When less is more: enhanced statistical learning of non-adjacent dependencies after disruption of bilateral DLPFC

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Abstract

Brain networks related to human learning can interact in cooperative but also in competitive ways in optimizing performance. The investigation of such interactive processes are rare in language and learning research. Previous studies have shown that manipulations reducing the engagement of frontal lobe processes can lead to improved statistical learning performance; however, most of these studies used correlational methods only. The aim of the present study was to test the causal role of the dorsolateral prefrontal cortex (DLPFC) in implicit statistical learning of non-adjacent dependencies. We applied 1 Hz inhibitory transcranial magnetic stimulation or sham stimulation over both the left and right DLPFC intermittently during the learning. The DLPFC stimulation group showed better performance compared to the sham group after a 24-hour consolidation period. This finding suggests that the disruption of DLPFC induced qualitative changes in picking up non-adjacent statistical regularities during learning, which support the less-is-more hypothesis.

Keywords: non-adjacent dependency; statistical learning; probabilistic sequence learning; dorsolateral prefrontal cortex (DLPFC); memory consolidation; Transcranial Magnetic Stimulation (TMS)

Introduction

Picking up statistical regularities is crucial for successfully adapting to the environment and for the learning of many fundamental skills, such as the language. Most of the time, the focus of research is on the roles of isolated brain functions and how their manipulation can modify the learning processes. Thus, a lack of knowledge remains in terms of the interaction between different cognitive processes and its relation to more complex mechanisms such as learning non-adjacent dependencies, which is the hallmark of language acquisition (Christiansen & Chater, 2015). Cumulating evidence shows that memory systems and their underlying brain structures might interact in a cooperative manner but can also compete with one another to optimize performance. Such an antagonistic, competitive relationship is theorized to exist between the attention-dependent hypothesis-testing processes, and the procedural system (Ashby, Alfonso-Reese, Turken, & Waldron, 1998; Henke, 2010; Poldrack et al., 2001). Statistical learning is a key mechanism of the brain (Daw, Niv, & Dayan, 2005; Fiser, Berkes, Orbán, & Lengyel, 2010) that extracts and represents the relationship between events of the environment based on frequency and rule-type information to make better predictions of future events. This statistical structure can be learnt by relying on model-free processes (where the complete knowledge is gained directly by actions of trials and error), thus without frontal lobe-mediated hypothesis testing and model-based processes (where an initial top-down model of the environment is acquired prior to action) (Daw et al., 2005; Fiser et al., 2010; Janacsek, Fiser, & Nemeth, 2012; Nemeth, Janacsek, & Fiser, 2013). Evidence for better statistical learning associated with lower frontal functions comes mostly from developmental observations (Janacsek et al., 2012), neuropsychological studies (Virag et al., 2015), electrophysiological studies (Tóth et al., 2017) and from studies that experimentally manipulate the engagement of the two systems (Filoteo, Lauritzen, & Maddox, 2010; Nemeth, Janacsek, Polner, & Kovacs, 2013). These findings match with the well-known theory of language acquisition namely *less-is-more* by Newport (Goldowsky & Newport, 1993; Newport, 1990). However, direct evidence linking reduced functionality of the dorsolateral prefrontal cortex (DLPFC) to increased probabilistic statistical learning is still missing. The aim of our study was to fill this gap by directly manipulating the involvement of dorsolateral prefrontal cortex (DLPFC) in temporally distributed statistical learning using repetitive transcranial magnetic stimulation (rTMS).

The neural basis of picking up probabilistic statistical regularities was investigated by multiple neuroimaging studies. For example, Simon, Vaidya, Howard, & Howard (2012) have used event-related functional magnetic resonance imaging during a probabilistic sequence learning task in younger and older adult subjects. The results emphasized the role of the hippocampus in the early stage of learning, and the caudate in later stages. In older subjects, frontal activity was also detected accompanied by worse performance compared to young adults. With the help of diffusion tensor imaging, Bennett, Madden, Vaidya, Howard, and Howard (2011) have found that the integrity of the neural tracks between the DLPFC and the hippocampus, and also between the DLPFC and the caudate nucleus are related to the degree of statistical learning. Stillman et al. (2013) have described increased functional connectivity measures between the caudate and the medial temporal lobe correlated with increased probabilistic statistical learning. Based on their findings, the authors have also stressed the potential mediating role of the DLPFC between other, statistical learning-related areas. Thus, we hypothesized that by being a part of the fronto-striatal network, DLPFC might be able to modulate statistical learning abilities.

