Role of Broca’s Area in Implicit Motor Skill Learning: Evidence from Continuous Theta-burst Magnetic Stimulation

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Abstract

Complex actions can be regarded as a concatenation of simple motor acts, arranged according to specific rules. Because the caudal part of the Broca’s region (left Brodmann’s area 44, BA 44) is involved in processing hierarchically organized behaviors, we aimed to test the hypothesis that this area may also play a role in learning structured motor sequences. To address this issue, we investigated the inhibitory effects of a continuous theta-burst TMS (cTBS) applied over left BA 44 in healthy subjects, just before they performed a serial RT task (SRTT). SRTT has been widely used to study motor skill learning and is also of interest because, for complex structured sequences, subjects spontaneously organize them into smaller subsequences, referred to as chunks. As a control, cTBS was applied over the vertex in another group, which underwent the same experiment. Control subjects showed both a general practice learning effect, evidenced by a progressive decrease in RT across blocks and a sequence-specific learning effect, demonstrated by a significant RT increase in a pseudorandom sequence. In contrast, when cTBS was applied over left BA 44, subjects lacked both the general practice and sequence-specific learning effects. However, surprisingly, their chunking pattern was preserved and remained indistinguishable from controls. The present study indicates that left BA 44 plays a role in motor sequence learning, but without being involved in elementary chunking. This dissociation between chunking and sequence learning could be explained if we postulate that left BA 44 intervenes in high hierarchical level processing, possibly to integrate elementary chunks together.

INTRODUCTION

The ability to arrange, learn over practice, and then perform structured sequences is critical in most behaviors, such as language, music, but also in skillful movements that make humans so distinctive (Fadiga, Craighero, & D’Ausilio, 2009). This ability has been regarded as an ultimate factor of the human cognitive development (Corballis, 2003; Conway & Christiansen, 2001; Keele & Curran, 1996; Greenfield, 1991) and is mostly noticeable in language. Indeed, words composing sentences are not arranged randomly but have to comply with a precise hierarchical organization based on grammatical rules—or syntax—allowing the production of meaningful sentences. Similarly, complex actions, which also result from merging several simpler units, also critically depend on the ability to arrange them into the appropriate order, according to certain rules. Recently, it has been suggested that language and action may share the same syntactic processor, possibly located in Broca’s area, which could act irrespective of the domain (language, action, music, calculation, etc.) and whatever the nature of the sequences (perceptual, motor, or cognitive; e.g., Bahlmann, Schubotz, Mueller, Koester, & Friederici, 2009; Clerget, Winderickx, Fadiga, & Olivier, 2009; Fadiga et al., 2009; Fazio et al., 2009; Bahlmann, Schubotz, & Friederici, 2008; Koechlin & Jubault, 2006; Tettamanti & Weniger, 2006; Dominey, Hoen, Blanc, & Lelekov-Boissard, 2003).

Whether Broca’s area is also involved in learning new rules has been addressed in linguistics by using artificial grammar learning (AGL) tasks, taking advantage of subjects’ ability to detect and acquire implicitly a set of new syntactic rules from experience (Reber, 1967, 1989). AGL tasks are typically divided into two phases: an acquisition phase, consisting of learning implicitly a new syntactic rule, and a classification phase, in which subjects have to detect violations of the newly acquired rule. Functional neuroimaging studies have shown that AGL tasks activate a large network of brain areas, including the pFC, ACC, inferior parietal cortex, and regions in the occipital and temporal cortices (Forkstam, Hagoort, Fernandez, Ingvar, & Petersson, 2006; Lieberman, Chang, Chiao, Bookheimer, & Knolton, 2004; Petersson, Forkstam, & Ingvar, 2004; Skosnik et al., 2002; Seger, Prabhakaran, Poldrack, & Gabrieli, 2000; Fletcher, Buchel, Josephs, Friston, & Dolan,
In addition, some of these studies have reported an activation of Broca’s area in AGL tasks, suggesting that it may be involved in extracting artificial rules from different types of sequences, an ability which could underlie the acquisition of natural languages (Forkstam et al., 2006; Lieberman et al., 2004; Petersson et al., 2004). This view about the role of Broca’s area is further supported by clinical studies showing that patients with a lesion of this brain region, resulting in an agrammatic aphasia, have difficulties in performing AGL tasks (Christiansen, Louise Kelly, Shallock, & Greenfield, 2010; Dominey et al., 2003). Additional evidence for the role of Broca’s area in acquiring new syntactic rules comes from studies showing that the application of either transcranial direct current stimulation (de Vries et al., 2009) or off-line repetitive TMS (Udden et al., 2008) over the Broca’s area during the acquisition phase of AGL tasks enhances the subject’s performance in the classification phase. Finally, Floel, de Vries, Scholz, Breitenstein, and Johansen-Berg (2009), using diffusion tensor imaging, have shown that the ability to extract grammatical rules depend on the integrity of the white matter fiber tracts originating from Broca’s area.

