Interference with Existing Memories Alters Offline Intrinsic Functional Brain Connectivity

Nitzan Censor, Silvina G. Horovitz, and Leonardo G. Cohen

1Human Cortical Physiology and Neurorehabilitation Section
2Human Motor Control Section
National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: censorn@ninds.nih.gov (N.C.), cohenl@ninds.nih.gov (L.G.C.)
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SUMMARY
The notion that already existing memories can be modified after their reactivation has received an increasing amount of experimental support, with empirical data accumulating across species and memory paradigms. However, there is no evidence for systems-level task-free intrinsic signatures of memory modification. Here, using a combination of behavioral, brain stimulation, and neuroimaging paradigms, we report that noninvasive transcranial magnetic stimulation interference with a reactivated motor memory altered offline task-free corticostratal interregional functional connectivity, reducing it compared to stimulation in which the reactivated memory was intact. Furthermore, the modulated functional connectivity predicted offline memory modification. This reduction in functional connectivity recovered after additional execution of the memorized task, and the interference did not affect control cerebellar-cortical functional connectivity. This demonstrates that intrinsic task-free offline brain activity can be modulated by noninvasive interaction with existing memories and strongly correlates with behavioral measurements of changes in memory strength.

INTRODUCTION
Modification of existing memories is a critical process required to improve skills and rehabilitate brain injuries and conditions like posttraumatic stress disorders (Schiller et al., 2010). Thus, after encoding and initial stabilization through consolidation (McGaugh, 2000; Robertson, 2012), memories are dynamic and have the potential to significantly change over time (Lee, 2008; Dudai, 2012; Nader et al., 2000; Walker et al., 2003; Robertson, 2012). Compelling evidence suggests that previously consolidated memories can be substantially modified after retrieval, during which the memories are reactivated. This modification can result in strengthening, disruption, or update of the memory (Nader and Hardt, 2009). The neural mechanisms of memory modification have been studied in animal models at the cellular level (Lee, 2008; Dudai, 2012) and evidence has been accumulating for similar processes in humans (Chan and LaPaglia, 2013; Forcato et al., 2007; Hupbach et al., 2007; Schiller et al., 2010; Schwabe et al., 2012; Censor et al., 2010; Walker et al., 2003). However, whether there are actual offline task-free intrinsic neural signatures of modified memories at a systems level is an open question in memory research. Here we show that noninvasive brain stimulation interference with a reactivated motor memory altered offline task-free corticostral interregional functional connectivity, reducing it compared to control stimulation in which the reactivated memory was intact. This identifies an intrinsic task-free neural signature of procedural memory modification that is associated with changes in memory strength.

Interfering with memories represents a valuable approach to acquire insight into the mechanisms of memory (Robertson et al., 2005; Walker et al., 2003; Censor et al., 2010; Robertson, 2012). Here, a combination of noninvasive techniques allowed us to test the effects of memory interference on human systems-level intrinsic brain functional connectivity. Our protocol utilized a motor learning paradigm, repetitive transcranial magnetic stimulation (rTMS), and resting-state fMRI. We used a well-defined behavioral paradigm that characterized modification of existing motor memories after their reactivation (Censor et al., 2010; Walker et al., 2003). In this paradigm, an already existing motor memory is reactivated by having the participants execute additional trials of the motor task. Interference is applied time locked to memory reactivation, resulting in significant memory modification relative to subjects who received control stimulation (Censor et al., 2010; Walker et al., 2003). Specifically, participants were first trained on a motor memory task. On a separate day, subjects were tested in the consolidated task and subsequently received rTMS interference or control stimulation synchronously with reactivation of the memory (additional trials of the task). On the following day, the memory was retested to determine whether it has been modified in the group that received rTMS interference compared to the group that received control stimulation. Thus, memory modification was measured as offline performance gains between test and retest (see Experimental Procedures) (Censor et al., 2010; Walker et al., 2003).

