NIH Public Access
Author Manuscript
Neuropsychologia. Author manuscript; available in PMC 2011 December 1.

Published in final edited form as:
Neuropsychologia. 2010 December ; 48(14): 4003–4011. doi:10.1016/j.neuropsychologia.2010.10.019.

A case for the involvement of phonological loop in sentence comprehension

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Abstract

The specific role of the phonological loop in sentence comprehension is still a matter of debate. We tested the behavioural consequences of activity disruption in left BA 40 and BA 44, key regions of the phonological loop, on language comprehension using 1 Hz rTMS. Comprehension was assessed by means of two tasks: a sentence-to-picture matching task, with sentences varying in length and syntactic complexity (Experiment 1), and a sentence verification task (Experiment 2). rTMS over left BA40 significantly reduced accuracy for syntactically complex sentences and long, but syntactically simpler sentences, while rTMS over left BA 44 significantly reduced accuracy only for syntactically complex sentences. rTMS applied over left BA40 also impaired performance on sentences in which word order was crucial.

We suggest that the neural correlates of the phonological loop, left BA40 and BA44, are both involved in the comprehension of syntactically complex sentences, while only left BA40, corresponding to the short-term store, is recruited for the comprehension of long but syntactically simple sentences. Therefore, in contrast with the dominant view, we showed that sentence comprehension is a function of the phonological loop.

Keywords

phonological loop; verbal short-term memory; sentence comprehension; TMS

Introduction

A defining property of natural languages is the widespread occurrence of long distance dependencies, namely links of various types between non-adjacent linguistic items, such as the link between the subject "the dog" and the predicate "is chasing the cat" in the sentence "The dog [that the boy is watching] is chasing the cat". Processing of long distance dependencies requires syntactic computation within a pool of memory resources. In

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principle, these resources may rely on working memory (Baddeley & Hitch, 1974; Baddeley, 2000), e.g. the central executive and/or the phonological loop (PL), which is used in non-syntactic tasks, like remembering lists of digits or words. Alternatively, memory resources involved in language comprehension may be a specialized subset. The choice between these two positions is still an open issue.

The latter position is held by supporters of the very influential *Separate Sentence Interpretation Resource* (SSIR) theory (cf. Caplan & Waters, 1999 for a presentation of this theory and Fedorenko, Gibson, & Rhode 2006 for a criticism). The SSIR theory suggests that a separate subsystem is responsible for the syntactic and semantic operations performed online when a sentence is interpreted. If this hypothesis is correct, the central executive and/or the PL may have a role (if any) only in a post-interpretative stage.

Support for this theory comes mainly from negative results, showing that the impairment of the PL would not necessarily result in a shortfall in sentence comprehension. Indeed, patients with a selective impairment of the PL do not show any *major* deficit in language comprehension (Waters & Caplan, 1996; Howard & Butterworth, 1989). However, when syntactically more complex sentences are considered, several behavioral (Waters, Caplan, & Hildebrandt, 1987; Waters, Caplan, & Yampolsky, 2003) and neuropsychological data (Caramazza, Basili, Koller, & Berndt, 1981; Friedrich, Glenn, & Martin, 1984; Papagno, Cecchetto, Reati, & Bello, 2007) strongly suggest that the PL *is* involved. Nevertheless, the co-occurrence of language comprehension deficits and impaired PL does not allow concluding for a causal relation. It is possible that the neural correlates of both functions are adjacent and therefore both regions can be damaged by the same lesion.

Transcranial Magnetic Stimulation (TMS) has been increasingly used in language research (Devlin & Watkins, 2007): this technique, by means of a magnetic field and its associated electrical field, can be used to non-invasively stimulate a specific cortical site and interfere with information processing. TMS can be particularly useful to disentangle the above-mentioned issue, namely the PL involvement in sentence comprehension. As an interference technique, it can be used to show that a region that is active while a given task is performed is also essential for task performance. In addition, TMS allows studying healthy subjects, who can be used as their own controls, thus increasing experimental power and retest reliability and avoiding the confounding effects of the diffuse impairment and compensatory cortical plasticity associated with brain lesions.

Neuropsychological reports (for a review see Vallar & Papagno, 2002) neuroimaging (Paulesu, Frith, & Frackowiak, 1993; Henson, Burgess, & Frith, 2000) and rTMS studies (Romero, Walsh, & Papagno, 2006) converge in suggesting that the neural correlates of the PL include two discrete regions in the left hemisphere, namely the inferior parietal lobule (BA40) for the phonological short-term store (STS), and the inferior frontal gyrus (BA44) for articulatory rehearsal.

If the PL is involved in sentence comprehension, we reasoned, a “virtual lesion” induced using 1 Hz TMS over the left BA44 and BA40 should interfere with sentence comprehension and could unveil distinctions on the relative contribution of the two subcomponents.

Since contrasting patterns of impairment have been found according to the type of sentence used, in a first experiment we tested subjects' performance on a sentence-to-picture matching task in which length and syntactic complexity were manipulated. We expected that TMS effects would depend on the syntactic complexity of sentences.

Another crucial point is the type of task used (Caplan & Waters, 1999). Indeed, any effect of TMS over the PL neural correlates in a sentence-to-picture matching task could be attributed to the post-interpretative operation of matching the meaning of the sentence with the corresponding picture. In order to address this issue we ran a second experiment using a different task, namely a sentence verification task, which did not involve a matching operation.

