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Article title: Motor sequence learning in the elderly: Differential activity patterns as a function of hand modality

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ABSTRACT

Previous research on motor sequence learning (MSL) in the elderly has focused mainly on unilateral tasks, even though bilateral coordination might be impaired in this age group. In this fMRI study, 28 right-handed elderly subjects were recruited. The paradigm consisted of a Novel and a simple Control sequence executed with the right (R), left (L) and both hands (B). Behavioral performance (Accuracy[AC], Inter-tap Interval[ITI]) and associated brain activity were assessed during early learning. Behavioral performance in the Novel task was similar between unilateral conditions whereas in the bimanual condition more errors and slower motor execution were observed. Brain activity increases during learning showed differences between Conditions: R showed increased activity in pre-SMA, basal ganglia and left hippocampus while B showed activity increments mainly in posterior parietal cortex and cerebellum. L did not show any activity modulation during learning. Performance correlates for AC (related to spatial success) and ITI (related to accurate timing) shared a cortico-basal-cerebellar network. However, it was found that the ITI regressor presented additional significant correlations with activity in SMA and basal ganglia in R. The AC regressor showed additional significant correlations with activity in more extended thalamic and cerebellar areas in B. The present findings suggest that, behaviorally, the spatial and temporal components of MSL are impaired in elderly subjects when using both hands. Additionally, differential brain activity patterns were found across hand modalities. The results obtained reveal the existence of a highly specialized network in the dominant hand and identify areas specifically involved in bimanual coordination.

Keywords: motor sequence learning, timing, accuracy, bimanual, fMRI.

BACKGROUND

According to Doyon et al. model (Doyon et al., 2003) motor learning is divided into motor sequence learning (MSL) and motor adaptation. The former involves the integration of serial steps into a coherent unit; the latter corresponds to previously learned movements and its change in response to an external input (King et al., 2013). While MSL is mostly preserved in older individuals (Daselaar et al., 2003; Rieckmann et al., 2010), when compared to young adults, previous work suggests that they may present some degree of dysfunction, especially when the sequence is explicit, but not implicit (Verneau et al., 2014) or associated to sequence complexity (Howard et al., 2004).

Neural correlates of early MSL in healthy subjects have been extensively studied (Doyon et al., 1998; Doyon et al., 2003; Doyon et al., 2009; Lehericy et al., 2005) and include cortical regions (supplementary motor area (SMA), pre-SMA, prefrontal cortex (PFC), motor cortex (M1), hippocampus), motor and associative basal ganglia (dorsal putamen, anterodorsal globus pallidus GP, thalamus, subthalamic nucleus) (Schendan et al., 2003) and the cerebellum (lobules V, VI). Striatal and medial temporal lobe (MTL) activity decreases have been reported during the slow learning phase in young adults, whereas this effect is not seen in elderly subjects. In the latter activity in the hippocampus remains intact, probably as the result of a compensating effect (Schendan et al., 2013).

The learning of many everyday motor skills, unlike in experimental settings, is acquired through visual input and the subsequent practice attempts. To have a successful execution of a new motor program, several factors have to be taken into account, including working memory and a correct timing of the motor sequence execution. Even when accuracy in unimanual MSL remains mostly intact in aging populations, slower reaction times and learning rates have consistently been reported (Bhakuni and Mutha, 2015a; Hoff et al., 2015). These results suggest that motor sequence learning might comprise two different components: a spatial component, which is in close relationship with accuracy, and the temporal component of correct finger movements.