Modification of memories has been demonstrated across different animal models and memory types and studied predominantly in nonhumans by invasive administration of protein synthesis blockers with memory reactivation (Nader et al., 2000). The noninvasive nature of rTMS makes it feasible to manipulate human memories. Therefore, rTMS enabled us to apply a virtual
lesion (Censor and Cohen, 2011; Dayan et al., 2013) to the primary motor cortex (M1). This approach is particularly advantageous for evaluating resting-state connectivity the day after rTMS application, since the neurophysiological aftereffects of rTMS per se are relatively short lasting, in the order of the stimulation duration (Chen et al., 1997; Cheeran et al., 2010; Eisenegger et al., 2008). Given that M1 is a key region for successful acquisition of motor memories (Karni et al., 1995; Muellbacher et al., 2002), we interfered with activity in this region during memory reactivation (Censor et al., 2010). The timing of interference relative to task performance is important since rTMS applied to M1 immediately after practicing the serial reaction time task does not block subsequent learning when tested after a night of sleep (Robertson et al., 2005). Given this previous literature, in our study interference was applied simultaneously with memory reactivation and the behavioral and connectivity effects of such interference were measured the following day, long after dissipation of the rTMS neurophysiological aftereffects (Chen et al., 1997; Cheeran et al., 2010; Eisenegger et al., 2008).

fMRI enables to gain insight into intrinsic functional connectivity while the brain is at rest (Albert et al., 2009; Fox and Raichle, 2007; Tambini et al., 2010; Vahdat et al., 2011), evaluating the effects of interference with existing memories on the resting human brain. Task-based fMRI measurements that attempted to address this issue faced the complication that comparable performance is required to evaluate task-related measurements of memory strength and online execution of a task may by itself affect the fMRI signal (Logothetis, 2008; Censor et al., 2013; Schwabe et al., 2012). Measurements of intrinsic resting-state functional connectivity represent a way to close this gap in knowledge without the confound of task-based fMRI (Albert et al., 2009; Dayan and Cohen, 2011; Vahdat et al., 2011). Such studies showing that motor learning can modulate subsequent activity within resting-state networks have highlighted this useful approach to study memory processes at the systems level. For example, such studies have shown that motor adaptation learning is associated with changes in frontoparietal and cerebellar resting-state networks (Albert et al., 2009; Vahdat et al., 2011).

Different anatomical and functional loops contribute to skill acquisition. Specifically, the corticostriatal loop plays a key contributing role in motor learning, as shown in animal (Hikosaka et al., 2002; Yin et al., 2009) and in human neuroimaging (Albouy et al., 2008; Dayan and Cohen, 2011; Debas et al., 2010; Lehéricy et al., 2005; Ungerleider et al., 2002) studies. For example, Yin et al. (2009) have used in vivo striatal recordings to show that the sensorimotor dorsolateral striatum is engaged in late learning. Ex vivo recordings from striatal neurons of trained mice revealed that the changes observed in vivo corresponded to training-specific changes in excitatory glutamatergic synaptic transmission in the dorsolateral striatum (Yin et al., 2009). Neuroimaging studies have provided support for the influential models of striatal involvement in motor learning (Hikosaka et al., 2002) in humans, showing that the sensorimotor striatum is engaged in late stages of learning (Lehéricy et al., 2005) and optimizes oculomotor and motor sequence learning after sleep (Albouy et al., 2008; Debas et al., 2010). Overall, previous studies have shown that within the dorsal striatum, the posterior putamen is involved in sensorimotor processing in connection with striatal-motor projections (Draganski et al., 2008; Lehéricy et al., 2006; de Wit et al., 2012). Accordingly, we focused our

