Tides of Change: Identifying the Neural Correlates of Motor Sequence Learning

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Abstract

Tides of Change: Identifying the Neural Correlates of Motor Sequence Learning

Christopher J. Steele

The present study combines behavioural and neuroimaging techniques to investigate the learning of a motor sequence task within- and across-day. The goals of this study were to identify behavioural changes during learning, determine the patterns of activity associated with learning, and investigate the relationships between behaviour and brain activity over the course of learning. Participants were trained on a relatively complex motor sequencing task and a simple baseline task. They performed these tasks in an fMRI scanner while behavioural and functional data were collected. Behavioural performance increased within, but not across, day. The initial learning network included activity in CB cortex, posterior BG, PMC, PL, and preSMA/SMA. Within-day changes included increased activity in anterior BG, CB nucleus, and STS; with decreased activity in M1/S1, SMA, and planum temporale. Across-day increases were found in the left anterior BG, no decreases were found across-day. These results may be interpreted as a shift in activity from the visual to the spatial corticostriatal loop, and are discussed within the context of two current theories of motor sequence learning.

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Introduction

Motor skill learning is an important, though often unrecognised, aspect of our lives. We are born able to perform a limited set of motor skills but, given time and practice, each of us develops an extensive repertoire for interacting with our environment. We are not born with the skill to hold a spoon, ride a bike, swing a bat, or play the piano – we acquire these skills through practice. Motor skill learning is an internal process that is inferred from improvements seen in performance on a particular task following a period of practice (Willingham, 1998). The last ten years have witnessed an increased interest in establishing and describing the neural bases of motor skill learning. A wide number of tasks, paradigms, and behavioural and imaging modalities have been used to study motor learning. In an effort to synthesise the results of various experimental paradigms in the area of motor sequence learning, at least two different models have been proposed. The first focuses on the stages involved in motor skill learning (Doyon & Benali, 2005) while the second puts more emphasis on specific processes involved in sequence learning (Hikosaka et al., 2002). Each model makes different predictions for the brain areas involved in motor sequence learning within and across days of learning. Though there is considerable support for each of these models, there has been no single study that has characterised the learning and expression of a motor sequence over two consecutive days of practice. Building upon previous work in our laboratory, the current study characterises the shifts in brain activity occurring within and across two days of practice.

Motor skill learning can be defined as the process that mediates the transition

from effortful to effortless movement with practice (Willingham, 1998). It can be broken down into at least three behaviourally relevant stages: early learning, late learning, and consolidation (Doyon & Benali, 2005; Korman, Raz, Flash, & Karni, 2003). Early learning is defined as the learning that occurs during a single session of practice, characterised by rapid within-session improvements in performance (e.g., Bapi et al., 2006). Late learning is the slow incremental improvement seen across multiple days of practice (e.g., Karni et al., 1995). The consolidation stage is the process that fixes the motor skill in memory and improves its resistance to interference (Krakauer & Shadmehr, 2006). Consolidation is commonly operationalised as either an observable increase in performance after a period of rest (over and above what would be expected with continued practice) or a maintenance in performance after a period of rest – both of which are disrupted by learning a similar motor skill within a consolidation window of four to six hours (Robertson, Pascual-Leone, & Miall, 2004). Consolidation has traditionally been detected by testing multiple groups of participants in an ABA interference paradigm and looking for temporally graded retroactive interference. However, consolidation can also be considered (somewhat less strictly) as the across-day transition from early to late learning. For simplicity, most studies of motor skill learning have tended to focus on changes that occur within a single session or day of practice; effectively ignoring two stages of motor learning. Of those studies that do provide more than one day of practice, most are focussed on evaluating aspects of consolidation (e.g., Brashers-Krug, Shadmehr, & Bizzi, 1996; Caithness et al., 2004; Fischer et al., 2005; Shadmehr & Brashers-Krug, 1997; Walker et al., 2003).

Motor skill learning can be studied through many different types of tasks. These tasks can be grouped into two major types: adaptation and sequencing. Motor adaptation tasks involve learning to perform a task while undergoing either kinematic or dynamic transformation while motor sequencing tasks require that participants learn to reproduce a well ordered set of movements. Adaptation tasks probe our ability to learn from and adapt to different environments while sequence tasks force us to form new ordered memories. Motor adaptation tasks include target pointing/reaching within a dynamic force field (e.g., Shadmehr & Brashers-Krug, 1997) and pointing/reaching under visuomotor rotation (e.g., Mattar & Ostry, 2006). Motor sequence tasks include sequential pointing, pinching, and serial reaction time variants with one, four, or eight fingers. In the last ten years there have been a large number of studies identifying the brain areas that mediate the learning and expression of motor skills (see Kelly & Garavan, 2005 for a review). Recent work in our laboratory utilised a temporal motor sequencing task (TMST), which emphasises accurate reproduction of a sequence of temporal intervals, to investigate sequence learning within- and across multiple days of practice (Penhune & Doyon, 2002, 2005; Savion-Lemieux & Penhune, 2005).

As a result of the large number of brain imaging studies investigating the neural correlates of motor skill learning, two major models of motor sequence learning have been proposed. The more recent of the two, championed by Doyon and colleagues, presents theories for motor adaptation and sequence learning (Doyon & Ungerleider, 2002; Doyon & Benali, 2005). Here we consider only the sequence learning aspect of this model. Doyon and Benali (2005) propose that motor sequence skills undergo a

progression through stages as they transform from novel to well learned. In this model, early learning occurs quickly (i.e., there are rapid behavioural improvements) and involves the cerebellum (CB), rostral striatum, motor cortical, prefrontal, and parietal cortical regions. The early learning stage includes a shift in activity from associative to motor regions of both the CB and the striatum. Late learning is thought to occur more slowly and involves the caudal striatum, motor cortical, and parietal cortical regions. This qualitative change from early to late learning is thought to be mediated through the consolidation stage. Crucially, following consolidation, activity in the striatum shifts from rostral to caudal regions and the CB is no longer necessary for the production of skilled motor responses. Thus, the performance of a well learned motor sequence in the late learning stage is represented within the caudal striatum and motor and partietal cortices.

Hikosaka and colleagues' model of motor sequence learning focusses on the interaction of two loop circuits rather than a progression through fixed stages (2002). They propose that motor sequence skills are represented as two sequences, one spatial and one motor. Each type of sequence is represented by a different loop and has different behavioural signatures and neural bases. The spatial sequence, or explicit ordering of the task, requires a high level of attention, is learned quickly, can be identified by rapid improvements in accuracy, and is encoded in the loops between the rostral basal ganglia (BG), prefrontal and parietal cortices, and the lateral CB. The motor sequence, or implicit dynamic movements of the task, requires little attention, is learned more slowly, can be identified through improvements in speed, and is encoded between the loops in the caudal

BG, motor cortex, and medial CB. The two loop circuits communicate from spatial to motor through the supplementary motor area (SMA), preSMA, and premotor cortex (PMC). While learning occurs in both loops simultaneously, the areas involved in representing the spatial sequence and communicating between the two circuits are more important early in learning (when explicit sequence knowledge is used and transformed into the motor sequence via the preSMA/SMA) and less important in later learning (where the areas involved in representing the motor sequence become more dominant). This framework predicts a gradual shift in activity from rostral BG – prefrontal – parietal – lateral CB loop to the caudal BG – motor cortex – medial CB loop as learning progresses (Hikosaka et al., 2002).

The two models presented here predict different patterns of activity for within- and across-day learning of a motor sequence task (see Figure 1). The model described by Doyon and Benali (2005) – the stage model – predicts that early learning will be represented by a network including the rostral striatum, CB cortex, and parietal, motor, and frontal cortices. Later within-day learning will be characterised by a shift from rostral to caudal striatum and an increase in CB nuclei activity, along with maintained activity in the other brain regions present in early learning. After consolidation, across-day learning will be characterised by an increase in activation in the parietal and motor cortical regions and the caudal striatum. The model described by Hikosaka and colleagues (2002) – the process model – predicts a fluid shift in activity within- and across-day. The initial network of brain areas involved in learning will predominantly involve the associative regions of the BG and CB, prefrontal and parietal cortices, and motor cortical regions.

Because the preSMA/SMA/PMC are involved in the transformation from spatial to motor coordinates, these areas will be especially active during early learning. Within-day learning is characterised by an increase in activity in the motor areas of the BG and CB, and the motor cortex. The areas involved in early learning will decrease as learning progresses within-day. Across-day learning is a continuation of this shift in activity to the motor cortex and motor BG and CB regions. Major differences between the two models include the stage model's inclusion of consolidation, emphasis on qualitative shifts, constant contribution of the parietal cortices, absence of the CB from late learning/recall, and lack of hypotheses for the PMC and preSMA/SMA. On the other hand, both models affirm that motor sequence learning is highly dependent on the CB and BG and that shifts in activity from associative to motor areas occur as learning progresses.

