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Frontal and Parietal Contributions to Probabilistic Association Learning

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Neuroimaging studies have shown both dorsolateral prefrontal (DLPFC) and inferior parietal cortex (iPARC) activation during probabilistic association learning. Whether these cortical brain regions are necessary for probabilistic association learning is presently unknown. Participants' ability to acquire probabilistic associations was assessed during disruptive 1 Hz repetitive transcranial magnetic stimulation (rTMS) of the left DLPFC, left iPARC, and sham using a crossover single-blind design. On subsequent sessions, performance improved relative to baseline except during DLPFC rTMS that disrupted the early acquisition beneficial effect of prior exposure. A second experiment examining rTMS effects on task-naive participants showed that neither DLPFC rTMS nor sham influenced naive acquisition of probabilistic associations. A third experiment examining consecutive administration of the probabilistic association learning test revealed early trial interference from previous exposure to different probability schedules. These experiments, showing disrupted acquisition of probabilistic associations by rTMS only during subsequent sessions with an intervening night's sleep, suggest that the DLPFC may facilitate early access to learned strategies or prior task-related memories via consolidation. Although neuroimaging studies implicate DLPFC and iPARC in probabilistic association learning, the present findings suggest that early acquisition of the probabilistic cue-outcome associations in task-naive participants is not dependent on either region.

Keywords: consolidation, dorsolateral prefrontal cortex, inferior parietal cortex, probabilistic association learning, repetitive transcranial magnetic stimulation

Introduction

While cognitive processes rely on multiple brain regions for normal function, some cognitive processes may depend critically on contributions from specific cortical or subcortical regions. Although the prefrontal and parietal cortices are found to be active during many cognitive tasks, it is often unclear whether these 2 brain regions make necessary contributions to specific cognitive processes or, alternatively, whether activity in either of these regions is simply correlated with a given cognitive process. Previous studies have suggested that the prefrontal cortex is essential for working memory and executive function (Milner et al. 1985; Paulesu et al. 1993; Goldman-Rakic 1996), and the parietal cortex is required during working memory (Paulesu et al. 1993), attention (Carter et al. 1995), and self-awareness (Weniger et al. 2009). Functional neuroimaging studies have shown that declarative and nondeclarative rule learning and set shifting tasks activate both the dorsolateral prefrontal and the posterior parietal cortices in humans (Monchi et al. 2001; Asari et al. 2005). In nonhuman primates, in which it is possible to make direct recordings of neural activity during cognitive processing, feedback learning has elicited changes in neural response patterns of the prefrontal cortex (Miller et al. 1996) and the posterior parietal cortex (Joelving et al. 2007) during delay periods when decisions regarding category membership are thought to be made.

Probability estimation (i.e., determining the likelihood that a particular event will occur) is a form of inductive reasoning that is related to categorization (Smith 1989) and is integral to normal thought processing that is central to daily function, for example, when determining whether or not one should prepare for a rainy day on the basis of the presence of dark clouds in the sky or when determining how to respond appropriately on the basis of social cues displayed by other people (Knowlton et al. 1994; Behrens et al. 2008). Probabilistic association learning refers to a gradual feedback-based learning of probabilistically related cue–outcome associations, without the necessity of conscious appreciation of the rules or strategies (Knowlton et al. 1994). The "weather prediction task" (Knowlton et al. 1994; Knowlton et al. 1996a) is one such computerized task in which participants learn the relationship between 2 equally occurring outcome variables (rain or shine) and combinations of 4 cue cards, each composed of simple geometric shapes. It requires feedback-based integration of categorical information presented on cue cards, and acquisition of the probabilistic associations depends at least in part on caudate nucleus function (Knowlton et al. 1996a, 1996b; Poldrack et al. 2001). Functional magnetic resonance imaging (fMRI) studies of probabilistic association learning in healthy adults have revealed activation of a neural network that includes the prefrontal cortex, parietal cortex, and the caudate nucleus (Poldrack et al. 1999; Fera et al. 2005; Weickert et al. 2009). However, it is presently unclear which parts of this network are critical for successful task performance. For example, relative to healthy young adults, healthy older adults demonstrating successful probabilistic association learning showed decreased prefrontal cortex and caudate nucleus activation in conjunction with increased inferior parietal cortex (iPARC) activation (Fera et al. 2005). Thus, although both prefrontal and parietal cortex activation is present during probabilistic association learning, it is presently uncertain whether either prefrontal and parietal cortex activation is necessary for probabilistic association learning.

To test whether different task-related cortical regions are required for probabilistic association learning, the present study used repetitive transcranial magnetic stimulation (rTMS) to selectively dissociate the contributions of cortical brain regions by disrupting neural activity in the given region under stimulation while the participant engaged in probabilistic association learning. Previous studies using low frequency stimulation (≤1 Hz) in healthy adults have shown that temporary disruption of the dorsolateral prefrontal cortex (DLPFC) can lead to a significant deterioration of working memory performance, which relies on prefrontal cortex activity (Pascual-Leone and Hallett 1994; Mottaghy et al. 2000; Robertson et al. 2001; Mottaghy et al. 2002). Conversely, high-frequency rTMS (> 1Hz) to the superior parietal lobule facilitates responding during working memory (Hamidi et al. 2008). Thus, low-frequency rTMS should provide an appropriate method to disambiguate the respective contributions of the prefrontal and parietal cortices during probabilistic association learning by disrupting cortical activity under the region of stimulation.