Figure 1. Experimental Design Enabling Testing of the Effects of Memory Interference on Intrinsic Resting Brain Functional Connectivity
The task required the subject to tap a five-digit sequence with performance measured as the number of correct sequences during each fixed 10 s trial. Participants were trained on a motor memory task (Figure S1). On a separate day, subjects completed the memory test and subsequently were divided into two groups. One group was stimulated with 1 Hz rTMS applied to the primary motor cortex (M1) synchronous with memory reactivation. The memory was reactivated by having the participants perform additional trials of the task. The control group received rTMS to a vertex position applied simultaneously with peripheral ulnar nerve stimulation, disrupting manual performance present when stimulating M1 but without disrupting memory modification. Both groups returned the following day for memory retest. Rest scans were performed before and after memory test and memory retest.
Intrinsic Signature of Memory Modification

**RESULTS**

We used a behavioral paradigm that characterizes modification of existing motor memories (see Experimental Procedures and Figure 1). The task required participants to perform a sequence of five finger movements with the nondominant left hand as quickly and accurately as possible during each 10 s trial. Participants (n = 20; mean age 26.1 ± 4.7; 7 males, 13 females) trained on a motor memory task (Figure S1 available online). On a separate day, after performing a memory test, participants were divided into two groups. In both groups, the memory was reactivated by having the subjects execute the memorized motor sequence task approximately 30 min after the test. In order to interfere with the motor memory, the interference group received 1 Hz rTMS to M1 synchronous with memory reactivation. In order to control for the interference with the memory induced by rTMS, a second group received 1 Hz rTMS to a vertex position, commonly used as a control site for TMS (see Experimental Procedures) (Censor et al., 2010). In addition, the control group received peripheral ulnar nerve stimulation at the wrist to mimic the disruption of manual performance present when stimulating M1 in the first group. In this way, online performance during reactivation trials was similarly disrupted in the two groups (p = 0.65, reduced compared to the memory test by 40.2% ± 3.3%, p = 0.37). Therefore, overall, there was a significant between-group memory modification difference (p < 0.02, in the absence of between-group test differences, p = 0.85, see Figure 2 and Figure S3). Memory interference altered preretest functional connectivity between dorsal striatum and M1 (Figures 3A and 3B), the crucial components of the corticostriatal loop engaged in motor learning (Albouy et al., 2008; Dayan and Cohen, 2011; Debas et al., 2010; Hikosaka et al., 2002; Lehe´ ricy et al., 2005; Unger-leider et al., 2002; Yin et al., 2009). Memory interference altered functional connectivity after additional execution of the memorized task, thus reaching equivalent functional connectivity strength to controls postretest (Figure 3C; H = 4.098, p < 0.05, see Experimental Procedures).

A crucial question is whether the identification of this offline intrinsic neural signature of motor memory modification relates to the behavioral measurements of changes in memory strength. Such association with the behavioral measurement in the absence of online task-confounding fMRI component would significantly strengthen the interpretation of the findings (Dayan and Cohen, 2011; Logothetis, 2008). Indeed, we found that pre- and retest functional connectivity between dorsal striatum and M1 predicted the magnitude of memory modification (performance gains between memory test and retest, see Experimental Procedures), showing a strong correlation between the behavioral measurement and resting functional connectivity (r = 0.58, p < 0.008; Figure 3D).

We also measured the resting functional connectivity after memory retest. Interestingly, corticostriatal functional connectivity in the interference group recovered after additional execution of the memorized task, thus reaching equivalent functional connectivity strength to controls postretest (Figure 3C; H = 0.037, p = 0.85). Such recovery in functional connectivity in the corticostriatal loop suggests that the memory trace after additional execution of the memorized task is restrengthened, in line with...
the engagement of this circuit in motor sequence learning (Albouy et al., 2008; Dayan and Cohen, 2011; Debas et al., 2010; Hikosaka et al., 2002; Lehericy et al., 2005; Ungerleider et al., 2002; Yin et al., 2009). Between-session connectivity changes were not significant for the interference (Friedman’s test, $\chi^2 = 3.684$, $p = 0.30$) and the control groups ($\chi^2 = 3.800$, $p = 0.28$).