In summary, two main points were investigated: whether the PL is involved in sentence comprehension and, if so, when this involvement takes place. These two issues were explored by means of rTMS in two different experiments. Previous studies have investigated the effects of event-related TMS on syntactic decision (Sakai, Noguchi, Takeuchi, & Watanabe, 2002) and of rTMS on an artificial grammar learning task (Udden et al., 2008). However, to the best of our knowledge, this is the first rTMS study focusing on natural language comprehension and PL.

Experiment 1

Material and Methods

Participants—Twelve healthy, right-handed, native English speaker volunteers participated in this experiment (5 males and 7 females; mean age: 30, SD 7, all graduated). Subjects were recruited through the National Institute of Health (NIH) Clinical Research Volunteer Program. Volunteers gave a written informed consent to participate in the study. Prior to recruitment, each subject underwent a complete physical and neurological exam and completed the TMS safety screen questionnaire (TASS; Keel, Smith, & Wasserman, 2001), to exclude any potential contraindication to the use of TMS. Handedness was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971): subjects with a Laterality Quotient less than +70 were excluded (mean score: + 96%, SD 9). The experiments were run in the laboratory of the Human Cortical Physiology and Stroke Neurorehabilitation Section, NINDS, NIH and at the Psychology Department of the University of Milano-Bicocca. The first study was approved by the National Institute of Neurological Disorders and Stroke (NINDS) Institutional Review Board and the second by the Local Ethical Committee of the University of Milano-Bicocca.

Before starting the experiment, subjects completed the Positive and Negative Affect Scales (PANAS) questionnaire, a 20-item self-report measure monitoring the affective state of the subjects (Watson, Clark, & Tellegen, 1988). The PANAS includes positive (PA) and negative affect (NA) factors with subjects rating the extent to which they feel a particular emotion on a 5-point scale. It was used to assess the presence of emotional distress, which might interfere with task performance. All the subjects enrolled in the experiment had a score in the NA (mean: 15, SD: 3) within one SD above the mean value of a reference population (Crawford & Henry, 2004).

Materials—A sentence to picture matching task was prepared using a limited set of high frequency lexical items (nouns: “boy”/“girl”, “man”/“woman”, “grandfather”/“grandmother”; verbs: “to give”, “to watch”, “to eat”). Four groups of English sentences were used, including 72 items each. Sentences in the four groups were characterised by an increasing complexity (see Supplemental Material).

1. Short sentences (Short): These were short sentences with no coordination or subordination: 24 were *active sentences* (e.g. “the dog is chasing the cat”), 24 *passive* (“the boy is kissed by the woman”), and 24 *dative* sentences (“the boy is giving the cake to the girl”).

2. Long sentences *without* long distance dependencies (Long): Length was increased by using coordination. There were 36 sentences with *noun phrase coordination* ("the girl is welcoming the man and the woman") and 36 with *sentential coordination* ("the boy is drinking milk and the girl is eating an apple"). These sentences although relatively long, contained no long distance dependency.

3. Sentences with long distance dependencies 1: relative clauses in right peripheral position (Complex1): Syntactic complexity was manipulated by selecting sentences with a relative clause, namely a type of subordination that introduces a computational burden (Grodner & Gibson, 2005). A relative clause contains a structural link (dependency) between two discontinuous positions. The position of the relative clause was right peripheral, i.e. it started after the end of the main sentence. In particular, we used subject and object relatives, i.e., the pronoun "that" (or "who"/"whom"), in the left periphery of the relative clause is linked to its canonical position as, respectively, the subject/object of the relative clause. The canonical position of an argument (called source position) is conventionally indicated with *e*, which stands for "empty category" or "gap", and the relative clause is enclosed by square brackets. Everything else being equal, the length of a dependency differs between subject and object relatives; in a subject relative such as "the man is watching the dog [that *e* is chasing the cat]" the dependency between the pronoun and its source position is shorter than a dependency involving an object, such as "the man is watching the cat [that the dog is chasing *e*]". However the two types of sentence were included in the same group as sentences involving a relative clause in the right periphery of the main clause.

The group included 24 *subject relatives in right peripheral position* (i.e. "the man is watching the dog that is chasing the cat"), 24 *object relatives in right peripheral position* (i.e. "the man is watching the cat that the dog is chasing") and 24 "*long*" *subject relatives* ("the boy is watching the man with a stick who is walking"). In the last type of sentence the distance between the noun modified by the relative clause and the gap has been made longer by adding some words ("with a stick"). The rationale for including the last type of sentence is having subject relatives in which the distance between the noun modified by the relative clause and its gap is roughly matched to the distance found in object relatives.

4. Sentences with long distance dependencies 2: relative clauses in centre embedded position (Complex2): In this group, complexity was further increased by changing the position of the relative clause: the relative clause was in a "centre embedded" position, in other words embedded within the main sentence instead of starting at its end. Centre embedded structures are particularly demanding for the syntactic parser, since they interrupt the processing of the main sentence (Gibson, 1998 for review).

The group comprised 36 *subject relatives in a centre embedded position* ("the dog that is chasing the cat is watching the girl") and 36 *object relatives in a centre embedded position* ("the man whom the woman is watching is eating pasta").

For each sentence a line drawing was created. Half of the line drawings correctly depicted the meaning of the sentence, half represented a picture with the same characters playing a different role in the same event (Figure 1).

For instance, in the case of a sentence such as "the man is pushing the dog that is biting the cat", the incorrect picture represented a man pushing a dog bitten by a cat. It was not possible to detect whether the picture was incorrect before the end of the stimulus sentence, since the incorrect part of the foil corresponded to the end of the sentence. All sentences pronounced by a native English speaker were digitized and played back for auditory presentation.