Although a lot of evidence claims the role of DLPFC in statistical learning, to date, its exact mechanism is still not clear. From a developmental point of view, hypofrontality (reduced DLPFC function, observed normally in children) is a result of a trade-off between the ability to maximize learning procedural skills (such as language or social skills) and to perform well in tasks demanding cognitive control (Goldowsky & Newport, 1993; Newport, 1990; Thompson-Schill, Ramscar, & Chrysikou, 2009). This theory is supported by the fact that children tend to perform better than adults on statistical learning tasks, while an inverse pattern is observable on executive function tasks (Janacsek et al., 2012). Several findings emphasize a possible negative relationship between DLPFC-dependent frontal functions and statistical learning abilities by studying populations with hypofrontality (Virag et al., 2015), by engaging executive functions to learning in healthy subjects (Filoteo et al., 2010) or by using hypnosis to disconnect the frontal lobe (Nemeth, Janacsek, Polner, et al., 2013). Moreover, weaker fronto-parietal functional connectivity in theta-band was found to be associated with increased statistical learning. Although evidence claiming an inverse relationship between frontal functions and statistical learning abilities is accumulating, the above-mentioned neuroimaging and behavioral findings rely mostly on correlational evidence only.

Employing non-invasive brain stimulation methods, we are able to reveal causal relationships between learning and the activation of distinct brain areas. To date, only a few studies aimed to investigate the effect of such methods on procedural learning tasks by stimulating the DLPFC. An early rTMS study by Pascual-Leone, Wassermann, Grafman, and Hallett (1996) have shown that 5 Hz rTMS over the contralateral DLPFC during a deterministic serial reaction time task (SRTT) impaired online learning. Galea, Albert, Ditye, & Miall (2010) have applied inhibitory continuous theta-burst stimulation (cTBS) over the DLPFC following practice on SRTT. After an 8-hour consolidation period, subjects were faster on sequence elements compared to random elements in the verum, but not in the sham stimulation group. Smalle, Panouilleres, Szmalec, and Möttönen (2017) have found increased learning on phonological sequences following cTBS over the left DLPFC. Contrary, Savic, Cazzoli, Müri, & Meier (2017) have reported null effects of different brain stimulation methods over the DLPFC on a deterministic sequence learning task, which requires the use of only one hand. It suggests that, in some cases, a strong interhemispheric competition might obscure the effects of the stimulation. More importantly, the mentioned studies all used deterministic sequences instead of non-adjacent probabilities. Thus, the causal role of the DLPFC in this complex form of statistical learning has not been tested yet.

In this study, we go beyond previous findings by disrupting the DLPFC during a non-adjacent statistical learning task using bilateral stimulation. We chose a widely used task for measuring probabilistic statistical learning, namely the alternating serial reaction time task (ASRT, Figure 1A), which has been used in experimental psychology studies (J. H. Howard & Howard, 1997; D. V. Howard et al., 2004; Kóbor, Janacsek, Takács, & Nemeth, 2017; Nemeth et al., 2010; Song, Howard, & Howard, 2007), developmental studies (Janacsek et al., 2012; Juhasz, Nemeth, & Janacsek, 2019; Nemeth, Janacsek, & Fiser, 2013), as well as neuroimaging studies (Bennett et al., 2011; Stillman et al., 2013). The ASRT task is a four-choice reaction time task in which predetermined stimuli alternate with random ones creating a probabilistic structure with more frequent versus less frequent stimulus triplets. Participants are able to pick up these non-adjacent statistical regularities, and over time, performance differences emerge between high- and low- frequency triplets without the participants becoming aware of the underlying structure (Howard Jr and Howard 1997; Howard et al. 2004). As the statistical pattern is hidden in noise, it mimics the real-life skill learning which also occurs under uncertainty, in a noisy environment

(Fiser et al., 2010). During the learning phase, we used 1 Hz repetitive TMS over the DLPFC, which has been shown to reduce blood flow in the stimulated region (Groiss, Ugawa, Paulus, & Huang, 2012), extending beyond the termination of the stimulation (Robertson, Théoret, & Pascual-Leone, 2003; Walsh & Cowey, 2000). As ASRT requires using both hands, a bilateral stimulation might be an ideal choice to control for the possible premotor response bias (by bilateral stimulation, high- and low-frequency triplets are affected to a similar extent). Therefore, a sequential, bilateral TMS protocol has been chosen in order to suppress the compensation of the non-stimulated hemisphere. Moreover, to study the effect of TMS on the whole learning process and being able to differentiate between intermediate, long-lasting and non-specific effects of the stimulation, offline retests at 10-min, 2-hour and 24-hour post-stimulation were implemented in the design. We hypothesized that disrupting the DLPFC functions bilaterally would increase statistical learning performance in the ASRT task.