In the present study, we applied low-frequency (1 Hz) rTMS to either the DLPFC or the iPARC during administration of the "weather prediction" probabilistic association learning test on separate sessions using a within-participant design to disrupt neural activity and determine whether prefrontal and/or parietal cortex function is necessary for probabilistic association learning. Based on fMRI studies revealing activity of prefrontal and parietal cortices in healthy adults during probabilistic association learning (Poldrack et al. 1999; Fera et al. 2005; Weickert et al. 2009), we hypothesized that if the prefrontal and/or parietal regions are critical to probabilistic association learning, then low-frequency rTMS to either prefrontal or parietal cortices would disrupt acquisition of the probabilistic cue–outcome associations. Results from the first experiment showed that only rTMS to the DLPFC negatively influenced acquisition of the probabilistic cue–outcome associations. However, given that stimulation was applied only following an initial baseline session, it was unclear whether the rTMS affected task-naive acquisition of the cue–outcome associations or whether it suppressed access to previously acquired strategies and associated task-specific memories afforded through practice (repetition) or consolidation following the baseline session, which may have facilitated an early performance enhancement on subsequent sessions. Therefore, 2 follow-up experiments were performed using independent samples of task-naive participants to clarify the extent to which low-frequency rTMS was capable of disrupting task-naive probabilistic association learning, as opposed to suppressing facilitation of learning dependent on multiple session practice or between session consolidation (as per Robertson 2009).

Experiment 1: The Effect of 1 Hz rTMS to the Left DLPFC or Left iPARC Compared with Sham in Individuals Familiarized with Weather Prediction Task

Materials and Methods

Participants

Twenty-six healthy volunteers participated in Experiment 1 (age range 18–40 years, mean age 24.2 years, standard deviation (SD) = 5.9, 12 males, 23 right-handed). Mean education level was 16.0 years (SD = 1.4), average IQ was 122.5 (SD = 11.4), based on a 4-subtest version of the Wechshler Adult Intelligence Scale 3rd edition (WAIS-III) (Wechsler 1997), including Picture Completion, Similarities, Digit Symbol Coding, and Arithmetic from which the full-scale IQ estimate was derived. The procedure was explained, and informed consent was obtained in accordance with a protocol approved by the University of New South Wales and the South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committees. Participants were required to complete a demographic and screening questionnaire, and all participants were interviewed by a medical practitioner for contraindications to transcranial magnetic stimulation (TMS) prior to stimulation. None of the participants had a history of seizures, psychiatric illness, or severe head injury nor any contraindications to rTMS (Wassermann 1998; Loo et al. 2008).

Procedure

Weather Prediction Task

Each volunteer participated in 5 separate testing sessions performing the weather prediction task: an initial session without stimulation (which will be referred to as "baseline"), followed by 1 active and 1 sham rTMS session each to the left DLPFC and the left iPARC, with the latter 4 sessions presented in pseudorandomized order such that equal numbers of participants received each of the presentation orders. Stimuli were 4 cue cards containing patterns of different geometrical shapes presented on a laptop computer screen. In any given trial, a stimulus consisted of 1, 2, or 3 cue cards (see Fig. 1 for an example of a trial). Participants were told that they should make a decision to predict rain or shine based on the presence or absence of the cue cards. They were also told that they should guess at first, but gradually, based on feedback provided, they would improve at determining which cue card combinations predict rain or shine. The relationship between cue cards and outcome variables was predetermined on a probabilistic basis (see Table 1 for an example of a cue–outcome probability schedule), and presentations were randomized with the constraint that identical cue combinations would not appear consecutively, and each outcome (rain or shine) was limited to 5 consecutive occurrences. All stimuli were displayed on

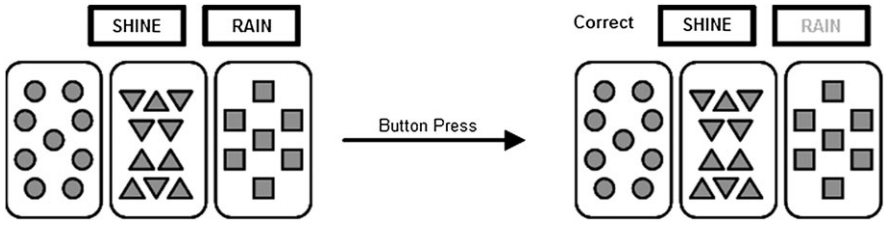

Figure 1. Schematic of the probabilistic association learning (weather prediction) test screen during a representative trial.

Table 1
Probability structure of probabilistic learning (weather prediction) task

	Cue					
Cue pattern	1	2	3	4	*P*(cue combination)	*P*(outcome)
1	0	0	0	1	0.133	0.150
2	0	0	1	0	0.087	0.385
3	0	0	1	1	0.080	0.083
4	0	1	0	0	0.087	0.615
5	0	1	0	1	0.067	0.200
6	0	1	1	0	0.040	0.500
7	0	1	1	1	0.047	0.143
8	1	0	0	0	0.133	0.850
9	1	0	0	1	0.067	0.500
10	1	0	1	0	0.067	0.800
11	1	0	1	1	0.033	0.400
12	1	1	0	0	0.080	0.917
13	1	1	0	1	0.033	0.600
14	1	1	1	0	0.047	0.857

Note: For any given trial, 1 of the 14 possible cue pattern combinations displayed above appeared on the computer screen with a probability indicated as: *P*(cue combination). As shown above, the probability of the cue combinations to predict "sunshine" (outcome 1) was set at *P*(outcome). Conversely, the probability of the above cue combinations to predict "rain" (or outcome 2) was equal to $1 - P$.

screen for 4.5 s with an intertrial interval of 0.5 s. Participants responded with a left mouse move and button press by their right hand to choose either rain or shine. After each response the words 'correct' or 'incorrect' appeared on screen as feedback to the participant. Missed trials were not included in the analyses. While cue cards were maintained across separate presentations, a different probability schedule was used at each testing session, with the different schedules presented in randomized order with the constraint that no probability schedule was presented more than once to a given participant.