Experimental tasks

Two tasks were prepared and run on a computer using E-Prime (Psychology Software tools Inc., Pittsburgh, PA).

Sentence-to-Picture Matching Task—Subjects heard the sentences through headphones and 1000 ms before the end of the auditory trace of the sentence, a picture was displayed on the computer screen for 3000 ms. In this way the onset of the picture was uniform across trials even though the length of the auditory trace was variable (i.e. auditory traces of sentences in Long, Complex1 and Complex2 groups were longer than those in the Short group). Subjects were asked to judge if the picture correctly represented the meaning of the sentence by pressing one of two response keys. The task was run in 4 blocks, including 72 trials each (18 sentences for each group). The order of blocks was counterbalanced across subjects. Within each block, sentences of the four groups were presented in a random order.

Visual task—This task was designed to control for nonspecific effects of rTMS in response selection. It was run in 4 blocks, including 18 trials each. In each trial subjects were presented with a picture for 3000 ms and had to decide whether the picture was centered or shifted to the right of the computer screen. The same pictures as in the sentence comprehension task were used.

Procedure

The experiment consisted of 4 experimental sessions performed on 4 different days, at the same time of the day (morning vs. afternoon). During the experiment, subjects sat comfortably on a chair. The Nexstim Navigated Brain Stimulation system (NBS) and eXimia software (Nexstim Co., Helsinki, Finland) were used to guide TMS coil positioning. The eXimia software first processed the individual structural MRI brain scans in order to release a 3-D rendering of subject's head, visible on a screen monitor. The system then enabled the 3-D rendering to be aligned to the subject's head with a match accuracy set at 4 mm. Once the alignment was complete, it was possible to monitor the coil position over the 3-D rendering, thus allowing optimal TMS targeting. For each subject, resting motor threshold (rMT) of the abductor pollicis brevis muscle was assessed by stimulation of the primary motor cortex (M1). rMT is defined as the lowest intensity required to elicit a MEP of at least 50μV in a minimum of 5 out of 10 trials (Rossini et al., 1994). MEPs were measured using Signal software (Cambridge Electronic Design Ltd, UK).

rTMS was applied using a Magstim Rapid high-frequency magnetic stimulator (Magstim Co., Whitland, South West Wales, UK) with a focal figure-of-eight coil (dual 70 mm coil) at a frequency of 1 Hz, at an intensity equivalent to the 90% of the individual rMT, for a duration of 30 minutes. Previous studies have shown that 1 Hz rTMS interferes with the cognitive processing of a targeted region beyond the duration of the train of stimulation itself (see Robertson et al., 2003 for a review), producing sustained physiological responses as measured with EEG (Chen et al., 2003;Kähkönen, Komssi, Wilenius, & Ilmoniemi, 2005; Thut & Pascual-Leone, 2010). More specifically, the effects of rTMS at 1 Hz are shown to last for a period of time equivalent at least to half of the duration of the stimulation train. Therefore, when applying rTMS for 30 minutes, we were expecting the interference effect to last for 15 minutes after the end of stimulation. Subjects were asked to perform the two experimental tasks immediately after the end of stimulation. The two tasks were completed in less than 15 minutes.

The sites of stimulation were left BA 44, left BA40 and Vertex (CZ) defined as a point midway between the inion and the nasion and equidistant from the left and right intertrachial notches (Figure 2). The experimental conditions were four: three corresponding to the stimulation sites and a sham TMS condition over CZ. In the Sham condition a specific sham-coil system (Magstim Co) was used, which mimics the popping sound of the discharge associated with a real stimulation, without inducing a magnetic field. The Sham and the CZ stimulation were used as control conditions.

The NBS is able to precisely locate (mismatch error≤ 4mm) the relative positions of the subject's head and brain and the TMS coil by means of an optical tracking system, thus allowing continuous monitoring of the stability of stimulation coordinates during the session (Thielscher & Kammerer, 2002).

The order of presentation of the two tasks was counterbalanced across the 4 experimental conditions. The order of the conditions was randomized and counterbalanced across subjects. In both tasks accuracy and reaction times (RTs) were recorded.

In addition, potential confounding variables such as sleep, tiredness, attention and discomfort of stimulation were controlled for. Before each experimental session, subjects were invited to report how many hours they had slept the night before, and to indicate their level of tiredness on a 10-point Likert scale (from 1 “not at all” to 10 “exhausted”), and level of attention on a 5-point Likert scale (from 0 “distracted” to 5 “very focused”). Subjects with less than 5 hours sleep, tiredness level > 6 and low level of attention (0-1) were excluded from the experiment.

In addition, at the end of each experimental session, subjects were asked to complete a questionnaire in which they evaluated the discomfort caused by the stimulation and the difficulty of the tasks on a 5-point Likert scale (from 1 “not at all” to 5 “very painful”).

Results

Sentence-to-picture matching task—An ANOVA for repeated measures was performed on mean percentage of correct responses and mean RTs with the experimental condition (4 levels: left BA44, left BA40, CZ and Sham) and type of sentence (4 levels: Short, Long, Complex1 and Complex2) as within subjects factors. RTs were excluded from the analysis when the response was incorrect. Data were tested for sphericity using the Mauchly Test, which showed no significance. Planned comparisons (Duncan post-hoc test) were performed. The level of significance was set at .05.