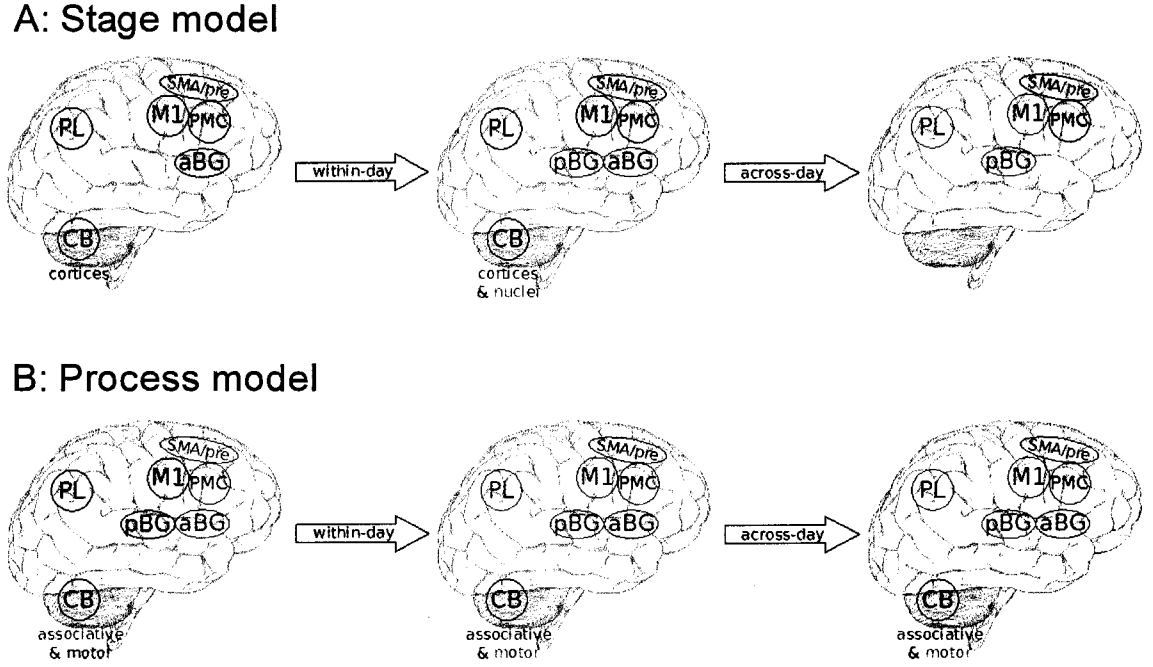

Figure 1. Schematic of two models of motor sequence learning. Each model predicts different patterns of brain activity for early, later within-day, and across-day learning. Panel A depicts the model of Doyon and Benali (2005), Panel B that of Hikosaka et al. (2002). Colour code for brain areas: black – basic network/no change; red – increasing activity; blue – decreasing activity (e.g., the PMC in the process model is predicted to decrease in activity from early to later within-day, and again across-day). Abbreviations: CB – cerebellum; PL – parietal lobe; M1 – primary motor cortex; PMC – premotor cortex; SMA/pre – supplementary motor area and presupplementary motor area; aBG – anterior basal ganglia; pBG – posterior basal ganglia. Appendix A contains the complete list of anatomical names and abbreviations used in this paper.

Though few, if any, studies have assessed the efficacy of both models, varying amounts of support for each can be found in the literature. In an early study using a four-finger sequencing task, Karni et al. (1995) found evidence for a transient decrease in primary motor cortical (M1) activity within-day when participants were naïve to the sequence – attributed to a habituation effect. After three weeks of training, the size of the activation map and cortical activity associated with the trained sequence increased relative to control (Karni et al., 1995). The same pattern of results were seen in a similar study (Karni et al., 1998), and provide support for the increasing role of M1 in later learning. Penhune and Doyon (2002) used the TMST in a PET scanner to assess learning on the first and fifth days of training, and at recall four weeks later. Largely consistent with the stage model, they found that activity in the CB cortices decreased across the three sessions while activity in M1, PMC, and parietal lobe (PL) increased. Activity in the BG and frontal cortices (FC) increased across the first two sessions, but did not change at recall (Penhune & Doyon, 2002). This study also partially supports the process model's role for the frontal and premotor cortices at intermediate stages of learning. A subsequent study by the same group found strong support for the stage theory with within-day decreases in the CB cortices and increases in M1, PL, and BG (Penhune & Doyon, 2005). Interestingly, correlational analyses revealed that improvements in performance associated with increases in M1 and preSMA activity were negatively correlated with activity in the CB cortices and dentate nucleus (DN) – lending further support for the role of the cortical-cerebellar system described by both models of motor sequence learning. Lehericy and colleagues (2005) used a two-finger serial reaction time

task to assess learning-related changes in the BG on days 1, 14, and 28 of practice. Consistent with both models, they found a shift in activity from the associative/premotor (rostrodorsal) to sensorimotor (caudoventral) areas of the BG as learning progressed. In another study with yet another motor sequencing task – learning to vary pinch force in a sequential manner – Floyer-Lea and Matthews investigated within-day and long-term skill learning (2004, 2005). As in the studies by Karni and colleagues (1995, 1998), participants had no knowledge of the sequence prior to scanning. A within-day network characterised by decreases in M1 (ipsilateral), dorsolateral prefrontal cortex (dlPFC), PL, CB cortex, and the caudate nucleus with increases in the thalamus, putamen, and CB DN was found (Floyer-Lea & Matthews, 2004). After three weeks of practice there were significant increases in contralateral M1 and ipsilateral BG – consistent with both the stage and process theories (Floyer-Lea & Matthews, 2005). An experiment designed by Bapi and colleagues (2006) in which participants learned a 2x6 pointing task (a sequence of six sets of two ordered choices on a 3x3 button matrix) and then practised the task in either visually or motorically rotated space strongly supported the process model. They found that learning under the spatially rotated condition was characterised by a shift in activity from the PL to PL-PMC, increases in the caudal BG and preSMA, a decrease in the dlPFC, and maintained activity in the CB and M1. In the motor rotation condition, there was a shift from PL-PMC to primarily PMC, decreases in CB cortex, thalamus, BG, and dlPFC, and maintained activity in M1 and somatosensory (S1) cortex (Bapi et al., 2006). This group's imaging results, in addition to their activity-behaviour correlations, strongly support the interpretation that two different neural processes are involved in the

learning of motor sequence skills. There is a large body of literature supporting each of these models and, due to a considerable amount of overlap in their predictions, quite a number that support both.

Though there have been many studies of motor sequence learning, few studies have investigated within- and across-day changes during the early and late stages of motor sequence learning. In an effort to better understand the neural bases of motor learning and consolidation both within- and across-day, the current experiment utilises a modified version of the TMST (Penhune & Doyon, 2002) to investigate changes in neural activity associated with the learning and performance of a motor sequence task. To isolate and assess learning rather than just performance, two different types of sequences were used: one that was difficult to learn and temporally irregular (LRN); and one that was identical in the number and type of motor movements, but simple to learn and temporally regular (ISO). To observe changes within- and across-day, this study evaluates participants over four runs spanning two fMRI sessions separated by twenty-four hours.

The goals of this experiment are threefold: 1) identify the behavioural changes associated with learning within- and across-day; 2) determine the pattern(s) of activation within- and across-day; and 3) investigate the relationships between behaviour and activity that characterise within- and across-day learning. First, we hypothesised that participants' performance will improve on LRN both within- and across-day while, conversely, there will be no change in performance on ISO within- or across-day. Second, neural activity associated with the learning of LRN within-day will evidence a

gradual decrease in cerebellar cortex involvement with increases in motor cortical, caudal BG, parietal, and cerebellar nuclei involvement. Third, consistent with the stage model, the pattern of change in activity seen within-day will not be preserved across-day – consolidation of the motor sequence will be apparent as a qualitative shift in activity across day. This experiment aims to identify shifts in the network of areas involved in motor sequence learning across two days of practice. It combines both behavioural and functional neuroimaging measures to better understand the direct contributions of the brain areas implicated in motor sequence learning.

Method

Participants

Fifteen participants (5 female) between the ages of 18 and 35 (M = 22.80; SD = 2.91 years) participated in this study. All participants were right handed (assessed using a Handedness Questionnaire adapted from Crovitz and Zener,1962; Appendix B), neurologically normal, and had less than three years of musical experience (assessed using a global Index of Musical Training and Experience, Penhune, 1999; Appendix C). Participants were either recruited from the Montréal community via advertisements posted on the McGill University Classifieds website or via word of mouth. All participants gave informed consent (Appendix D) and completed an MR safety screening form to assess their suitability for study in the MR environment (Appendix E). Participants were asked to refrain from drinking alcohol twenty-four hours prior to each session. The experimental protocol was approved by the McGill University MNH/I Research Ethics Board and the Concordia University Human Research Ethics Committee. At the completion of the study, participants were debriefed and remunerated for their time.

Apparatus

Stimulus delivery and response recordings were controlled by software developed in house using Microsoft Visual C# [by Mr. Alejandro Endo]. Two Dell Inspiron ME051 1.80 GHz (Intel Pentium M) notebook computers were used to record participants' behavioural responses. Behavioural responses were collected via standard PC USB mouse during training and non-MR sessions, and via a custom designed fibre-optic

mouse and signal processing box during MR sessions (Hollinger et al., 2007). The fibre-optic mouse was fashioned from a PC mouse to retain the same feedback characteristics as the PC USB mouse. During training and non-MR sessions, participants were seated 60cm from the notebook computer screen (resolution, 1280x800). While in the MR scanner, participants viewed a projected image by way of a mounted mirror (resolution, 1024x768).