rTMS Setup

In order to mark the locations for rTMS application, a tightly fitting lycra swimming cap was fitted on each participant's head. A Medtronic MagPro (MagVenture) stimulator and a figure eight-shaped water-cooled coil (70 mm outer diameter) were used to administer rTMS. Prior to the first rTMS session, each individual's resting motor threshold was determined as the minimum intensity that induced a visible movement of the right abductor pollicis brevis muscle as agreed by 2 experimenters on 6 of 10 trials. The motor cortex was initially defined as 5 cm lateral and 2 cm anterior from the vertex and then adjusted slightly for each individual, based upon the optimal site for eliciting muscle movement as above. TMS was applied in single pulses with an interstimulus interval of at least 7 s in order to avoid "carry-over" effects (Mottaghy et al. 2002). During probabilistic association learning, rTMS was applied at 110% of each participant's motor threshold. The average motor threshold was 60% of the maximum stimulator output (range 39–75%). Each individual participated in 4 stimulation sessions as described above. Sessions were scheduled at least 2 days apart (mean days between sessions 5.7, SD = 5.2). During the active sessions, participants received 1 Hz rTMS over either the left DLPFC, defined as 5 cm anterior to the motor cortex (as used in the majority of previous studies stimulating DLPFC; George et al. 1995; Pascual-Leone et al. 1996), or the left iPARC, defined as 4 cm posterior to the motor cortex. The stimulation was applied by placing the flat surface of the coil tangentially on the participant's scalp, at the site of interest, with the handle of the coil oriented posterolaterally. For each sham session, an active coil was placed over 1 of the stimulation sites of interest (at exactly the same parameters described above) but angled at 90 degrees to the scalp. Two trains of active rTMS or sham were administered while participants simultaneously completed 2 blocks of the weather prediction task, each block consisting of 75 trials with a 2-min break between blocks. Each rTMS train consisted of 375 pulses and lasted for 6.25 min. All participants and experimenters wore earplugs and earmuffs during each of the 4 rTMS sessions.

Statistical Analyses

In order to assess the learning curve over the course of the task, we performed a repeated measures analysis of variance (ANOVA) on the cumulative mean percentage correct over 150 trials. The main analysis of interest concerns the condition by trial interaction, which shows differences in the progression of learning over the course of the task among the rTMS conditions (baseline vs. DLPFC rTMS vs. iPARC rTMS vs. sham). For all statistical analyses, the 2 sham conditions were averaged, as there were no significant differences between sham rTMS applied to the DLPFC or iPARC. Upon obtaining a significant interaction, we performed Fischer's least significant difference (LSD) post hoc tests to assess differences in cumulative percent correct among the 4 conditions.

Mean reaction times were calculated for each of the conditions over the initial 25 trials, indicative of the acquisition phase, and across the final 50 trials, indicative of asymptotic learning. A repeated measures ANOVA was conducted on these reaction times (RTs), and planned contrasts were used to assess differences between active and sham conditions.

Results

Learning Curves

The repeated measures ANOVA on the cumulative mean percentage correct using within-subjects factors "condition" and "trial" revealed a near significant main effect of condition, $F_{3,72} = 2.42$, $P = 0.07$, and a highly significant main effect of trial number, $F_{149,3576} = 11.60$, $P < 0.001$, indicating a gradual increase in the percentage correct over the course of the task across all conditions. Importantly, the interaction of condition by trial was also highly significant, $F_{447,10728} = 1.17$, $P = 0.009$, indicating that acquisition differed among conditions.

Figure 2*a* shows the significant condition by trial interaction for percent correct across all 150 trials. Since we had a specific hypothesis regarding acquisition, Figure 2*b* focuses on the acquisition phase during the first 25 trials showing significant differences in the baseline and DLPFC conditions relative to iPARC and sham stimulation. Results of Fischer's LSD post hoc tests to assess differences in cumulative percent correct among the 4 conditions revealed DLPFC rTMS significantly suppressed the percentage correct compared with sham rTMS during trials 1 to 8 (all *P*s < 0.05) and compared with iPARC rTMS during trials 1–5 and 7 (all *P*s < 0.05), see Figure 2*b*. There were no significant differences between DLPFC and baseline. Conversely, baseline percent correct was significantly less than iPARC rTMS percent correct during trials 2–3 (all *P*s < 0.01), with near significant levels during trials 7–8 ($0.05 < P$s < 0.10). Baseline percent correct was significantly less than sham percent correct on trials 1–3 (all *P*s < 0.01).