Materials and methods

Participants

Thirty-two participants took part in the experiment. All of the participants were right-handed; their visual acuities were normal or corrected to normal. None of the participants reported previous history of neurological or psychological disorders, drug or alcohol abuse, had no metal implants and were not taking regular medication relevant to the study. Written informed consent has been acquired from all participants. All of them tolerated the experimental procedures, and none withdrew because of discomfort with TMS. All participants were students of the University of Jena and participated in exchange for partial course credits or monetary compensation. One participant was excluded from the final sample because of poor performance on the Berg Card Sorting Test (% of preservative errors = 31.25%; % of correct responses: 51.56%, the scores are more than 3 standard deviations above the mean, see group averages in the Experimental Procedure section). Thus, the final sample contained data of 31 participants (16 in the DLPFC stimulation group and 15 in the sham group, 4 males, M_{age} = 22.16 years, SD_{age}: ± 3.01). The experiment was conducted in accordance with the guidelines of the Declaration of Helsinki and with the approval of the ethics committee of the University of Jena.

Alternating Serial Reaction Time Task

Statistical learning was measured using the Alternating Serial Reaction Time (ASRT) Task (D. V. Howard et al., 2004; Song et al., 2007) In this task, a stimulus (a dog's head) appeared in one of the four horizontally arranged empty circles on the screen. The participants were instructed to press the corresponding key (Z, C, B and M on a QWERTY keyboard), using both hands, as quickly and as accurately as they could (Figure 1A). The target remained on the screen until the participant pressed the correct button. The response to stimulus interval was set to 120 ms. Stimuli were presented in blocks of 85 trials. The first five trials of each block were random elements and were for practice purposes only (not analyzed further). After these five practice trials, an eight-element alternating sequence was repeated ten times in a block (e.g., 2*r*4*r*3*r*1*r*, where 1–4 indicate the target locations from left to right, and *r* indicates a randomly selected position out of the four possible ones) (Figure 1A). After each TMS stimulation phase, five blocks of ASRT were presented to the participants, which took approximately 5 minutes to complete. Similarly, five blocks of ASRT were administered after 10 minutes, 2 hours and 24 hours (Figure 1B) to test long-term effects of stimulation on behavior.