However, whether Broca’s area is also involved in learning complex actions requiring syntactical processing remains puzzling. By analogy with AGL tasks, serial RT task (SRTT) could be used to address this issue because subjects learn a motor sequence which, without subject’s knowing, is repeated several times in consecutive blocks (Nissen & Bullemer, 1987). This procedure leads to a gradual decrease in response time across blocks, regarded as evidence for implicit learning. More importantly, because learning such a task relies, under certain circumstances, on the segmentation of the main sequence into several sub-sequences, known as “chunking” (Miller, 1956), SRTT seems appropriate to investigate, in combination with the TMS technique, the neural correlates of hierarchical/syntactic processing in the motor domain. Previous investigations in Broca’s aphasic patients have shown that such patients with left peri-sylvian lesions are still able to perform SRTT but, in these studies, no particular attention was paid to the chunking strategy used by participants (Dominey et al., 2003; Goschke, Friederici, Kotz, & van Kampen, 2001).

Our working hypothesis is that Broca’s area is involved in this chunking process, a view further supported by the results of some neuroimaging studies showing an activation of Broca’s area during SRTT (Bapi, Miyapuram, Graydon, & Doya, 2006; Bischoff-Grethe, Goedert, Willingham, & Grafton, 2004). Although this activation is clearly marginal when compared with that of the other cortical and sub-cortical structures (Bapi et al., 2006; Poldrack et al., 2005; Seidler et al., 2005; Bischoff-Grethe et al., 2004; Schendan, Searl, Melrose, & Stern, 2003; Willingham, Salidis, & Gabrieli, 2002; Grafton, Hazeltine, & Ivry, 1998; Hazeltine, Grafton, & Ivy, 1997), it is possible that the recruitment of Broca’s area varies during the course of the learning process, leading to a rather weak activation when averaged over a long period (Bapi et al., 2006). If this holds true, it is clear that functional imaging is not the most appropriate approach to determine the possible involvement of Broca’s area in SRTT. In addition, functional neuroimaging studies do not allow us to determine the causal contribution of a given area to the process under investigation (Bolognini & Ro, 2010; Walsh & Cowey, 2000). One way to circumscribe these limitations is to investigate the consequences of a transient inhibition of Broca’s area, as induced by continuous theta-burst TMS (cTBS), on SRTT performance.

In the present study, we investigated implicit learning in SRTT in two groups of subjects, in which cTBS was applied either over the caudal part of Broca’s area (left BA 44) or over the vertex (control group). We assessed the learning effects classically reported in SRTT (Robertson, 2007; Nissen & Bullemer, 1987), and we also quantified the chunking strategy used by the participants. Our prediction was that an inhibition of left BA 44 will impair the chunking pattern, leading to a deficit in sequence learning.

**METHODS**

**Subjects**

Seventeen healthy volunteers (nine women, age = 20–42 years, mean age = 27 years) participated in the experiment. They were all right-handed, as assessed by the Edinburgh Inventory (Oldfield, 1971) and were pseudo-randomly assigned to either the left BA 44 group (left BA 44 stimulation, n = 8) or to the control group (vertex stimulation, n = 9). All participants had normal neurological functions and met the safety criteria for TMS (Wassermann, 1998). The procedure was approved by the Ethic Committee of the Université Catholique de Louvain.