To determine the specificity of the effects induced by rTMS interference with the motor memory, we then measured resting functional connectivity within the visuomotor cerebellar-cortical network, between M1 and the anterior cerebellum. Previous studies have suggested that this network plays an important role in early motor learning and that it is involved in movement execution and initial motor skill acquisition (Dayan and Cohen, 2011; Hikosaka et al., 2002; Ungerleider et al., 2002). Memory interference did not alter preretest M1-cerebellar functional connectivity at rest measured on the following day, with no significant difference compared to controls (Figure 4; $H = 0.02$, $p = 0.97$). Functional connectivity in the interference group increased after additional execution of the memorized task; however, there were no significant differences between the groups postretest ($H = 0.67$, $p = 0.41$). This nonsignificant increase may reflect resetting of the cerebellar-cortical circuit after memory interference, consistent with its involvement in initial skill acquisition, thereby allowing within-session memory strengthening (Dayan and Cohen, 2011; Hikosaka et al., 2002; Ungerleider et al., 2002). Between-session connectivity changes were not significant for the interference (Friedman’s test, $\chi^2 = 1.400$, $p = 0.71$) and the control groups ($\chi^2 = 3.132$, $p = 0.37$). An additional set of exploratory analyses showed no effects of interference using additional regions of interest based on previous studies (Albert et al., 2009; Vahdat et al., 2011) (see Tables S1 and S2).
DISCUSSION

The question of how existing memories are modified over time has received extensive attention over the last decade, specifically following the boost of seminal studies on reconsolidation of fear memories (Lee, 2008; Nader et al., 2000). These studies utilized predominantly invasive techniques such as administration of protein synthesis blockers with memory reactivation, in order to identify possible memory modification mechanisms. Recent efforts have focused on extending the knowledge gained from animal studies to reveal the mechanisms underlying memory modification in humans. Indeed, significant progress has been made at the behavioral level, with studies in humans spanning from episodic memory to fear memories and motor memories (Forcato et al., 2007; Hupbach et al., 2007; Schiller et al., 2010; Walker et al., 2003). However, while the molecular signatures of memory modification have been studied extensively, evidence for system-level intrinsic task-free neural signatures of human memory modification is still needed. A limitation to acquire this knowledge has been that the use of invasive approaches is not generally feasible in humans. Here, we used a combination of noninvasive techniques to address this question.

Interference with memory modification altered interregional corticostriatal functional connectivity in the resting brain. The day after interference, corticostriatal functional connectivity was reduced compared to controls and predicted the magnitude of memory modification. After additional execution of the memorized motor task, reduced connectivity returned to control values. Motor memory interference did not affect control cerebellar-cortical functional connectivity. These results provided a causal demonstration that intrinsic offline neuronal representations can be modulated by noninvasive interference with procedural memories and are strongly associated with behavioral measurements of changes in memory strength.

Recent studies have demonstrated that experience can modify functional connectivity within resting-state networks (Albert et al., 2009; Vahdat et al., 2011) and predict subsequent memory strength (Tambini et al., 2010). Training with a motor task can modulate activity within resting-state networks the same day (Albert et al., 2009), the day after training (Vahdat et al., 2011), and at even longer time periods after practice (Taubert et al., 2011). Our finding, consistent with this literature, indicates that resting corticostriatal connectivity predicts memory modification and provides evidence for task-free involvement of this loop in offline modification of motor memories. This conclusion is consistent with previous work showing dynamic reorganization within striatal circuits during skill acquisition in rodents (Yin et al., 2009), nonhuman primates (Miyachi et al., 1997), and humans (Albouy et al., 2008; Debas et al., 2010; Lehéricy et al., 2005; Ungerleider et al., 2002). The finding that modulation of functional connectivity was detected the next day, after a night of sleep, supports the notion that this modulation required an interaction with offline processes such as sleep or memory reconsolidation (Diekelmann and Born, 2010; Dudai, 2012; Nader and Hardt, 2009).