On the other hand, plenty of research has been conducted in bimanual coordination tasks both in young and older participants, suggesting that it might be impaired in the latter (Bangert et al., 2010; Hoff et al., 2015; Seidler et al., 2010; Serrien et al., 2000). Despite these findings, few studies have made a direct comparison in the young between unimanual and bimanual learning (Aznarez-Sanado et al., 2013; Wang and Sainburg, 2009) or finger-tapping tasks (Jäncke et al., 1999; Nair et al., 2003; Sadato et al., 1997; Serrien, 2009a; Serrien, 2008; Walsh et al., 2008). Among these, there is still controversy, since some authors have reported significant differences in behavior between both modalities (Serrien, 2009b; Serrien, 2008) while others have not (Aznarez-Sanado et al., 2013; Nair et al., 2003; Sadato et al., 1997). However, no previous studies have focused on MSL and hand modality in the elderly.

Based on previous findings, we hypothesized that: 1) Early motor sequence learning in healthy elderly adults would show worsened performance in the bimanual condition when compared to the unimanual conditions; 2) Hand modalities (dominant hand, non-dominant hand and bimanual) would be related to different neural activation patterns in MSL; 3) Spatial and timing components of MSL would be processed by different brain areas. For this purpose, a novel, complex and non-guided motor learning paradigm was developed. Specifically, the aim of this work was twofold: 1) To assess motor performance and its associated neural correlates during early learning in a group of elderly subjects, when using the right, left and both hands respectively and 2) To map the spatial and timing components of successful motor execution during early learning.

METHODS

Participants

28 healthy (17 males) elderly (60.2 (SD: 8.6) years old) subjects were recruited. All of them were right-handed (mean dexterity score 47.4 (SD: 3.2) out of 50) as determined by the 10 item Edinburgh handedness inventory (Oldfield, 1971). Each item was scored from 1 to 5. The total score was calculated by adding the individual item scores. A total high score indicates right-handedness and a total low score, left-handedness. Exclusion criteria included those associated with MRI use, left-handedness and the presence of cognitive dysfunction. The experimental protocol was approved by the University of Navarra Research Ethics Committee. Subjects signed written informed consent before participating in the study.

Experimental Setup

Subjects lay supine inside the scanner. Images were projected onto a screen and then reflected by a mirror system attached to the head coil into the subjects' field of vision. Motor finger responses were collected from two 4-key response pads (Current Designs, Inc.), one for each hand, placed on the subjects' abdomen. Stimuli design, presentation and data collection was done using Cogent (Cogent 2000, UCL, London) and Matlab.

Experimental Task

Subjects were requested to visualize and memorize a visual sequence which represented an eight finger movement sequence. First, colored circles, indicating targets of finger movements, were presented on a screen. After the eight targets had been shown, a fixation cross appeared to announce the sequence reproduction period, which was 12.5 seconds long. The subjects were requested, during the fixation cross period, to reproduce the same sequence of eight sequential finger movements that had been previously visually presented, by pressing the buttons on the response pad. The four buttons of the response pad corresponded to index (1), middle (2), ring (3) and little (4) fingers respectively. Subjects had to memorize and execute three

different Novel sequences with the right (R), left (L) and both hands (B) respectively. Subjects underwent three different scanning sessions. Each session corresponded to the learning paradigm of R, L or B movements (i.e. Session 1: B, Session 2: R and Session 3: L). The order in which participants performed R, L or B sessions was randomized. The Novel sequence to be learned for R condition was 2-3-1-3-4-2-1-4, for L condition 3-2-4-2-1-3-4-1 and for B condition 1-3-4-3-2-4-2-1. Bimanual movements required symmetrical simultaneous finger button presses. Following the Novel trials, a Control sequence was presented, consisting of successive finger movements (4-3-2-1-4-3-2-1) for all hand conditions.

Both tasks (Novel and Control) were presented in an event related design in blocks of two. At the end of Control trials, a four second rest period was added, where subjects were asked to relax and look at the fixation cross. Visual stimuli were presented sequentially on the screen at a frequency of 0.67 Hz (inter-stimulus interval of 1500 ms) and a random jitter was included (0-500 ms) at the beginning of each sequence. Subjects were asked to reproduce each trial at the same frequency as in presentation. Novel and Control trials were executed 16 times each per session, which were divided in two Learning phases of 8 trials each (L1, L2) corresponding to the first and second halves of each run. No feedback on performance was provided to subjects. All subjects executed the Control sequence (up to three trials) but not the Novel sequence, before entering the scanner, in order to become familiarized with the task.