**Task and Experimental Procedure**

We used a classical SRTT (Nissen & Bullemer, 1987) in which subjects had to learn implicitly a motor sequence by associating four possible visual cues to a particular finger movement. In this task, the visual cues are, without subject’s knowing, presented in a fixed order, which is also repeated several times during a given block. This procedure led to a gradual decrease in RT across blocks, which is typically regarded as evidence for implicit motor skill learning.

In the present study, the visual cue was a white rectangle (9.55° wide and 13.37° high) displayed on a 21-in. computer screen (ViewSonic P227f, ViewSonic Corporation, Taiwan) at one of four positions arranged horizontally. Each screen position, designated as 1–4 from left to right, corresponded to a given response button on a computer keyboard (F5–F8) and, therefore, to the movement of a particular finger (Fingers II–V) of the right hand (see Figure 1A). Each visual cue was displayed until a key was pressed and the subsequent cue was displayed after an RSI of 250 msec, whatever the response was...
correct or not (see Figure 1B); this short RSI was chosen because it has proven to enhance sequence learning (Soetens, Melis, & Notebaert, 2004; Destrebecqz & Cleeremans, 2001). Participants were told to keep each finger on the appropriate response button during the block and to respond to each cue presentation as quickly and as accurately as possible. When an error occurred or when the RT was longer than 1000 msec, the screen background became red for 50 msec. At the end of each block, the subjects received a feedback about their speed (mean RT for the correct trials) and accuracy (number of correct responses in the block); those two values were displayed on the screen.

The experiment was controlled by a PC running a program written in Matlab (The Mathworks, Inc., Natick, MA). To minimize the measurement errors in RT because of the timing uncertainty of the operating system (Windows, Microsoft, Redmond, WA), we built a device to detect, with millisecond accuracy, the display of each frame on the computer screen and the subsequent key-press.

To maintain a high level of motivation throughout the whole experiment (Wachter, Lungu, Liu, Willingham, & Ashe, 2009), the participants were told that they will be rewarded proportionally to their performance. To do so, at the end of each block, a score was calculated based on the difference between the mean RT in Block 1 and mean RT in the current block (1 point/msec); this score was also affected by the number of errors (−0.5 point/error).

However, irrespective of their actual scores, all subjects received the same amount of money at the end of the experiment.

Finally, after the experiment, participants were informed about the existence of a repeated sequence in the blocks and they were asked to reproduce the sequence, or part of it, by pressing the response buttons (see below).

**Experimental Design**

The whole experiment contained eight blocks, seven blocks (Blocks 1–6 and 8) consisting of five repetitions of the same structured sequence of 20 elements and one block (Block 7) consisting of five repetitions of a 20-element pseudorandom sequence. In Blocks 1–6 and 8, subjects were presented with the following sequence: 314241342131234, in which 1, 2, 3, and 4 refer, respectively, to the four visual cues, from left to right. The order of the elements of the sequence was carefully determined so that it could be chunked as follows: the first eight items could be chunked into two subsequences (3142–2413), with the second one being the reverse of the first one. The six following items were two \( n - 2 \) repetitions (424-131) and the six last trials consisted of two triplets of contiguous digits (321-234). This chunking pattern was corroborated by the results of a pilot study performed on seven subjects, showing that they actually chunked...
The RT variation for each item with respect to its position in the sequence, which allowed us to determine the chunking strategy used by the participants. Indeed, chunks can be identified because the RT to items belonging to the same chunk should be different. Indeed, when compared with the first element of a chunk, the
subsequent elements of that chunk should, because they become more predictable, lead to a decrease in RT (Koch & Hoffmann, 2000; Rosenbaum, Kenny, & Derr, 1983).

Finally, as already mentioned, at the end of the experiment, participants were informed about the presence of a 20-item structured sequence repeated five times in Blocks 1–6 and 8. Participants were then asked to recall the sequence as accurately as possible by pressing the correct response buttons. In this free recall test, we scored both the length of the reported sequence and the number of elements correctly placed in that sequence. The latter is considered as an indicator of explicit knowledge of the sequence.