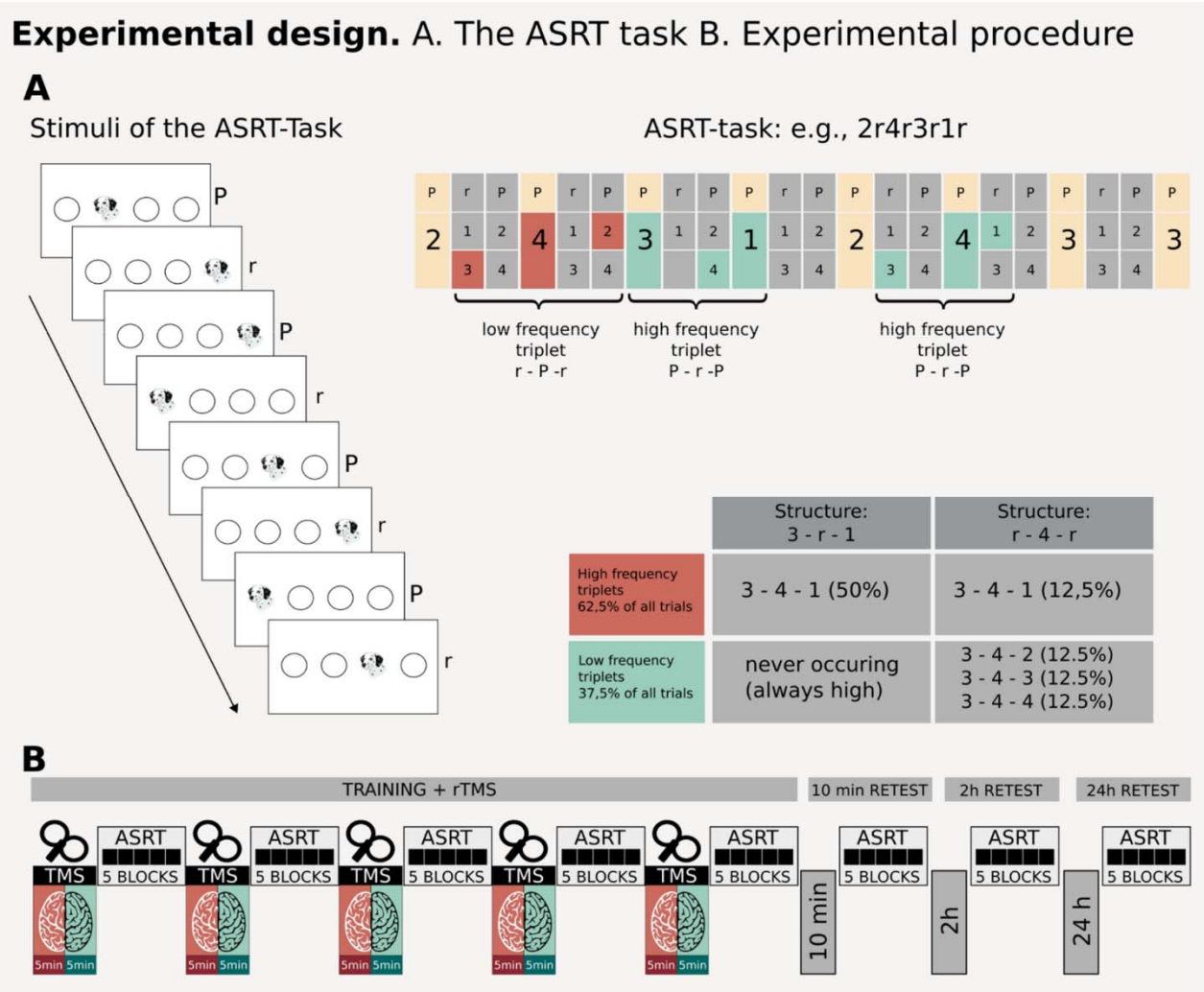

Figure 1. Experimental procedures. (A) Stimuli of the Alternating Serial Reaction Time (ASRT) task. Repeating elements (P – pattern) alternate with random events (r – random). Due to this structure of the sequences, some triplets (i.e. three consecutive events) occur more frequently (high-frequency triplets) than others (low-frequency triplets). Implicit statistical learning is measured as the RT difference between these two triplet types. (B) Five minutes of 1 Hz rTMS of both DLPFCs was administered before each of the five learning blocks, with the order of the stimulated hemispheres counterbalanced inter-participants. The volunteers performed five ASRT blocks 10 minutes, 2 hours and 24 hours post-learning, as well.

Structural MRI and Neuronavigated TMS

Structural MRI scanning was performed in a Siemens Magnetom Trio 3T MRI scanner at the Institute for Diagnostic and Interventional Radiology, University of Jena. High-resolution sagittal T1-weighted images for the 3D head and brain meshes were acquired using a magnetization EPI sequence (MP-RAGE; TR = 2300 ms; TE = 3.03 ms; 1 mm isotropic voxel size). For the purposes of neuronavigated TMS stimulation, the 3D-head and brain models were created from the participants' individual MRI scans. Coordinates for the DLPFC were taken from a meta-analysis by Cieslik et al. (2013), which corresponds to the dorsal part of the DLPFC, which is

revealed to be connected to distinct neural networks, and to be especially involved in executive control and working memory processes (MNI coordinates: x = 37, y = 33, Z = 32). For sham TMS stimulation the coil was oriented perpendicularly, facing away from the skull (Lisanby, Gutman, Luber, Schroeder, & Sackeim, 2001).

TMS stimulation was delivered using a PowerMag 100 Research Stimulator (MES Forschungssysteme GmbH). Neuronavigation was carried out using a PowerMag View (MES Medizintechnik GmbH) Neuronavigation system. Magnetic pulses were delivered with 1 Hz, at 55% maximum stimulator output of the stimulator. A single intensity was used on the basis of previous studies (Figner et al., 2010; Silvanto, Cattaneo, Battelli, & Pascual-Leone, 2008). TMS was applied before each of the five learning blocks, i.e. before the first block and in the inter-block intervals (300 pulses, 5 minutes per hemisphere). The order in which the two hemispheres were stimulated was counterbalanced inter-participants.

Experimental procedures

Participants were seated in a dimly lit room; their heads fixed using a chinrest, 60 cm viewing distance away from the stimulus presentation monitor. After giving informed consent, the volunteers performed an ASRT practice run to familiarize themselves with the task and the keyboard layout. In the Learning/rTMS Phase, the participants received 1 Hz rTMS for both left and right hemispheres sequentially (5 minutes, 300 TMS pulses for each hemisphere, thus 5 minutes for the left and after that for the right, in a counterbalanced order inter-participants), then performed five blocks of the ASRT task, lasting ca. 5 minutes. This procedure was repeated five times. Therefore, a total of 25 blocks of ASRT were completed, which provides enough trials for the learning to manifest as showed by previous studies (Janacsek et al., 2012; Nemeth et al., 2010; Vekony et al., 2019). The order in which the two hemispheres were stimulated was assigned randomly, remained the same for each individual participant. To test the causal role of DLPFC in statistical learning in both the acquisition and the consolidation, we tested ASRT performance multiply times after the stimulation as well. In the post-learning/stimulation phases, the volunteers performed five blocks of ASRT task 10 minutes, 2 hours, and 24 hours after the completion of the learning phase. The exact retest times were defined by previous literature (Janacsek, Ambrus, Paulus, Antal, & Nemeth, 2015). The aim of the 10 minutes retest session was to test the immediate after-effect of TMS *without* stimulation. With the help of the 2 hours

retest session, we could test the long-term effects of the stimulation. The performance after 24 hours can provide information about the effects of the stimulation on consolidation.