Stimuli

The task used in this experiment was the TMST that has been used in a number of previous behavioural and neuroimaging experiments (Penhune & Doyon, 2002, 2005; Savion-Lemieux & Penhune, 2005). The TMST requires that participants produce a timed sequence of finger taps in synchrony with visual stimuli. The stimuli were sequences of coloured squares (100x100 pixels) presented sequentially in the center of the screen on a neutral grey background. Sequences were made up of short (300ms) and long (600ms) elements with a constant interstimulus interval (ISI) of 300ms (Figure 2, panel A). They were arranged into six trial types – three used for training and three for training and task (Figure 2, panel B). Trial types were: Long (LL: ten long), Short (SS: ten short), Mixed (MM: two long and two short repeated three times), Isochronous baseline (ISO: five long followed by five short), Perceptual baseline (PER: five long followed by five short), and Learn (LRN: a mixture of five long and five short, the TMST sequence of interest). LL, SS, and MM, epochs were used only during training, while ISO, PER, and LRN epochs made up the task conditions. A Rest condition (RST) of 40s of black fixation cross, including the presentation of a condition/epoch cue, was also used

during testing. LRN was created to conform to a temporally regular, though non-standard, musical rhythm – having no more than two repeated elements with seven transitions from short to long. Each trial was preceded by a trial cue (40x40 pixel black square of 500ms duration). Trials were grouped into epochs consisting of four identical trials preceded by an epoch cue prior to the first trial cue. Each trial had a total length of 10s, with 2.5s of cue time and 7.5s of stimuli presentation. The epoch cues were presented within the 2.5s cueing window of the first trial of the epoch, and were the words "Practice", "Attend", "Learn", and "Rest" for ISO, PER, LRN, and RST epochs respectively (Figure 2, panels C and D illustrate trial and epoch arrangement respectively).

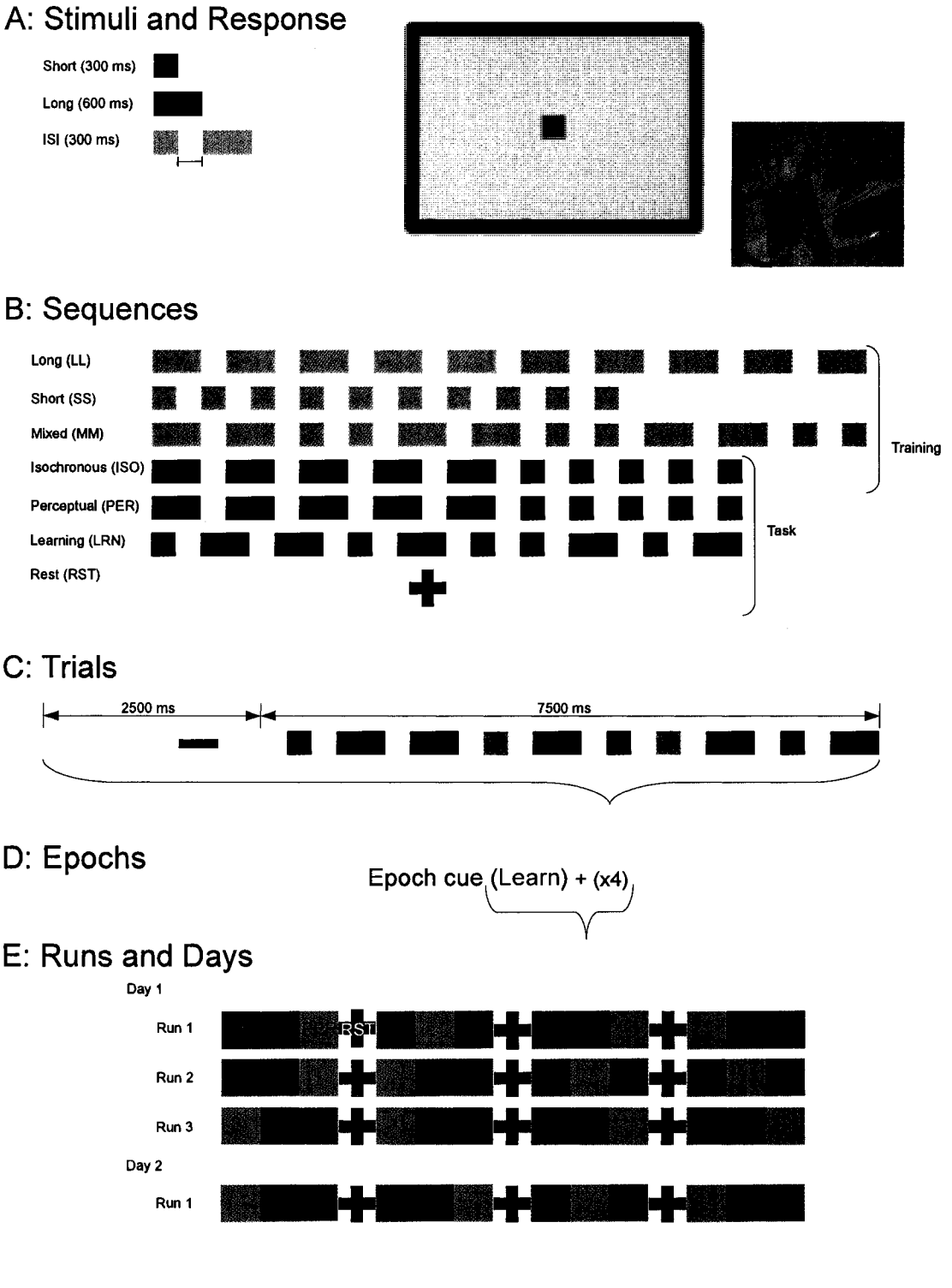

Figure 2. Experimental stimuli, method, and procedure. Panel A: Individual short and

long stimuli elements and constant ISI, schematic computer display, and fMRI compatible mouse. Panel B: The six sequences and rest stimulus. Panel C: Example trial arrangement depicting a single 10s LRN trial, including the trial cue. Panel D: The organisation of epoch cue plus four identical trials into a single epoch. Panel E: Pseudorandom arrangement of epochs in each run and day – each coloured square represents an epoch of trials of the same colour as those depicted in Panel B.

Procedure

Day 1

At the beginning of the first day of testing, participants were familiarised with the general procedure for the day and completed the first day of the Sleep Questionnaire (Appendix F).

Training: Stage I. Participants were seated in front of the computer and placed their right hand on the mouse. Participants were shown an example presentation of the LL practice trial, then asked to "synchronise your mouse-button presses with the onset and offset of the squares for four more trials." This procedure was repeated for the SS, MM, and ISO trials. The M and SD for responses to the long and short elements were calculated on-line, filtered, then used to score both the second stage of training and the behavioural results obtained from the testing sessions.

Training: Stage II. During the second stage of training, participants performed LRN trials until their accuracy reached a criterion of 90% over three consecutive trials. Accuracy was calculated on-line by the computer on a per element basis by comparing the button-press duration to the baseline mean long or short element response from the first stage of training. If the duration fell between two standard deviations above or below the mean (specific to the element type) it was considered correct. Learn trials were presented individually and feedback indicating which part(s) of the trial were correct/not correct was provided when necessary. After reaching criterion, participants were shown examples of the ISO, PER, LRN, and RST conditions. They were instructed to "press the mouse button in synchrony" with Practice (ISO) and Learn (LRN), "watch and

concentrate on the timing" during Attend (PER) and "fixate on the cross" during Rest (RST). Participants were then given an overview of the testing session and prepared for the MR environment.

Testing. Participants were placed in the scanner and a high-resolution anatomical scan was collected. The fibre-optic mouse assembly was then placed at the participants right hand and their arm fixed in a fully extended, though comfortable, position. They were again shown examples of the ISO, PER, LRN, and RST trials and reminded that their primary task was to "press the mouse button in synchrony with Practice, the green squares, and Learn, the blue squares; watch and concentrate on the timing during Attend, the grey squares; and fixate on the cross during Rest." Three runs of functional images were then acquired. Each run was composed of 4 epochs each of LRN, ISO , and PER arranged randomly about three Rest epochs as shown in Figure 2, panel E.

Day 2

The second day of the experiment was a subset of the first. Participants filled out the second day of the Sleep Questionnaire, were reminded of the experimental task, completed the first stage of training (identical to that done the previous day), were presented with examples of all testing sequences, and prepared for the MR environment. A lower-resolution anatomical image was collected, followed by the acquisition of a single functional run.

Image Acquisition

Images were acquired using the Montreal Neurological Institute's Brain Imaging Centre's Siemens 3 Tesla TRIO whole-body Magnetic Resonance Imaging scanner

equipped with an eight-channel head coil. A whole-brain anatomical image of 160 slices giving a $1mm^3$ resolution was acquired (time to repeat - TR, 23ms; time to echo - TE, 7.4ms; field of view - FOV, 256mm; flip angle, 30°; matrix, 256x256). A lower resolution 2x1x1mm anatomical scan was collected on the second day to reduce scan time. Slices were acquired parallel to the Anterior Commissure-Posterior Commisure (AC-PC) line. A total of 265 T2* weighted functional frames were acquired for each run. Functional data was acquired in 36 interleaved slices with an isotropic resolution of 4mm; TR, 2500ms; TE, 30ms; FOV, 256mm; flip angle, 90°; matrix, 64x64. Slices were acquired at an angle of approximately 30° to the AC-PC line. Angulation was adjusted individually, though maintained for each participant across runs and days, to maintain full brain coverage while attempting to reduce possible artifact caused by the eyes, orbital socket, and nasal cavities.

Data Analysis

Behavioural

Many motor sequence learning tasks utilise decreases in reaction time (RT) as the primary measure for the behavioural assessment of learning. However, the TMST emphasises the synchronisation of key-presses with the onset and offset of the stimulus such that decreases in RT will not necessarily correspond to improvements in performance. As such, learning was assessed by changes in accuracy and asynchrony. Accuracy reflects the more explicit component of the task – learning the correct order of long and short elements that make up the sequence; while asynchrony is a measure of the more implicit timing components of the task – learning the precise timing of key-press

and release relative to the visual stimuli. The first stage of each participant's daily training was used to calculate these measures. Each participant's mean (M) and standard deviation (SD) for the long and short elements was first calculated, then all response durations that fell between M +/- 2SD were used to calculate the baseline mean and standard deviation for each element type. This baseline mean and standard deviation were then used in the second filter described below.