**Statistical Analysis**

First, to compare the initial performance between the two groups of subjects (control group vs. left BA 44 group), we performed a one-way ANOVA on RT for Block 1 (Statistica, StatSoft Inc., Tulsa, OK).

General practice and sequence-specific learning effects were analyzed by using repeated measures ANOVA (ANOVARM) with Group (control group vs. left BA 44 group) as between-subject factor and Block as within-subject factor. According to the various issues we wanted to investigate, the factor Block was composed as follows: to assess learning from Block 1 to Block 2, the Block factor had two levels (Block 1 vs. Block 2); when analyzing general practice learning, the Block factor had five levels (Blocks 2–6); to address the issue of the sequence-specific learning, the Block factor had two levels (Block 7 vs. Blocks 6 and 8). To analyze the chunking pattern at the end of learning, we performed an ANOVARM with Group as between-subject factor and Position (1, 2, 3, …, 20) as within-subject factor on averaged data from Blocks 6 and 8. Additionally, to characterize the progressive emergence of the chunks across blocks, we also performed an ANOVARM with Group as between-subject factor and Position (1, 2, 3, … , 20) as within-subject factor for each block separately, and we determined, in each block, the number of significant chunk(s). Then, the number of significant chunks was plotted against the block number. Lastly, to evaluate the effectiveness of chunks on motor performance, for Blocks 6 and 8, we computed the RT benefit for items INSIDE chunks (RTINSIDE) when compared with items OUTSIDE chunks (RTOUT). When appropriate, a Fisher’s least significant difference post hoc test ($p < .05$) was performed.

Finally, a one-way ANOVA was performed on the data of the free recall test (the number of elements recalled and the length of the correct sequence).

**RESULTS**

To ensure that the initial performance of subjects from both groups was identical, we compared the mean RT gathered for Block 1. The mean RT for Block 1 was $411 \pm 68$ msec (mean $\pm$ SD, $n = 8$) in the control group and $395 \pm 40$ msec ($n = 8$) in the left BA 44 group (Figure 3A). A one-way ANOVA with Group (control group vs. left BA 44 group) as between-subject factor confirmed that the two groups were not different in terms of RT ($F(1, 14) = 0.33, p = .57$).

**General Practice Learning**

First, an ANOVARM with Block (Block 1 vs. Block 2) as within-subject factor and Group as between-subject factor showed a main effect of Block ($F(1, 14) = 39.93, p < .01$), but no main effect of Group ($F(1, 14) = 0.46, p = .51$) on RT and no interaction between these factors ($F(1, 14) = 0.02, p = .89$), indicating that the performance increase from Block 1 to Block 2 was comparable in both groups (Figure 3A). The absence of difference
between groups was even more remarkable when RT was expressed in relative value with respect to Block 1 RT (Figure 3B).

Then to assess the general practice learning across blocks, we performed an ANOVA with Block (Blocks 2–6) as within-subject factor and Group as between-subject factor. This analysis revealed a significant main effect of Block ($F(4, 56) = 4.33, p < .01$) but no main effect of Group ($F(1, 14) < 0.01, p = .98$) on RT; it also showed a significant interaction between Block and Group ($F(4, 56) = 2.81, p = .03$). A post hoc test indicated that, only for the control group, RT for Block 2 was statistically different from RT in all other blocks (Blocks 3–6) ($p$s < .03) and RT in Block 3 was different from that in Block 6 (not illustrated, $p = .02$). These results show that, whereas control subjects gradually improved their performance with practice, subjects in the left BA 44 group failed to do so ($p$s > .33). This difference in the evolution of performance between both groups was even more noticeable when the RT change across blocks was expressed in relative value with respect to Block 1 RT (Figure 3B).

