchers have found V5 activation related to memory and learning in pursuit and the fact that V5 was more active during the short sequences, then our suggestion would support these earlier findings and suggest a principal role for this area early in the learning pathway, during the acquisition of an efference copy of the target information, that becomes less important as learning progresses. Indeed, supporting this suggestion is that the correlations between the t_{COR} values (eye movements) and the fMRI beta weights suggests a closer correspondence with the shorter when compared to the longer sequence lengths. More research into the role of V5 in learning is needed to make further conclusions.

Cingulate cortex: The cingulate cortex is suggested to be a higher order structure involved in motor memory and planning (Schmid et al., 2001) and related to both saccadic and pursuit eye movements (Gaymard et al., 1998; Haller, Fasler, Ohlendorf, Radue, & Greenlee, 2008). In pursuit studies, the role of anterior cingulate (ACC) is in the motor planning and memory of stimulus (timing and trajectory), and very important for predictive eye movements (Burke & Barnes, 2008; Ding et al., 2009; Schmid et al., 2001). Interestingly, single cell recordings from Procyk, Tanaka and Joseph (2000) revealed selective activation of neurons within the ACC for new learning and also for learnt behaviour. Furthermore, Ding et al (2009) found that both the DLPFC and the ACC increased in activity during visuomotor tracking of occluded targets. However, they also found a stronger correlation between the ACC and the FEF in the more predictable conditions (i.e., when tracking visible targets in a predictable sinusoidal path), whereas higher activation was observed in the DLPFC when the target was occluded and working memory requirements were higher. Schmid et al (2001) also found an increase in ACC activation when tracking a more predictable stimulus presentation. Our data supports these previous findings as we too find DLPFC more active for

shorter sequences, and the ACC/FEF active for complex sequence acquisitions. In addition, as a novel contribution to the literature we also find shorter sequences tend to active more posterior cingulated regions (PCC) suggesting a shift in activity for simpler (posterior) to more complex (anterior) learning in this frontal-cingulate loop.

Conclusion

Our data collectively highlights distinct brain areas involved in motor sequence learning for the longer versus the shorter sequence options. These results provide new evidence for the parallel model suggesting that short-term motor learning is an adaptive process that shifts from prefrontal activation in simpler shorter sequences, to more motor areas when longer sequence of movements are required, but is independent of number of repetitions as suggested by earlier studies. This process is possibly equivalent to the initial stages of learning a new motor skill when individuals are exposed to movements with differing complexity.

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References

Alexander, G. E., DeLong, M. R., & Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience*, *9*, 357–381. https://doi.org/10.1146/annurev.ne.09.030186.002041

Baddeley, a. (2000). The episodic buffer: a new component of working memory? *Trends in Cognitive Sciences*, *4*(11), 417–423.

Barnes, G. R., Barnes, D. M., & Chakraborti, S. R. (2000). Ocular pursuit responses to repeated, single-cycle sinusoids reveal behavior compatible with predictive pursuit. *Journal of Neurophysiology*, *84*(5), 2340–55.

Barnes, G. R., & Donelan, S. F. (1999). The remembered pursuit task: evidence for segregation of timing and velocity storage in predictive oculomotor control. *Experimental Brain Research*, *129*(1), 57–67.

Barnes, G. R., & Schmid, A. M. (2002). Sequence learning in human ocular smooth pursuit. *Experimental Brain Research*, *144*(3), 322–35. https://doi.org/10.1007/s00221-002-1050-8

Barton, J. J., Simpson, T., Kiriakopoulos, E., Stewart, C., Crawley, A., Guthrie, B., … Mikulis, D. (1996). Functional MRI of lateral occipitotemporal cortex during pursuit and motion perception. *Annals of Neurology*, *40*(3), 387–398. https://doi.org/10.1002/ana.410400308

Bennett, S. J., & Barnes, G. R. (2003). Human ocular pursuit during the transient disappearance of a visual target. *Journal of Neurophysiology*, *90*(4), 2504–20. https://doi.org/10.1152/jn.01145.2002

Brainard, D. H. (1997). The Psychophysics Toolbox. *Spatial Vision*, *10*(4), 433–436.