To ensure that the two experimental groups did not differ in executive functions performance, the short-form of Berg Card Sorting Test (Fox, Mueller, Gray, Raber, & Piper, 2013) and the Counting Span test (Case, Kurland, & Goldberg, 1982; Conway et al., 2005; Engle, Laughlin, Tuholski, & Conway, 1999) were administered after the completion of the ASRT in the last session. We observed no significant differences in performance between the two experimental groups (Berg Card Sorting Test, percent correct responses: DLPFC: M = 81.06, SD = 5.90, Sham: M = 80.42, SD = 7.76, p = 0.797, percent perseverative errors: DLPFC: M = 10.16, SD = 4.56, Sham: M = 11.67, SD = 4.83, p = 0.377, percent non-perseverative errors: DLPFC: M = 8.79, SD = 5.59, Sham: M = 7.92, SD = 5.06, p = 0.652; Counting span, mean of three runs: DLPFC: M = 4.06, SD = 1.11, Sham: M = 3.62, SD = 0.85, p = 0.228).

As a part of the post-experimental debriefing, the participants filled out a questionnaire assessing their levels of discomfort, tiredness, and perceived task difficulty, measured on a ten-point scale.

The Learning Phase (with informed consent) lasted ca. 2 hours, the 10 min and 2 hours Test Phases lasted 5 minutes each, and the 24 hours Retest Phase (with the control tasks) lasted ca. 30 min.

Statistical analysis

Only correct responses were considered for the ASRT analysis, and stimulus repetitions (e.g. 333, 444) and trills (e.g. 313, 121) were also excluded (J. H. Howard & Howard, 1997; D. V. Howard et al., 2004). Trials with reaction times (RTs) more than 2.5 standard deviations above or below the mean of the given epoch were also eliminated (separately for each participant). After that, we calculated the mean RTs for high- and low-frequency triplets separately. Implicit statistical learning was assessed using a triplet-learning index, by subtracting the RTs for low-frequency triplets from those for high-frequency triplets. A higher score thus means faster responses for high- than for low-frequency triplets, i.e. better statistical learning performance.

The triplet-learning index was calculated in each phase of the experiment for each participant. We conducted a 2 (TMS Group: 1. DLPFC, 2. Sham) × 4 (Phase: 1. Learning/rTMS,

2. 10 minutes post-stimulation Phase, 3. 2 hours post-stimulation Phase, 4. 24 hours post-stimulation Phase) mixed design ANOVA to compare statistical learning performance between the two stimulation groups along the course of the experiment. Greenhouse-Geisser corrections were applied where necessary. Significant main effects and interactions were followed up using Fisher's LSD tests.

As we aimed at investigating the effects of rTMS on both hemispheres, the possibility of the observed effect being due to the stimulation of the hemisphere before the ASRT block had to be ruled out. Thus, we conducted a 2 (TMS condition: Right Start, Left Start) × 4 (Phase) ANOVA on the triplet learning index to ascertain if our results were due to the effect of stimulation order.

All analyses were two-tailed and were conducted with a significance level of $p < 0.05$.

Results

The ANOVA conducted on the triplet learning indices (i.e., RTs for low-frequency stimulus triplets minus RTs for high-frequency stimulus triplets) revealed a significant main effect of experimental Phase ($F_{3, 87} = 6.521$, $p < 0.001$, $\eta^2_p = 0.184$). It indicates an overall increase in performance in the three retest phases when compared to the Learning/rTMS Phase (10 min post-stimulation: $p < 0.001$, 2 hours post-stimulation: $p < 0.001$, 24 hours post-stimulation: $p = 0.002$). There was no main effect of Stimulation Group ($F_{1, 29} = 0.752$, $p = 0.393$, $\eta^2_p = 0.025$), but the interaction between experimental Phase and Stimulation Group was shown to be significant ($F_{3, 87} = 4.194$, $p = 0.008$, $\eta^2_p = 0.126$). In the sham group, post-hoc comparisons revealed an increasing performance up to 2 hours compared to the Learning/rTMS Phase (10 min post-stimulation: $p < 0.001$; 2 hours post-stimulation: $p = 0.015$). We also found a trend-level decrease in learning scores from 10 min to 24 hours post-stimulation ($p = 0.096$). Performance in the DLPFC stimulation group also increased with time compared to the Learning/rTMS Phase (10 min post-stimulation: $p = 0.035$; 2 hours post-stimulation: $p = 0.002$), and contrary to the sham group, the difference remained significant at 24 hours post-stimulation ($p < 0.001$). A trend-level increase from 10 min to 2 hours post-stimulation ($p = 0.089$) was also found. It should also be noted that the performance of the DLPFC group at 24 hours post-stimulation was not only markedly better than the performance in the Learning Phase but differed significantly from

performance indices of the 10 min post-stimulation Phase ($p = 0.011$) (Figure 2A). Importantly, the performance of the DLPFC stimulation group in the 24 hours Retest Phase was significantly higher than the performance of the Sham group ($p = 0.023$), indicating the boosting effect of bilateral rTMS on the performance that was evident after a consolidation period (Figure 2B).