Behavioral Data

In this study, three behavioral variables were assessed: Accuracy (AC), which was defined as the average number of correct movements executed per trial. For the bimanual condition, this was only true when the correct button was pressed with both hands. Inter-tap Interval (ITI) was defined as the average time interval between each of the finger taps across trials. In the bimanual modality this value was calculated by taking into account the average of the right and left hand corresponding time intervals between finger taps. A Synchrony Index (SI) was calculated in the B condition as the average difference between finger pressing times of each hand (right hand ITI – left

hand ITI). AC and ITI were computed and analyzed by Condition (R, L, B), Task (Novel, Control) and Learning phase (L1, L2) for every subject. Behavioral differences were evaluated using a three-way full factorial repeated-measures ANOVA with factors Task, Condition and Learning phase for AC and ITI respectively. In order to analyze SI differences between tasks and learning phases, a two-way ANOVA with factors Task (Novel, Control) and Learning phase (L1, L2) was carried out in the B condition. Post hoc comparisons were assessed using Tukey's multiple comparisons test.

fMRI Data Acquisition and Analysis

Studies were performed on a 3.0 Tesla MR scanner (Siemens TRIO, Germany) using a 12-channel head coil. A total of 295 volumes using a T2*-weighted gradient echo-planar imaging (EPI) sequence (repetition time/echo time [TR/TE] = 3000/30ms, field of view [FOV] = 192x192 mm², flip-angle = 30°, 49 slices, resolution = 3x3x3 mm³) were acquired during each session. The anatomical image was obtained using a T1-weighted MPRAGE sequence (TR/TE = 1620/3ms, inversion time (TI) = 950ms, FOV = 250x187x160 mm³, flip-angle = 15°, 160 slices, resolution = 1x1x1 mm³).

Data analysis was done using SPM12 (Wellcome Department of Imaging Neuroscience, UCL, London). First, EPI images were realigned to the first volume of the series and co-registered to the anatomical image. Normalization and segmentation were done using the DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie algebra) toolbox, which has demonstrated a better performance than traditional SPM non-linear registration (Klein et al., 2009). A three-dimensional Gaussian smoothing kernel of 8 mm full-width at half maximum was applied to the EPI images.

In the first-level analysis, tasks (Novel, Control) were modeled for every condition (R, L, B) and subject. Additionally, each task was divided in two learning phases (L1, L2) consisting of 8 sequence repetitions each. In separate analyses, values corresponding to the analyzed behavioral variables (AC, ITI) were introduced as parametric modulators in different matrices. The general

linear model (GLM) was implemented in SPM12. Six motion regressors were included to control any head movement generated during image acquisition.

Second-level analyses included a 2x2x3 full factorial ANOVA design, with factors Learning phase (2 levels: L1, L2), Task (2 levels: Novel, Control) and Condition (3 levels: R, L, B) in order to assess differences and commonalities among hand conditions between the first and second halves of each run. Additionally, each of the two regressors' related activity was studied separately using one-way ANOVAs with factor Condition (3 levels: R, L, B) using first level Novel as the contrast of interest. All analyses were corrected by post-hoc procedures (FWE-cluster level corrected, cluster defining threshold $p=0.001$).

To improve the cerebellar and brainstem structural and functional registration, the Spatially Unbiased Atlas Template (SUIT) toolbox for SPM was employed (Diedrichsen, 2006). With this tool, each subject's cerebellum and brainstem were initially isolated from its structural image by generating probability maps. These maps were later normalized to the SUIT template. Functional images were then resliced to their own isolated and normalized image in order to render the data into the SUIT atlas space.