A custom-designed scoring program was developed to calculate accuracy and asynchrony. Accuracy was defined as the percentage of key-press responses that passed two criteria: 1) the key-press was initiated between 300ms before the stimulus and the end of the stimulus and 2) the duration of the key-press was less than M +2SD (for short elements) or greater than M -2SD (for long elements). The first criterion allowed the inclusion of anticipatory responses and the second ensured that if participants polarised the durations of short and long elements during the task, their responses would still be scored correctly. Only correct responses were used in the calculation of asynchrony and all scores were calculated on a per-element basis. Asynchrony was defined as the ratio of actual stimulus duration to the sum of the absolute lag between stimulus on and key-press on, and stimulus off and key-press release. Accuracy and asynchrony measures were calculated for each trial of both LRN and ISO, averaged across runs, and converted into percentages. Differences across runs were assessed using repeated-measures analysis of variance (ANOVA) with Greenhouse-Geisser correction. Performance changes over the three runs of day 1 were assessed to determine within-day learning; performance changes over the last run of day 1 and the run on day 2 were assessed to determine the effects of

across-day learning; differences between LRN and ISO were assessed both within- and across-day to assess overall differences between the two sequences. Significant differences were analysed using tests of simple main effects, Bonferroni corrected for multiple comparisons ($p < .05$).

Imaging

The last eleven frames of functional data were discarded as they corresponded to frames acquired after the completion of the experiment. Functional runs were realigned to the third frame of the series, motion corrected, and smoothed with an 8mm full-width half-maximum isotropic Gaussian kernel with fmr_preprocess (available with the MINC software package at: http://www.bic.mni.mcgill.ca/software/). Each participant's daily anatomical and functional images were transformed into common Talairach and Tournoux stereotaxic space with an affine transformation to the ICBM 152 template (Collins, Neelin, Peters, & Evans, 1994). Statistical analyses were conducted using the General Linear Model as instantiated in fMRISTAT, with the timecourse of each condition (PER, ISO, LRN) used as predictors of Blood Oxygen Level Dependent (BOLD) signal change (Worsley et al., 2002; available at www.math.mcgill.ca/keith/fmristat/). PER, ISO, and LRN served as the active conditions in the contrasts, while RST was used as the baseline for all conditions. PER was included as a predictor in the model, but not included in the analyses as it was not considered a measure of interest. Individual statistical maps of each condition and contrast of interest were calculated, then combined across participants using a mixed-effects model. The significance threshold was set at $p < .05$, after correcting for multiple comparisons with

the whole brain as the search space (Worsely, 2005; Worsley et al., 2002). Locations of peak activity were identified using atlases and/or previously established criterion (Chiavaras et al., 2001; Dimitrova et al., 2002; Mayka et al., 2006; Picard & Strick, 2001; Rademacher et al., 2001; Schmahmann et al., 2000; Talairach & Tournoux, 1988).

Initial network. The initial network of brain areas involved in the learning of the TMST was identified by contrasting LRN with ISO. LRN and ISO were designed to be identical except for the arrangement of the long and short elements within the sequence; therefore, the LRN-ISO contrast represents the brain activity in LRN that is unique to the sequencing or complex timing of the sequence.

Performance-related changes. A covariation analysis was conducted to identify the changes in BOLD response directly related to changes in percent asynchrony (PASY). PASY was chosen for its greater sensitivity to change over the course of the experiment. This allowed the identification of voxels where activity changed with changes in performance. Each participant's effect maps of LRN were correlated with their respective PASY scores on each run to produce covariation maps within- (runs 1,2,3) and across-day (runs 3,4). In the resulting map, positive t-values represent voxels whose activity increases with improvements in performance and negative t-values represent voxels whose activity decreases with improvements in performance.

Results

Behavioural

As no sex differences were found, all results and analyses were collapsed across sex. All participants reached the 90% criterion level for performance on the TMST sequence (LRN) within the second stage of training. On average, performance reached criterion after 41 response trials (SD = 24).

Within-day

Overall, participants' performance on LRN was poorer than on ISO across the three runs of practice. This was true for both the accuracy, as percent correct (PCOR) (PCOR, $F(1,28) = 10.56$, $p < .01$, $\eta^2 = .43$) and percent asynchrony measures of performance (PASY, $F(1,28) = 6.46$, $p < .05$, $\eta^2 = .32$). Performance on ISO for both measures did not change across runs, while performance on LRN improved. Accuracy increased across practice (PCOR, $F(2,28) = 3.77$, $p < .05$, $\eta^2 = .27$) and PASY decreased (PASY, $F(2, 28) = 8.99$, $p < .01$, $\eta^2 = .39$). Planned pairwise comparisons of PASY across the three runs revealed a significant difference between runs 1-2 and 1-3 such that run 1 was greater than both runs 2 and 3 ($p < .05$), indicating that participants' responses became more synchronised with the stimuli across runs. Figures 3 and 4 represent the percent correct (PCOR) and percent asynchrony (PASY) measures respectively.

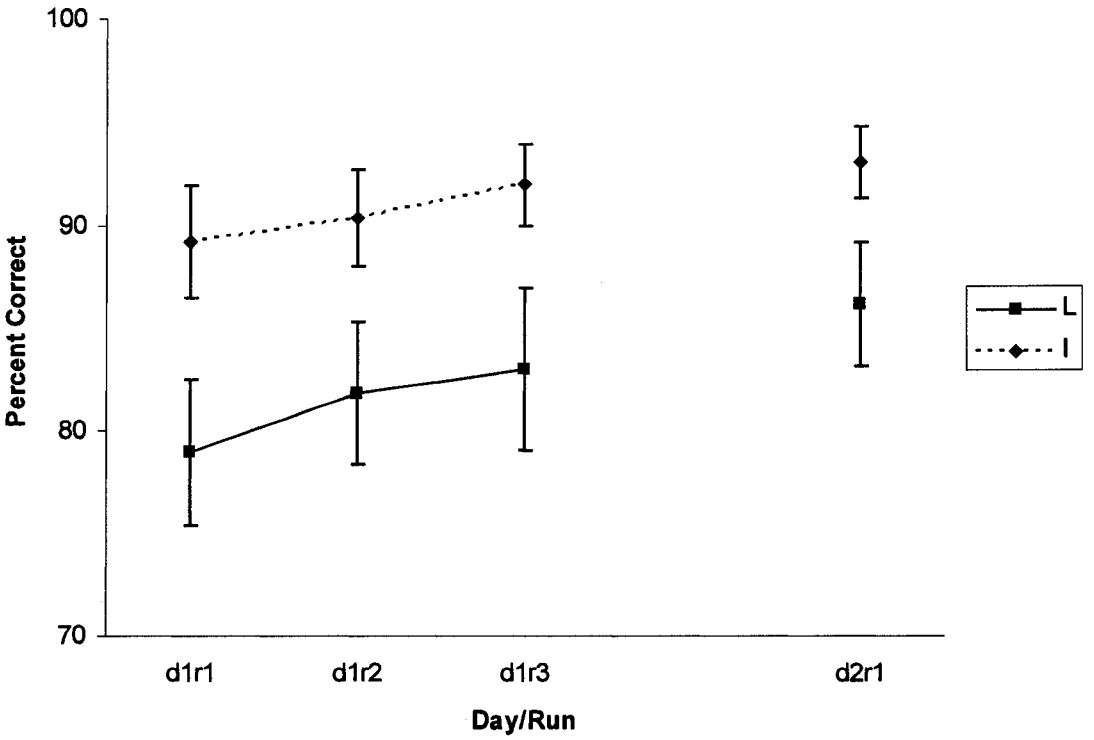

Figure 3. Percent correct on LRN and ISO over the four runs. There was a main effect of run for LRN within-day, with a trend towards improvement in percent correct, but no such effect in ISO. Participants showed significantly better within- and across-day performance on ISO than LRN, on average, across runs.

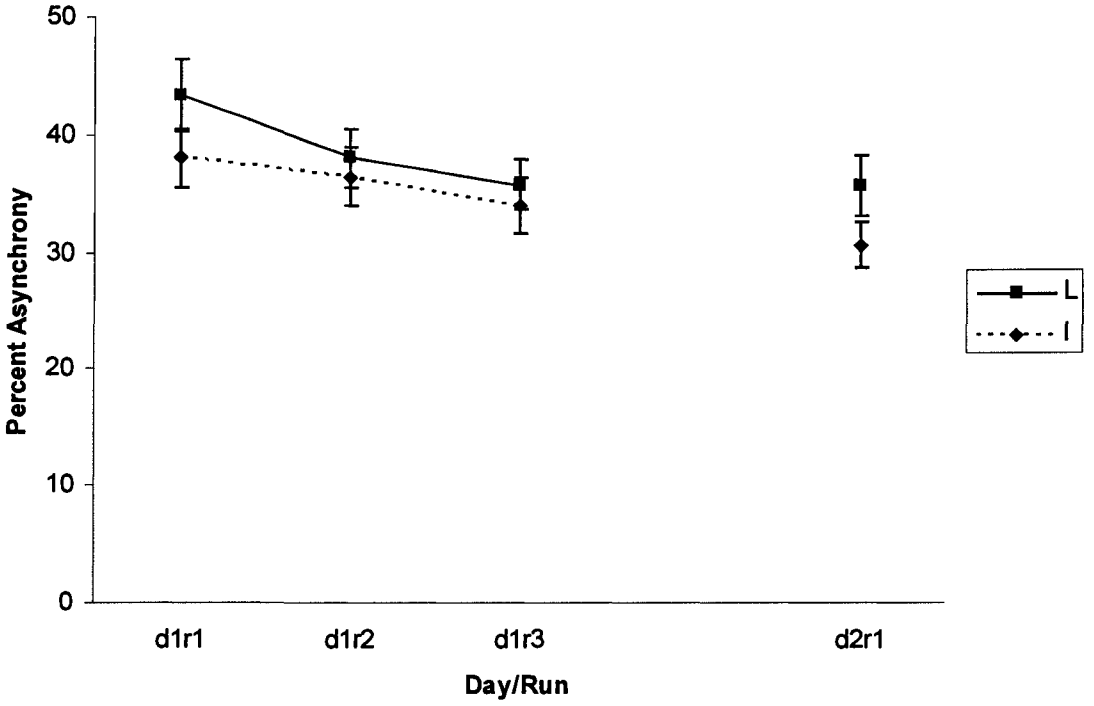

Figure 4. Percent asynchrony on LRN and ISO over the four runs. Participants' performance significantly improved on LRN within-day, with planned pairwise comparisons indicating statistical differences between runs 1-2 and 1-3. There was no significant effect of run for ISO. Participants showed significantly better within- and across-day performance on ISO than LRN, on average, across runs.

