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The inferior frontal cortex in artificial syntax processing: An rTMS study

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ABSTRACT

The human capacity to implicitly acquire knowledge of structured sequences has recently been investigated in artificial grammar learning using functional magnetic resonance imaging. It was found that the left inferior frontal cortex (IFC; Brodmann's area (BA) 44/45) was related to classification performance. The objective of this study was to investigate whether the IFC (BA 44/45) is causally related to classification of artificial syntactic structures by means of an off-line repetitive transcranial magnetic stimulation (rTMS) paradigm. We manipulated the stimulus material in a 2×2 factorial design with grammaticality status and local substrings familiarity as factors. The participants showed a reliable effect of grammaticality on classification of novel items after 5 days of exposure to grammatical exemplars without performance feedback in an implicit acquisition task. The results show that rTMS of BA 44/45 improves syntactic classification performance by increasing the rejection rate of non-grammatical items and by shortening reaction times of correct rejections specifically after left-sided stimulation. A similar pattern of results is observed in fMRI experiments on artificial syntactic classification. These results suggest that activity in the inferior frontal region is causally related to artificial syntax processing.

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1. Introduction

Humans are equipped with acquisition mechanisms that, as a consequence of information processing, have the capacity to implicitly extract structural regularities from experience. In his seminal work, [Reber \(1967\)](#) showed that the human brain is able

to implicitly acquire knowledge about formal (artificial) grammars and is able to discriminate between grammatical and non-grammatical items based on intuitive judgment in a grammaticality classification task after having been exposed to grammatical exemplars only and without performance feedback during implicit acquisition. The participants acquired this capacity

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without any explicit knowledge of the underlying grammar and Reber (1967) suggested that this implicit learning process is intrinsic to natural language acquisition.

The artificial grammar learning (AGL) paradigm is typically used to investigate higher order implicit learning (Forkstam and Petersson, 2005) and includes one or more implicit acquisition session followed by one or more classification sessions. Typically a sample of symbol sequences generated from a formal grammar is presented to the subjects for example as a short-term memory task during implicit acquisition. In the standard AGL paradigm, participants are subsequently informed that the acquisition sequences were generated according to a complex rule system, while not providing any information about the actual rules, and they are asked to classify novel items as grammatical or not based on their immediate intuitive impression (i.e., guessing based on 'gut feeling'). The robust finding that subjects discriminate reliably above chance between grammatical and non-grammatical items exemplifies the capacity for implicit acquisition of structured knowledge (Meulemans and Van der Linden, 1997). A complementary theoretical perspective suggests that the AGL model is useful in investigating the role of implicit learning in natural language acquisition (Gomez and Gerken, 2000; Petersson et al., 2004).

Recent fMRI studies of implicit AGL, in the sense briefly outlined above, have shown that the left inferior frontal cortex (IFC; Brodmann's area (BA) 44 and 45) is specifically sensitive to artificial syntactic violations (Forkstam et al., 2006; Petersson et al., 2004). The possibility that the grammaticality effect (for a recent reviews see Pothos, 2007; Forkstam and Petersson, 2005) depends on local substring familiarity (e.g., bigram and trigram acquisition frequency) has been raised by several researchers (Dulany et al., 1984; Perruchet and Pacteau, 1990; Pothos, 2007; Servan-Schreiber and Anderson, 1990). For example, classification strings containing substrings that occur frequently in the acquisition set might induce recognition or a feeling of familiarity that promotes subjects to endorse the string as grammatical independent of its actual grammaticality status. By controlling substring familiarity in balanced associative chunk strength (ACS) designs, studies have shown that substring familiarity has some effect on endorsement rates (e.g., Folia et al., 2008, submitted; Forkstam et al., 2006, 2008; Meulemans and Van der Linden, 1997). However, these studies show that the ACS effect is much smaller than the grammaticality effect (Folia et al., 2008, submitted; Forkstam et al., 2006; Forkstam et al., 2008) and more importantly that the grammaticality effect on classification performance is independent of substring familiarity (Folia et al., 2008, submitted; Forkstam et al., 2006; Forkstam et al., 2008; Meulemans and Van der Linden, 1997). It is also worth emphasizing that the grammaticality effect is independent of substring familiarity by the nature of the 2×2 factorial experimental design. Furthermore, fMRI studies show that medial temporal lobe is active when contrasting strings with high vs. low substring familiarity (Forkstam et al., 2006; Lieberman et al., 2004), while this region is not sensitive to grammaticality. In contrast, the left inferior frontal region (BA 44/45) is sensitive to grammaticality but not substring familiarity, while the right BA 44/45 is sensitive to both (Forkstam et al., 2006). In addition, when contrasting gram-

matical vs. non-grammatical strings, caudate nucleus activation is observed (Forkstam et al., 2006; Lieberman et al., 2004). This suggests that grammaticality is processed by a fronto-striatal network while local substring familiarity is processed by the medial temporal memory system. This is consistent with theoretical views that have characterized implicit learning in terms of four characteristics: (1) no or limited explicit access to the acquired knowledge; (2) the acquired knowledge is more complex than simple associations or exemplar-specific frequency-counts; (3) is an incidental consequence of information processing; and (4) does not rely on declarative memory (e.g., Forkstam and Petersson, 2005).