Cerebral and cerebellar activation patterns were localized using the SPM Anatomy toolbox.

RESULTS

Behavioral Motor Results

AC showed significant main effects of Task ($F[1,90]=121.8$, $p<0.001$) and Condition ($F[2,90]=8.36$, $p<0.001$), the latter revealing significant differences between the R-B ($AC_{RNov}=6.29\pm0.85$ ($\pm$ Standard Deviation), $AC_{BNov}=5.08\pm1.3$, $q=5.88$, $p=0.001$) and L-B ($AC_{LNov}=6.29\pm1.23$, $AC_{BNov}=5.08\pm1.3$, $q=5.85$, $p=0.001$) but not the R-L conditions in the Novel task (Fig. 1). Interactions between the factors Task and Condition were also significant ($F[2,90]=3.61$, $p=0.0311$). Consequently, in order to assess the effect of this interaction, further post-hoc analyses were done, revealing significant differences between tasks for all three conditions: right ($AC_{RCont}=7.85\pm0.17$, $AC_{RNov}=6.29\pm0.85$, $q=7.55$, $p<0.001$), left ($AC_{LCont}=7.81\pm0.19$, $AC_{LNov}=6.29\pm1.23$, $q=7.34$, $p<0.001$), and bimanual ($AC_{BCont}=7.58\pm0.3$, $AC_{BNov}=5.08\pm1.3$, $q=12.11$, $p<0.001$). A significant interaction Task x Learning phase was found, and post-hoc multiple comparisons revealed that L2 differed from L1 for Novel ($AC_{L1}=5.07\pm1.68$, $AC_{L2}=6.7\pm1.79$, $q=8.08$, $p<0.001$), but not for the Control task. AC scores were higher over time during the second phase of the experiment for the Novel task (Fig. 2). No significant Task x Condition x Learning phase interaction was found.

Figure 1. Box-plot for Accuracy (AC) and Inter-tap Interval (ITI) variables among Conditions. Boxes indicate the mean and the 25th to 75th percentile. Whiskers represent the minimum and maximum values. Different boxes are represented for every task (Novel, Control) and Condition (R, L, B). * Indicates

significant differences (after Tukey's correction). For every significant comparison the obtained p-value is $p < 0.001$.

For the ITI variable significant main effects of Task ($F[1,90]=355.1$, $p < 0.001$) and Condition ($F[2,90]=32.47$, $p < 0.001$) were found. Significant differences were observed only between R-B ($ITI_{RNov}=1130 \pm 103.8$, $ITI_{BNov}=1389 \pm 173$, $q=11.18$, $p < 0.001$) and L-B ($ITI_{LNov}=1130 \pm 75.13$, $ITI_{BNov}=1389 \pm 173$, $q=11.2$, $p < 0.001$) for the Novel task (Fig. 1). A significant Interaction Task x Condition was found ($F[2,90]=12.65$, $p < 0.001$). Post-hoc multiple comparison tests showed that all conditions reported significant differences between tasks: right ($ITI_{RCont}=815 \pm 23.14$, $ITI_{RNov}=1130 \pm 103.8$, $q=13.57$, $p < 0.001$), left ($ITI_{LCont}=862.5 \pm 21.42$, $ITI_{LNov}=1130 \pm 75.13$, $q=11.52$, $p < 0.001$) and bimanual ($ITI_{BCont}=900.3 \pm 66$, $ITI_{BNov}=1389 \pm 173$, $q=21.07$, $p < 0.001$). Also, a significant Interaction Task x Learning phase revealed lower ITI times during L2 for the Novel task ($ITI_{L1}=1307 \pm 376.42$, $ITI_{L2}=1126 \pm 346.1$, $q=3.65$, $p=0.006$, Fig. 2) but not for the Control task. No significant interaction Task x Condition x Learning phase was found.