Across-day

Performance on LRN was poorer than that of ISO across runs 3 and 4 of the across-day analysis. This was true for both measures (PCOR, $F(1,14) = 7.17$, $p < .05$, $\eta^2 = .34$; PASY, $F(1,14) = 5.45$, $p < .05$, $\eta^2 = .28$). There were no changes in LRN or ISO performance across-day.

Imaging

Initial network

The contrast of the LRN and ISO conditions on the first run of the first day revealed the initial network of brain regions involved in early learning of the TMST. As LRN was the condition of interest, areas that showed increases in activity relative to the ISO baseline condition are reported. Results show significant activation in the medial and bilateral CB cortex (VI/Cr I and X laterally; IV, V, VII/VIII medially), parietal lobe, thalamus, and preSMA/SMA. This is in agreement with the pattern of results found in a previous PET study using the same task (Penhune & Doyon, 2005). In addition, the present results showed significantly greater activity in the body and tail of the caudate nucleus bilaterally. Representative peaks are shown in Figure 5 and listed in Table 1 (the complete list can be found in Appendix G). To identify qualitative shifts in the initial network of learning across-day, the LRN contrast of run 4 was contrasted with that of run 3. No significant differences were found.

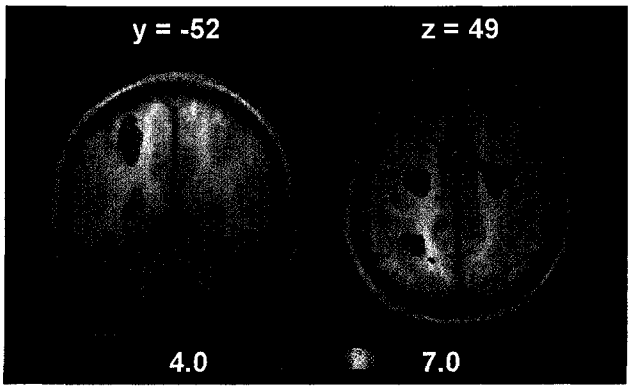

Figure 5. The early learning network. LRN-ISO Contrast depicting the basic network of activity on day 1, run 1. Images are t-maps representing voxels that showed significantly greater activity in the LRN condition than the ISO condition. The image on the left presents an illustrative view of cerebellar activations. Bilateral VI/CR I & medial IV are significantly active while bilateral VIIIa are just below threshold. The image on the right shows the significant activations in the preSMA/SMA, bilateral PMC, and left sPL.

Table 1

Select Locations of Significant Peaks for the LRN vs. ISO Contrast on day 1, run 1

	x	y	z	t-value
L PMC	-24	-14	56	6.19
R PMC	24	-8	48	5.99
Medial preSMA/SMA	2	4	48	6.66
L BG Caudate nucleus (body)	-16	-16	20	5.35
L BG Caudate nucleus (tail)	-18	-28	16	5.36
	-20	-30	16	5.42
R BG Caudate nucleus (body)	16	-8	24	4.75
R BG Caudate nucleus (tail)	20	-28	18	5.23
Thalamus	4	-18	18	6.29
R CB X	26	-38	-46	5.67
L CB VI/Cr I	-38	-56	-30	5.87
R CB VI/Cr I	36	-52	-28	5.74
Medial CB IV	2	-54	-4	5.08
Medial CB VI/VII	0	-60	-22	5.59
Medial CB V	2	-64	-10	5.42
L Superior parietal lobule	-28	-50	52	5.77
L Inferior parietal lobule	-30	-34	40	4.71
R Inferior parietal lobule	42	-34	38	4.93
L Insula	38	16	4	5.71
R Insula	-32	16	2	5.46

Note. See Appendix G for the complete listing of significant peak coordinates and t-values. R – right hemisphere; L – left hemisphere.

Performance-related changes

Within-day. Covariation of PASY with BOLD response provides a direct picture of which brain areas are involved in the learning of the TMST. Within-day results showed that better task performance was correlated with greater activity in three motor areas: left CB V, left CB interposed nucleus, and bilateral head of the caudate nucleus (Figure 6). Significant increases were also found in the right iPL, PMC, and occipito-parietal boundary. Areas in which activity decreased with improvements in performance included the left M1, S1, preSMA/SMA, and right M1/dPMC. In addition, there were significant decreases in the right sPL and bilateral occipital poles and planum temporale (Figure 7). Table 2 lists significant peaks and their associated t-values for the within-day covariation analysis.

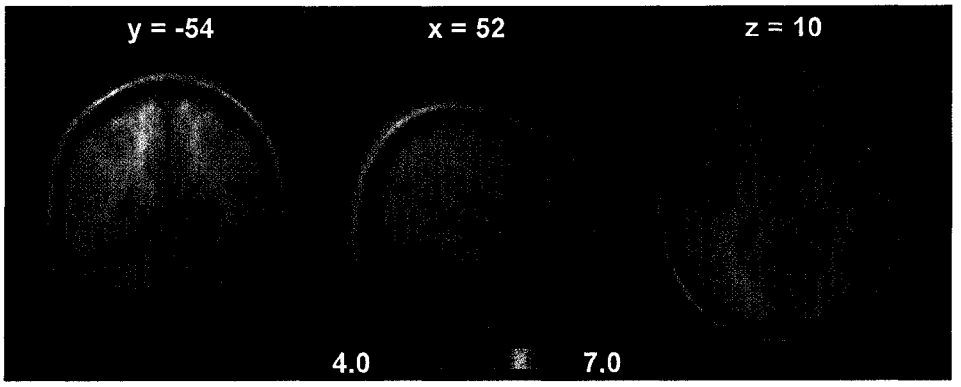

Figure 6. Within-day increases. Areas positively correlated with performance improvements Within-day. Images are t-maps representing voxels where increases in activity correlated significantly with increases in performance. From left to right, the coronal slice shows the left lateralised significant correlation in CB V and interposed nucleus; the saggital slice shows auditory association cortex (BA 22); and the axial slice shows bilateral head of the caudate nucleus.

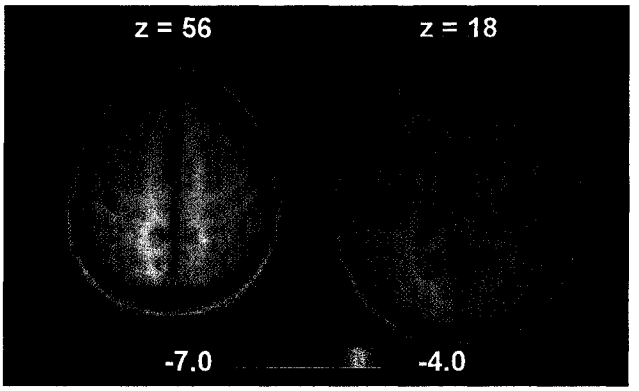

Figure 7. Within-day decreases. Voxels negatively correlated with performance improvements within-day. Images are t-maps representing voxels where decreases in activity correlated significantly with increases in performance. The image on the left shows the significant negative correlation between performance and BOLD signal in the left M1/S1, SMA, and right dPMC. That on the right shows activity in the right and left planum temporale.

Table 2

Locations of Significant Peaks of the Within-day Covariation of LRN with PASY

	x	y	z	t-value
Increasing with Improving Performance				
R PMC (BA 6/8)	24	18	34	4.85
L BG Caudate nucleus (head)	-12	12	8	7.00
R BG Caudate nucleus (head)	10	10	12	5.79
	12	12	10	5.70
L CB V	-22	-54	-16	6.59
L CB Interposed nucleus	-8	-54	-32	5.37
R Inferior parietal lobule	40	-52	20	5.36
	42	-48	18	4.89
R Superior temporal sulcus (BA 22)	52	-20	0	5.27
	50	-22	-2	5.19
R Occipito-parietal	54	-62	20	4.83
Decreasing with Improving Performance				
L M1/S1	-38	-20	54	6.37
	-40	-16	50	6.20
	-38	-18	50	6.12
	-38	-20	48	6.05
	-22	-28	68	5.41
R M1/Dorsal PMC	48	-10	52	4.76
L SMA	-4	-6	54	4.76
R Superior parietal lobule	60	-32	48	4.75
L TL Planum temporale	-64	-28	16	6.50
	-62	-28	14	6.48
R TL Planum temporale	70	-24	16	6.92
	68	-22	18	6.43
	72	-24	14	6.41
L Posterior fusiform (BA 18/19)	-24	-74	-16	4.95
	-26	-72	-16	4.90
L Occipital lobe	-22	-104	2	5.14
R Occipital lobe	22	-102	-6	6.55

Note. R – right hemisphere; L – left hemisphere.