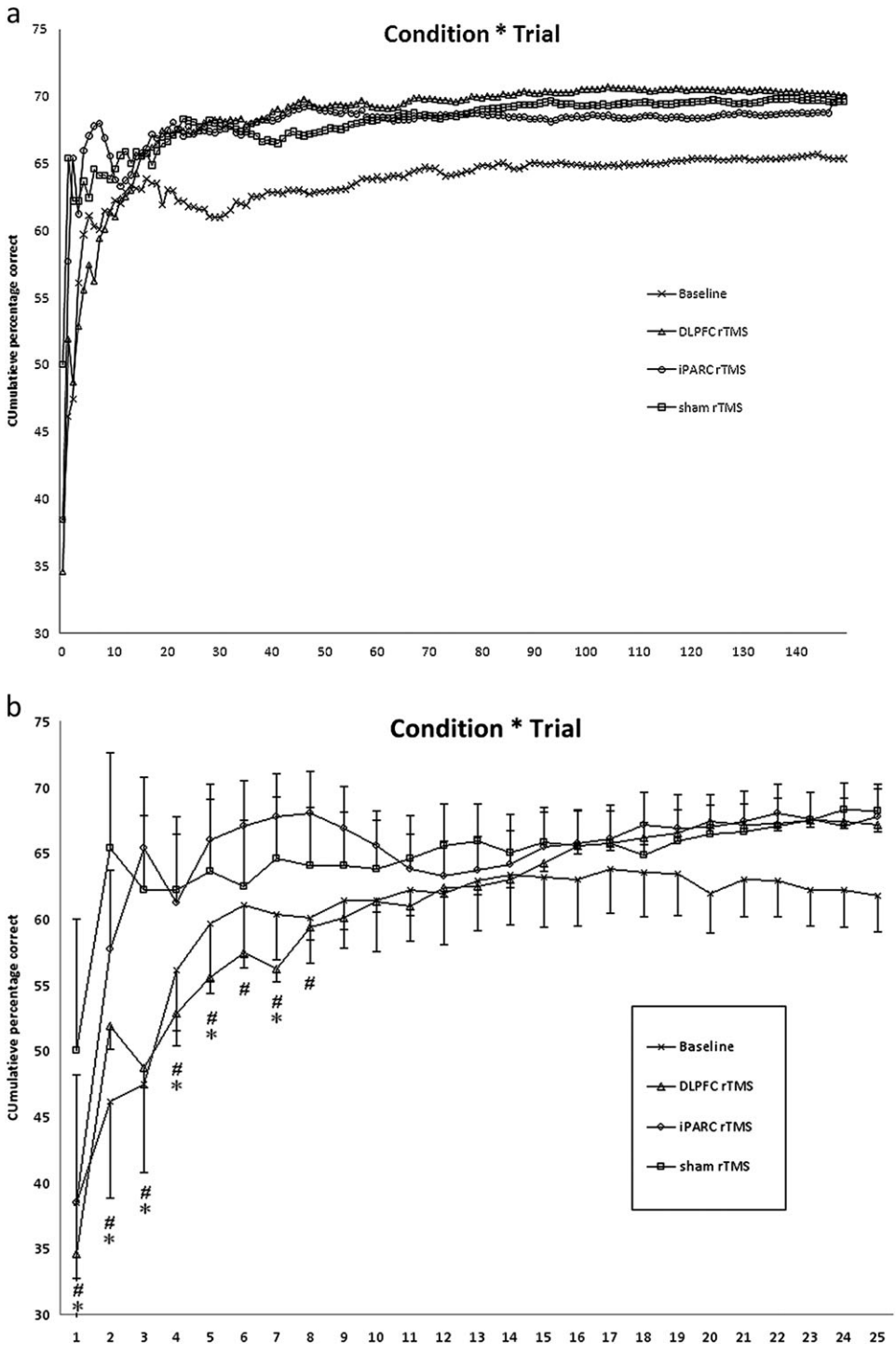

Figure 2. (*a*) Learning curves across 150 trials for each of the conditions (baseline, DLPC rTMS, iPARC rTMS, and sham rTMS) during probabilistic association learning, showing the significant condition by trial interaction across all trials. (*b*) Learning curves across the first 25 trials of the probabilistic association learning task, showing a significant suppression of learning in the DLPFC rTMS session compared with the iPARC rTMS session (indicated by asterisk) and compared with the sham rTMS session (indicated by hash). Vertical bars denote standard error.

During later trials (100–150), the learning curve reached asymptote. We therefore calculated the average of the cumulative percentage correct over the final 50 trials to assess differences in the asymptotic performance level over the different task conditions. A repeated measures ANOVA on the average percent correct across the last 50 trials, with task condition as a within-subjects variable, revealed a significant main effect of condition, $F_{3,75} = 4.43$, $P = 0.006$. Planned contrasts were conducted to assess the differences among the conditions. Compared with baseline, each of the subsequent conditions was characterized by a higher asymptotic level attained: DLPFC rTMS, $F_{1,25} = 12.54$, $P = 0.002$; iPARC rTMS, $F_{1,25} = 5.21$, $P = 0.031$; sham, $F_{1,25} = 6.88$, $P = 0.015$. There were no significant differences between active rTMS and sham stimulation conditions during the asymptotic phase.

Reaction Times

A repeated measures ANOVA was conducted on the mean RTs for the different conditions over all 150 trials, revealing a highly significant main effect of condition, $F_{3,75} = 20.96$, $P < 0.001$ (see Fig. 3), with RT at baseline being significantly slower than RT during all other conditions. There were no significant differences among active rTMS or sham conditions with respect to RT.

Examining the acquisition and asymptotic phases revealed a significant main effect of condition both on the first 25 trials, $F_{3,75} = 18.39$, $P < 0.001$, and the final 50 trials, $F_{3,75} = 11.86$, $P < 0.001$. Planned contrasts revealed a significant difference in mean RT between the baseline session and each of the subsequent sessions (all P's <0.001), but no significant differences among rTMS stimulation and sham conditions.

Discussion

One hertz rTMS to the DLPFC, but not the iPARC-disrupted acquisition of probabilistically, related cue–outcome associations on subsequent exposures to revised probability schedules. Neither active nor sham stimulation affected latter trials and ultimate overall performance levels. Previous fMRI studies suggest of a role for the prefrontal cortex in the acquisition of probabilistic associations by showing prefrontal cortex activity during cue–outcome association learning (Poldrack et al. 1999; Fera et al. 2005; Weickert et al. 2009). The current finding that rTMS to the DLPFC suppresses the acquisition of the probabilistic cue–outcome associations during a subsequent session suggests that DLPFC activity may facilitate acquisition of rearranged cue–outcome associations on subsequent exposure. Participants became familiar with the task and may have retained some memories for the cards, previous cue–outcome associations, or strategies during a baseline assessment session. Although different probability schedules were administered over multiple sessions with the cue–outcome associations varied, the cue cards remained identical across sessions. In the initial presentation of the novel task, participants have to rely on a guessing strategy since no information about the different cue–outcome associations is originally available. If participants are able to access memories of learning strategies on subsequent exposures that use rearranged cue–outcome probabilities, then acquisition may be initially facilitated. DLPFC access to strategy memories from previous exposure would obviate the need for gradual acquisition of the cue–outcome associations as evidenced by the "hockey stick-" or "hinge-"like function (Hasselblad et al. 1976) shown during the early trials of subsequent sessions (Fig. 2*b*) with the exception of DLPFC rTMS which displayed the more characteristic gradual learning curve similar to baseline. Functional neuroimaging studies have also found a relationship between increased performance during early strategy use and greater DLPFC activity in healthy adults (Della-Maggiore and McIntosh 2005). However, as the task progresses within the first 25 trials, on subsequent presentations, some gradual acquisition occurs as participants adjust to learn the rearranged cue–outcome probabilities. Thus, although some familiarity with the task may provide some acquisition benefit during subsequent sessions, the rearranged probabilistic structure requires additional learning.