Taken together these results suggest that grammaticality cannot be reduced to substring familiarity and that the neural architecture subserving the processing of grammaticality and substring familiarity differ. However, fMRI characterizes the correlation between behavior, cognition, and the measured blood oxygenation level dependent (BOLD) signal, and it is in principle possible to argue that some of the observed activations potentially might be epiphenomenal to concurrent processing and not necessarily causally related to task performance. The main objective of the present study was to test the hypothesis that activity in the inferior frontal cortex (IFC; BA 44/45) is causally related to artificial syntactic classification by means of an MRI guided off-line 1Hz repetitive transcranial magnetic stimulation (rTMS) paradigm. In principle, any modulation of classification performance can be taken as evidence consistent with a causal involvement of the IFC in artificial syntax processing. The fact that the detailed effects of various rTMS parameters on cognition/behavior are not well-understood at present makes it difficult to predict in detail the behavioral outcome of any given rTMS paradigm. For example, the factors that determine whether TMS will improve or degrade task performance are currently not known (cf., Discussion). Moreover, no previous TMS study of the AGL paradigm has been reported and little is known about the role of the inferior frontal cortex in the type of implicit AGL paradigm we investigate. However, based on our previous fMRI studies (Forkstam et al., 2006; Petersson et al., 2004), it is reasonable to predict that rTMS of the left IFC will modulate the grammaticality effect, in particular the rejection rate of non-grammatical items, and not the effect of local substring familiarity, while rTMS of the right IFC will affect both.

One of the benefits of stimulating the brain off-line (i.e., when the participant is not engaged in an experimental task) is that this minimizes the unspecific effects of stimulation on task performance, including muscle twitches, peripheral nerve stimulation, the sharp TMS clicking noise, and the constrained position of the subject. Low frequency (0.5–4Hz) rTMS of the frontal cortex during 15–30min at 90–130% of the resting motor threshold have previously been shown to influence cognition (Mottaghy et al., 2002; Naeser et al., 2005; Nahas et al., 2001; Tanaka et al., 2005) and to produce sustained physiological responses measured with EEG (Chen et al., 2003; Kähkönen et al., 2005). In addition, effects at the receptor level have been observed, including the activation of the NMDA receptor, associated with reduced intra-cortical inhibition after low frequency rTMS (Ziemann et al., 1998). The effects of low frequency rTMS last for approximately half the time of the period of stimulation. In the present AGL study, we

manipulated the stimuli material in a 2×2 factorial design using local substring familiarity (ACS) and grammaticality as independent factors. After 5 days of implicit acquisition, subjects participated in a morning and afternoon classification session with rTMS. One target region (left or right BA 44/45) was stimulated in each session and the order was balanced across subjects. Classification sessions were administered before and immediately after each off-line rTMS stimulation. The results from a control experiment suggest that the rTMS results we report here are specific to the rTMS treatment. For a detailed description of all the experimental procedures see the final section, Experimental procedures, of this paper.

2. Results

2.1. Localization precision

The stimulation site in the left and right inferior frontal cortex (BA 44/45; centre of mass at MNI coordinates $[x, y, z] = [\pm 48, 16, 20]$ mm) was selected based on the averaged overlap of the left inferior frontal activation observed in two recent fMRI studies of artificial syntax processing (Forkstam et al., 2006; Petersson et al., 2004). The stimulation site is localized to BA 45, close to the border with BA 44 (Amunts et al., 1999; Eickhoff et al., 2005). A high-resolution structural MR volume was acquired for each subject and each MR image was spatially normalized to the standard MNI space in order to mark the rTMS sites. The marked subject anatomy was subsequently inversely transformed into the subject specific anatomical space by inverting the normalization transformation. We monitored the 3D coil position continuously during rTMS and the localization precision of the hand-held figure-eight TMS coil was in the order of millimeters (right–left axis: mean $\pm$ standard error = 1.3 ± 0.8 mm; anterior–posterior: 1.6 ± 1.0 ; superior–inferior: 2.5 ± 1.6). The TMS coil was always in permanent contact with the scalp. The mean scalp-to-cortex distance was comparable for the two rTMS conditions with an estimated precision of the marked sites in the order of ~ 1 –2 mm. Altogether, the achieved localization precision is well within the localization precision in average group anatomy after normalization, which is on the order of 10 mm (cf. Brett et al., 2002; Petersson et al., 1999).