Regarding accuracy, the mean percentage of correct response was 89.7% at Sham which can be considered the baseline, 87.8% at CZ, 84.5% and 83.8% when stimulating left BA44 and left BA40, respectively. This relatively high percentage of correct responses is due to the fact that although rTMS is often used to cause a so-called “temporary virtual lesion” on a stimulated area, actually it does not inactivate a region in the same way that a lesion does – instead it introduces a random, transient neural firing into the current computation (i.e. “noise”), thus leading to mild behavioural effects when applied to healthy subjects (Devlin & Watkins, 2006).

The main effect of condition was significant [$F(3,33) = 4.68$, $p<.005$, $p\eta^2 = .29$] with a higher number of errors when rTMS was applied over BA44 and BA40 in comparison to CZ and Sham conditions (Figure 3). The main effect of sentence type was also significant [$F(3,33) = 46.95$, $p<.0001$, $p\eta^2 = .81$], with a higher accuracy for Short and Long than Complex1 and Complex2. Finally, the interaction between condition and type of sentence was significant [$F(9,99) = 2.41$, $p< .05$, $p\eta^2 = .17$]. Since this value refers to an interaction effect, it should not be considered as modest (see McClelland & Judd, 1993).

Post hoc analyses showed that, in the case of Short, no significant differences were found among the 4 conditions. In Long, rTMS over left BA40 significantly reduced accuracy in comparison to left BA44 ($p<.05$), CZ ($p<.005$) and Sham ($p< 0.05$) stimulation, while rTMS over left BA 44 did not produce any effect (a paired t-test between BA44 and CZ mean number of correct responses in long sentences was not significant either [$t(11) = -1.3$, $p = .91$]). In the case of Complex1, accuracy was significantly reduced after rTMS over left BA44 and BA40 in comparison to CZ ($p< .005$ and $p<.05$, respectively) and Sham ($p<.05$) conditions. The number of correct responses did not vary significantly among left BA44 and left BA40, as well as among CZ and Sham. In Complex2, the number of errors increased after stimulation of left BA44 and BA40 in comparison to CZ ($p<.001$ and $p<.0001$, respectively) and Sham ($p<.05$). No difference in accuracy was found between left BA44 and left BA40. Similarly, no significant difference in accuracy was found among CZ and Sham.

As explained, object relatives contain a longer dependency than subject relatives. Accordingly, differences between subject and object relatives typically emerge (with an advantage for subject relatives) when the relative is centre-embedded (Gibson, 1998; Fedorenko et al., 2006; King & Just, 1981). For this reason, we ran a separate ANOVA for repeated measures on mean percentage of correct responses in subject and object relatives of complex 2 group with type of relative (2 levels: subject and object) and condition (4 levels: BA44, BA40, Cz and Sham) as within subjects factors. As expected, the main effect of relative type was significant [$F(1,11) = 5.34$, $p <.05$], with a lower accuracy for object relatives. The main effect of condition also was significant [$F(3,33) = 3.79$, $p <.05$], with a lower accuracy for left BA44 and leftBA40. The interaction between type of relative sentence and condition was not significant [$F(3,33) = .82$, p = n.s.].

Regarding RTs, the main effect of type of sentence was significant [$F(3,33) = 78,5$ $p <.001$, $p\eta^2 =.88$] with RTs increasing in Complex1 and Complex2 with respect to Short and Long.

Condition [$F(3, 33) = 0.7$, p = n.s.] was not significant, nor was the interaction between condition and type of sentence [$F(9, 99) = 0.8$, p = n.s.].

Visual Task—A repeated measure ANOVA on mean number of correct responses and RTs was performed with condition (4 levels: left BA44, left BA40, Cz and Sham) as within subject factor. The main effect of condition was not significant for both accuracy [$F(3, 33) = 0,65$, p = n.s.] and RTs [$F(3, 33) = 0.5$, p = n.s.].

Hours of sleep, level of tiredness and level of attention, discomfort of the stimulation and perceived difficulty of the task—For each of these variables, a repeated measures ANOVA was performed on the scores given by the subjects, with condition (4 levels: left BA44, left BA40, CZ and Sham) as within subjects factor. No difference was found among the four conditions either in the hours of sleeping, or in the level of tiredness or attention. Stimulation of left BA44 was rated as producing the significantly higher level of discomfort as compared with the other conditions. The sentence comprehension task was always rated as more difficult than the visual task (see Table 1 for a summary of the analyses)

Duration of the effect of TMS—To double check whether the effect of the TMS lasted until the end of the experimental session, we controlled for the number of errors made at the beginning vs end of the session for each stimulation condition (BA44, BA40, CZ) in the sentence comprehension task. Indeed, in each session the order of the two tasks (visual and sentence comprehension) was balanced. To verify whether the effect of TMS on sentence comprehension was still evident at the end of a temporal window of 15 minutes, we compared the blocks in which the sentence comprehension task was performed at the beginning of the session vs. the end. T-test were not significant in any experimental condition either for RTs (BA44: $t(5) = -.81$, p = n.s.; BA40: $t(5) = .32$, p = n.s.; CZ: $t(5) = -.37$, p= n.s.) or accuracy (BA44: $t(5) =.84$, p = n.s.; BA40: $t(5) = -.59$, p = n.s.; CZ: $t(5) = -.75$, p= n.s.; SHAM: $t(5) = 2.4$, p = n.s.)

Control Experiment—Since the sentence-to-picture matching task was rated as significantly more difficult than the visual task, a further control experiment was designed to balance the level of difficulty. Indeed, the percentage of correct responses in the sham condition, which can be considered as the baseline, was 95% in the visual task, and 84% and 82% in Complex1 and Complex2, respectively. Discrepancies in the level of difficulty were eliminated by checking that the two tasks (control task and sentence comprehension) had a comparable percentage of correct responses at the baseline. Six of the 12 subjects who took part in the previous experiment were called back to perform two additional experimental sessions.