Clerget et al. 85

**Chunking Pattern**

To investigate how the subjects actually chunked the sequence independently of its intrinsic structure, we computed the mean RT for each item of the sequence (20 items per sequence) for each block (5 sequences per block) and for each group. Because the chunking pattern is supposed to be more robust—and therefore more detectable—at the end of the experiment, we started our analyses on averaged results from Blocks 6 and 8 (Figure 4); another reason for incorporating Block 8 data in this analysis was to control for any possible unspecific effect of fatigue. An ANOVA on these averaged RT was performed with Position (1, 2, 3, ..., 20) as within-subject factor and Group (control group vs. left BA 44 group) as
between-subject factor. This analysis did not show a main GROUP effect ($F(1, 14) = 0.13, p = .72$) nor an interaction ($F(1, 19) = 0.61, p = .90$) but revealed a significant main effect of Position ($F(1, 19) = 14.21, p < .001$), indicating that the RT varied as a function of the item position in the sequence. Because a chunk is characterized (1) by a longer RT for the first item and (2) by a significant decrease in RT for the subsequent item(s) belonging to the same chunk (Koch & Hoffmann, 2000; Rosenbaum et al., 1983), we only concentrated on results from post hoc analyses showing a significant effect of Position for adjacent items (item $n$ vs. $n + 1$); when a significant difference between RT was found for a given pair of neighboring items, we then tested the difference between the RT for item $n$ vs. $n + 2$, and so on. By using this approach, we found five chunks in Blocks 6 and 8, starting at Positions 1, 3, 6, 11, and 18 and for which at least the subsequent item showed a significant decrease in RT ($all p < .008$); three of these five chunks were triplets (see Figure 4). This finding indicates that subjects used a chunking strategy different from that we expected on the basis of the results of our pilot study (see Methods). However, more importantly, an ANOVA showed neither a main effect of Group nor a Group × Position interaction, indicating that subjects from both groups used the same chunking strategy.

To investigate the progressive emergence of chunks across blocks, we applied the same analysis we used for Blocks 6 and 8 to each individual block. These ANOVA confirmed the main effect of Position on RT ($all F(1, 19) > 5.82, all p < .001$) and, as previously, we identified the chunks based on the post hoc analysis results. Figure 5A shows the number of significant chunks for each block, clearly illustrating the progressive emergence of chunks across blocks, starting with one chunk in Block 1 to reach a plateau of 5 chunks in Blocks 5, 6, and 8. This analysis did not show the presence of chunks in Block 7.

**Figure 5.** Chunking pattern emergence across blocks. (A) Number of chunks across blocks. The number of chunks was determined in each block as shown in Figure 4 (see Methods, for details). The progressive chunk increase across blocks was best fitted with a four-parameter sigmoid ($R^2 = 0.9989$). Only one sigmoid was computed because the ANOVA (with GROUP as between-subject factor and POSITION (1, 2, 3, …, 20) as within-subject factor) did not reveal a main effect of GROUP nor an interaction between GROUP and POSITION. Because Block 7 consisted of a pseudorandom sequence, no chunk was present in this block and data from Block 7 were not incorporated in this analysis; this is symbolized by a dashed line between Blocks 6 and 8. (B) Evolution of the chunk formation across blocks. Block number is represented along the y axis. Each dot along the x axis represents one item position in the sequence. Colored dots connected to each other indicate that they belong to the same chunk; gray dots designate items outside a chunk. The five chunks (Items 1–2, 3–4, 6–8, 11–13, and 18–20) are depicted by different colors (respectively: dark blue, orange, purple, pink, and light blue). The first two chunks to appear were those including items situated at the two extremities of the structured sequence. There was no chunk in the pseudorandom block (Block 7). Along the x axis, for each item position (from 1 to 20), the actual item number (from 1 to 4) is also indicated (white number in black circles).
increase in chunk numbers across blocks was best fitted with a sigmoid function ($\hat{R}^2 = 0.99, \text{Figure 5A}$).

To characterize further the chunking pattern, we investigated the emergence of the different chunks across blocks (Figure 5B). Interestingly, in both groups, the first chunk that appeared was the first two-element chunk (Items 1 and 2, dark blue), already present in Block 1, then the last three-element chunk (Items 18–20, light blue, Block 3), followed by the two others chunks (Items 3–4 and 6–8, respectively, orange and purple, Block 4), and finally the last three-element (Items 11–13, pink, Block 5).

These results corroborate the well-known observation that the chunking process builds up gradually with practice (Sakai, Hikosaka, & Nakamura, 2004; Sakai, Kitaguchi, & Hikosaka, 2003) and show that the first chunks to emerge were those at the extremities of the sequence.