Burke, M. R., & Barnes, G. R. (2007). Sequence learning in two-dimensional smooth pursuit eye movements in humans. *Journal of Vision*, *7*(1), 5. https://doi.org/10.1167/7.1.5

Burke, M. R., & Barnes, G. R. (2008). Brain and behavior: a task-dependent eye movement study. *Cerebral Cortex*, *18*(1), 126–35. https://doi.org/10.1093/cercor/bhm038

Collins, C. J., & Barnes, G. R. (2005). Scaling of smooth anticipatory eye velocity in response to sequences of discrete target movements in humans. *Experimental Brain Research*, *167*(3), 404–13. https://doi.org/10.1007/s00221-005-0044-8

Culham, J. C., Cavanagh, P., & Kanwisher, N. G. (2001). Attention response functions: characterizing brain areas using fMRI activation during parametric variations of attentional load. *Neuron*, *32*(4), 737–45.

Ding, J., Powell, D., & Jiang, Y. (2009). Dissociable frontal controls during visible and memory-guided eye-tracking of moving targets. *Human Brain Mapping*, *30*(11), 3541–52. https://doi.org/10.1002/hbm.20777

Drew, A. S., & van Donkelaar, P. (2007). The contribution of the human FEF and SEF to smooth pursuit initiation. *Cerebral Cortex*, *17*(11), 2618–24. https://doi.org/10.1093/cercor/bhl169

Dukelow, S. P., DeSouza, J. F., Culham, J. C., van den Berg, A. V., Menon, R. S., & Vilis, T. (2001). Distinguishing subregions of the human MT+ complex using visual fields and pursuit eye movements. *Journal of Neurophysiology*, *86*(4), 1991–2000.

Fukushima, K., Fukushima, J., Warabi, T., & Barnes, G. R. (2013). Cognitive processes involved in smooth pursuit eye movements: behavioral evidence, neural substrate and clinical correlation. *Frontiers in Systems Neuroscience*, *7*, 4. https://doi.org/10.3389/fnsys.2013.00004

Fukushima, K., Yamanobe, T., Shinmei, Y., & Fukushima, J. (2002). Predictive responses of periarcuate pursuit neurons to visual target motion. *Experimental Brain Research*, *145*(1), 104–20. https://doi.org/10.1007/s00221-002-1088-7

Gagnon, D., Paus, T., Grosbras, M.-H., Pike, G. B., & O'Driscoll, G. A. (2006). Transcranial magnetic stimulation of frontal oculomotor regions during smooth pursuit. *The*

Journal of Neuroscience, *26*(2), 458–66. https://doi.org/10.1523/JNEUROSCI.2789-05.2006

Gaymard, B., Rivaud, S., Cassarini, J. F., Dubard, T., Rancurel, G., Agid, Y., & Pierrot-Deseilligny, C. (1998). Effects of anterior cingulate cortex lesions on ocular saccades in humans. *Experimental Brain Research*, *120*(2), 173–83.

Georgopoulos, A. P. (1994). New concepts in generation of movement. *Neuron*, *13*(2), 257–268.

Gonzalez, C. C., Billington, J., & Burke, M. R. (2016). The involvement of the fronto-parietal brain network in oculomotor sequence learning using fMRI. *Neuropsychologia*, *87*, 1–11. https://doi.org/10.1016/j.neuropsychologia.2016.04.021

Haller, S., Fasler, D., Ohlendorf, S., Radue, E. W., & Greenlee, M. W. (2008). Neural activation associated with corrective saccades during tasks with fixation, pursuit and saccades. *Experimental Brain Research*, *184*(1), 83–94. https://doi.org/10.1007/s00221-007-1077-y

Halsband, U., & Lange, R. K. (2006). Motor learning in man: a review of functional and clinical studies. *Journal of Physiology, Paris*, *99*(4–6), 414–424. https://doi.org/10.1016/j.jphysparis.2006.03.007

Heide, W., Kurzidim, K., & Kömpf, D. (1996). Deficits of smooth pursuit eye movements after frontal and parietal lesions. *Brain: A Journal of Neurology*, *119 (Pt 6)*, 1951–1969.

Hikosaka, O., Miyashita, K., Miyachi, S., Sakai, K., & Lu, X. (1998). Differential roles of the frontal cortex, basal ganglia, and cerebellum in visuomotor sequence learning. *Neurobiology of Learning and Memory*, *70*(1–2), 137–149. https://doi.org/10.1006/nlme.1998.3844

Hikosaka, O., Nakahara, H., Rand, M. K., Sakai, K., Lu, X., Nakamura, K., … Doya, K. (1999). Parallel neural networks for learning sequential procedures. *Trends in Neurosciences*, *22*(10), 464–471.