Previous studies (Knowlton et al. 1996a, 1996b; Poldrack et al. 1999, 2001) have shown that the striatum (especially, the caudate nucleus) is important for the acquisition of the probabilistic cue–outcome associations. The accumulation of cue–outcome associations occurs over time, putatively takes place without conscious awareness, and is akin to gradually acquired habit learning, which depends on dorsal striatal functioning, as seen in experimental animals (Packard et al. 1989). As described above, this would apply throughout each session in the baseline and DLPFC rTMS conditions but not

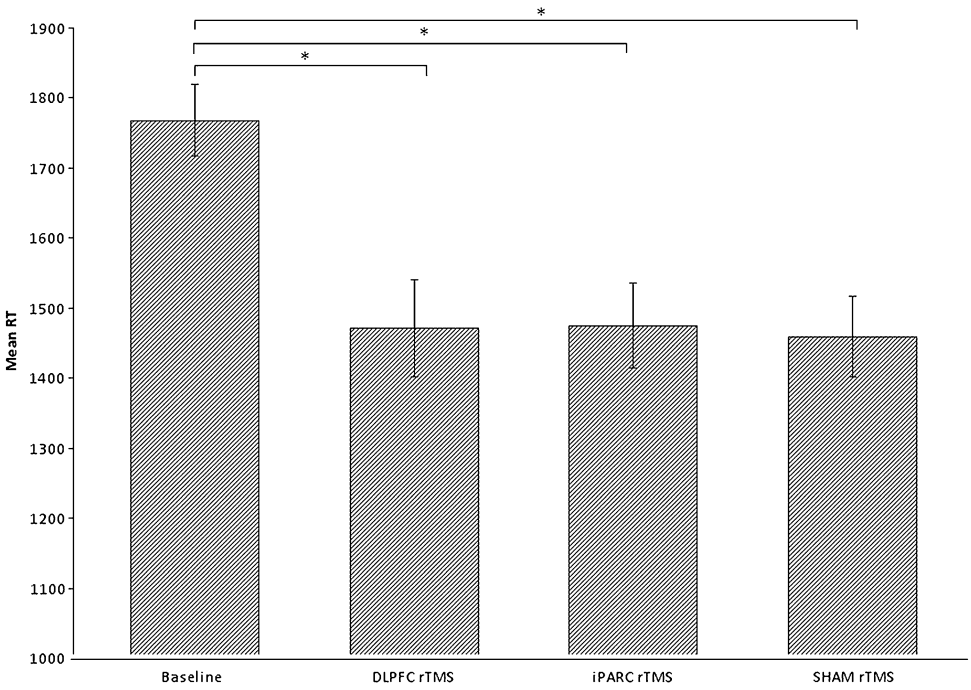

Figure 3. Mean RTs for the 4 conditions across all trials. There was a significant difference between the baseline session and each of the subsequent sessions (indicated by asterisk), but no significant difference among the subsequent sessions, indicating faster response times in all subsequent sessions following baseline assessment.

necessarily during early acquisition of subsequent administrations.

Regarding the role of the iPARC in probabilistic association learning, functional neuroimaging studies have shown active engagement of this area and positive associations between iPARC activity and enhanced performance in older adults (Poldrack et al. 1999; Fera et al. 2005; Weickert et al. 2009). However, in the present study, we found no evidence of a disruption in acquisition nor in the ultimate performance level due to iPARC stimulation. Thus, it appears that the contribution of the iPARC is not critical in learning the cue–outcome associations. Weickert et al. (2009) and Fera et al. (2005) provide evidence suggesting that the iPARC may have a compensatory role in groups in which the DLPFC and caudate nucleus are compromised. Patients with schizophrenia, who typically show prefrontal deficits, failed to sufficiently activate DLPFC and caudate nucleus during probabilistic association learning (Weickert et al. 2009). However, those people with schizophrenia who did perform the task well showed increased activation in a compensatory network of brain regions that included the iPARC. This further supports the idea that the underlying neural substrate to learn the probabilistic associations can vary. Unlike the DLPFC, in healthy adults, the iPARC does not seem to provide access to prior strategy or memory of prior task-related information. However, chronically ill patients who have had time to develop cortical reorganization may exhibit a compensatory mechanism via iPARC.

The ultimate performance level, following presentation of a sufficient number of trials, is indicative of how well these cue–outcome associations are acquired. Interestingly, in each of the rTMS stimulation or sham conditions, the ultimate performance level surpassed that attained during the baseline session when participants were task-naive. This is in line with the finding that task skill consolidation between sessions may positively influence performance on subsequent encounters with the same task, which has been previously demonstrated for procedural and declarative learning (Cohen et al. 2005; Brown and Robertson 2007; Robertson 2009). This was achieved through a combination of a rapid elevation in performance during the early trials (made possible via DLPFC access to previous memories of task-relevant information) and gradual acquisition of the revised cue–outcome associations across latter trials. Through consolidation, a motor skill or memory may undergo both qualitative and quantitative changes: It can be enhanced, leading to an increase in performance, or stabilized, leading to reduced susceptibility to interference, and in terms of qualitative changes, there can be a shift in strategy or an emergence of awareness for what has been learned earlier (Robertson et al. 2004; Walker 2005). Consolidation of an acquired skill occurs in memory systems that are characterized by reciprocal interactions during wakefulness but are disengaged during sleep. This disengagement during sleep allows the simultaneous processing of procedural and declarative knowledge and may increase the computational power of memory processing. During sleep, the brain's capacity to reorganize and reveal "hidden patterns" becomes especially potent (Robertson 2009). That is, the capacity to bypass intermediary steps increases following consolidation during sleep by forming associations between early steps and the final solution of a problem (Wagner et al. 2004). It thus seems that this type of implicit probabilistic association learning assessed by the weather prediction test is a skill that is amenable to improvement and can be consolidated over days.