Finally, to evaluate the benefit of the chunking strategy, for Blocks 6 and 8, we performed an ANOVA with Group (control group vs. left BA 44 group) as between-subject factor and Item (in vs. outside chunks) as within-subject factor. This analysis showed a main effect of Item on RT ($F(1, 14) = 32.12, p < .001$) but no main effect of Group and no interaction between factors. The post hoc analysis indicated that $\text{RT}_{\text{IN}}$ (mean $\pm SD$: 332 $\pm$ 44 msec) was significantly shorter than $\text{RT}_{\text{OUT}}$ (mean $\pm SD$: 355 $\pm$ 47 msec, $p < .001$), showing that chunking had a global benefit on subject performance.

**Free Recall Test**

As mentioned in the Methods, at the end of the experiment, each subject was asked to recall and perform the sequence. A one-way ANOVA failed to show any difference for the factor Group (control group vs. left BA 44 group) both for the length of the reported sequence (5.9 $\pm$ 2.3 elements for the left BA 44 group and 6.1 $\pm$ 2.8 for the control group; $F(1, 14) = 0.04, p = .85$) and for the number of elements correctly positioned in that sequence (2.5 $\pm$ 1.9 for the left BA 44 group and 3.5 $\pm$ 1.8 for the control group; $F(1, 14) = 1.22, p = .29$). These results indicate that all subjects, irrespective of their group, remained largely unaware of the existence of a structured sequence repeated across blocks.

**DISCUSSION**

The aim of the present study was to determine whether the caudal part of Broca’s area (left BA 44) plays a role in learning motor sequences, in particular when such a learning requires to process the hierarchical relationship between different subcomponents of the sequence. To address this issue we used cTBS to inhibit temporarily left BA 44 in healthy subjects before they performed a SRTT. We used this task because, besides allowing us to investigate implicit motor skill learning (Robertson, 2007; Nissen & Bullemer, 1987), it also constraints subjects to organize spontaneously complex sequences into several subsequences—or chunks—characterized by a simple hierarchical relationship. This process has been proved critical for learning difficult sequences because the presence of a relational pattern between the items of the sequence improves sequence-specific learning (Kirsch, Sebald, & Hoffmann, 2010; Sakai et al., 2003; Koch & Hoffmann, 2000). Because it has been shown that Broca’s area is involved in processing hierarchically organized behaviors, we hypothesized that a transient inhibition of this area would impair the chunking process and, therefore, affect sequence learning.

Accordingly, we found that a temporary inhibition of left BA 44 altered implicit motor learning. In contrast to the result gathered in the control group, when cTBS was applied over left BA 44, subjects failed to show a decrease in RT across blocks, a parameter usually regarded as a measure of general practice learning (Jimenez, 2008; Robertson, 2007; Dominey et al., 2003; Goschke et al., 2001; Koch & Hoffmann, 2000; Nissen & Bullemer, 1987). A more specific measurement of sequence learning is typically obtained by contrasting the RT gathered for the blocks containing the structured sequence—when the general practice learning has reached a plateau—against the RT gathered in a block containing a pseudorandom sequence. The present study shows that, following a transient inhibition of left BA 44 induced by cTBS at the beginning of SRTT, this contrast failed to reveal a difference between RT; this lack of sequence-specific learning differs from what we found in control subjects and from results reported in the literature in patient with left peri-sylvian lesion (Dominey et al., 2003; Goschke et al., 2001). Because the results of the recall test performed at the end of the experiment showed that subjects from both groups were equally unaware of the existence of a structured sequence, the hypothesis that an inhibition of left BA 44 may have modified the level of explicitness of the sequence can be ruled out. In addition, the present results clearly indicated that the consequence of a momentary inhibition of left BA 44 on both general practice and sequence-specific learning cannot be explained by a difference in initial performance between groups (see Figure 3A) or an effect of cTBS on the performance improvement occurring between Blocks 1 and 2 (see Figure 3B). Altogether, the present results suggest that left BA 44 is causally involved in implicit skill motor learning as investigated with SRTT.