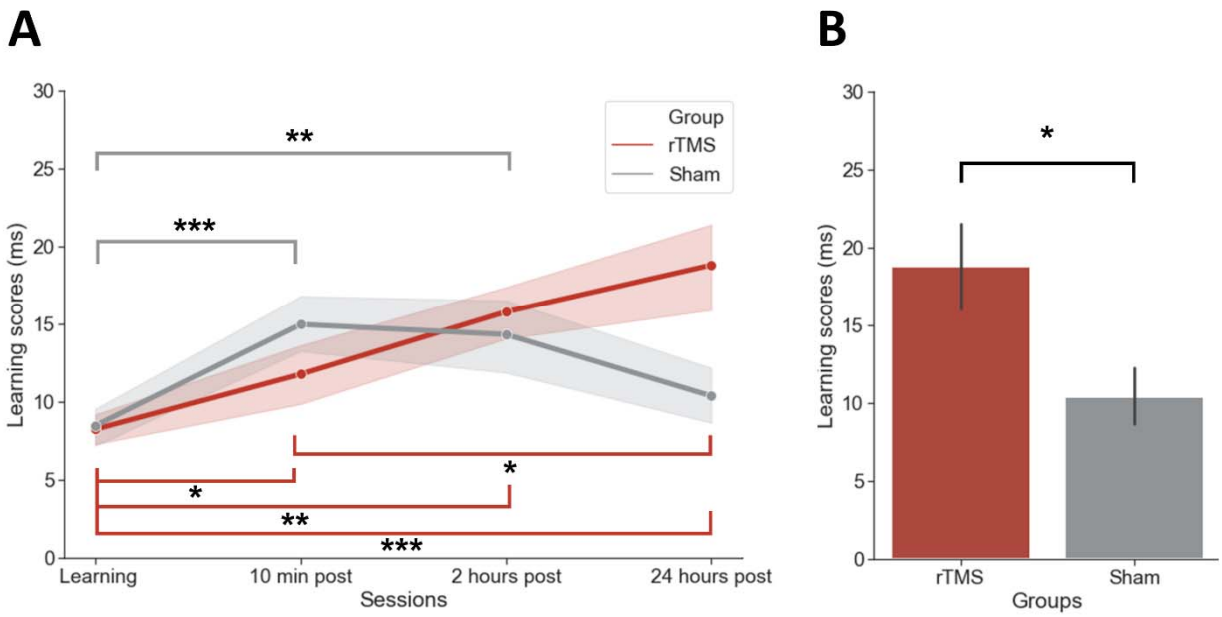

Figure 2. (A) The triplet-learning index in the two experimental groups along the course of the experiment and (B) the comparison of the triplet learning indices between groups on the last session. We observed a continuous increase in performance in the active DLPFC stimulation group, while performance in the sham group was only significantly better in the 10-minutes and 2 hours post-stimulation phase (A). After 24 hours, the performance of the active DLPFC stimulation group was statistically higher compared to the sham control group (B). The error bars denote SEM. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

The observed group differences are unlikely to be due to a general effect of the stimulation on arousal level since general reaction time (Phase × Stimulation Group interaction: $F_{3,87} = 0.392$ $p = 0.759$, $\eta^2_p = 0.013$) and response accuracy (Phase × Stimulation Group interaction: $F_{3,87} = 0.383$, $p = 0.766$, $\eta^2_p = 0.013$) was not statistically different between the two groups. Furthermore, the level of discomfort ($p = 0.568$), tiredness ($p = 0.829$), perceived task difficulty ($p = 0.237$), assessed as a part of the post-experiment debriefing, was also not different between the DLPFC stimulation and the Sham groups (see Methods).

Moreover, the order of the hemispheres stimulated, assessed by the two-way interaction of a 2 (TMS condition: Right Start, Left Start) × 4 (Phase) mixed-design ANOVA, had no effect on statistical learning performance ($F_{3,42} = 0.151$, $p = 0.928$, $\eta^2_p = 0.011$).

Discussion

To date, only a few studies investigated the causal role of DLPFC in statistical learning (Galea et al., 2010; Pascual-Leone et al., 1996; Savic et al., 2017; Smalle et al., 2017); however, none of them tested the causal role of the DLPFC in the acquisition of non-adjacent dependencies, which are known to be crucial for language skills (Christiansen & Chater, 2015). Here we aimed to fill this gap by administering bilateral rTMS over the DLPFCs during learning of non-adjacent statistical regularities. We went beyond previous studies in three aspects. First, instead of deterministic sequences, we tested the effect of rTMS on the acquisition of non-adjacent probabilities. Second, we applied bilateral stimulation to control for the possible interhemispheric compensation of the non-stimulated hemisphere. Third, we tested the effect of the used TMS protocol throughout the learning phase, as well as 10 minutes, 2 hours and 24 hours post-stimulation. Our results show that the disruption of the DLPFCs bilaterally during the learning phase had a beneficial effect on the statistical learning performance after the 24 hours offline period.