A visual pattern task was prepared using checkerboards, with half of the squares black and half white. The task was divided in 2 blocks. Each block included 18 trials. In each trial a first checkerboard was presented on a computer screen for 500 ms. This was followed by a 2000 ms interval with a blank screen, after which a second checkerboard was presented, which could be identical to the previous or different for the position of one square (Figure 4). Subjects were asked to judge whether the two checkerboards were the same or not by pressing one of two response keys. The size of the checkerboards used in the task was established based on the results of a preliminary test, performed prior to the experiment. In this test, checkerboards of increasing size were used: 3×4, 4×5, 5×6, 5×7 and 5×8. For each of the 6 possible sizes, 15 trials were presented. The biggest checkerboard correctly matched in 85% of trials was then used in the experiment. In this way, the performances on the visual task and on the complex sentence comprehension task were equivalent at the baseline.

rTMS at 1 Hz, at an intensity of 90% of individual rMT was applied for 30 minutes over two sites: left BA40 and Cz. Left BA40 was chosen since its stimulation led to the most disruptive effects, thus being the best candidate to test whether rTMS effects were simply due to the difficulty of the task per se. Cz was the control condition. The two sites were stimulated in different experimental sessions, run in different days. The order of sites was counterbalanced across subjects. Immediately after stimulation, subjects were required to perform one block of the visual task. The time required to complete one block was less than 15 minutes; therefore the effect of stimulation was present until the end of the task.

Accuracy and RTs were recorded.

A paired T-test was performed on mean percentage of correct responses and mean RTs for the two conditions (BA 40 and CZ). No significant difference was found between the two conditions in both accuracy [$t(5) = 0.88$, $p > .05$] and RTs [$t(5) = 0.51$, $p > .05$]

Moreover, an ANOVA for repeated measures on the mean scores on the questionnaire concerning the perceived difficulty showed a significant main effect of task suggesting that the visual pattern task was regarded as more difficult than the sentence comprehension task [$F(1,5) = 17.5$, $p < .05$]. However, since only 6 out of 12 subjects took part in this experiment and only two sites were stimulated, we re-analysed the results of the same six subjects on sentence comprehension only for BA40 and Cz. The main effect of condition reached significance [$F(1,5) = 6.50$, $p = .05$], with a lower accuracy for left BA40 than for Cz; the effect of sentence was also significant [$F(1,5) = 12.05$, $p<.001$, $p\eta^2 = .70$], since accuracy was lower for Complex1 and Complex2 as compared to Short and Long. Finally, the interaction was significant [$F(3,15) = 3.74$, $p<.05$, $p\eta^2 = .43$].

Interim Comment: The results of Experiment 1 suggest that while processing of syntactically complex sentences makes use of both components of the phonological loop, processing of long but syntactically simpler sentences relies on the phonological STS only. On the other hand, short sentences do not require the intervention of either rehearsal or phonological STS. However, it could still be possible that the PL is required only in a post-interpretative stage when subjects match a sentence to the corresponding picture, as suggested by Rochon et al. (2000). In order to address this issue, we ran a second experiment not involving picture matching, namely a sentence verification task using material and procedure similar to those employed in Vallar and Baddeley (1984)'s study. In that study a patient with a defective STS showed a degraded performance in a sentence verification task with long sentences in which word order was crucial for a correct judgement (for example “the world divides the equator into two hemispheres: the northern and the southern”). Since Vallar and Baddeley used sentences controlled for length but not for syntactic complexity, only BA 40 was stimulated; yet, comprehension of long sentences is disrupted by BA 40 stimulation, irrespective to syntactic complexity, while BA 44 stimulation disrupts *only* syntactically complex sentences.

Experiment 2

Participants—Eight native English speakers (3 males and 5 females, mean age: 26.2; two college students and 6 graduate students) volunteered to the experiment. The same TMS parameters were used (rTMS at 90% rMT, 1Hz, 30 minutes).

Methods

Experimental task—A sentence verification task was prepared using two types of sentence, including 60 sentences each, 30 true and 30 false. All items were statements about the world and their truth/falsity could be determined on the basis of general knowledge. Group 1 was composed of long sentences in English (average: 19 words, range: 14-26). The false items were created inserting a semantically implausible element (for example “North America can be said with justification to be edible and nobody would be in disagreement with this statement”). Group 2 comprised English sentences of comparable length [$t(118) = -1.7$, $p >.05$], but their truth value depended on the positioning of two relevant items (for example “the order of days in the week is such that Tuesday is immediately followed by Monday, isn't it?”). Whereas in group 1 the statements were falsified by a semantic mismatch between two relevant items, in group 2 the falsity depended on a specific word order. All sentences were recorded by a female native English speaker and played back. The task was divided in two blocks, each run at the end of the stimulation train. Subjects heard the sentence through headphones and were asked to judge whether it was true or false by pressing one of two response keys. Subjects were explicitly required to be as fast as possible. Accuracy and RTs were recorded.

Procedure—rTMS at 1 Hz, at an intensity of 90% of individual rMT was applied for 30 minutes over two sites: left BA40 and Cz. The two sites were stimulated in different experimental sessions. The order of the sites was counterbalanced across subjects. Each block was performed immediately after rTMS stimulation. The time required to complete each block was within the 15 minutes window in which the rTMS effects would be expected to be present.