However, the present study failed to support our working hypothesis that the Broca’s area makes a significant contribution to the chunking process. Indeed, because it is well known that Broca’s area plays a role in processing hierarchically organized behaviors (Fadiga et al., 2009; Koechlin & Jubault, 2006; Tettamanti & Weniger, 2006; Dominey et al., 2003), it was sensible to assume that its temporary inhibition could actually impact the chunking strategy and, therefore, sequence-specific learning. The chunking process consists of splitting a large sequence...
into smaller subsequences of consecutive items, easier to memorize. Chunking was originally defined to account for the memory span (Miller, 1956) and is regarded as a strategy to enhance the amount of information stored in STM. Importantly, according to Miller, a chunk could refer to either digits, words, or any other meaningful units. In the context of motor control, the chunking process can be regarded as a way to split complex actions into simpler units or motor acts, each one being executable as individual motor program, which can be merged together to form a meaningful action (Rhodes, Bullock, Verwey, Averbeck, & Page, 2004; Rosenbaum et al., 1983; Lashley, 1951). In the present study, surprisingly, we found that inhibitory cTBS applied over left BA 44 altered both general practice and sequence-specific learning but left the chunking strategy unchanged and indistinguishable from that observed in control subjects.

Before discussing further this dissociation between chunking and learning, it is worth mentioning that our results are at odds with two previous studies performed in frontal aphasic patients (Dominey et al., 2003; Goschke et al., 2001). Indeed these two studies showed that these patients still present both general practice and sequence-specific learning. Several factors can explain this discrepancy. First, in these two aforementioned studies, the lesion location was not clearly documented. Second, it cannot be excluded that, following a stroke, a significant reorganization process had occurred, which might have contributed to a partial recovery. Finally, this discrepancy could also be explained by a difference in task difficulty. Indeed Goschke and collaborators (2001) used a sequence of 10 elements, repeated 40 times (Experiment 1) and an eight-element sequence, repeated 60 times (Experiment 2); Dominey and collaborators (2003) used a 12-element sequence, repeated 45 times. In contrast, in the present study, we used a longer sequence (20 elements) repeated only 50 times.

Nevertheless, the dissociation between chunking and sequence learning we reported in the present study is somehow puzzling because several authors have suggested a causal relationship between learning in various tasks, including SRTT, and chunking process (De Kleine & Verwey, 2009; Kirsch et al., 2010; Sakai et al., 2003; Verwey & Eikelboom, 2003; Koch & Hoffmann, 2000; Rosenbaum et al., 1983). In contrast, in the present study, we used a longer sequence (20 elements) repeated only 50 times.

Our conclusion about the contribution of left BA 44 to motor skill learning is consistent with the current view that Broca’s area is involved in integrating elementary components into higher-order hierarchical sequences (Koechlin & Jubault, 2000; Petersson et al., 2004; Gelfand & Bookheimer, 2003). Indeed, Koechlin and collaborators have suggested that a prefrontal network, including BA 44 and BA 45, processes hierarchically structured behaviors (Koechlin & Jubault, 2000; Koechlin, Ody, & Kouneiher, 2003). The finding that left BA 44 is causally involved in learning implicitly motor sequences is
reminiscent of the involvement of Broca’s area in learning artificial rules as tested in AGL paradigms (Christiansen et al., 2010; de Vries et al., 2009; Floel et al., 2009; Bahlmann et al., 2008; Udden et al., 2008; Forkstam et al., 2006; Petersson et al., 2004). Indeed, these studies provided evidence that Broca’s area, besides its well-known contribution to syntactic processing of “natural” language (Grodzinsky & Friederici, 2006; Gough, Nobre, & Devlin, 2005), also plays a role in detecting and using new artificial rules. More precisely, Broca’s area could be involved in the abstraction process that enables to comply with sequences of different nature. Consistently with this view, a patient study has shown that agrammatic aphasics with Broca’s area lesions are impaired in extracting the abstract structure in both linguistic and nonlinguistic sequences during learning (Dominey et al., 2005).