Figure 2. Accuracy (AC), and Inter-Tap Interval (ITI) averaged values across Novel sequence repetitions for every condition (Right, Left, Bimanual). Upper and lower limits represent the standard-error (SE).

Differences in SI were also measured (Fig. 3). The B condition required simultaneous symmetrical finger presses. Obtained mean SI values in the study were always negative, indicating that on average subjects tapped with the right hand before the left hand when executing a bimanual movement. Participants

showed greater variability in timing button presses with both hands. Significant main effects of Task ($F[1,108]=6.23$, $p=0.014$) and Learning phase ($F[1,108]=4.93$, $p=0.028$) were obtained, with no significant interactions between them ($F[1,108]=2.71$, $p=0.095$). To note, the Novel task presented an initial 100 ms fluctuation, an effect not observed in the Control task. This difference might be due to a “start-up” effect which is only seen during the first Novel sequence block, and removing it from the analysis eliminates the main effect of Learning phase.

Figure 3. Synchrony Index (SI) across sequence repetitions for the Novel and Control tasks. Upper and lower limits represent the standard-error (SE). * Indicates significant differences between L1 and L2 in the Novel task ($p=0.01$, after Tukey’s correction).

Functional Imaging Results

Significant main effects of Learning phase, Task and Condition were found. The Novel>Control contrast revealed areas usually associated with motor sequence learning, including pre-SMA, DLPFC, posterior parietal cortex, bilateral anterior insula, thalamus and cerebellum (Supplementary Fig. 1).

Since a significant Learning x Condition interaction (Supplementary Fig. 2) was found and we were interested in evaluating early motor learning activity increases ($L2>L1$) across the three conditions. Three $L2(\text{Novel})>L1(\text{Novel})$ contrasts for the R, L and B conditions were created (Fig. 4 and Supplementary Table 2). $R[L2(\text{Novel-Control})>L1(\text{Novel-Control})]$ showed significant activity in

pre-SMA, bilateral anterior putamen and left hippocampus ($p < 0.001$, FWEc-based correction $k = 663$). $L[L2(\text{Novel}) > L1(\text{Novel})]$ did not show any significant brain activations. Finally, $B[L2(\text{Novel}) > L1(\text{Novel})]$ showed significant activity in the dorsal precuneus, cuneus and the cerebellum ($p < 0.001$, FWEc-based correction $k = 805$). A global conjunction analysis was performed among these three contrasts in order to test for common areas, independent of hand modality, related to early motor learning. This analysis revealed activity in the premotor cortex, left caudate and thalamus, bilateral anterior putamen, cuneus and cerebellum (Fig. 5, $p < 0.001$, FWEc-based correction $k = 337$).

Figure 4. $[L2(\text{Novel}) > L1(\text{Novel})]$ contrast the R (red) and B (green) conditions ($p < 0.001$, FWEc-based correction $k_R = 663$, $k_B = 805$).

Figure 5. Conjunction analysis for L2(Novel)>L1(Novel) ($p < 0.001$, FWEc-based correction $k=337$).

In order to study the neural correlates of sequence performance, two different behavioral regressors were used. AC regressor corresponded to the number of correct key presses for each repetition of the Novel sequence. Significant positive correlations –higher BOLD signal for number of correct taps- were found between AC and neural activity. Conversely, no significant negative correlations –higher BOLD signal for lower number of correct taps- were found.

A common network was significantly correlated with the AC regressor for the three conditions (conjunction analysis, $p < 0.001$, FWEc-based correction $k=351$) which included the left somatosensory cortex, bilateral basal ganglia (BG), left hippocampus, middle cingulate cortex (MCC) and cerebellar lobules I-IV and VIII bilaterally along with the right cerebellar lobule V (Fig. 6 and Supplementary Table 3).

When testing for the main effects of Condition in the AC regressor (Fig. 7, $p < 0.001$, FWEc-based correction $k=415$, Supplementary Table 1), differences were found in the left caudate and thalamus, as well as the cerebellum.