Accuracy and RTs were recorded.

Results

An ANOVA for repeated measures with Duncan's multiple range test for post hoc comparisons was performed on mean percentage of correct responses with condition (2 levels: left BA40 and CZ) and sentence type (2 levels: group 1 and group 2) as within subjects factors. Regarding accuracy, the main factor of condition was not significant [$F(1,7) = 4.5$, p = n. s.]. On the contrary, the main effect of sentence was significant [$F(1,7) = 12.4$, $p < .05$, $p\eta^2 = .64$], with a higher number of correct responses for group 1 than group 2. The interaction between condition and sentence was also significant [$F(1,7) = 7.0$, $p < .05$, $p\eta^2 = .50$] (Figure 5).

Post hoc analyses showed that left BA40 stimulation significantly increased the number of errors in group 2 sentences as compared to group 1 ($p < .001$), while in CZ there was no difference. The number of errors did not differ between left BA40 and Cz for group 1 sentences, whereas in the case of group 2, subjects produced significantly more errors after rTMS on left BA40 than on CZ ($p < .001$).

Regarding RTs, the main effect of condition was significant [$F(1,7) = 11.6$, $p < .05$, $p\eta^2 = .62$], with RTs increasing after left BA40 rTMS in comparison to CZ. The main effect of sentence was not significant [$F(1,7) = 2.7$, p = n.s.]. The interaction was not significant [$F(1,7) = .00$, p = n.s.].

Discussion

In the present study rTMS was used to investigate the role of the PL in sentence comprehension. Two experiments were run. Experiment 1 was designed to explore the PL involvement according to the type of sentence; to this purpose a sentence-to-picture matching task was used including sentences varying in length and syntactic complexity (containing or not long distance dependencies). rTMS was applied over two sites, namely left BA44 and left BA40, in order to tap the rehearsal and the phonological STS subcomponents of the PL. Experiment 2 aimed at ruling out the possibility that the PL involvement was due to the post-interpretative picture matching operation. To this purpose, a sentence verification task was used, including two types of long sentence, in one of which word order was crucial.

Comprehension of sentences containing long distance dependencies (Complex1 and Complex2) was impaired when rTMS was applied both over left BA40 and BA44, while comprehension of Long (long sentences *without* long distance dependencies) was disrupted only when TMS was applied over left BA40. Therefore, two main conclusions can be drawn. First, the PL (BA 40 and 44) seems to be involved in the comprehension of syntactically complex sentences, but not in the comprehension of Short (short sentences that are active, passive or dative); second, the phonological STS (BA 40), but not rehearsal (BA 44), seems to be involved in the comprehension of long, but syntactically simple, sentences (noun phrase coordination and sentential coordination). The first result is in line with neuropsychological studies showing that a deficit of the PL, due to damage to either the left frontal or the temporal cortex, does impair the comprehension of syntactically demanding sentences (Caramazza et al., 1981; Friedrich et al., 1984; Waters, Caplan, & Hildebrandt, 1991; Papagno et al., 2007). For instance, patient EA (Martin, 1987), whose lesion included the posterior temporal lobe, supramarginal and angular gyri, was impaired with centre embedded clauses, when the embedded clause was either a passive or an object relative. MC (Papagno et al., 2007), suffering from a selective deficit of the rehearsal component due to a lesion in the posterior part of the left frontal gyri, made a significantly higher number of errors in a sentence to picture matching task with object relatives in right peripheral position, as well as with subject and object relatives in centre embedded position. An additional patient, BO (Waters et al., 1991) was at chance level in complex structures like object relatives with written presentation and fall to 25% correct when she was given a limited viewing (15 seconds or less).

One crucial feature of Complex1 and Complex2 sentences is the presence of a long distance dependency. However, it could be suggested that the picture matching task is simply harder, regardless of any memory load these sentences may impose. Yet, the results of the control experiment showed that rTMS over left BA40 does not affect a visual task of the same level of difficulty. Similarly, it is unlikely that the discomfort caused by the stimulation interfered with task performance, since an off-line paradigm was used and discomfort did not outlast stimulation. Furthermore, left BA44 stimulation was evaluated as the condition with the highest level of discomfort, but rTMS over left BA40 impaired comprehension of Long (long sentences without long distance dependencies) significantly more than left BA44 stimulation. Moreover, the results of the questionnaire completed at the beginning of each experimental session rule out any aspecific effect of tiredness, hours of sleeping or attention.

The second result of Experiment 1 was the impaired comprehension of Long, when rTMS was applied over BA40. This is in line with MC's performance, whose deficits were attributable to a lesion involving BA44, sparing BA40: accordingly, her performance on long sentences without long distance dependencies was comparable to controls'.

Taken together, our results and MC's pattern on the same type of sentence suggest that while processing syntactically complex sentences requires both components of the PL, long sentences that are syntactically easier rely on the phonological STS only. Short sentences do not require any PL subcomponent.