2.2. Classification performance

The classification performance prior to rTMS was significantly above chance in all conditions (50% correct expected by chance; mean = $74 \pm 16\%$, one sample t -test $P < 0.01$) consistent with previous findings (Folia et al., 2008, submitted; Forkstam et al., 2006, 2008; Meulemans and Van der Linden, 1997; Petersson et al., 2004; Reber, 1967). There was no significant response bias before ($T(21) = -0.9$, $P = 0.4$) or after ($T(21) = -0.02$, $P = 0.98$) rTMS, and no significant interaction between the criterion characterizing response bias and the experimental manipulations ($P > 0.23$). The d -prime analysis on endorsement rates showed a significant increase in sensitivity to grammaticality over test occasion ($F(1,21) = 5.7$, $P = 0.03$). This was also reflected in the endorsement rates ($F(1,21) = 6.2$, $P = 0.02$, Fig. 1). The main effect of grammaticality ($F(1,21) = 134$, $P < 0.001$)

Fig. 1 – Percentage of endorsed items (i.e., perceived as grammatical) for grammatical and non-grammatical strings before and after 20min 1Hz rTMS. The results show a significant interaction of test and grammaticality ($P = 0.02$), driven by a significant improvement in the rejection rate of non-grammatical strings ($P = 0.04$). Error bars correspond to standard error of the mean.

and ACS ($F(1,21) = 7.0$, $P < 0.05$) were significant. The endorsement of non-grammatical strings significantly decreased after rTMS (before vs. after rTMS: ($F(1,21) = 4.6$, $P < 0.05$; planned comparison). Post hoc tests showed that rTMS did not affect the endorsement of grammatical strings ($P = 0.78$) and that the effect of test occasion (i.e., before vs. after rTMS) in the non-grammatical strings was significant after rTMS of the left Broca's region ($P = 0.03$) and only borderline significant after right rTMS ($P = 0.05$). No other interaction reached significance. Testing for the factor time of day (morning/afternoon) did not yield any significant main effect or interaction (overall $P > 0.50$).

We then added the factor block to the analysis, dividing each test into a first and last 5-min block. The main effect of block was significant ($F(1,21) = 5.31$, $P = 0.03$). Post hoc tests showed that the block effect was only significant when analyzing the tests related to left-sided rTMS ($P = 0.03$) and not right-sided ($P = 0.22$). Moreover, further post hoc tests showed that there was no significant block effect before ($P = 0.72$) but only after left-sided rTMS ($P = 0.03$). This suggests that the main effect of block resulted from the TMS treatment of the left inferior frontal region. Post hoc tests showed that the effect of test occasion in the non-grammatical strings was significant when we compared the first 5-min block of each test ($P = 0.04$) and non-significant during the last 5-min block ($P = 0.39$) which suggests that the TMS effect decreased over the 10 min of classification. Testing for the factor time of day (morning/afternoon) did not yield any significant main effects or interaction (overall $P > 0.10$). Taken together, these results suggest that rTMS of BA 44/45 resulted in improved classification accuracy mainly due to an increased correct rejection rate of non-grammatical items independent of substring familiarity. This effect was more pronounced after left BA 44/45 compared to right BA 44/45 rTMS.

Fig. 2 – Mean response times of correctly classified items. Low ACS strings in black and high ACS strings in grey before and after left (solid line) and right (dashed line) rTMS. The response time difference due to ACS is stable or decreasing after left BA 44/45 stimulation and increasing after right BA 44/45 stimulation. This is in line with fMRI results (Forkstam et al., 2006) showing that the right IFC is sensitive to ACS. No significant interaction with grammaticality was found. Error bars correspond to standard error of the mean.

2.3. Response times

The response time analysis showed a significant main effect of ACS ($F(1,21) = 37.7$, $P < 0.001$). The response times were in general shorter for low than high ACS strings (high = 1.84 ± 0.01 s; low = 1.67 ± 0.01 s). The main effect of grammaticality was non-significant but the interaction between grammaticality and ACS was significant ($F(1,21) = 20$, $P < 0.001$) and post hoc tests showed that there was a significant response time effect due to grammaticality for the low ACS strings ($P < 0.01$), with grammatical strings showing shorter reaction times than non-grammatical. This was not the case for high ACS strings ($P = 0.30$). The interaction of stimulation side, test occasion, and ACS was significant ($F(1,21) = 5.2$, $P = 0.03$, Fig. 2). The response time difference was due to a larger ACS effect after rTMS to the right side (test occasion $\times$ ACS: $F(1,21) = 5.47$, $P = 0.03$; planned comparison), while the rTMS to the left side did not modulate the ACS effect (test occasion $\times$ ACS: $F(1,21) = 0.8$, $P = 0.4$). No other main effects or interactions reached significance.