Analyzing by Condition (Fig. 8, Supplementary Table 3), AC regressor correlated positively with activity in the MCC, SMA, left medial cingulate cortex, pericentral areas and cerebellum in the R condition ($p < 0.001$, FWEc-based correction $k=516$); it correlated positively with activity in right motor and somatosensory cortex and left cerebellum in L ($p < 0.001$, FWEc-based correction $k=594$); and it correlated positively with activity in the thalamus and bilateral cerebellum in B ($p < 0.001$, FWEc-based correction $k=377$).

Figure 6. Qualitative overlap between AC (red) and ITI (blue) positive correlation conjunction maps ($p < 0.001$, FWEc-based correction $k_{AC}=351$, $k_{ITI}=245$).

Figure 7. Accuracy main effect of Condition ($p < 0.001$, FWEc-based correction $k=415$).

Figure 8. Accuracy positive correlation map partitioned by Condition ($p < 0.001$, FWEc-based correction $k_R = 534$, $k_L = 594$, $k_B = 377$). Red=Right; Blue=Left; Green=Bimanual; Yellow = Right+Bimanual; Cyan = Left+Bimanual.

For the ITI regressor, significant positive correlations -longer ITI times- and negative correlations -associated with shorter ITI times- with neural activity were obtained. A common neural network was significantly correlated with the ITI regressor for the three conditions (conjunction analysis, $p < 0.001$, FWEc-based correction $k = 245$) which included the right somatosensory cortex and right basal ganglia, as well the cerebellar lobules VI and VIII bilaterally and right lobule V (Supplementary Table 3).

A main effect of Condition was found for the ITI regressor (Fig. 9, $p < 0.001$, FWEc-based correction $k = 307$, Supplementary Table 1), showing differences in brain activity among conditions specifically in bilateral M1, SMA, pre-SMA, left putamen and cerebellum. When dissociated by Condition (Fig. 10, Supplementary Table 4), ITI regressor correlated negatively with activity in the bilateral motor and somatosensory cortex, medial cingulate cortex extending to SMA, bilateral basal ganglia and cerebellum ($p < 0.001$, FWEc-based correction $k = 434$) in the R condition; it correlated negatively with activity in the right motor cortex and left cerebellum ($p < 0.001$, FWEc-based correction $k = 551$) in L; and it correlated negatively with the bilateral motor cortex and cerebellum ($p < 0.001$, FWEc-based correction $k = 500$) in B. Positive correlations were only significant for the L condition showing activity in the DMPFC, bilateral DLPFC and bilateral anterior insula ($p < 0.001$, FWEc-based correction $k = 436$, see Supplementary Table 5).

Figure 9. ITI main effect of Condition ($p < 0.001$, FWEc-based correction $k=307$).

Figure 10. ITI negative (a) and positive (b) correlation maps partitioned by Condition ($p < 0.001$, FWEc-based correction $k_R^+ = 434$, $k_L^+ = 551$, $k_B^+ = 500$, $k_L^- = 436$). Red=Right; Blue=Left; Green=Bimanual

DISCUSSION

Behaviorally, healthy elderly subjects' performance while executing the learning task (Novel task) was significantly more inaccurate and slower than when executing a simple task (Control task), irrespective of the hand Condition. Of interest, significant differences in performance for the Novel task were found between bimanual (B) and right and left hand conditions (R and L), but not between the latter. These results differ from what was found previously in young healthy adults with the same task and increased number of repetitions, where no behavioral differences among the unimanual and bimanual conditions were found (Aznarez-Sanado et al., 2013). Although it has been stated that several factors can impair motor sequence learning in the elderly, such as sequence complexity (Howard et al., 2004) or explicit knowledge of the sequence (Verneau et al., 2014), this effect has been studied mostly while using the dominant hand, with no studies comparing unimanual and bimanual tasks in this age group. In the present study, however, we demonstrate that motor sequence learning in the elderly is worsened when using both hands, in comparison with performance when using the dominant and the non-dominant hand. Therefore, the involvement of bimanual coordination should also be considered when accounting for differences in MSL at all ages.