An alternative interpretation is that the PL is required in a post-interpretative stage when subjects need to recover the content of the sentence and match it to a picture (Rochon, Waters, & Caplan, 2000). This interpretation would be compatible with the SSIR. We have provided evidence against this interpretation, though. First, in our experiment, the line drawing appeared on the screen 1000 ms before the end of the auditory trace of the sentence, so picture matching and on-line sentence parsing partially overlapped; moreover, the instructions were to be as fast as possible in answering, since RTs were recorded and it is likely that subjects started to process the drawing as soon as they could. Second, if rTMS had mainly an effect on picture matching, the interference should have been modulated also by picture complexity, with more complex pictures triggering a less efficient performance. It is intuitive that complexity of a picture depends on the number of elements a subject has to evaluate. However, the pictures (correctly or incorrectly) representing centre embedded relatives (see Figure 1) include three elements, as pictures representing a dative sentence. In a similar vein, pictures corresponding to dative sentences may be considered more complex than pictures corresponding to active sentences (involving only two elements). However, there was no evidence that dative sentences were harder to match than active ones. Indeed, an ANOVA for repeated measures on mean percentage of correct responses within the Short group showed no effect of condition [$F(3,33) = 0.93$], type of sentence (active, passive and dative: $F(2,22) = 1.98$), or significant interaction [$F(6,66) = 0.61$]. The third argument against considering the TMS effect only post-interpretative comes from patient MC (Papagno et al., 2007), who had a deficit of the rehearsal component and was impaired when tested with the same material used in Experiment 1 in an *on line* sentence comprehension task (self pace listening). A final argument is provided by Experiment 2, where we found an rTMS effect without a picture matching task.

We conclude by touching on a complex issue, namely the possibility of disentangling syntactic processing from working memory. Neuropsychological and neuroimaging studies have associated Broca's area with syntactic processing (Friederici, 2002 for a review) and, more specifically, activation of left BA44/45 has been observed for sentences with a non canonical order of words, such as object relatives (Cooke et al., 2001; Just, Carpenter, Keller, Eddy, & Thulborn, 1996), comparable to those used in this study. Therefore, it might be argued that rTMS interfered with syntactic competence rather than with the PL. Several considerations make this hypothesis unlikely, though.

First, left BA44 is involved in verbal short-term memory (STM) tasks, such as digit span, that do not require syntactic computation (Romero et al., 2006). Second, it has been suggested in functional neuroimaging studies that the increase of processing demand, due to the non-canonicity and long dependencies, is responsible for Broca's area activation (Friederici, 2006). Thus, during language processing, left BA44/45 comes into play whenever a reconstruction of sequential input at some level of representation is necessary. In this view, Broca's area activation in syntactic processing is consistent with the possibility that it might reflect also the involvement of a STM component. Accordingly, recent functional imaging data (Uchiyama et al., 2008) revealed that the processes for structure and memory operate separately but co-operatively in the left inferior frontal gyrus.

Processing a sentence involves a computation that, on one hand, is governed by (partially language specific) rules and, on the other hand, relies on various types of auxiliary resources, crucially including the PL. While it makes sense to distinguish the abstract knowledge of syntactic rules from the PL, memory resources are a defining part of any actual syntactic computation, at least for long/complex enough sentences. Having clarified this, the possible interference of rTMS might apply in two ways. The first possibility is that rTMS interferes with the abstract knowledge of syntactic rules. While this is logically possible, it is unlikely, since the overall level of accuracy of our subjects remained well beyond chance for all types of sentences, including object relatives in centre-embedded position, on which aphasic subjects, who have a damaged syntax or a difficult access to syntactic information, typically fail (Martin, 2006 for a review). Furthermore, the hypothesis that rTMS disrupts syntactic knowledge would not explain why stimulation over BA40, which is not associated to syntactic processing, has similar effect as stimulation over BA44.

The second possibility is that rTMS interferes with the actual syntactic computation. We think that this is what happens, and that is due to the rTMS detrimental effects on the PL, which is involved in sentence comprehension (contrary to what argued by the dominant SSIR theory). More specifically, we argued that the phonological STS (BA 40) is involved in the comprehension of long sentences, and both components, STS (BA 40) and rehearsal (BA 44), are necessary for the comprehension of syntactically complex sentences. Therefore, an additional task of the PL, beyond vocabulary acquisition, is complex sentence comprehension. Future researches combining different online paradigms should be used to corroborate our results. For instance, TMS-EEG technique, increasing TMS temporal resolution, would help in placing major constraints on results interpretation on processing, such as language comprehension, in which timing is crucial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by a grant from the MUR to CP and by the Intramural Research Program of NINDS, NIH.

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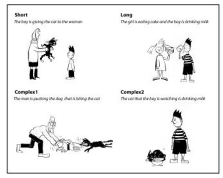

Figure 1.
Examples of the pictures used in the sentence comprehension task for each group of sentences. The pictures on the left side (pictures for Short and Complex1) provide an example of an incorrect matching to the sentence; the pictures on the right side (pictures for Long and Complex2) provide an example of a correct matching to the sentence.

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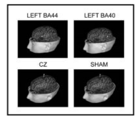

Figure 2.
Lateral view of a subject's 3-D head reconstruction, showing the stimulation sites for the four conditions. The location of left BA44 and left BA40 sites of stimulation were, on average, centred on Talairach coordinates, respectively, X = -46, Y = 2, Z = 16 and X = -44, Y = -32, Z = 24. The location for CZ and Sham condition was defined as a point midway between the inion and the nasion and equidistant from the left and right intertrachial notches.

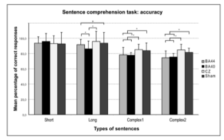

Figure 3.
Average accuracy in the four conditions (leftBA44, leftBA40, CZ and Sham) for the four groups of sentences used in the sentence comprehension task (Short, Long, Complex1, Complex2). Error bars represent standard deviations.
* indicates that leftBA44 and left BA40 rtMS significantly reduced accuracy as compared to CZ and Sham condition for complex1 and complex2. For Long group leftBA40 rTMS significantly reduced accuracy in comparison to leftBA44, CZ and Sham conditions.