Across-day. The across-day covariation with PASY revealed only a single area of significance, activity in the head of the left caudate nucleus increased as performance improved (Table 3). However, it should be noted that activity in the right caudate nucleus was present at levels just below the threshold for significance. As LRN was the only condition used during these analyses, the results directly reflect brain areas that increased or decreased in activity with variations in performance on the TMST.

Table 3

Locations of Significant Peaks of the Across-day Covariation of LRN with PASY

	x	y	z	t-value
Increasing with Improving Performance				
L BG Caudate nucleus (head)	-6	8	10	4.84
	-4	6	10	4.81
	-6	10	8	4.80
	-8	12	6	4.78
Decreasing with Improving Performance				
<none>				

Note. R – right hemisphere; L – left hemisphere.

Discussion

The current study utilised a combined behavioural and fMRI approach to investigate the within- and across-day changes in brain activity during motor sequence learning. Behavioural measures indicated that participants were able to learn the TMST and showed improvements in performance within-day. The LRN-ISO contrast of the first run of the first day revealed the initial network of brain areas recruited for the TMST. Covarying behaviour with BOLD signal identified the brain areas that vary with learning. Taken together, these results present an intriguing glimpse into the neural changes associated with learning a motor sequence across two days of practice.

Behavioural changes

The first goal of this study was to identify the behavioural changes that characterise within- and across-day sequence learning. We collected two behavioural measures of learning, accuracy and asynchrony, during two different conditions, LRN and ISO. Accuracy was operationalised as the percentage of correct key-presses in the sequence, synchrony as the percentage difference between the response and stimulus. As hypothesised, participant responses on ISO (the simple sequence) were both more accurate and better synchronised than those on LRN (the complex TMST sequence). There was also an improvement in both measures on LRN within-day, but no such improvement on ISO. The across-day behavioural analysis found no evidence for increases in performance that would be consistent with previous behavioural measures of consolidation. However, there was also no significant decrease in performance across-day, implying that the learning acquired on day 1 was retained for expression on day 2.

Though not satisfying the strict definition of consolidation, it is apparent that motor memory was acquired and stored across-day. In addition, by the end of the study, performance on LRN had still not reached that of ISO – indicating that there was still room for continued learning. This finding is interesting, especially given the fact that previous studies using the same task found higher initial accuracy and a greater rate of within-day gain on the TMST (Penhune & Doyon, 2002, 2005). There is one methodological difference that may help to explain these discrepancies: previous TMST studies presented conditions in long continuous blocks. The pseudorandom presentation of conditions in 40s epochs in the current experiment is likely to be the cause of the depressed learning curves, and may be a clue as to why we did not find behavioural improvement across-day. This idea is supported by research showing that within-day performance on two learning tasks is greater when presented in blocks of practice rather than randomly (e.g., Shea & Morgan, 1979). The current design randomly interleaves LRN, ISO, and PER epochs about rest epochs, thus creating a random practice design. Participants may not have had enough interference-free practice on the TMST to be able to show an increase in performance across-day.

Learning-related changes in brain activity

The second and third goals of this experiment were to identify the patterns of brain activity that characterise motor sequence learning within- and across-day, and to investigate the relationship between brain and behaviour. These goals were attained by combining analyses of brain activity on the first run of the first day with behavioural covariation analyses conducted within- and across-day. The initial LRN-ISO contrast

allows us to describe the basic network of brain areas involved in early learning. It therefore represents the relative starting point for changes described by the covariation analyses. The within-day covariation analysis identified brain areas that became more or less active as the skill was learned, and thus represent within-day learning-related shifts in activity. Covariation across-day identified the brain areas most important for skill learning across-day. It is important to keep in mind that the results of these covariation analyses represent brain areas that increase/decrease with learning – and that they cannot provide information about areas that either do not change, or change in ways that are not correlated with the behavioural measure. For this reason, the early learning contrast serves as a starting point for the interpretation of the covariation analyses and, in combination, these analyses provide a comprehensive picture of the shifts in brain activity related to motor sequence learning across two days of practice.

In the initial run on day 1, we compared BOLD activity during LRN to that of ISO to determine the basic network of brain areas involved in early learning. Since ISO only differed from LRN on the arrangement of sequence elements, the resulting network of activity represents those areas that contribute to the learning of the TMST over and above the simple sequence. Regions showing greater BOLD activity during early learning included the preSMA/SMA, PMC, PL, BG, thalamus, and CB cortex. The preSMA has been implicated in sequence initiation/chunking with memory retrieval (Kennerley, Sakai, & Rushworth, 2004), visuo-motor association (Sakai et al., 1999), and more recently, response conflict (Nachev et al., 2007). The PMC and PL are thought to provide a form of stimulus-response association with the PL being responsible for transforming

from sensory to motor coordinates and the PMC, with direct ties to M1, in preparing the motor response tied to a particular stimulus. The ventral PMC (vPMC) may be involved in direct visuomotor transformations and the dorsal PMC (dPMC) in indirect visuomotor transformations (Hoshi & Tanji, 2006). In this view, the vPMC directly matches properties of the target stimulus with the appropriate motor response while the dPMC is involved in higher-order aspects of motor response such as coordinating and timing (Davare et al., 2006). In the TMST, the vPMC could be involved in the selection of either long or short key-press responses while the dPMC may be helping to organise long and short responses into the appropriate sequence. The body and tail of the caudate nucleus, part of the BG's input nuclei, were also found to be active during early learning. The tail of the caudate nucleus functions as part of the 'visual' corticostriatal loop and is thought to integrate visual sensory input into environmentally relevant motor output (Lawrence et al., 1998; Seger, 2006). The activity seen here during early learning could represent the association of new visual categories with the complex temporal sequence. Interestingly, activity in the caudate nucleus during early learning was not seen in another functional imaging study of the TMST, perhaps due to the methodological differences cited above or the relatively poor spatial resolution of PET (Penhune & Doyon, 2005). The location of cerebellar cortical activity identified in the present study is consistent with that found in other motor learning studies and reflects areas that have anatomical connections with M1 and PFC in monkeys (Kelly & Strick, 2003). Cerebellar activation during early learning is consistent with its role as a feedback controller for the correction of error, and is well supported in the literature (Doyon & Benali, 2005).

Within-day learning-related changes were identified by correlating PASY with BOLD signal change across runs 1, 2, and 3. This analysis is specifically designed to highlight brain areas that directly contribute to the increases and decreases seen in individuals' performance. Thus allowing specific conclusion about brain-behaviour relationships to be made. Within-day learning was characterised by activity within more specific areas than those involved in early learning. Regions showing increases included head of the caudate nucleus, PMC, CB cortex and output nucleus, and BA 22. The shift in activity from the CB cortices to include the CB nuclei has been well supported in the literature and may be related to changes in connectivity and synaptic strength which occurs with practice and the formation of motor memory (e.g., Kleim et al, 2004).

Brain areas that showed decreasing activity with improving performance included M1/S1, M1/dPMC, SMA, planum temporale, and BA 18/19. Though there is a considerable amount of evidence indicating that activity in the primary motor cortex increases with motor sequence learning, some studies have also shown decreases in activity. Two PET studies by Karni and others (1995, 1998) detected decreases in M1 activity in the first session of learning on a novel motor sequence. However, after two or three weeks of practice the well learned sequence was represented by enhanced activity in M1. Floyer-Lea and Matthews (2004) also found decreases in M1 activity, though ipsilateral, during novel sequence learning and Doyon, Song, Karni, Lalonde Adams, and Ungerleider (2002) found no changes in M1 activity during learning. The present experiment is similar to these studies in that it may represent very early (novel) sequence learning. Combined with the pseudorandom presentation of conditions, learning appears

to have been significantly retarded. We expect that continued practice and subsequent functional imaging scans would reveal enhanced M1 activity on this task. Across-day learning was characterised by an increase in activity in the head of the caudate nucleus. The head of the caudate nucleus is part of the 'spatial' corticostriatal loop described by Lawrence and colleagues (1998) and thought to have a role in encoding the spatial aspects of a motor task (Hikosaka et al., 2002). Though the TMST may not contain an immediately obvious set of spatial movements, it is apparent that spatial sensory information must still be processed and transformed into motor commands in order to perform the task. Participants must still be aware of where their hand/finger is in relation to the mouse button to be able to plan to press or release the button. Improvements in this awareness and positioning in the midst of the TMST sequence is likely responsible for the between day increase in activity observed in the head of the caudate nucleus. There were no other significant increases or decreases across-day.

There were also two auditory brain areas that showed significant shifts in activation throughout the learning of the TMST. Increases in the STS were paralleled by decreases in the planum temporale – indicating a shift from auditory association areas to those involved in multimodal processing. This shift is an indication that participants may be recoding the visually presented stimuli into an auditory sequence; an interpretation that is consistent with participants' reports of 'playing' or 'hearing' the sequence in their heads as they performed the task.