A possible alternative explanation for the effects observed during the early phases of DLPFC rTMS is that stimulation to the prefrontal region is more likely to produce side-effects such as facial muscle twitching, which may interfere with task performance and which could affect the participant's ability to register an adequate response. However, it is unlikely that the DLPFC effect observed in the accuracy data is merely due to the direct distracting effects of the stimulation itself since the reaction time data showed that both during acquisition as well as in the asymptotic phase, RTs were similar across all stimulation conditions. In addition, if rTMS application was due to distracting side effects, then we would expect performance to fall below the level of the baseline session, during which no stimulation at all was present; however, this was not the case. Furthermore, such a disturbance would be consistent throughout the task not differentially affecting acquisition. Thus, it is more likely that the findings are specific to the influence on local brain activity and not due to general rTMS-induced side effects. Alternatively, 1 Hz rTMS may facilitate neural activity, and the increased DLPFC activity may impair acquisition; however, in the majority of previous studies (Pascual-Leone and Hallett 1994, 1996; Mottaghy et al. 2000, 2002; Robertson et al. 2001) low-frequency (<1 Hz) rTMS decreases cortical excitability.

In summary, 1 Hz rTMS to the DLPFC was found to impair only the acquisition phase of probabilistic association learning during subsequent presentations while ultimate learning was unaffected by either DLPFC or iPARC stimulation. Speed of responding was not disrupted by rTMS to either DLPFC or iPARC. Although functional neuroimaging studies routinely report increased activity in DLPFC and iPARC during probabilistic association learning, the present findings suggest that disruption of DLPFC activity during subsequent presentation of revised probability structures may inhibit or retard access to consolidated strategies or memories from previous exposure to the task. Neither DLPFC nor iPARC activation appear to be critical for the ultimate acquisition of the probabilistic cue–outcome associations in healthy young individuals.

Since rTMS was applied only during subsequent administrations, on the basis of Experiment 1, it was not clear whether DLPFC disruption via low-frequency rTMS would impair acquisition of the probabilistic cue–outcome associations during the first administration in task-naive participants. Thus, in order to determine whether disruption of DLPFC interferes with acquisition of the probabilistic cue–outcome associations during the first administration in task-naive participants, we conducted Experiment 2 in which rTMS or sham was administered to the DLPFC during the first administration of the weather prediction test in an independent sample of task-naive participants. If the DLPFC contributes critically to acquisition of the probabilistic cue–outcome associations, then applying 1 Hz rTMS to the DLPFC during the first administration in task-naive participants should disrupt acquisition of cue–outcome associations. Additionally, since all subsequent sessions were administered with 1 or more intervening night's sleep, it was not clear whether practice (simple repetition) or consolidation was responsible for early acquisition improvement during subsequent sessions. Thus, we conducted Experiment 3 in which a third independent sample of 9 healthy participants who were naive to the weather prediction

test received 2 consecutive administrations of the weather prediction test on the same day with different probability schedules at each administration without brain stimulation in order to confirm that accelerated early acquisition on subsequent administrations is related to consolidation as opposed to practice (repetition) effects.

Experiment 2: The Effect of 1 Hz rTMS to the DLPFC in Task-Naive Participants

Participants

Fifteen healthy volunteers naive to the probabilistic learning test participated in Experiment 2 (age range 19–28 years, mean age 21.8 years, SD = 2.6, 7 males, all right-handed). Mean education level was 15.1 years (SD = 1.3), average IQ was 120.2 (SD = 9.9), based on a 4-subtest version of the WAIS-III (Wechsler 1997). Consent was obtained in the same manner as in Experiment 1. Participants were required to complete a demographic and screening questionnaire, and all participants were interviewed by a medical practitioner for contraindications to TMS prior to stimulation. None of the participants had a history of seizures, psychiatric illness, or severe head injury or any contraindications to rTMS (Wassermann 1998; Loo et al. 2008).

Procedure

Weather Prediction Task

Each volunteer participated in 2 separate testing sessions, performing 75 trials of the weather prediction task described above. The sessions consisted of 1 active and 1 sham rTMS session to the left DLPFC in pseudorandomized order such that half the participants received active rTMS during the first session. A different probability schedule was used at each testing session, presented in pseudorandomized order such that no probability schedule was presented more than once to a given participant.

rTMS Setup

Motor threshold determination was conducted as described above for Experiment 1. The average motor threshold was 53% of the maximum stimulator output (range 47–64%). Each individual participated in 2 rTMS sessions scheduled at least 2 days apart (mean days between sessions 6.7, SD = 6.0). During the active rTMS sessions, participants received 1 Hz rTMS over the left DLPFC. Sham rTMS was achieved by tilting the coil by 90 degrees away from the scalp, at the same location. Localization and application of rTMS were performed as described above, for Experiment 1, with the exception of only a single train of active rTMS or sham being administered while participants completed 1 block of the weather prediction task, consisting of 75 trials. The rTMS train consisted of 375 pulses and lasted for 6.25 min.

Statistical Analyses

In order to assess the rate of learning over the course of the task, we performed a repeated measures ANOVA using within-subjects factors "condition" (active rTMS vs. sham) and "trial block" and a between-subjects factor of "condition order" (active rTMS first vs. sham first) on the cumulative mean percentage correct over 75 trials. The main analysis of interest was the condition by trial interaction, which shows differences in the learning curves over the course of the task between the 2 conditions (DLPFC rTMS vs. sham).

Mean reaction times were calculated for each of the conditions, over the initial 25 trials, indicative of acquisition, and across the final 50 trials, indicative of asymptotic learning. A repeated measures ANOVA was conducted on these RTs, with task condition (DLPFC rTMS vs. sham) and trial as the within-subject variables.

Results and Discussion

Learning Curves

The repeated measures ANOVA on percent correct revealed a highly significant effect of trial block $F_{74,814} = 4.13$, $P < 0.001$, indicating a gradual increase in the percentage correct over the course of the task across both conditions. However, neither the effects of conditions nor the interactions of conditions by trial block were significant, indicating that the rate of learning between active and sham conditions was similar (see Fig. 4) and no order effect. There was also no significant main effect of condition order and no significant interaction.