Age-related declines in bimanual coordination have been reported before (Bangert et al., 2010; Hoff and Ragert, 2015; Seidler et al., 2010; Serrien et al., 2000). On the other hand, recent studies in a bimanual serial reaction time task show that older adults can learn as much as younger adults, with the only difference being slower response times (Bhakuni and Mutha, 2015b; Hoff and Ragert, 2015). Due to this controversy, we believe that a proper comparison (i.e. employing the same learning paradigm) between young and elderly populations in MSL is still needed.

Regarding brain related activity during early motor learning in the elderly, a common neural network was found for the three conditions, including visual primary areas (V1-3), SMA, BG, and posterior parietal areas. These results are in accordance with previous findings (Doyon et al., 1998; Doyon et al., 2003; Doyon et al., 2009; Lehericy et al., 2005) and stress the relevance of these

structures in early motor learning, independently of hand modality. However, it is worth noting that no learning related decreases in activity ($L1 > L2$) were obtained. These results contrast with those found by previous studies in young adults (Aznarez-Sanado et al., 2013; Doyon et al., 2002; Fernández-Seara et al., 2009; Lehericy et al., 2005; Müller et al., 2002) where prefrontal regions diminished their activity as learning progressed. These differences could be attributed to variations in the motor task or the number of repetitions performed by the subjects. Alternatively, these results may indicate the existence of a compensatory mechanism, where elderly subjects would need more brain resources in order to carry out a new motor sequence (Graziadio et al., 2015; Reuter-Lorenz and Cappell, 2008).

Differential learning phase related increases ($L2 > L1$) across conditions were found. The dominant hand (right hand) showed the largest activity modulation during early learning, mainly in the PFC, bilateral BG and left hippocampus. These last two structures are closely related to motor sequence learning. However, striatal activity increases and hippocampal activity decreases have been previously reported in young adults during learning, whereas both areas show increased activation in older adults (Rieckmann et al., 2010). These areas also collaborate in a sleep-dependent striato-hippocampal memory system, which appears to be involved in similar motor sequence learning tasks, at least in young adults (Albouy et al., 2015; 2008; Fernández-Seara et al., 2009). Interestingly, no significant activity increases during learning were associated with the left hand. This finding may represent a lack of cortical specialization of the non-dominant hand during the early learning phase, resulting in a slower sensorimotor integration. In the bimanual condition, posterior parietal and occipital cortex and cerebellum activity increases were detected. It is worth noting that, to our knowledge, no previous study has reported these early learning activity increases during bimanual sequence learning. In bimanual movement generation, activation in the occipital and parietal cortex –the dorsal visual stream- is evoked by externally generated stimuli (Swinnen and Wenderoth, 2004). Also, the cerebellar lobule VI and several vermal areas have been reported to become more active when movements are executed in synchrony to a specific rhythm (for a review see (Swinnen and Wenderoth,

2004)). In part, the activity changes obtained in this study for the bimanual condition could be behaviorally explained by the improvement in ITI observed during the second half of the experiment.

AC and ITI performance regressors were introduced with the intent to separate the spatial and temporal components of our motor learning task. Behavioral and neural grounds related to these parameters were studied in a similar fashion to what it has previously been carried out in other motor learning paradigms (Ghilardi et al., 2009; Muller et al., 2002; Steele and Penhune, 2010; Wadden et al., 2013).

Common to all three Conditions, improvements in AC correlated positively with activity in BG, hippocampus, cerebellum and pons, in accordance with previous findings (Gheysen et al., 2010). However, the B condition showed additional brain activity correlates (when compared to those of R and L) located in bilateral paravermal lobule VIIIA/B along with the thalamus. These regions might play a differential role when accurately coordinating the spatial components of a bimanual motor task (Debaere et al., 2004a; Steele and Penhune, 2010).