The shifts in brain activity seen within- and across-day in the present experiment, as illustrated in Figure 8, are not completely consistent with the stage and process models

that have been discussed. M1, PL, and SMA activity decreased within-day and showed no change across-day. Lobule V and the interposed nucleus of the CB, and the PMC, increased within-day and maintained their activity across-day. The caudate nucleus of the BG showed early activity in the body and tail followed by increases in activity in the head within- and across-day. Activity in the preSMA did not change over the course of the experiment. These patterns of activity appear significantly different from those described for the stage and process models of motor sequence learning (Figure 1). However, it is possible that the network of learning presented here represents an earlier stage than that described by the models. Indeed, there are four lines of evidence that contribute to this interpretation: 1) behaviourally, participants had not reached ceiling and were performing at lower levels than in previous experiments; 2) activity in M1 decreased within-day; 3) activity in the body and tail of the caudate nucleus was present at early learning; and 4) activity in the head of the caudate nucleus was found after early learning. The first piece of evidence implies that our procedure made the task significantly harder to learn than in previous studies. That we have, in effect, slowed down the learning process. This interpretation is further supported by the decrease in M1 activity seen within-day in this experiment; an effect previously only associated with novel sequence learning. The presence in early learning of the body and tail of the caudate nucleus suggests that the visual corticostriatal loop is participating in improved performance. If we consider that the identification of visual stimuli and association with relevant responses is a necessary precursor for the planning and executing a motor sequence, then it follows that the visual learning system should be active. The visual

corticostriatal loop, with its role in associating visual stimuli with relevant motor responses (Seger, 2006), is therefore a necessary actor in the early learning of visually-presented motor tasks. Interestingly, positing that our first day's results provide a window into an earlier stage of motor learning can lead us to a number of predictions. As we have hypothesised that our study has captured an earlier period of learning than has previously been reported, we expect that continued learning on the TMST would result in shifts in activity consistent with the early learning networks of the stage and process models. The fourth line of evidence, the presence of activity in the head of the caudate nucleus, is consistent with the early learning network described by both the stage and process models. A second prediction is that, in accordance with the two models, M1 activity will increase as participants continue to learn the TMST. Thus, the within- and across-day network of brain areas identified in the current study appear to precede and overlap the motor sequence learning models described by Doyon and Benali (2005) and Hikosaka (2002).

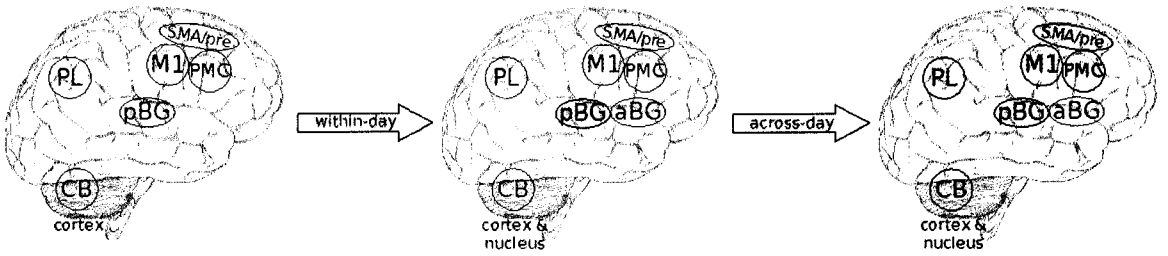

Figure 8. Schematic of within- and across-day patterns of motor-related brain activity found in the current study. Early learning was identified by the LRN-ISO contrast, later within-day and across-day learning-related changes by covariation analyses. Colour code for brain areas: black – basic network/no change; red – increasing activity; blue – decreasing activity

Our ability to perform motor sequencing tasks is an oft undervalued aspect of our daily lives. It is difficult to imagine one facet of our interaction with our environment that does not involve, at least partly, a sequential motor event. From holding a spoon or riding a bike to voicing the syllables of the word 'sequencing', we are continuously organising discrete motor events in time. Understanding the behavioural and neural foundations of motor sequence learning is; therefore, a compelling area of research. The current study expands upon our knowledge of within- and across-day motor sequence learning by suggesting that there is a behaviourally and neurally distinct period of learning preceding 'early learning'. And that this period is characterised by a distinct network of activity that, with continued learning, shifts to areas previously defined as 'early learning'. Though this study is a step in the right direction, much future research is required to clarify the complex interactions between brain areas involved in the process of motor learning.

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Appendix A

Anatomical Names and their Abbreviations

Abbreviation	Full Name
BG	basal ganglia
aBG	anterior basal ganglia
pBG	posterior basal ganglia
CB	cerebellum
I-VIII, X	lobule I-VIII, X
Cr I	crusate I
DN	dentate nucleus
FC	frontal cortex
M1	primary motor cortex
PFC	prefrontal cortex
dlPFC	dorsolateral prefrontal cortex
PL	parietal lobe
iPL	inferior parietal lobule
sPL	superior parietal lobule
PMC	premotor cortex
dPMC	dorsal premotor cortex
vPMC	ventral premotor cortex
S1	primary somatosensory cortex
SMA	supplementary motor area
SMA/pre	SMA and preSMA
preSMA	presupplementary motor area
STS	superior temporal sulcus

Appendix B

Handedness Questionnaire

ID #: _____

Handedness Questionnaire

Stated Hand Preference

Ra - right hand always (1/5)
Rm - right hand most of the time (2/4)
E - both hand equally often (3)
Lm - left hand most of the time (4/2)
La - left hand always (5/1)
X - do not know which hand (0)

Which hand do you normally use to:	**1-5**	**5-1**
1. hold scissors when cutting	_____	
2. throw a ball	_____	
3. hold a slice of bread when buttering		_____
4. hold a watch when winding it		_____
5. hold a drinking glass when drinking	_____	
6. hold a needle when threading		_____
7. hold a dish when wiping		_____
8. insert a key into a lock	_____	
9. hold a pencil when writing	_____	
10. hold a comb when combing hair	_____	
11. hold a bottle when removing cap		_____
12. hold a potato when peeling		_____
13. hold a tooth brush when brushing teeth	_____	
14. dial a telephone number	_____	
15. hold a pitcher when pouring out of it	_____	
16. turn on a water faucet	_____	
17. hold a loaf of bread when cutting with a knife		_____
18. hold nail when hammering		_____
Total :	_____	_____

Appendix C

Index of Musical Training

ID #: _____

Interviewer: _____ **Date (day/month/year):** _____

Time: _____

SCREENING FORM

I am going to ask you some questions about yourself. This information will help us learn more about the people participating in our research project. Like all the information in our study, the information on this questionnaire will be kept *strictly confidential.*

1) Musical Training/ Experience

Have you ever played a musical instrument (including voice/dance)?
☐ **YES** ☐ **NO**

(The following questions are letter coded with respect to the first question, e.g. years of playing for instrument "a", instrument "b", etc.)

If yes, which instrument(s) (including voice) in order of concentration:
a) _____, b) _____

How old were you when you first started playing/singing/dancing?
a) _____, b) _____

How did you learn to play/sing/dance?
a) _____, b) _____

For how many years did you play/sing/dance?

0-3 yrs:	a) ☐	b) ☐
4-8 yrs:	a) ☐	b) ☐
9-13 yrs:	a) ☐	b) ☐
14 + yrs:	a) ☐	b) ☐

ADD YRS: _____

If stopped, at what age did you stop?
a) _____, b) _____

Are you currently practicing? a) YES ☐ NO ☐ b) YES ☐ NO ☐

(What is important here is that the person is excluded if they are currently practicing a musical instrument or have had more than three years of musical experience)

- **If they are currently practicing or have 3+ years musical experience then again tell them that for this study we aren't using people with music experience because we know that they perform better than people without musical experience on this task...perhaps they would like to come in for another study**

2. Contact information

Telephone numbers:
Home: _____
Cell: _____
Work: _____
Other: _____

Most convenient time for us to call you: _____

May we leave a message on your answering machine? ☐ **YES** ☐ **NO**

Email: _____

3. Personal Information

Full Name: _____

Date of birth (day/month/year):
_____

Age: _____

Sex: _____

I would like to ask if you have any challenges, special needs, or problems that we should be aware of.

Any serious medical concerns or problems? (*if so*, what?) _____
_____

Did you ever have a head injury for which you were hospitalized?
_____

Were you ever unconscious for more than 24 hours?
_____

Were you ever diagnosed with a learning disability?
_____

Were you ever diagnosed with a behavioural problem (e.g., ADD/ADHD)?
_____

Were you ever diagnosed with a pervasive developmental disorder or an autism spectrum disorder (e.g. Asperger's)?
_____

Do you have any cognitive challenges? (*if so*, what?)
_____

Have you ever been to see a psychologist or therapist? (*if so*, why?)

Do you take any medication? (*if so*, what and what for?)

Other comments:

Appendix D

Informed Consent Form

Cerebellum, Basal Ganglia, and Motor Cortex Interactions During Motor Learning

Principle Investigator: Dr. Virginia Penhune, Concordia University
Investigator: Chris Steele, Concordia University

INFORMED CONSENT FORM

MONTREAL NEUROLOGICAL INSTITUTE AND HOSPITAL
McConnell Brain Imaging Centre

1. TITLE OF PROJECT

Cerebellum, Basal Ganglia, and Motor Cortex Interactions During Motor Learning

2. REASON FOR THE STUDY

The aim of this study is to understand the brain areas involved in the learning and performance of a motor skill. Your participation in this study will help us to learn which areas of the brain are involved in the stages of motor learning and motor performance in healthy people.