We then added the factor block (i.e., 1st and 2nd 5-min block of each test) to the statistical analysis and we found a significant interaction between side, test, and grammaticality present ($F(1,6436) = 5.97$, $P = 0.02$). Post hoc test showed that this effect was driven by a significant effect of test for the non-grammatical items on the left side ($P = 0.01$) not present on the right side ($P = 0.99$) or in grammatical items on either side ($P > 0.43$; Fig. 3). Thus the response time results suggest that the effect of TMS varied dynamically, with the first 5min showing a significant three way interaction between side, test, and grammaticality ($F(1,3120) = 4.62$, $P = 0.03$; planned comparison) for which was not present during the last 5min ($F(1,3295) = 1.75$, $P = 0.19$). This is consistent with the endorsement results which also showed a trend suggesting that the TMS effect decreased over the 10min of classification. We also analyzed the data collected in the morning separately from the data collected in the afternoon, as a between subject comparison.

The interaction between side, test and grammaticality did not reach significance in the morning ($F(1,2925) = 1.56$, $P = 0.21$) but in the afternoon ($F(1,3480) = 4.96$, $P = 0.03$), showing that the interaction was also present in a subset of the data from the same time of the day. Testing for the factor time of day (i.e., morning/afternoon) did not yield any significant main effects or interactions (overall $P > 0.50$).

Taken together, these results suggest that response time for correct rejections were modulated by side of stimulation and left-sided stimulation shortened while right-sided stimulation prolonged the response time. The response times were also modulated by substring familiarity and that this effect was greater after right compared to left rTMS consistent with previous fMRI results which showed that the right inferior frontal region was sensitive to ACS while the left (BA 44/45) was only sensitive to grammaticality and not ACS (Forkstam et al., 2006).

2.4. Control experiment

In addition to testing for the factor time of day (i.e., morning/afternoon), which did not yield any additional significant main effect or interaction suggesting that the performance pattern for both endorsement rates and response times in the two sessions were similar, we also controlled that subjects starting with rTMS of the left IFC behaved similar to those that started with rTMS of the right IFC. Overall they performed similarly ($P > 0.50$). Furthermore, in order to further investigate potentially confounding time effects in the pre-post rTMS design we analyzed independent behavioral data from a control experiment (cf., Control experiment). The control experiment was closely matched to the TMS experiment and comprised five consecutive days of implicit acquisition with

Fig. 3 – Mean response times of correctly classified NG items before and after left (grey solid line) and right (black dashed line) rTMS. This shows that NG items were driving the 3-way interaction between side, test and grammaticality, here depicted as the significant two way interaction: shorter response times after left and longer response times after right-sided rTMS. The interaction between grammaticality and test is significant after rTMS of the left ($P < 0.001$) but not the right IFC ($P = 0.97$). This suggests that the effect of TMS is regionally specific and related to left IFC processing of artificial syntactic violations as predicted by earlier fMRI results. Error bars correspond to standard error of the mean.

the same instruction and stimulus material as in the TMS experiment. On the fifth day the subjects participated in a grammaticality classification task which was divided into two equal pre- and post-session with a ~ 20min break in between as in the TMS experiment. We analyzed the endorsement rates by a repeated measures ANOVA in the same way as for the TMS data in order to examine whether there were any general effects related to the pre-post design of the TMS experiment. We first established that the subjects acquired knowledge of the underlying grammar in a similar manner as in the TMS experiment. We observed a significant effect of grammaticality ($F(1,31) = 59.9$, $P < 0.001$) and local substring familiarity ($F(1,31) = 17.2$, $P < 0.001$), while the interaction between grammaticality and ACS was non-significant ($F(1,31) = 1.9$, $P = 0.18$). These results are in general agreement with the TMS and previous behavioral results (e.g., Petersson et al., 2004; Forkstam et al., 2006). We then investigated potential effects related to the pre-post design. There was no main effect of test on performance ($F(1,31) = 0.3$, $P > 0.56$) and no significant interaction between test and other factors in the design. Thus, there was no evidence at any level for a test effect in the control experiment, suggesting that the effects observed in the TMS experiment was specific to the rTMS treatment.