Reaction Times

Repeated measures ANOVA revealed no significant difference in the mean RT's between the 2 conditions (DLPFC rTMS vs. sham) over the first 25 trials, indicative of acquisition rate, or the final 50 trials, indicative of asymptotic performance.

Experiment 3: The Effect of Consecutive Administration of Probabilistic Learning Test

Participants

Nine healthy volunteers naive to the probabilistic learning test participated in Experiment 3 (age range 20–38 years, mean age 26.4 years, SD = 5.7, 3 males, 8 right-handed). Mean education level was 17.0 years (SD = 1.7). Consent was obtained in the same manner as in Experiment 1.

Procedure

Weather Prediction Task

Each volunteer participated in 2 separate testing sessions administered consecutively, performing 150 trials of the weather prediction task described above during each session. A different probability schedule was used at each testing session presented in pseudorandomized order such that no probability schedule was presented more than once to a given participant.

Statistical Analyses

In order to assess the rate of learning over the course of the task, we performed a repeated measures ANOVA using within-subjects factor "first versus second administration" on the cumulative mean percentage correct over 150 trials. The main analysis of interest was the condition by trial interaction that shows differences in the learning curves over the course of the task between the 2 administrations (first vs. second).

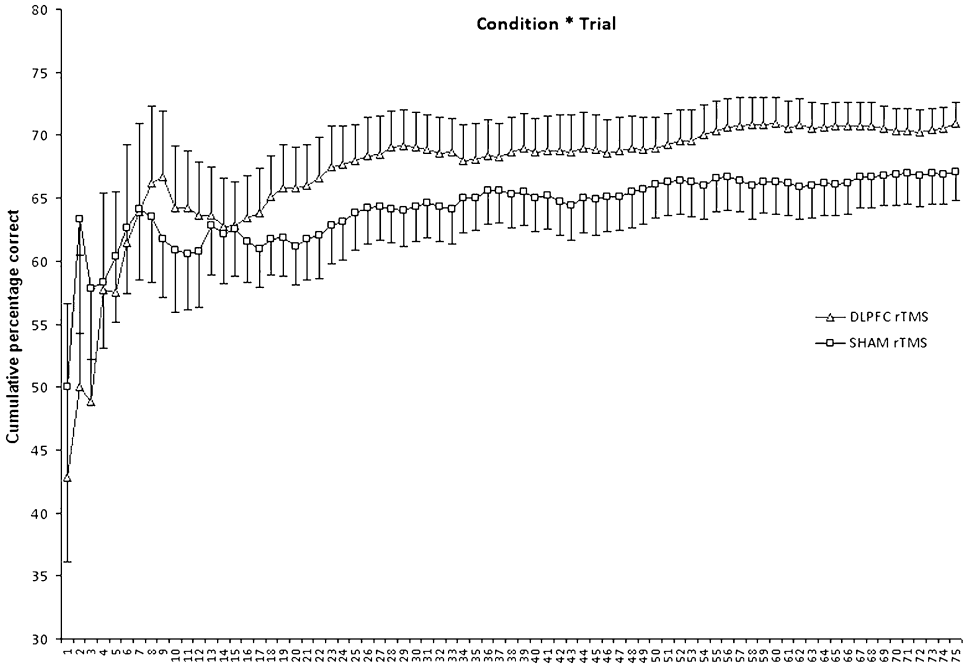

Figure 4. The learning curves for the 2 conditions (DLPC rTMS and sham rTMS) during initial probabilistic association learning session showing no significant differences across the 75 trials of the task. Vertical bars denote standard error.

Results and Discussion

Learning Curves

The repeated measures ANOVA on percent correct revealed no significant main effect of administration (first vs. second administration), a highly significant effect of trial block, $F_{9,72}$ = 2.82, $P < 0.007$, indicating a gradual increase in the percentage correct over the course of the task across both conditions, and a significant trial block by administration (first vs. second administration) interaction, $F_{9,72} = 2.14$, $P = 0.04$, indicating an effect of administration on the learning curve. Post hoc LSD comparisons revealed significant differences between first and second administrations only during the first trial block, $P = 0.004$ (see Fig. 5), that is, significant interference during the early acquisition of the second administration.

General Discussion

Results from the second experiment confirm that when participants are task-naive, disrupting left DLPFC activity by means of 1 Hz rTMS does not impair the acquisition of cue–outcome associations during probabilistic association learning. There were no differences in the learning curves between the 2 conditions (DLPFC rTMS vs. sham) during the acquisition phase or in the asymptotic learning phase. Reaction times were also unaffected by rTMS administration. Although learning rates in the weather prediction task have been shown to be enhanced among those with higher levels of executive function, and thus presumably adequate PFC functioning (Price 2005), another study revealed that acquisition of probabilistic cue–outcome associations was in fact not impaired in people with prefrontal cortex lesions (Knowlton et al. 1996a). The current series of experiments suggests that prefrontal cortical activity is not integral to initial acquisition of the probabilistic associations. Our results also fit with a previous finding that executive function may be associated with explicit category learning, but not "implicit" category learning (DeCaro et al. 2008), which apparently constitutes the basis of gradual and nondeclarative learning of novel cue–outcome associations in a probabilistic design such as the weather prediction task.