Our findings, as predicted, are in line with the competition model that posits an antagonistic relationship between the fronto-striatal networks that mediate executive functions and implicit statistical learning. Without providing mechanistic and causal insight, previous studies have demonstrated the inverse relationship of these two systems on the behavioral level. For instance, Virag et al. (2015) have shown a negative correlation between frontal-lobe related executive functions and implicit statistical learning. Filoteo et al. (2010) have found that implicit category learning improved with the addition of a secondary working memory task, reducing the contribution of the frontal lobe functions by overly engaging working memory processes. Using hypnosis, Nemeth, Janacsek, Polner, et al. (2013) experimentally reduced frontal-lobe connectivity, reducing competition from the attention-based frontal lobe processes, and found increased statistical learning performance compared to an alert, awake state. Additionally, several developmental studies have found that children perform better than adults on statistical learning tasks (Janacsek et al., 2012; Juhasz et al., 2019; Zwart, Vissers, Kessels, & Maes, 2019). Supporting the competition model, the degree of learning drops with the onset of adolescence, coinciding with the maturation of the frontal lobe. Besides the above-mentioned behavioral studies, Tóth et al. (2017) have found evidence for this inverse relationship at the level of neural oscillations. Namely, they have found increased statistical learning to be associated with weaker

fronto-parietal connectivity in theta frequency which band has a crucial role in memory access (Düzel, Penny, & Burgess, 2010). These previous studies provided only correlational evidence; however, our recent findings are causal proofs for the role of DLPFC in non-adjacent statistical learning. This type of learning can be boosted by tuning down frontal lobe-mediated model-based hypothesis testing processes, and thus strengthen the data-driven, model-free processes (Daw et al., 2005; Fiser et al., 2010; Janacsek et al., 2012; Nemeth, Janacsek, & Fiser, 2013).

To date, only a few studies have investigated the causal role of DLPFC on procedural learning skills by using TMS protocols. With the intention of disrupting cortical processing by 5 Hz rTMS during deterministic SRTT, Pascual-Leone et al. (1996) have found that stimulation over contralateral DLPFC impaired online learning. It should be noted, however, that since the publication of this study, 5 Hz rTMS has been found to induce excitatory effects on cortical excitability (Matsunaga et al., 2005; Peinemann et al., 2004), thus the performance decrease in this study might be better explained by the facilitation of DLPFC functions. To test the role of the DLPFC in the consolidation of sequential knowledge, Galea et al. (2010) have applied disruptive cTBS after the execution of deterministic SRTT. They have found offline improvement following the inhibition of the right but not the left DLPFC. However, based on these results, we cannot decide whether a disrupted DLPFC during learning can affect the initial learning. Smalle et al. (2017) have gone beyond Galea et al (2010) by applying disruptive stimulation over the left DLPFC prior to learning. Increased learning on phonological sequences has been found on the Hebb repetition paradigm. To control for the possible compensation of the non-stimulated hemisphere, Savic et al. (2017) have tested the effect of brain stimulation over the DLPFC on a deterministic sequence learning task, which requires the use of only one hand. In their analysis, no stimulation effect has been found over either of the hemispheres (however, as different protocols have been used above for each of the hemispheres, results are hard to interpret). Taken together, the few above-mentioned studies were the only ones that used TMS to manipulate the involvement of DLPFC during and/or after procedural learning. Most importantly, all of them focused on deterministic sequences; hence, they provide no information about the acquisition of non-adjacent statistical regularities. Nevertheless, the previous results point to the same directions: facilitating stimulation of the DLPFC hinders, while inhibitory stimulation improves the learning of new sequences, new patterns, or new statistics.

The previous studies revealed a possible methodological issue: the interhemispheric compensation might obscure effects following unilateral stimulation. This effect might have played a role in the negative result of Savic et al. (2017). Galea et al. (2010) have found left hemisphere advantage, but it does not indicate that the activation of the right hemisphere cannot interfere with the results. Several TMS studies have proved that lateralization does not necessarily indicate that the function is completely eliminated from the other hemisphere, even in the case of well-known lateralized functions as language processing (e.g. Hartwigsen et al., 2010) or working memory (Mottaghy, Döring, Müller-Gärtner, Töpper, & Krause, 2002; Vékony et al., 2018). This means that even if the dominating hemisphere is stimulated, the other can have confounding effects on the results. Thus, we eliminated this possible confounding factor by using bilateral brain stimulation to disrupt the involvement of both DLPFCs during learning. Future studies could benefit from using both unilateral and bilateral stimulation in one experimental design to get a complete picture of the role of DLPFC in statistical learning.