3. Discussion

The results of the present study show that rTMS applied to the inferior frontal region (BA 44/45) improve syntactic classification performance by shortening response times after rTMS of the left inferior frontal region and by increasing the rejection rate of non-grammatical items after rTMS to the inferior frontal regions bilaterally. The specificity of both effects (improved correct rejection rate of non-grammatical but not of the acceptance rate of grammatical items; and shortened response times for correct rejections but not for the hits) and the regional stimulation specificity of the response time effect argues against an unspecific rTMS effect and is consistent with the results from the control experiment. The rTMS results suggest that the inferior frontal cortex (IFC), in particular the left IFC, is causally involved in artificial syntax processing. The same regional specificity is also observed in fMRI experiments on classification of artificial syntactic structures showing significant unilateral left (Petersson et al., 2004) and bilateral (Forkstam et al., 2006) activations of IFC (BA 44/45) specific for artificial syntactic violations. The response times were significantly modulated by substring familiarity and the finding that this was influenced more after right than left rTMS is also consistent with recent fMRI findings (Forkstam et al., 2006) showing that the right IFC is sensitive to both grammaticality and substring familiarity, while the left IFC is only sensitive to grammaticality but not substring familiarity. Thus, these results support the view that the left IFC is causally involved in artificial syntax processing and the relevance of the AGL as a model for implicit structural acquisition and related aspects of natural language processing (Petersson et al., 2004).

With the present experimental design, it is at least in principle conceivable that the rTMS effect on the endorsement

rates might be due to an order effect. In order to rule this out we analyzed the behavioral data from a control experiment. The result from the control experiment showed no order or any other time effects at any level of analysis. Moreover, control tests for a morning/afternoon effect suggested that the performance pattern was similar in both TMS sessions and comparisons between those subjects who initially received left to those who received right IFC rTMS showed no difference. In addition, we observed similar improvements for the first and second rTMS session. Taken together, this suggests that the specific increase in correct rejection rate observed in our experiment is an effect of rTMS of the IFC. Moreover, it is possible that contra lateral effect of the right IFC stimulation can explain the endorsement rate effect seen after right-sided stimulation. This is consistent with the result suggesting that the effect was more pronounced after left IFC compared to right IFC rTMS. Moreover, since the reaction time results showed two interactions: stimulation side and grammaticality as well as stimulation side and substring familiarity, the rTMS effect was sufficiently localized in this respect. In other words, the rTMS effect was not non-localized to such an extent that the interaction between left- and right-sided stimulation could not be detected.

In the present context and from a theoretical vantage point, we note that it is possible to translate the transition graph of the Reber machine (Fig. 4) into a framework based on lexical unification grammars (Hagoort, 2005; Jackendoff, 2007; Vosse and Kempen, 2000; Forkstam and Petersson, 2005). In this picture, unification of structured representations correspond to state transitions in the underlying computational machine (Petersson et al., 2005). These are equivalent to computational steps of a dynamical system, corresponding to the grammar, acquired by a recurrent neural network (Petersson, 2005, 2008). One might speculate that the neural implementation of the corresponding dynamical system is a mechanism in which lateral inhibition enables the system to parse structured

Fig. 4–The computational machine corresponding to the Reber grammar here given by its transition graph. This is a visualization of the set of rules that determines the underlying structure of the stimuli. A grammatical string is generated if the letters naming valid transitions (arrows) while going from the start node to the end node are concatenated in the order they are generated. The #-sign is the empty end of string character.

sequences and select the appropriate responses during classification (Petersson et al., 2005). For example, if it is assumed that rTMS inhibit excitatory neurons in the IFC, a general increase in inhibitory functionality might enhance classification performance by more efficiently rejecting non-grammatical strings, which would be consistent with the results reported here. We note that part of the dynamics in the computational language model of Vosse and Kempen (2000) also is based on lateral inhibition. In this model, lexical frames (i.e., lexically anchored elementary syntactic trees) are activated and selected by the input and sentence-level syntactic structures are created by unification of lexical frames under competitive inhibition in an on-line unification space.

The finding that rTMS of the IFC (BA 44/45) resulted in a performance improvement in artificial syntax processing replicates and extends earlier TMS findings that show performance improvements during natural syntax processing (Sakai et al., 2002). Improved probabilistic classification performance of implicitly acquired patterns has also been found after transcranial direct current stimulation of the prefrontal cortex (Kincses et al., 2004). In particular, we observed a performance improvement on measures of accuracy in addition to modulations of response times and both of these effects have been observed in natural syntax processing (Sakai et al., 2002). It is important to note that the response time and the endorsement rate both show improvement after rTMS and are thus unlikely to reflect potential strategic changes in processing, including for example speed–accuracy trade-offs.