Without prior exposure to the task, participants cannot rely on previously acquired and consolidated strategies to enhance performance early during the acquisition phase when new probabilistic cue–outcome associations are being presented. Thus, the rTMS-elicited deficit during early trials in the first experiment appears to have been due to disruption of DLPFC-mediated rapid access to consolidated task-specific skills, memories, or strategies accrued from previous exposure to the task. Experiment 1 was designed in such a way that at least 2 days (and 2 nights) passed between the baseline session and any subsequent session. This time delay may have allowed for consolidation of task skills before any active or sham rTMS session was performed. Those skills may have been both declarative, which would have enhanced performance in the early trials of subsequent exposures (due to strategic responding and conscious memories of prior task experiences), and non-declarative, which may have enhanced the gradual acquisition of the revised cue–outcome associations as the task progressed. In the second experiment, effects of prior task exposure were eliminated by administering randomized active or sham stimulation during the first exposure in task-naive participants and using different probabilistic association schedules. Interference due to prior exposure to distinct probabilistic cue–outcome association schedules in Experiment 3 and the lack of any early disruption by DLPFC rTMS during the first administration of the probabilistic association learning test to task-naive participants in Experiment 2 suggest that DLPFC rTMS during subsequent administrations induces a disturbance of neocortical processing which disrupts access to the previously stored and consolidated skills, memories, or

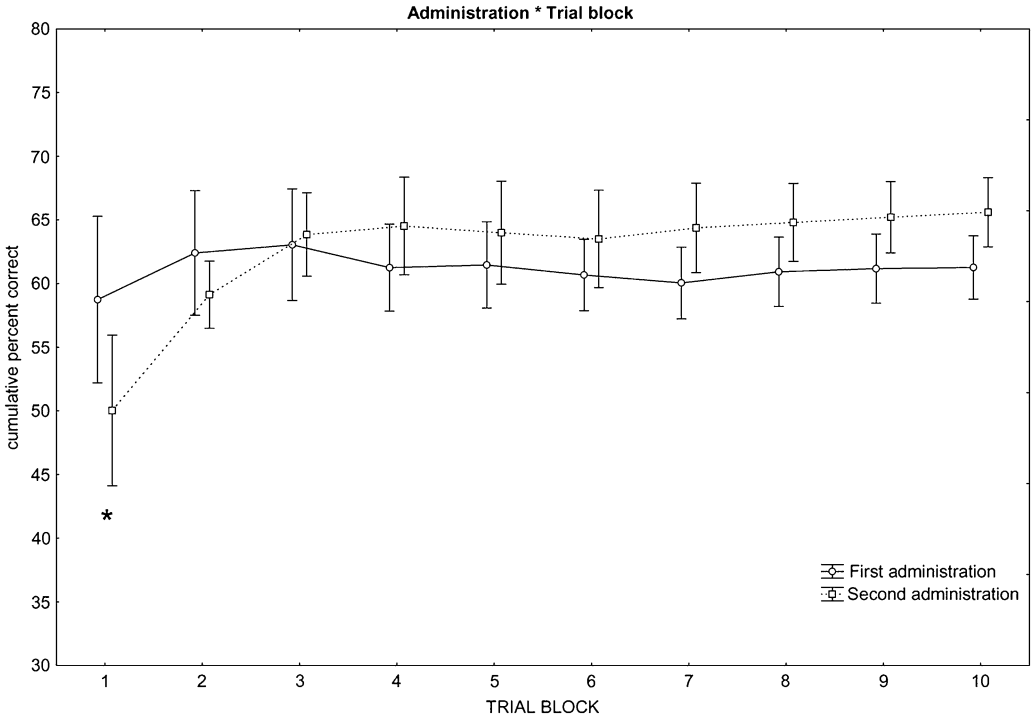

Figure 5. Learning curves for repeated administration of probabilistic association learning test using different probabilistic cue–outcome association schedules in task-naive participants showing significant (based on LSD post hoc tests with $P = 0.004$ and indicated by asterisk) diminution of learning between first and second administration. Vertical bars denote standard error.

strategies, thereby necessitating a complete reacquisition de novo of the probabilistic cue–outcome associations.

One alternative explanation for the lack of an observable disruption in the acquisition of cue–outcome associations during Experiment 2 is that rTMS stimulation was applied unilaterally. Interhemispheric compensatory mechanisms may circumvent the potential interfering effects of rTMS applied to the left DLPFC in isolation. Also, fMRI studies have revealed that both the DLPFC and the iPARC are simultaneously activated during probabilistic association learning; hence, either 1 of these 2 regions may compensate at least partially for disruption of the other as suggested by increased iPARC activity with comparable probabilistic learning performance in older adults (Fera et al. 2005). However, other rTMS studies have observed disrupted performance on cognitive tasks, including working memory, by means of rTMS application to the left DLPFC in isolation (e.g. Osaka et al. 2007), which indicates that compensatory mechanisms may not be sufficient for optimal task performance when a critical region is disrupted. However, Experiment 1 in the present study did demonstrate a detrimental effect of unilateral DLPFC rTMS on acquisition during subsequent exposure to the task. Conversely, with respect to cortico-cortical connectivity, the DLPFC and medial temporal lobe (MTL) are strongly associated with memory encoding and retrieval; thus, DLPFC rTMS may have produced indirect effects on MTL-related declarative processes during early acquisition as per Poldrack et al. (2001).

Since identical cues were used during subsequent administrations, there was some potential for interference from previous exposure that could negatively influence performance on subsequent administrations, and DLPFC activity may have inhibited such interference from previous exposure. Given this interpretation, DLPFC rTMS may have prevented the DLPFC from inhibiting the interference effects from previous sessions. Finally, those participants displaying increased conscious awareness of the rules may have been more susceptible to the effects of DLPFC rTMS. However, Gluck et al. (2002) have shown that self-report of strategies used to solve the weather prediction test are inconsistent with people's own performance even in healthy participants.

In sum, evidence from 3 experiments, in which rTMS was used to disrupt processing in discrete brain regions previously associated with performance on probabilistic association learning suggests a lack of a critical contribution from either the DLPFC or iPARC to initial acquisition of novel probabilistic cue–outcome associations. Disrupting DLPFC function affected the early acquisition phase of probabilistic association learning but only during subsequent sessions that included at least 1 or more intervening night's sleep. These results suggest that the DLPFC plays a role in accessing consolidated memories and cognitive skills to improve performance when acquiring novel probabilistic based cue–outcome associations during subsequent sessions of probabilistic association learning. However, initial learning of probabilistic cue–outcome associations may be more generally dependent on other brain regions such as the striatum.

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