Hikosaka, O., Nakamura, K., Sakai, K., & Nakahara, H. (2002). Central mechanisms of motor skill learning. *Current Opinion in Neurobiology*, *12*(2), 217–222.

Hikosaka, O., Rand, M. K., Miyachi, S., & Miyashita, K. (1995). Learning of sequential movements in the monkey: process of learning and retention of memory. *Journal of Neurophysiology*, *74*(4), 1652–1661.

Hikosaka, O., Rand, M. K., Nakamura, K., Miyachi, S., Kitaguchi, K., Sakai, K., Shimo, Y. (2002). Long-term retention of motor skill in macaque monkeys and humans. *Experimental Brain Research*, *147*(4), 494–504. https://doi.org/10.1007/s00221-002-1258-7

Jenkins, I. H., Brooks, D. J., Nixon, P. D., Frackowiak, R. S., & Passingham, R. E. (1994). Motor sequence learning: a study with positron emission tomography. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *14*(6), 3775–3790.

Jueptner, M., Stephan, K. M., Frith, C. D., Brooks, D. J., Frackowiak, R. S., & Passingham, R. E. (1997). Anatomy of motor learning. I. Frontal cortex and attention to action. *Journal of Neurophysiology*, *77*(3), 1313–24.

Karni, A., Meyer, G., Jezzard, P., Adams, M. M., Turner, R., & Ungerleider, L. G. (1995). Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature*, *377*(6545), 155–158. https://doi.org/10.1038/377155a0

Koch, K., Wagner, G., von Consbruch, K., Nenadic, I., Schultz, C., Ehle, C., … Schlösser, R. (2006). Temporal changes in neural activation during practice of information retrieval from short-term memory: an fMRI study. *Brain Research*, *1107*(1), 140–50. https://doi.org/10.1016/j.brainres.2006.06.003

Kowler, E., & McKee, S. P. (1987). Sensitivity of smooth eye movement to small differences in target velocity. *Vision Research*, *27*(6), 993–1015.

Lancaster, J. L., Woldorff, M. G., Parsons, L. M., Liotti, M., Freitas, C. S., Rainey, L., … Fox, P. T. (2000). Automated Talairach atlas labels for functional brain mapping. *Human Brain Mapping*, *10*(3), 120–31.

Lee, D., & Quessy, S. (2003). Activity in the supplementary motor area related to learning and performance during a sequential visuomotor task. *Journal of Neurophysiology*, *89*(2), 1039–56. https://doi.org/10.1152/jn.00638.2002

Lekwuwa, G. U., & Barnes, G. R. (1996). Cerebral control of eye movements. I. The relationship between cerebral lesion sites and smooth pursuit deficits. *Brain*, *119 (Pt 2)*, 473–490.

Lencer, R., Nagel, M., Sprenger, A., Zapf, S., Erdmann, C., Heide, W., & Binkofski, F. (2004). Cortical mechanisms of smooth pursuit eye movements with target blanking. An fMRI study. *The European Journal of Neuroscience*, *19*(5), 1430–6. https://doi.org/10.1111/j.1460-9568.2004.03229.x

Lencer, R., & Trillenberg, P. (2008). Neurophysiology and neuroanatomy of smooth pursuit in humans. *Brain and Cognition*, *68*(3), 219–28. https://doi.org/10.1016/j.bandc.2008.08.013

MacAvoy, M. G., Gottlieb, J. P., & Bruce, C. J. (1991). Smooth-pursuit eye movement representation in the primate frontal eye field. *Cerebral Cortex (New York, N.Y.: 1991)*, *1*(1), 95–102.

Miller, E. K. (2000). The prefrontal cortex and cognitive control. *Nature Reviews. Neuroscience*, *1*(1), 59–65. https://doi.org/10.1038/35036228

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual Review of Neuroscience*, *24*, 167–202. https://doi.org/10.1146/annurev.neuro.24.1.167

Müri, R. M., & Nyffeler, T. (2008). Neurophysiology and neuroanatomy of reflexive and volitional saccades as revealed by lesion studies with neurological patients and transcranial magnetic stimulation (TMS). *Brain and Cognition*, *68*(3), 284–292. https://doi.org/10.1016/j.bandc.2008.08.018

Nagel, M., Sprenger, A., Hohagen, F., Binkofski, F., & Lencer, R. (2008). Cortical mechanisms of retinal and extraretinal smooth pursuit eye movements to different target velocities. *NeuroImage*, *41*(2), 483–92. https://doi.org/10.1016/j.neuroimage.2008.02.058