Several other natural language processing studies have reported performance improvements after TMS. For example, rTMS applied to the left prefrontal cortex improved analogical reasoning (Boroojerdi et al., 2001), while applied to Wernicke's region it improved language perception (Andoh et al., 2006) and picture naming performance (Mottaghy et al., 2006; Töpper et al., 1998). Findings of enhanced phonological memory, by eradication of the phonological similarity effect, after TMS of the left inferior parietal region has also been reported (Kirschen et al., 2006). Moreover, Naeser et al. (2005) showed that rTMS applied to the right IFC, as a 2–8-month treatment of Broca's aphasia, improved picture naming. These findings suggest that TMS perturbation of a neural network does not necessarily lead to impaired performance. On the other hand, there are also studies on language where TMS applied to the left prefrontal cortex produced impaired performance: reaction time increases were observed in a production task with verbs but not nouns (Shapiro et al., 2001) and in a semantic but not a phonological control task (Devlin et al., 2003). The latter dissociation was recently extended to a double dissociation between anterior and posterior left IFC (Gough et al., 2005). In addition, impairments in the ability to read aloud (Epstein et al., 1999) and to match pictures to words (Flitman et al., 1998) have also been reported after TMS stimulation of the left IFC. Taken together, these results seem to suggest that the factors determining whether TMS will improve or degrade task performance are currently not well-understood.

In the current study, we found opposite response time changes after rTMS of the left and the right IFC. This is consistent with the inter-hemispheric inhibition theory

(Chiarello and Maxfield, 1996). The response time results show an effect of grammaticality which is significantly modulated by left-, but not right-sided stimulation as predicted by fMRI results (Petersson et al., 2004; Forkstam et al., 2006). However, since the results also show a lateralized effect to the right in relation to substring familiarity, one might speculate that the opposite pattern reflects a specialization of the right IFC. The right IFC has been implicated in successful response inhibition (Aron et al., 2003). It is therefore possible that a high substring familiarity induces an initial bias to endorse a string but that this response is inhibited by the right IFC in order to ensure high classification performance. This would be consistent with the longer response times observed for strings with high substring familiarity after rTMS of the right IFC compared to the left. The hemispheric specificity of the reaction time effect suggests that the response time measure might be a sensitive measure of hemispheric specialization. Reaction times can be seen as dependent not only on IFC but also on the interactions between IFC, lower level perceptual systems, and systems generating motor responses, while the endorsement rate might reflect the syntactic processing itself. This provides a tentative explanation for why the regional specificity is more pronounced in the response times compared to the endorsement rates, which is consistent with previous fMRI results (Petersson et al., 2004; Forkstam et al., 2006).

4. Conclusion

The results of the current study provide support for the hypothesis that the left inferior frontal region (BA 44/45) is causally related to artificial syntax processing. In addition, our results provide support for the finding that the right inferior frontal cortex (BA 44/45) is more sensitive to substring familiarity compared to its left homotopic Broca's region as reported in previous fMRI studies.

5. Experimental procedures

5.1. Participants

Twenty five right-handed healthy native Dutch university students volunteered to participate in the study (18 females, 7 males, mean age $\pm$ standard deviation = 23 ± 3 years; mean university education = 3.7 ± 1.6 years). They were all pre-screened for relevant medical history, medication use, drug abuse, head trauma, neurological or psychiatric illness, and family history of neurological or psychiatric illness. All subjects had normal or corrected-to-normal vision. All participants gave written informed consent according to the Declaration of Helsinki. All subjects were explicitly informed that they could stop their participation at any time without having to give any explanation for this and we encouraged the subjects to do so if that was the case. The local medical ethics committee at the UMC St. Radboud approved the study. Three subjects were excluded from further analysis on the basis of technical problems with the TMS equipment, overheating TMS coils, or heat and noise during the TMS session.

Fig. 5 – Localization of TMS target sites. Left: the bilateral inferior frontal target regions (BA 44/45) of stimulation. Right: the left inferior frontal stimulation site.

5.2. Stimulus material

We generated 569 grammatical (G) strings from the Reber grammar with a string length of 5–12 symbols from the alphabet {M, S, V, R, X}, see [Figure 4](#). We calculated the specific associative chunk strength (ACS, cf., [Knowlton and Squire, 1996](#); [Meulemans and Van der Linden, 1997](#)) for each string in relation to the complete set of 569 strings. In an iterative procedure 100 strings were randomly selected and tested with respect to its ACS content in order to generate an acquisition set which was representative in terms of ACS in comparison to the complete string set. The classification sets were subsequently derived from the remaining 469 grammatical (G) strings and for each of these a non-grammatical (NG) string was derived by a switch of letters in two non-terminal positions; these strings were selected to match the grammatical strings in terms of both terminal and complete string position ACS (i.e., collapsed over order information within strings). The strings were further classified in terms of their ACS status independent of grammatical status. For example, high ACS classification strings are composed of common bi- and trigrams in the acquisition set while low ACS strings are composed of uncommon bi- and trigrams, for both terminal and complete string position (for further technical details see [Forkstam et al. 2006](#); [Meulemans and Van der Linden, 1997](#)). Finally, 4 sets of 100 strings each were randomly selected from the 469 grammatical and their matched 469 non-grammatical strings in an iterative procedure, such that for a given