Resting corticostriatal connectivity improved with additional execution of the memorized task, consistent with the involvement of the corticostriatal loop in motor learning (Dayan and Cohen, 2011; Censor et al., 2013; Hikosaka et al., 2002; Ungerleider et al., 2002). The extent to which this improvement in connectivity relates to rebuilding of the memory remains to be determined. The framework of reconsolidation would predict that additional exposures to the task without further rTMS would result in rebuilding of the memory. This issue requires investigation since it may be relevant to the design of interventional strategies to avoid rebuilding of maladaptive memories as in posttraumatic stress disorders. Purposeful modulation of memories seems to require balancing the need for frequent interference sessions (rather than a “one-time” shot at the reactivated maladaptive memory) and the need to minimize exposure to the original memory to prevent it from rebuilding.

These results raise considerations for future research. First, previous work using longer training sessions demonstrated within-session modulation of activity and functional connectivity (Albouy et al., 2008; Dayan and Cohen, 2011; Debas et al., 2010; Lehéricy et al., 2005; Albert et al., 2009; Vahdat et al., 2011), not seen here using a much shorter period of practice. The influence of length of training is probably an important factor in modulating memories. Second, it remains to be established at a systems level the differences and similarities between the mechanisms of consolidation and those of memory modification (Dudai, 2012). Finally, while rTMS interference was applied during memory reactivation, it could have acted during memory...
reactivation or immediately after rTMS application (Robertson, 2012).

Beyond the identification of an intrinsic task-free brain activity signature of memory modification, our results demonstrate a biological link between such brain activity and interindividual variability in memory-related behavior. The ability to noninvasively modulate intrinsic offline neuronal representations, which in this case predict changes in memory strength, may provide a powerful approach to study neuronal architectures of learning and memory, possibly applicable to other modalities (Censor et al., 2012). Furthermore, in order to modulate offline brain activity as performed here, noninvasive interaction with existing memory traces may lay the foundations for future attempts to facilitate network connectivity to improve brain function in humans with neurological disorders and memory deficits.

EXPERIMENTAL PROCEDURES

Subjects
Twenty naive right-handed healthy subjects (mean age 26.1 ± 4.7) having a normal neurological examination gave their written informed consent to participate in the project, which was approved by the Combined Neuroscience Institutional Review Board of the National Institutes of Health (NIH). Participants reported at least 6 h of sleep at night before each experimental session and were able to perform and learn the motor task without being an active musician (Censor et al., 2010; Korman et al., 2007).

Task and Experimental Design
The experimental design is outlined in Figure 1. Resting-state fMRI scans were performed immediately before (pretest) and after (posttest) the motor memory test. Thirty minutes after the test, the memory was reactivated by having participants perform additional trials of the memorized task while applying inhibitory rTMS or control stimulation. On the following day, resting-state fMRI scans were performed immediately before (pretest) and after (posttest) the motor memory retest.

Subjects performed a sequential finger-tapping task (Censor et al., 2010; Korman et al., 2007; Walker et al., 2003). All sessions were performed before 3 p.m. Using a four-key response pad, participants were instructed to repeatedly tap with their nondominant left hand a sequence of five finger movements (4-1-3-2-4) as quickly and accurately as possible during each 10 s trial, followed by a 10 s intertrial interval. Each key press produced a dot on the screen. During the trial, the sequence was displayed on a monitor in front of the subject. Subjects trained in 36 trials of the motor task (see Figure S1). Their motor skill was tested on a separate day with nine trials. After 30 min, subjects were divided into two groups. The interference group (n = 10) performed nine trials to reactivate the memory with rTMS applied over M1 to interfere with subsequent memory modification (reactivation trials). The control group (n = 10) performed the reactivation trials with rTMS applied over the vertex. Concurrently, controls received peripheral nerve stimulation (PNS) to the ulnar nerve at the wrist in order to mimic disruption of manual performance present when stimulating M1. While performing test trials in the absence of rTMS, reactivation trials were performed under the influence of M1 stimulation to interfere with subsequent memory modification (or control vertex/PNS stimulation; Censor et al., 2010). On the following day, both groups received rTMS at the frequency of 1 Hz for 15 min while performing the reactivation trials. rTMS was applied to M1 (to interfere with memory modification) or to the vertex (which did not interfere with memory modification) control site. Stimulus intensity was adjusted for each individual in order to elicit five out of ten motor-evoked potentials (MEPs) greater than 1 mV in the left first dorsal interosseous (FDI) muscle (Censor et al., 2010). Surface electromyogram (EMG) was recorded from surface Ag-AgCl electrodes positioned on the skin overlaying the FDI muscle (band-passed 25 Hz to 1 kHz, sampled at 2 Hz). The signal was processed via Signal 4 and Microw1401 mkII (Cambridge Electronic Design) and displayed on a monitor. A 70 mm Magstim (Magstim Company) standard double coil was connected to a rapid rate magnetic Magstim stimulator. The coil was kept in position (right M1 or vertex) using a frameless stereotactic brain navigation system (Brainsight, Rogue Research) and each subject’s MRI. To elicit equivalent disruption of manual performance during reactivation trials as in the M1-stimulated group, subjects receiving vertex stimulation also received 1 Hz ulnar nerve stimulation at the left wrist during the nine memory reactivation trials.