On the other hand, in all Conditions ITI times correlated negatively with BOLD activity in the sensorimotor cortex, right BG and motor cerebellum (i.e. higher activity for shorter ITIs). M1 activity has been previously reported to correlate positively with improvements in a similar timing measure (Steele and Penhune, 2010). Altogether, and in accordance with Doyon MSL model (Doyon et al., 2003), these areas belong to the cortico-striatal and cortico-cerebellar pathways which are involved in error correction, internal model formation, stimulus-response association and sequence representation (Penhune and Steele, 2012). Condition related differences for the ITI regressor were notable in R, showing a strong positive activity modulation of SMA, BG and bilateral cerebellar lobules V-VI. In contrast, L and B showed activity modulation only on their expected cortico-cerebellar pathway plus cerebellar lobule VIIIA/B, a structure involved in the spatiotemporal component of other bimanual tasks (Debaere et al., 2004b). These results suggest an alternative functional specialization of the dominant hand when executing timed precise movements. Furthermore, according to this idea, positive correlations with brain activity (i.e. higher activity for longer ITI

times) were found only for L in the bilateral VMPFC, DLPFC and anterior insulae. These findings might indicate that when subjects execute movements with the non-dominant hand they are more variable and need to employ more attentional and planning resources to overcome this situation (Muller et al., 2002).

It is interesting to note that both AC and ITI regressors share a common “good performance” network that is related to correct –matching the presented key sequence- and rhythmic –isochronic- movements across conditions (Fig. 6). Common areas to both regressors were mainly located in the thalamus and cerebellum. Although this is only a qualitative comparison, it is relevant how these structures are essential for accurate motor performance (spatially and temporally) independently of the hand Condition.

Besides showing activity increases during early learning, it is important to highlight how cerebellar lobules V, VI and VIII activity also correlates consistently with accurate motor performance. These areas belong to the so called “sensorimotor” cerebellum (Stoodley and Schmahmann, 2009) and are connected structurally and functionally to cortical motor areas via the dentate nucleus and thalamus (O'Reilly et al., 2010; Schulz et al., 2015). These results reinforce the idea that as learning progresses, a well-executed sequence becomes purely a motor task and stops employing cognitive cerebellar, as well as cortical resources. On the other hand, lobules VI, VII and crus 1, which are thought to be involved in working memory tasks as part of the “cognitive” cerebellum (Chen and Desmond, 2005; Luis et al., 2015) were found to be active for the B condition as learning progressed, suggesting a higher cognitive load during bilateral learning tasks.

In conclusion, right-handed elderly subjects' performance during early learning was similar when using the dominant and non-dominant hand, whereas bimanual learning was significantly slower and less accurate. Early learning was associated with activity increases for the dominant hand condition, mainly located in BG and left hippocampus and for the bimanual condition, mainly located in posterior parietal, occipital cortex and cerebellum. However, the non-dominant hand showed no activity gains during learning. Brain activity related to

accurate motor performance shared a cortico-basal-cerebellar network. However, bimanuality was associated with the employment of additional cerebellar resources in order to coordinate both hands. Additionally, the L condition showed recruitment of attentional brain resources when dealing with worsened motor performance. These results suggest that the non-dominant hand requires the use of additional brain resources in order to obtain the same results in motor performance. On the other hand, the results obtained with the dominant hand (additional recruitment of SMA and BG in order to optimize sequence timing) indicate the existence of an alternative network to improve performance in motor sequence learning. We also highlight the role of the cerebellum in MSL across all hand modalities. Overall, the present findings reveal new insights into early motor sequence learning in the elderly, providing evidence about the uniqueness associated to the use of the dominant, the non-dominant hand or both hands simultaneously.

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