classification test set (1) the high ACS strings did not differ significantly in terms of ACS compared to either the acquisition set or the high ACS strings in the other test sets, and (2) the low ACS strings differed significantly compared to both the acquisition set and the high ACS strings in the same and in the other test sets. Thus the classification material was organized in a 2×2 factorial design with the factors grammatical status (grammatical/non-grammatical) and ACS status (high/low), and each classification set included 25 strings from each category: high ACS grammatical (HG), low ACS grammatical (LG), high ACS non-grammatical (HNG), and low ACS non-grammatical (LNG).

5.3. Procedure

The complete experiment spanned 5 days with one implicit acquisition session each day. On the first day a high-resolution anatomical MR image was acquired for stereotactic localization of the TMS coil in relation to the subject specific target regions. The subject specific motor threshold was determined (cf., below). After the acquisition session on the last day (day 5) a morning and an afternoon rTMS session followed, both including two classification sessions. One target region (left or right IFC BA 44/45, see [Fig. 5](#)) was stimulated in each rTMS session and the order was balanced across subjects. Classification tests were administered before and immediately after each off-line rTMS stimulation. Each classification test lasted 10 min.

5.3.1. Implicit acquisition task

The acquisition task (~ 25 min) was presented as a short-term memory recall task to the subjects. During the acquisition task, each string was presented for 4 s (whole string presentation), centrally placed on a computer screen using the Presentation software (nbs.neuro-bs.com). After the string disappeared from the screen, subjects recalled the string by typing on a keyboard in a self-paced fashion. Subjects were allowed to correct themselves but no performance feedback was provided. The subjects were only exposed to grammatical examples and the presentation order of the 100 grammatical strings in the acquisition set was randomized over the acquisition sessions.

5.3.2. Classification task

Two 10 min classification tests (before and after rTMS) were administered in the morning and the afternoon rTMS sessions ([Fig. 6](#)). Subjects were informed about the existence of a complex system of rules used to generate the acquisition strings, but they were not informed about the actual rules,

Fig. 6 – An anatomical MRI for subject specific target region registration was acquired on day 1 and the subjects' resting motor threshold (MT) to TMS stimulation was determined. Five acquisition sessions (short-term memory task with acquisition string set; grammatical strings only without performance feedback) were administered separated into five consecutive days. On day 5, the subjects participated in an off-line rTMS experiment where their ability of making grammaticality classifications was manipulated by left- and right-sided rTMS of the target region (BA 44/45).

before the first classification session. They were then instructed to, as correct and as fast as possible after string onset, classify novel strings as grammatical or not based on their immediate intuitive impression (i.e., guessing based on 'gut feeling'). One hundred strings (50/50% G/NG and independently 50/50% H/L) were presented, one at a time, on the computer screen. The whole string was presented for 2.5s followed by an inter stimulus interval of 2–2.5s. Thus, participants had 4.5–5s to make their decision and push the corresponding key with their left or right index finger. The response hand was balanced across classification tests and across subjects. The order of presentation of the 4 classification sets was balanced over subjects.

5.3.3. MRI data acquisition and TMS localization

The stimulation site in the left and right inferior frontal cortex (BA 44/45; centre of mass at MNI coordinates $[x, y, z] = [\pm 48, 16, 20]$ mm) was selected based the averaged overlap of the left inferior frontal activation observed in two recent fMRI studies of artificial syntax processing (Forkstam et al., 2006; Petersson et al., 2004). A high-resolution structural MR image volume was acquired using a T1-weighted 3D magnetization-prepared rapid gradient-echo pulse sequence (MPRAGE; volume TR = 2250ms, TE = 3.68ms, 15° flip-angle, 176 sagittal slices, slice-matrix size = 256×256 , slice thickness = 1mm, isotropic voxel size = 1mm³). Each MRI image was spatially normalized to the standard MNI space using SPM 2 (www.fil.ion.ucl.ac.uk/spm). This was done in order to mark the normalized subject anatomy with the part of the left and right inferior frontal cortex (BA 44/45) that showed specific activation to artificial syntactic violations in our previous fMRI experiments (Forkstam et al., 2006; Petersson et al., 2004). The marked subject anatomy was subsequently inversely transformed into the subject specific anatomical space by inverting the normalization transformation.