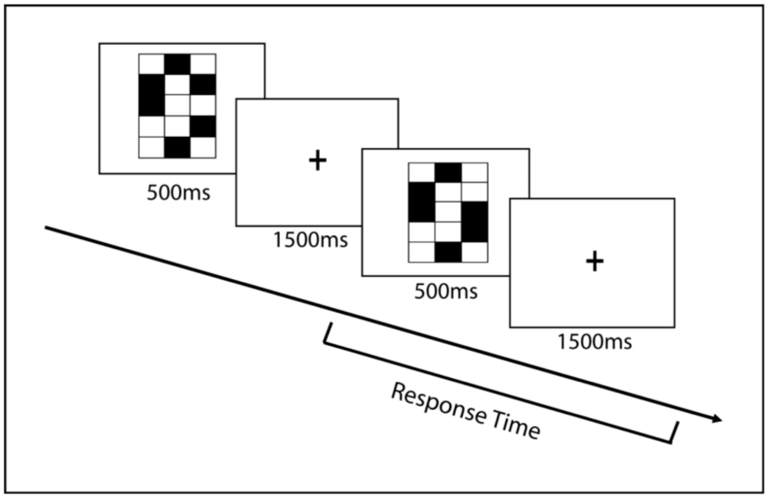

Figure 4.
Control study: visual pattern span. A first checkerboard appeared on the screen for 500 ms, followed by a 2000-ms interval. Then a second checkerboard was presented for 500 ms. In the following 1500ms the subjects had to respond whether the two checkerboards were the same or different by pressing one of two buttons on the keyboard.

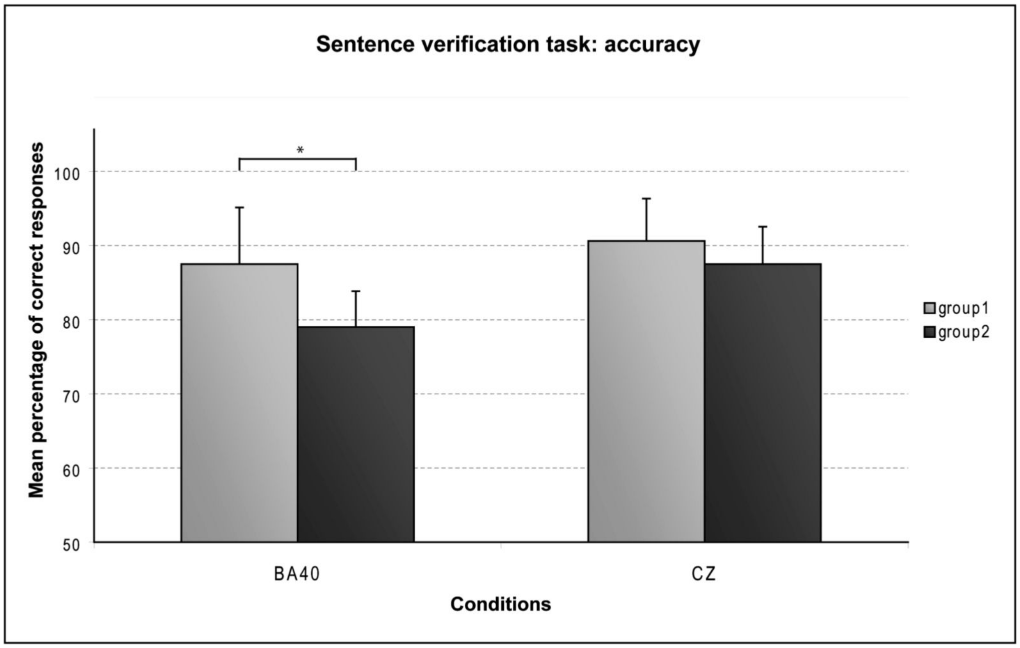

Figure 5.
Experiment 2: Sentence verification task. Average accuracy in the two conditions (left BA40 and CZ) for group1 (long sentences in which the truth/falsity depended on a semantic match/mismatch between two relevant items) and group2 (long sentences in which the truth/falsity depended on the word order. Error bars represent standard deviations.
* indicates that left BA40 rTMS significantly reduced accuracy for group2 in comparison to group1.

Table 1

CONDITIONS	Hours of sleep		Level of tiredness		Level of attention		Discomfort of the stimulation		Perceived difficulty of the task	
									Sentence comprehens.	Visual Task
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean (SD)	Mean (SD)
BA44	7.1	0.7	3.8/10	1.5	3.2/5	0.7	2.7/5*	0.6	2.8 (0.7)*	1.6 (0.9)
BA40	6.8	0.9	4.1/10	1.5	3.3/5	0.7	1.8/5	0.9	2.5 (0.6)*	1.8 (0.8)
CZ	6.4	1	4.1/10	1.6	3.3/5	0.7	1.8/5	0.9	3.0 (0.7)*	1.7 (0.9)
SHAM	6.5	1	4.0/10	1.8	3.4/5	0.5	1.4/5	0.5	3.0 (0.5)*	1.8 (0.9)

Mean scores and Standard Deviations (SD) for the 5 questionnaires checking for confounding variables are reported. The questionnaires were administered before (hours of sleep, level of tiredness and attention) or after (discomfort of the stimulation and difficulty of the task) each experimental session. Each session corresponds to one stimulation condition.

* Level of significance was set at $p < .05$. BA44 condition was rated as significantly more uncomfortable than the other conditions. In all conditions, the sentence comprehension task was perceived as significantly more difficult than the visual one.