Alternatively, another possible explanation for the dissociation between chunking and sequence learning we reported in the present study is that a left BA 44 inhibition leads to deficit in motor performance rather than in learning. However, this hypothesis predicts that, in the two groups, the RT profile across blocks should be the same with a typical RT increase for the pseudo-random block (Block 7) and that the only difference between groups should be an upward shift of the RT profile for left BA 44 group when compared with the controls. Because we failed to observe a main group effect in the general practice learning analysis, the present results do not support this hypothesis although previous studies have indicated that left BA 44 may play a role in controlling the motor performance. Indeed, it has been shown that, in patients suffering from an apraxia of speech consequent to a left hemisphere stroke, presumably involving a lesion of Broca’s area (Hillis et al., 2004), the so-called “study time” (Immink & Wright, 1998, 2001) was significantly increased in tasks involving either finger or speech sequences (Maas, Robin, Wright, & Ballard, 2008). In the present study, the study time could not be computed because SRTT does not allow us to measure this parameter, but if we assume that Broca’s area plays a role in arranging the different elements of the sequence before its execution, its temporary inhibition should lead to longer response time in SRTT. This conclusion is consistent with a recent TMS study, in which we demonstrated an increase in preparation time induced by left BA 44 inhibition in subjects learning explicitly a motor sequence by observation (Clerget et al., submitted). However, this hypothesis about an increase in study time following left BA 44 inhibition cannot account for the lack of RT increase in the pseudo-random block. Therefore, although plausible, this explanation does not render null and void our conclusion that left BA 44 is involved in sequence-specific learning.

As mentioned in the Introduction, several neuroimaging and TMS studies have already tried to identify the different cortical and subcortical centers involved in SRTT. However, the respective contribution of the different structures found activated in these tasks remains puzzling for several reasons. First, the results from the literature are still largely discrepant. For instance, whereas an rTMS study has shown that the contralateral dorso-lateral pFC plays a key role in learning motor sequences (Pascual-Leone, Wassermann, Grafman, & Hallett, 1996), others studies have failed to reproduce these results (Wilkinson, Teo, Obeso, Rothwell, & Jahanshahi, 2010; Koch et al., 2006). Similarly, for the SMA, although this area has been found activated in SRTT (Seidlert et al., 2005; Grafton et al., 1998; Hazeltine et al., 1997; Grafton, Hazeltine, & Ivry, 1995), its causal involvement in learning has been questioned because of a lack of confirmation from TMS studies (Wilkinson et al., 2010; Pascual-Leone et al., 1996). Second, it is now clear that distinct networks are recruited during the different stages of learning (Bapi et al., 2006; Doyon & Benali, 2005; Press, Casement, Pascual-Leone, & Robertson, 2005; Toni, Ramnani, Josephs, Ashburner, & Passingham, 2001; Toni, Krams, Turner, & Passingham, 1998) and that a given network can show plasticity during learning (Steele & Penhune, 2010). As far as the BA 44 activation in SRTT is concerned, functional imaging studies indicate that its activation, if any, is very weak (Bapi et al., 2006; Bischoff-Grethe et al., 2004). One possible explanation for this finding is that these studies did not use hierarchically structured sequences soliciting the contribution of Broca’s area; alternatively, it has been suggested that the frontal regions, including the inferior frontal gyrus, could be mainly involved in the early phase of the learning process (Grol et al., 2007; Doyon & Benali, 2005; Toni et al., 2001) when the different chunking levels have to be implemented together.

Among the other structures found activated in SRTT, it has been suggested that the BG are involved in the chunking process as demonstrated in both animals (Levesque et al., 2007; Jog, Kubota, Connolly, Hillegaart, & Graybiel, 1999; Aldridge & Berridge, 1998; Graybiel, 1998; Cromwell & Berridge, 1996; Berridge & Whishaw, 1992) and humans (Tremblay et al., 2010; Boyd et al., 2009). A similar assumption has been made for the hippocampus, because its lesion impairs associative learning in SRTT (Curran, 1997), a finding confirmed in rodents (Ergorul & Eichenbaum, 2006). Further experiments will be required to bring together these results, but it could be assumed that, whereas subcortical structures could be responsible for low-level chunking, left BA 44 may play a critical role in higher-order chunking processes.

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