5.3.4. TMS stimulation protocol

The marked subject specific MRI images were co-registered with the figure-eight TMS coil and the subject's head using the BrainSight Frameless Stereotactic System (Rogue Research, Montreal, Canada). During the delivery of the rTMS pulses, the 3D position of the coil was continuously monitored and recorded. This information was used to quantify the localization precision of the rTMS stimulation. The distance between the actual and the target coil position was used as a measure of the localization precision of the rTMS stimulation. The second rTMS session was performed > 5h after the first in order for any carry over effects to wash out (Berpohl et al., 2005; Oliveri et al., 2004).

The off-line rTMS stimulation consisted of a 20min continuous biphasic pulse train at 1Hz. The intensity was set to 110% of the subject's resting motor threshold (MT). The MT was defined as the intensity required to evoke a motor potential > 50μV in the right first dorsal interosseus muscle in at least 5 out of 10 trials. This level of stimulation was well-tolerated by all participants and the concurrent stimulation of facial muscles and the trigeminal nerve was limited and mainly related to the masseter muscle. Repetitive TMS was delivered through a hand-held focal figure-eight double coil with a 70mm wing diameter (Magstim Co., Whitland, Dyfed, UK) held tangentially

to the scalp with the focal point of the coil as close as possible to the marked target position and always in contact with the scalp of the subject. We used the TMS equipment in the biphasic pulse mode. Because of the figure-eight double coil, the current flow is both clock- (left-wing) and anti-clockwise (right-wing). On the coil midline the left- and right-wing coils overlap, and the coil current flow is towards the handle during the first phase of the biphasic pulse, which reverses during the second phase. The induced cortex current is opposite the current flow in the TMS coil.

5.4. Data analysis

Repeated-measures ANOVA was used for the analysis of the data using SPSS 14.0 and a significance level of $P < 0.05$ were used throughout. Tukey's HSD post hoc test was used for further analysis, except for three planned comparisons where clear prior hypotheses were present, which are explicitly noted in the results section. We analyzed the classification performance with endorsement rate as the dependent variable using stimulation side (right/left), test occasion in relation to rTMS (before/after), grammaticality (G/NG) and ACS (high/low) as within factors. The endorsement rate is defined as the number of strings classified as grammatical independent of their actual status, divided by the total number of recorded answers for each factor level (Meulemans and Van der Linden, 1997). D-prime and response bias were calculated using standard signal detection theory (Hochhaus, 1972). The mean accuracy and reaction time was calculated and is reported together with the standard error of the mean. The response time was calculated from the presentation onset until the time of response. Due to the high performance rate the response time analysis was performed on correctly classified strings only. In further analyses, we also included the factor block, the first and the last 5min block of each pre- and posttest. The reason for this was to investigate the potential dynamic nature of the TMS effect and possible within-test changes in the TMS effect. With respect to the planned comparisons, the repeated measures ANOVA, and subsequent post hoc tests, we used a sequential hypothesis testing approach. Logically, the planned comparisons precede the repeated measures ANOVAs and the subsequent post hoc tests. We use a well-known approach for controlling the level of specificity entailed by the multiple comparisons in sequential hypotheses testing devised by Holm (1979) and Dunnett (1955).

5.5. Control experiment

In order to further investigate potentially confounding time effects in the pre-post-rTMS design we analyzed independent behavioral data (part of a larger AGL study; Folia et al., submitted). Thirty-two new right-handed (16 females, mean age $\pm$ SD = 22 ± 3 years; mean years of education = 16 ± 2) healthy Dutch university students were included in the experiment. They were all pre-screened and none of the subjects used any medication, had a history of drug abuse, head trauma, neurological or psychiatric illness, or a family history of neurological or psychiatric illness. All subjects had normal or corrected-to-normal vision. Written informed consent was

obtained from all participants according to the Declaration of Helsinki and the local medical ethics committee approved the study.

As in the TMS experiment, we randomly selected 100 grammatical items for the acquisition set from the same 569 grammatical strings used in TMS experiment. Again, these strings were comparable in terms of ACS to the complete string set and the non-grammatical items were derived from each remaining 469 G items by a switch of letters in two non-terminal positions, and these were selected to match the G items in terms of both terminal and complete string ACS. Finally, we randomly selected 2 sets of 60 strings each from the 469 grammatical and their matched 469 non-grammatical items in order to generate 2 classification sets (balanced over subjects) consisting of 50% G and NG items, as well as 50% high and low ACS items. Again, the classification sets were used for the 2×2 factorial design of the classification task. The control experiment comprised five consecutive days of implicit acquisition with the same instruction as in the TMS experiment. On the fifth day the subjects participated in a grammaticality classification task which was divided into two equal pre- and post-session with a ~ 20min break in between as in the TMS experiment.

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