Nakahara, H., Doya, K., & Hikosaka, O. (2001). Parallel cortico-basal ganglia mechanisms for acquisition and execution of visuomotor sequences - a computational approach. *Journal of Cognitive Neuroscience*, *13*(5), 626–647. https://doi.org/10.1162/089892901750363208

Nyffeler, T., Pierrot-Deseilligny, C., Felblinger, J., Mosimann, U. P., Hess, C. W., & Müri, R. M. (2002). Time-dependent hierarchical organization of spatial working memory: a transcranial magnetic stimulation study. *The European Journal of Neuroscience*, *16*(9), 1823–1827.

Pascual-Leone, A., Grafman, J., & Hallett, M. (1994). Modulation of cortical motor output maps during development of implicit and explicit knowledge. *Science (New York, N.Y.)*, *263*(5151), 1287–1289.

Paus, T. (1996). Location and function of the human frontal eye-field: a selective review. *Neuropsychologia*, *34*(6), 475–483.

Penhune, V. B., & Doyon, J. (2002). Dynamic cortical and subcortical networks in learning and delayed recall of timed motor sequences. *The Journal of Neuroscience*, *22*(4), 1397–406.

Penhune, V. B., & Steele, C. J. (2012). Parallel contributions of cerebellar, striatal and M1 mechanisms to motor sequence learning. *Behavioural Brain Research*, *226*(2), 579–91. https://doi.org/10.1016/j.bbr.2011.09.044

Pierrot-Deseilligny, C., Müri, R. M., Ploner, C. J., Gaymard, B., Demeret, S., & Rivaud-Pechoux, S. (2003). Decisional role of the dorsolateral prefrontal cortex in ocular motor behaviour. *Brain*, *126*(Pt 6), 1460–73.

Pierrot-Deseilligny, C., Müri, R. M., Rivaud-Pechoux, S., Gaymard, B., & Ploner, C. J. (2002). Cortical control of spatial memory in humans: the visuooculomotor model. *Annals of Neurology*, *52*(1), 10–9. https://doi.org/10.1002/ana.10273

Procyk, E., Tanaka, Y. L., & Joseph, J. P. (2000). Anterior cingulate activity during routine and non-routine sequential behaviors in macaques. *Nature Neuroscience*, *3*(5), 502–508. https://doi.org/10.1038/74880

Rand, M. K., Hikosaka, O., Miyachi, S., Lu, X., Nakamura, K., Kitaguchi, K., & Shimo, Y. (2000). Characteristics of sequential movements during early learning period in monkeys. *Experimental Brain Research*, *131*(3), 293–304.

Sakai, K., Hikosaka, O., Miyauchi, S., Takino, R., Sasaki, Y., & Pütz, B. (1998). Transition of brain activation from frontal to parietal areas in visuomotor sequence learning. *The Journal of Neuroscience*, *18*(5), 1827–40.

Schmid, A., Rees, G., Frith, C., & Barnes, G. (2001). An fMRI study of anticipation and learning of smooth pursuit eye movements in humans. *Neuroreport*, *12*(7), 1409–14.

Seidler, R. D., Purushotham, A., Kim, S.-G., Ugurbil, K., Willingham, D., & Ashe, J. (2005). Neural correlates of encoding and expression in implicit sequence learning.

Experimental Brain Research, *165*(1), 114–124. https://doi.org/10.1007/s00221-005-2284-z

Shi, D., Friedman, H. R., & Bruce, C. J. (1998). Deficits in smooth-pursuit eye movements after muscimol inactivation within the primate's frontal eye field. *Journal of Neurophysiology*, *80*(1), 458–464.

Toni, I., Krams, M., Turner, R., & Passingham, R. E. (1998). The time course of changes during motor sequence learning: a whole-brain fMRI study. *NeuroImage*, *8*(1), 50–61. https://doi.org/10.1006/nimg.1998.0349

Wells, S. G., & Barnes, G. R. (1999). Predictive smooth pursuit eye movements during identification of moving acuity targets. *Vision Research*, *39*(16), 2767–2775.

Wu, T., Kansaku, K., & Hallett, M. (2004). How self-initiated memorized movements become automatic: a functional MRI study. *Journal of Neurophysiology*, *91*(4), 1690–1698. https://doi.org/10.1152/jn.01052.2003