3. PROCEDURES

Your participation in this study will involve five sessions lasting approximately 6 hours in total. Each session will take place over five consecutive days at approximately the same time on each day. On each day, you will learn to reproduce a sequence in sync with visual stimuli presented by a computer. You will reproduce the sequence on a mouse while lying down and viewing the computer-generated stimuli. Your responses will be recorded by a computer. On the first, second, and fifth day you will reproduce the sequence while in the MRI scanner. You will lie on a bed that will be moved into a cylindrical opening where pictures of your brain will be taken for approximately one hour. The MRI scanner will be quite noisy during the scan, but you will have headphones on to attenuate the sound. You will also be required to remain as still as possible since any movement will cause the pictures of your brain to become blurred. You will be able to communicate with the experimenter and technicians during the entire procedure. Over the course of the study you will spend approximately three hours in the MRI scanner. Before training on all days, you will complete a sleep questionnaire to assess your general level of alertness. On the third and fourth days you will be asked to perform the sequence by itself outside of the MRI scanner while your responses are recorded by a computer. In addition, you will be asked to complete the WAIS-III Vocabulary Subtest (where you will give word definitions), a Handedness questionnaire and the Grooved Pegboard Test (where you will place sticks in slots). In total, you will spend approximately three hours over these two days.

4. **CONTRAINDICATIONS**

The following are contraindications for a magnetic resonance study:

- Pacemaker
- Aneurysm Clip
- Heart/Vascular Clip
- Prosthetic Valve
- Transdermal Patches (Must be removed prior to scanning. Subject is advised to bring an additional patch to reapply post scanning.)
- Metal Prosthesis
- Pregnancy
- Claustrophobia
- Metal fragments in body

5. **ADVANTAGES OF THE PROPOSED STUDY**

Your participation in this study will result in no advantages. MRI is not a treatment. Your participation in this study may help us understand some functions of the human brain and may help the diagnosis and treatment of some neurological diseases.

6. **DISADVANTAGES OF THE PROPOSED STUDY**

During this study you will be exposed to a strong magnetic field. No short- or long-term negative side effects have been observed from studies involving MRI. As mentioned previously, there will be a lot of noise in the MRI scan environment. You will have headphones on that will help attenuate some of this noise. You will be able to communicate with the technician operating the MRI machine at all times. As also mentioned above, you may experience claustrophobia once inside the scanner. If this is so, and for any other reasons of discomfort, please let us know and you may withdraw from the study.

7. **CONFIDENTIAL NATURE OF THIS STUDY**

Individual identifying information will be kept confidential and only authorized personnel within the PI's lab will have access to it. The data from the study, devoid of identifying information, will be maintained in the laboratory's files, and used for purposes of analysis and comparison with other data, and for scientific dissemination and publication.

8. **DISCONTINUATION OF THE STUDY BY THE INVESTIGATOR**

The investigator has the right to stop the study and withdraw the subject, for any reason, and at any time during the experiment.

9. **WITHDRAWAL FROM THE STUDY**

Participation in this study is entirely voluntary and you have the right to withdraw from the study at any time, including during the procedure. Data accumulated up to the time of your withdrawal will be kept in use for research purposes.

10. **INCIDENTAL FINDINGS**

Research scans are not subject to clinical review. However, in the event that there are incidental findings pertaining to your health, we will inform you of them, and upon your request, inform your physician.

11. EFFECTS OF PARTICIPATION IN THIS STUDY

Magnetic resonance imaging does not interfere with any treatment or other diagnostic tests.

12. SUBJECT'S AGREEMENT TO BE CONTACTED BY THE RESEARCH ETHICS BOARD

I._____ agree to be contacted by a member of the Research Ethics Committee and/or a Quality Assurance Officer, at the discretion of the committee.

13. COMPENSATION FOR PARTICIPATION IN THE STUDY

Upon completion of the study, you will be compensated with $250.00 for your time and inconveniences. If the study cannot be completed in full for any reason, compensation will be adjusted accordingly.

14. CONTACT INFORMATION FOR SUBJECT

You may contact the investigator, Chris Steele, at any time should you have inquiries about the study. He may be reached at 514-848-2424 x7567. Alternatively, you may also contact the Principle Investigator, Dr. Virginia Penhune, at 514-848-2424 x7535. If you have any questions about your rights as a research subject or a complaint about the study, you may contact the Patients' Committee (a group established to protect the rights of patients and research subjects) at 514-398-5358.

Cerebellum, Basal Ganglia, and Motor Cortex Interactions During Motor Learning

Principle Investigator: Dr. Virginia Penhune

Investigator: Chris Steele

DECLARATION OF CONSENT

MONTREAL NEUROLOGICAL INSTITUTE AND HOSPITAL

McConnell Brain Imaging Centre

I, _____, have reviewed the project with one of the investigators, _____.

I fully understand the procedures, advantages and disadvantages of the study which have been explained to me. I freely and voluntarily consent to participate in this study.

Further, I understand that I may seek information about each test either before or after it is given, that I am free to withdraw from the testing at any time if I desire, and that my personal information will be kept confidential.

SIGNATURE _____ _____ _____

SUBJECT DATE CONTACT NO.

SIGNATURE _____ _____ _____

INVESTIGATOR DATE CONTACT NO.

Appendix E

MR Safety Screening Form

Cerebellum, Basal Ganglia, and Motor Cortex Interactions During Motor Learning
Magnetic Resonance Imaging
MONTREAL NEUROLOGICAL INSTITUTE AND HOSPITAL
McConnell Brain Imaging Centre

*It is of the **utmost importance** for the subject that this questionnaire be completed by the **subject and investigator**.*

1. Previous surgery (type and date)

_____

2. Does the subject have any of the following?	***YES***	***NO***
Cardiac pacemaker	____	____
Surgical clip on an aneurysm or other vessel	____	____
Surgical clip or valve on the heart	____	____
Prostheses (please specify type and location) _____	____	____
Implants (please specify type and location) _____	____	____
Metal or metallic fragments in any part of the body (please specify) _____	____	____
3. Is the subject pregnant?	____	____

All of my questions regarding this exam have been satisfactorily answered.

SIGNATURE _____ _____ _____
SUBJECT DATE CONTACT NO.

SIGNATURE _____ _____ _____
INVESTIGATOR DATE CONTACT NO.

Appendix F

Sleep Questionnaire

ID #:_____

SLEEP QUESTIONNAIRE

To be completed on Day 1:
Date (D/M/Y) ___/___/___
Time (HH:MM) ___:___

1)At what time do you usually go to sleep? _____

2)At what time do you usually wake up? _____

3)In general, what is the quality of your sleep?
poor average good

1)Do you usually take naps during the day? yes no (circle one)
If yes, for how long is the nap and when is it during the day? _____

- At what time did you go to sleep last night? _____
- At what time did you wake up this morning? _____
- What was the quality of your sleep last night? poor average good
- Did you take any naps during the day? yes no (circle one)

If yes, how long was it and when was it? _____

ID #:_____

- Choose the number of the description that best fits your current state:

1 feeling alert, active and wide awake

2 although not at peak, still feeling able to concentrate and function at high level

3 relaxed, but not at full alertness

4 a little foggy, not at peak

5 fogginess, starting to lose in interest in staying awake

6 sleepiness, fighting sleep and would rather be lying down

7 almost asleep, lost struggle to remain awake

8 asleep

ID #:_____

To be completed on Days 2, 3, 4, & 5:

	Day 2	Day 3	Day 4	Day 5
Date (D/M/Y)				
Time (HH:MM)				

- At what time did you go to sleep last night?

- At what time did you wake up this morning?

- What was the quality of your sleep last night?
  poor average good

- Did you take any naps during the day? **Yes/No**

If yes, how long was it and when was it?

Yes/No				
How Long				

- As in 9), choose the number of the description that best fits your current state:

Appendix G

List of Significant Peaks for the LRN vs. ISO Contrast on d1r1

Complete list of peaks for the LRN-ISO contrast, $p < .05$, corrected for multiple comparisons.

x	y	z	t-value
2	2	50	6.66
2	4	48	6.62
0	8	46	6.52
4	-18	18	6.29
4	-18	10	6.20
4	-20	16	6.20
-24	-14	56	6.19
-24	-12	52	6.04
24	-8	48	5.99
-38	-56	-30	5.87
-28	-50	52	5.77
36	-52	-28	5.74
38	16	4	5.71
38	16	0	5.69
30	16	8	5.69
26	-14	52	5.69
-28	18	2	5.67
26	-38	-46	5.67
0	-60	-22	5.59
26	-36	-44	5.58
0	-62	-20	5.56
32	18	4	5.56
34	-54	-26	5.54
-32	16	2	5.46
2	-64	-10	5.42
-20	-30	16	5.42
-20	-28	18	5.41
-16	-20	18	5.41
-2	-68	-32	5.41
0	-66	-12	5.40
2	-60	-28	5.38
-22	-30	18	5.37
-18	-28	16	5.36
-16	-16	20	5.35
24	-12	64	5.35
-28	-46	38	5.34
26	-32	-42	5.33
-28	-44	40	5.33

-22	-28	20	5.31
-2	-72	-12	5.28
-20	-26	20	5.28
26	-14	64	5.27
20	-28	18	5.23
18	-26	18	5.22
-34	14	0	5.22
-22	-26	22	5.19
-18	-16	22	5.19
30	-42	-50	5.12
-20	-24	22	5.09
2	-54	-4	5.08
2	-56	-6	5.07
-30	-58	-26	5.00
42	-34	38	4.93
30	-30	-34	4.89
-12	-14	12	4.83
6	-6	66	4.82
-24	-30	-44	4.81
0	-10	64	4.76
0	-8	62	4.75
16	-8	24	4.75
12	-10	12	4.75
-30	-34	40	4.71
-34	-36	38	4.67