Imaging Data Acquisition
Each resting-state scan was 5 min long. Subjects were instructed to lie still inside the scanner, awake with their eyes closed (Tambini et al., 2010). Scanning was performed on a 3T MRI scanner (GE Excite HDx, with a standard head coil, T1-weighted high-resolution (1 × 1 × 1 mm, MPRAGE) sequence) anatomical images were acquired for each subject to allow for volume-based statistical analysis and neuronavigation of the TMS coil. BOLD signal was obtained with a gradient-echo echo-planar imaging sequence (EPI, repetition time = 2,000 ms; echo time = 35 ms; flip angle = 90°; matrix size 64 × 64; field of view 240 × 240; 3.00 × 3.00 × 3.75 mm3 resolution). The scanned volume included 34 axial slices of 4 mm thickness (including a 0.25 mm interslice gap). The first six volumes of functional images were discarded to ensure that the experimental data were acquired after the scanner reached steady-state magnetization (resulting in 150 time points for analysis).

Data Analysis
The imaging data analyzed with BrainVoyager QX (Brain Innovation) was preprocessed. This procedure involved the correction of movement artifacts, high-pass filtering to remove low-frequency artifacts (up to five cycles per experiment, resulting in a cutoff of 0.01 Hz; Tambini et al., 2010), high-frequency fluctuations removal by a 4 s full-width at half-maximum (FWHM) Gaussian kernel (0.1 Hz) (Meindl et al., 2010), and spatial smoothing using a Gaussian kernel of 4 mm FWHM. The functional images were then superimposed on 2D anatomical images and incorporated into the 3D data sets through trilinear interpolation. The complete data set was transformed into Talairach space (Talairach and Tournoux, 1988). Reference time courses were extracted from right M1 and dorsal striatum regions of interest (ROIs), which were localized using a baseline measurement of tapping versus rest BOLD contrast (for details see Table S1; Figure 3A; M1 center of mass Talairach coordinates x = 32, y = −27, z = 47; dorsal striatum x = 20, y = 1, z = 3; Figure 4A; center cerebellar ROI x = −18, y = −50, z = −26; 1,000 voxels per ROI). Correlations between these reference time courses were then calculated for each subject. Only significant correlations were considered for further analysis (p < 0.05 resulting in r > 0.135 for a sample size of n = 150 time points, which resulted in distributions requiring nonparametric between-group correlations comparisons [Howell, 1997]). Accordingly, data from 17/20 subjects in whom there were at least three significant resting-state correlation measurements were included for further behavioral and corticostral imaging analysis. Nine subjects were in the interference group and eight in the control group, in all cases, at least three significant correlations per subject were present in the cerebellar-cortical analysis.
**SUPPLEMENTAL INFORMATION**

Supplemental Information includes three figures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.10.042.

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