Beyond the methodological aspects, our results open a new theoretical perspective in interpreting the role of DLPFC in statistical learning. The DLPFC might have a role in top-down processes, in accessing the existing models or long-term memory representations, which weakens the learning of new patterns (for example, the weaker theta-band connectivity in Tóth et al. (2017) also support this idea). If the access to these model-based processes, to the priors, and to the existing knowledge are limited, then the learning process will move towards a model-free approach, and thus, the learning of entirely new patterns will be enhanced. This framework explains well not only the results of the previously mentioned TMS studies about statistical learning but also our results on the consolidation. Namely, as we disrupted the DLPFC, we found better performance after the 24-hour consolidation period. The above-mentioned framework involves the interaction between model-free and model-based processes (Daw et al., 2005; Janacsek et al., 2012; Nemeth, Janacsek, & Fiser, 2013). When the DLPFC is fully functioning, the model-free processes extract the statistical information from the stimulus stream, and the DLPFC mediated model-based processes combine top-down information with these statistics. In the offline period, this mixed information consolidates (Figure 3). However, the brain stimulation on the DLPFC possibly interrupts the top-down information flow and its combination with data-driven extraction of pure statistical information. This pure statistical information consolidates, which is optimal when the brain faces the challenge to learn entirely new regularities. Our results

and the theory of limited access to long-term memory processes – thus the volume-down of top-down processes - fit in well with the *less-is-more* hypothesis of Newport about language acquisition (Goldowsky & Newport, 1993; Newport, 1990). The theory posits that children are better at learning languages because they have fewer available cognitive resources. Because of their limitations, children start by acquiring the small parts of a complex system (like a new language), and they proceed with the more complex constructions later. In contrast, adults cannot easily restrain from analyzing the whole complexity, and it will unavoidably lead to difficulties in mastering complex systems like languages. We can speculate that the mechanism behind the children's superiority in acquiring languages can be the same as the effect of TMS over the DLPFC: switching off our previous knowledge and limiting our resources can help liberate our model-free approaches to learn new skills. Future studies directly examining connections between the two theories seem warranted.

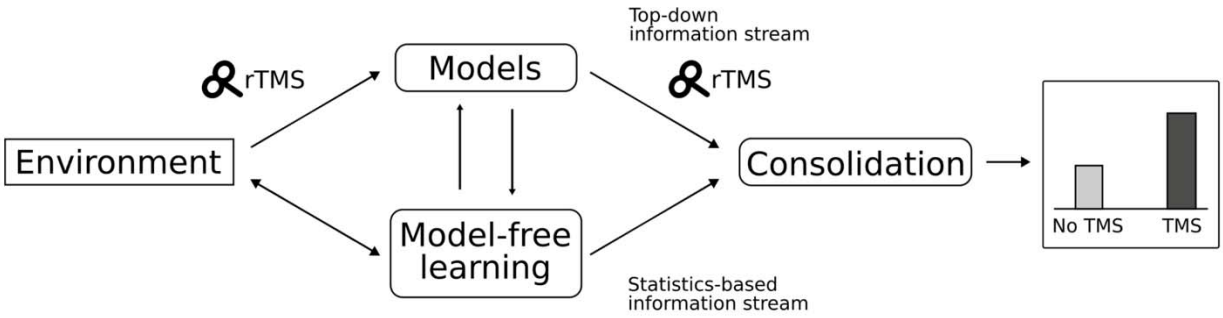

Figure 3. The schematic figure of the interpretation of our results. Internal models strongly modulate the interpretations of observed statistics of the input**.** This helps in extracting complex relations but relatively impairs detecting and learning raw probabilities. rTMS disrupts the involvement of these internal models leading to a better consolidation of the newly detected non-adjacent dependencies.

To sum up, we observed that the bilateral disruption of the DLPFCs during the learning phase had a beneficial effect on non-adjacent statistical learning performance that was observable after a 24-hour offline period. Our findings are significant in three aspects. First, this finding provides mechanistic level causal evidence for the models positing an antagonistic relationship between the model-based and the model-free processes, i.e. that DLPFC does have a causal role in implicit statistical learning. Second, from a methodological viewpoint, previous investigations using external brain stimulation methods stimulated only one hemisphere at a time. Therefore, the taking-over of the lost function by the contralateral hemisphere cannot be ruled out in earlier studies (Janacsek et al., 2015; Savic et al., 2017). Here we showed that the sequential application

of 1 Hz rTMS before learning blocks over both hemispheres establishes and sustains the inhibitory effect. The finding that no effect of stimulation order was observed supports the viability and practicality of this approach and may form the basis of future research requiring bi-hemispherical/multi-site intervention. Third, and most importantly, our results raise a new possible theoretical framework explaining the role of the DLPFC in statistical learning processes, which extends the *less-is-more* hypothesis. In spite of investigating separate neurocognitive processes, our results shed light on the importance of exploring the possible interactive approaches underlying learning, which can help us more deeply understand the exact mechanism of skill acquisition and consolidation.

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